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Intraspecific Variation in Mercury Contamination of Alligator Snapping Turtles (*Macrochelys temminckii*)

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Abstract: *Macrochelys temminckii* (alligator snapping turtle) is an aquatic turtle endemic to the southeastern United States that was proposed for listing under the Endangered Species Act in 2021. In the present study we analyzed total mercury (THg) concentrations in skeletal muscle, tail clips, and nail tissue of 93 *M. temminckii* sampled from 14 waterbodies in eastern Texas (USA). Our objectives were to assess (1) the degree of correlation between internal tissue (skeletal muscle and tail clip samples) and keratin (nail samples), (2) the influence of ecological factors (turtle size and waterbody/sampling site) on THg concentrations, and (3) whether THg concentrations were high enough to pose a risk to human consumers. The mean (±SE) THg concentrations of muscle and nail were 1.16 ± 0.08 μg/g dry weight and 4.21 ± 0.24 μg/g dry weight, respectively, and THg concentrations were highly dependent on the sampling site. The THg concentrations of nails were correlated with muscle concentrations ($R^2 = 0.56$, $p < 0.001$). The effect of body size on THg concentrations varied by sampling site, indicating that size is not a good predictor of Hg concentration across sites. Finally, THg concentrations in *M. temminckii* of eastern Texas were high enough to pose a potential risk to human health based on US Environmental Protection Agency dietary guidelines. *Environ Toxicol Chem* 2024;43:1903–1913. © 2024 SETAC. This article has been contributed to by U.S. Government employees and their work is in the public domain in the USA.

Keywords: Bioaccumulation; Body size; Heavy metals; Reptiles; Site-specific conditions; Wildlife toxicology

INTRODUCTION

Of the anthropogenic contaminants in aquatic systems, mercury (Hg) is particularly noteworthy due to its pervasive occurrence across the globe (Chen & Driscoll, 2018) and its propensity to contaminate and accumulate in biota (Rodrigues et al., 2019). At high concentrations, Hg is teratogenic, embryotoxic, and neurotoxic to exposed animals (Hopkins et al., 2013a; Wiener et al., 2003), making it a contaminant of significant epidemiological and ecological concern (Chen & Driscoll, 2018). The majority of Hg currently circulating in the environment was released by anthropogenic activities that have occurred since the 1500s (Streets et al., 2019; UN Environment Programme [UNEP], 2019). Following deposition of Hg from the atmosphere, anaerobic bacteria in aquatic systems methylate inorganic Hg into an organic form (methylmercury [MeHg]; Bishop et al., 2020; UNEP, 2019). Methylmercury concentrates in bacteria and algae, and consumers are exposed through their diet. Methylmercury is capable of bioaccumulating within individual consumers as they age and biomagnifying in food webs, reaching high concentrations in high-trophic-level organisms (Chumchal & Hambright, 2009; Yoshino et al., 2020).

Compared with other vertebrate taxa, our understanding of Hg contamination in aquatic reptiles (e.g., turtles) is relatively limited (Haskins et al., 2020; Rainwater et al., 2005; Schneider et al., 2013). Most turtles are omnivorous, and species at higher trophic positions have high total Hg (THg) concentrations (Bergeron et al., 2007). Within species, larger turtles can have relatively high THg concentrations (Hopkins et al., 2013b; Schneider et al., 2009), but this pattern is not always observed (Golet & Haines, 2001; Hopkins et al., 2013b; Schneider et al., 2010; Turnquist et al., 2011).

Macrochelys temminckii (alligator snapping turtle), the largest freshwater turtle in North America, is a relatively long-lived

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species (at least 70 years in captivity [Ernst & Lovich, 2009]) endemic to the southeastern United States. *Macrochelys temminckii* occupies a high trophic position and has a varied diet including piscivorous fishes (Denton et al., 2023; Elsey, 2006). *Macrochelys temminckii* is currently proposed for listing as a threatened species by the US Fish and Wildlife Service (Environmental Conservation Online System [ECOS], 2021), in part due to historical commercial harvests, contemporary recreational harvest pressure that is legal in some US states (i.e., Mississippi and Louisiana), and illegal poaching (ECOS, 2021). A closely related species, *Chelydra serpentina* (common snapping turtle) has been found to be contaminated with relatively high THg concentrations (e.g., a range of 0.05–32.29 μg/g dry wt muscle THg; Hopkins et al., 2013a), but Hg concentrations in *M. temminckii* muscle and keratin tissue have not been assessed.

We assessed THg concentrations in nail (i.e., keratin) and skeletal muscle tissue of 93 *M. temminckii* sampled from 14 waterbodies throughout eastern Texas (USA). Our objectives were to assess (1) the relationship between THg concentrations in nail tissue and THg concentrations in muscle, (2) the influence of body size and waterbody on the Hg concentrations of individual turtles, and (3) whether THg concentrations in turtle muscle were high enough to pose a risk to the humans that consume them.

METHODS

Study area and sampling

The present study was conducted in east Texas. The high forest cover, large number of impoundments, and wetland habitat of eastern Texas enhance Hg deposition, transport, and methylation (Drenner et al., 2013, 2022), and many waterbodies in the region have fish consumption advisories due to high Hg concentrations (Texas Parks and Wildlife, 2022).

Our efforts to acquire *M. temminckii* THg samples occurred from March through September 2021 within the major watersheds of eastern Texas, which included the San Jacinto, Trinity, Neches, Sabine, and Mississippi watersheds (Rosenbaum et al., 2023a, 2023b). The Mississippi watershed contains the Red, Sulphur, and Cypress watersheds, which confluence in Louisiana, while the other watersheds are contained in Texas and drain directly into the Gulf of Mexico (Figure 1). Within these watersheds, we surveyed for *M. temminckii* in 31 waterbodies of various habitat types including reservoirs, oxbows, sloughs, river mainstems, and tributaries. These sites were often directly connected to extensive bottomland and herbaceous wetland habitat conducive to Hg methylation (Drenner et al., 2013). We collected tissue from *M. temminckii* at all waterbodies where we detected the species ($n = 14$), with at least one waterbody representing each of the major watersheds surveyed (Figure 1 and Table 1). We assumed independence between all sampled waterbodies. The minimum distance between sampled locations was approximately 4 km and 9 river km. The mean linear home range of individual *M. temminckii* in Texas has been documented to be 575.4 m (Munscher et al., 2023), so we consider the probability of turtle movements between sampled waterbodies within the same watersheds to be negligible.

We deployed single-funnel, finger-throated, four-hoop traps (hoop diameter = 1.2 m; mesh size = 2.54 cm) to sample *M. temminckii*. Each trap was baited with fish held within a holding canister (constructed from 7.6- × 30.5-cm polyvinyl chloride pipe with 36 1.3-cm-diameter holes) suspended from the rear hoop of each trap. Site sampling efforts were standardized to 45 trap nights (15 hoop traps deployed for three consecutive nights). However, during seven site visits, logistical constraints (e.g., trap theft and flooding) resulted in fewer trap nights. We preferentially deployed traps upstream of aquatic structures, undercuts, or low-energy pools, because these areas provide microhabitat preferred by *M. temminckii* (Harrel et al., 1996). We checked traps and removed captured animals every morning (approximately every 24 h) during surveys.

For all captured turtles, we measured midline straight carapace length to the nearest 0.1 cm with tree calipers (sensu Method D of Iverson & Lewis, 2018), and we measured mass to the nearest 0.1 kg with a hanging digital scale. There are no reliable methods to age live *M. temminckii*, and growth rates among individuals can vary drastically (Rosenbaum et al., 2023a), so we did not attempt to estimate ages. We determined the sex of adults by examining the length of each turtle's tail from the posterior terminus of the plastron to the vent (plastron-to-vent length) relative to body size; adult males have a greater relative plastron-to-vent length than females. This dimorphism is not apparent in sexually immature individuals, so we categorized small individuals whose sex was unclear (all of which were less than 40 cm in carapace length) as juveniles. The species also exhibits male-biased sexual size dimorphism, with females and males in our study area averaging 42.8 and 51.6 cm in carapace length, respectively (Rosenbaum et al., 2023a). We did not consider sex as a covariate of THg concentration in analyses because we could not confirm the sex of small individuals, and the three resultant demographic categories (juveniles of undetermined sex, adult females, and adult males) were effectively three partially overlapping size classes. Carapace length was used as the measure for body size in statistical analysis.

For *M. temminckii* greater than 5 kg in body mass, we used a single-use 6-mm-diameter biopsy punch to collect a skeletal muscle sample from the ventrolateral aspect of the tail, posterior from the cloacal aperture (Dutton & Balazs, 1995; St. Andrews et al., 2021). Acquiring muscle required rotation of the biopsy punch through dermal and adipose tissue. After removing superficial tissue and reaching muscle, the punch and isopropanol-sterilized forceps were used to separate and remove a small muscle sample. Thick adipose deposits prevented muscle sampling from two large adult males. Average muscle sample wet mass was 108.9 ± 4.2 mg (mean ± standard error [SE]; $n = 85$). The tails of individuals less than 5 kg in body mass ($n = 6$) were too narrow in diameter to allow biopsy of muscle; therefore, we removed the entire distal 0.5 cm of tail with a single-use biopsy punch. Average tail clip sample wet mass was 78.1 ± 16.1 mg (mean ± SE; $n = 6$). Wounds were immediately treated with povidone-iodine, and pressure was applied to stanch bleeding. Tail samples from smaller turtles were used as proxies for muscle tissue in statistical analysis, and

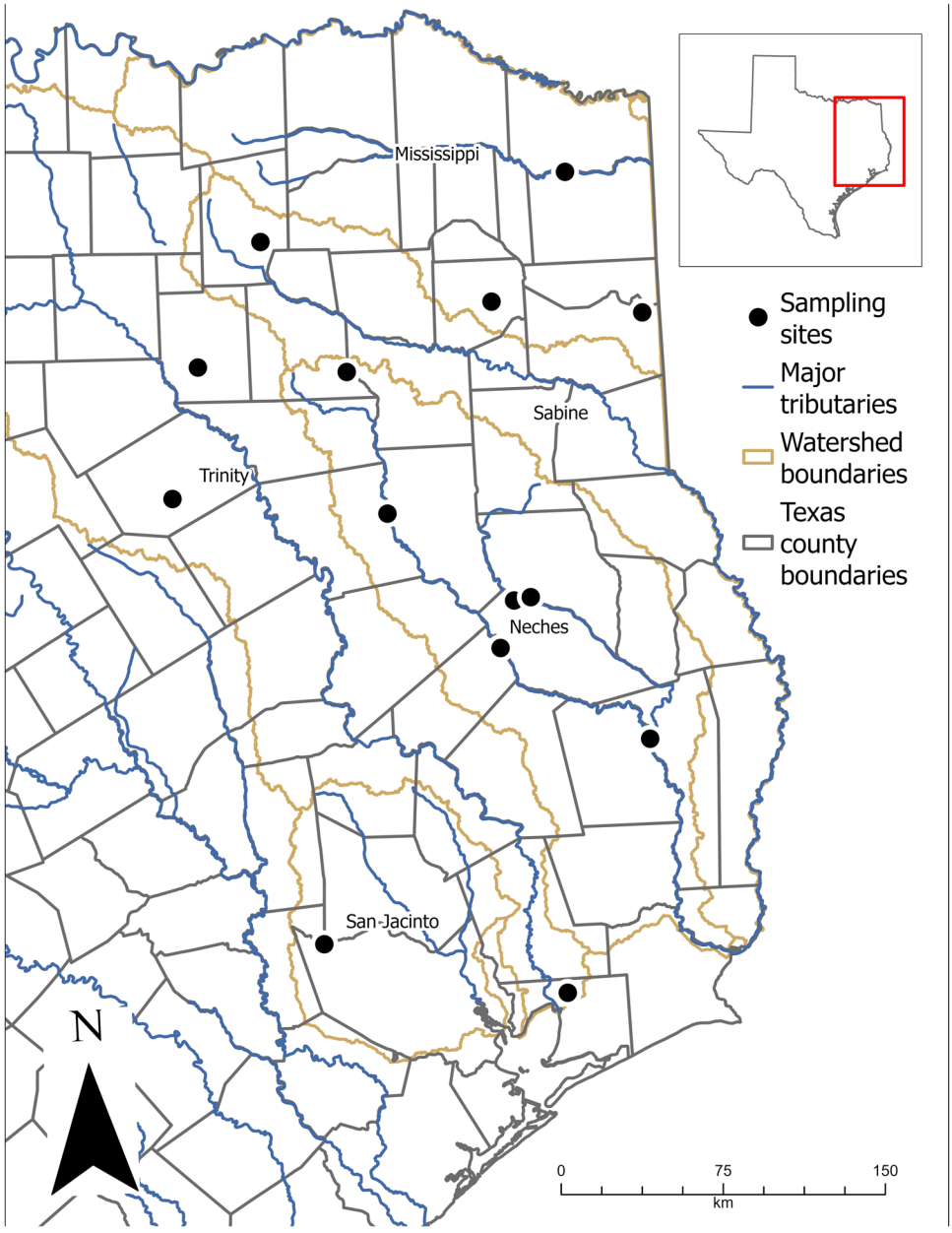

FIGURE 1: Approximate locations of 14 waterbodies in eastern Texas (USA), where *Macrochelys temminckii* (alligator snapping turtle) tissue was sampled in the present study. All the watersheds that the species is confirmed to occupy in Texas, which are labeled on the map, were sampled.

further references to muscle in the present study refer to both muscle biopsies and tail clips unless otherwise specified. After sampling internal tissue from individuals, we sampled two–three distal nail clippings from the hind left limb of turtles using isopropanol-sterilized wire cutters. Nails of *M. temminckii* increase in length and circumference with body size, so to ensure adequate sample material, we sampled more nails from small turtles to compensate for the lower mass of each nail. Fresh mass of nail clippings from each turtle was 82.9 ± 5.1 mg (mean ± SE; $n = 91$).

All sampled turtles ($n = 93$) were released at their points of capture. Field work, animal handling, and sample collection complied with a Texas Parks and Wildlife Department Scientific Permit for Research (SPR-0519-087) and Stephen F. Austin State University Institutional Animal Care and Use Committee project approval number 2019-11.

All field samples were stored on ice, and then transported to the laboratory at Stephen F. Austin State University, where they were stored in a −20 °C freezer until further processing. In the laboratory, we recorded the wet weight of all samples to the nearest 0.1 mg. These samples were rinsed with deionized water to remove any sediment or particulate residue that remained from field collection. All samples were then dried at 60 °C for 48 h, after which dry weights were recorded, which enabled calculation of percentage water within each sample. On average, *M. temminckii* muscle and tail samples consisted of 72.6% and 53.2% water weight, respectively, whereas nail averaged 22.0% water.

TABLE 1: Number of *Macrochelys temminckii* captured by hoop trap sampling from 14 sites across eastern Texas in 2021

Watershed	County	Waterbody type	Dates of sampling	Trap nights	No. of *M. temminckii* captured	No. of samples (muscle, nail) analyzed
Mississippi	Cass	Slough	July 16–19	45	3	3, 3
	Harrison	Stream	April 2–5	45	5	5, 5
	Upshur	Stream	July 26–29	45	2	2, 2
Neches	Angelina	River	March 5–8	45	1	1, 1
	Cherokee	Slough	May 12–15	45	15	15, 14
	Jasper	Slough	May 11–14	45	5	4, 5
	Nacogdoches	Stream	September 11–12	15	5	5, 5
	Trinity	Reservoir	July 6–9	45	8	8, 8
	Van Zandt	Stream	June 21–24	45	3	3, 3
Sabine	Hunt	River	June 11–14	45	16	15, 16
San Jacinto	Waller/Harris	Stream	June 2–4	38	9	9, 9
Trinity	Kaufman	Stream	June 7–10	45	1	1, 1
	Chambers	River	May 8–10	45	11	11, 10
	Navarro	River	July 11–14	45	10	9, 9

All five major watersheds in the state known to be occupied by the species were represented in samples. Surveyed sites are identified by county and type of waterbody. Sampling effort, denoted by the number of trap nights, is a product of the number of traps at each site and the number of nights they were deployed. Samples were not successfully obtained and analyzed from all captured individuals; final counts of the number of samples of each tissue type are noted in the last column.

Mercury analysis

We analyzed tissues of *M. temminckii* for THg concentrations using a direct mercury analyzer (DMA-80; Milestone) at Texas Christian University (Ft. Worth, TX, USA). This instrument thermally decomposes samples, and Hg in the resulting gas is collected via gold amalgamation and quantified with atomic absorption spectroscopy using US Environmental Protection Agency method 7473 [USEPA], 1998). Quality assurance included analysis of blanks, certified reference materials (CRMs), and duplicate samples at regular intervals. We used National Research Council of Canada DORM-4 (fish protein) and PACS-2 (marine sediment) as CRMs for samples with relatively low and high concentrations of THg, respectively. The mean (±SE) THg percentage recovery of DORM-4 references was 92.6% ± 2.76% ($n = 8$). The THg percentage recovery of PACS-2 references was 99.9% ± 3.64% ($n = 21$). Blank samples analyzed with muscle samples contained 0.47 ± 0.09 ng THg with a range of 0.08 to 0.72 ng ($n = 6$), and those analyzed with nail samples contained 0.31 ± 0.10 ng THg with a range of 0.07 to 1.02 ng ($n = 9$). All measures of the amount of THg within tissue and reference samples were above values measured in blank samples. The mean (±SE) percentage difference between duplicate *M. temminckii* muscle and tail samples was 40.3% ± 10.6% (range 0.07%–85.4%; $n = 8$). The mean (±SE) percentage difference between duplicate *M. temminckii* nail samples was 5.74% ± 1.79% (range 1.46%–12.7%; $n = 7$). Discrepant duplicate concentrations exhibited by *M. temminckii* muscle samples are likely attributable to differences in lipid concentrations between duplicates, because samples were not homogenized prior to THg analysis.

Statistical analyses

We calculated summary statistics for THg_{dw} (dry wt) and THg_{ww} (wet wt) concentrations within each sample type. Tail clips and muscle samples were pooled for summary statistics because they were used in conjunction in subsequent analysis to correlate internal concentrations with nail concentrations, but stand-alone mean values of the sample types were also calculated (Table 2). To assess the degree to which nail samples were capable of predicting muscle THg concentrations in turtles, we regressed *M. temminckii* nail THg_{dw} concentrations against respective muscle or tail clip THg_{dw} concentrations. Nail THg_{dw} concentration was used as the dependent variable in subsequent analyses because associated duplicate samples had a lower percentage difference, indicating that a greater proportion of residual error in modeling efforts using nail may be assumed to be attributable to ecological factors, as opposed to variability in lipid concentrations of muscle samples.

We conducted linear mixed effects regression with body size (i.e., carapace length) of individuals as a fixed predictor of their nail THg_{dw} concentrations. Prior to modeling, the variable carapace length was standardized to a mean of 0 and standard deviation of 1. To account for the lack of independence

TABLE 2: Summary statistics (mean and standard error [SE]) of dry weight (dw) and wet weight (ww) total mercury (THg) concentrations of skeletal muscle, distal tail clip, and nail clip samples obtained from *Macrochelys temminckii* (alligator snapping turtle) sampled from eastern Texas, USA, in 2021

	Muscle	Tail	Muscle and tail	Nail
Sample size	85	6	91	91
THg μg/g dw (mean ± SE)	1.201 ± 0.078	0.580 ± 0.161	1.160 ± 0.075	4.212 ± 0.244
Range (dw)	0.147–4.011	0.148–1.254	0.147–4.011	0.228–15.615
THg μg/g ww (mean ± SE)	0.315 ± 0.018	0.265 ± 0.070	0.312 ± 0.017	3.266 ± 0.197
Range (ww)	0.033–0.910	0.073–0.556	0.033–0.910	0.142–12.760

Tail and muscle tissues were both combined to be used as a predictor variable of nail THg concentration in regression analysis.

between individuals sampled at the same site and to represent the hypothesis that THg concentrations are site dependent, we incorporated random intercepts for each of the 14 sampled sites. This model is expressed as:

$$THg_{ij} = \beta_{0j} + \beta_1 \text{body size}_{ij} + \varepsilon_{ij}$$

$$\beta_{0j} = \gamma_{00} + \mu_{0j}$$

where the dependent variable THg concentration of individual *i* sampled from site *j* is a function of the site *j*-specific intercept β_0 and body size of individual *i*; γ_{00} is the fixed intercept averaged across all sites, and μ_{0j} is the difference in the average THg concentration at site *j* from the fixed average. We fitted a model that also incorporated random slopes (i.e., a maximal model) to represent the hypothesis that the relationship between body size and *M. temminckii* THg concentrations varied due to site-specific factors. This model may be written as:

$$THg_{ij} = \beta_{0j} + \beta_{1j} \text{body size}_{ij} + \varepsilon_{ij}$$

$$\beta_{0j} = \gamma_{00} + \mu_{0j}$$

$$\beta_{1j} = \gamma_{10} + \mu_{1j}$$

where the effect of body size of turtle *i* at site *j* on its THg concentration deviates from the global fixed slope γ_{10} by μ_{1j}. Lastly, to represent the null hypothesis that body size does not influence internal THg concentrations, we constructed a model that did not account for body size but allowed intercepts to vary by site. Maximum likelihood estimation was used to fit all models, and their relative goodness of fit was assessed using Akaike's Information Criterion (AIC), which enabled assessment of whether body size may influence THg concentrations and whether the body size–THg concentration relationship exhibits site-specific variation. The absolute goodness of fit for each model was assessed using marginal R^2 (i.e., the proportion of variation accounted for by fixed effects alone) and conditional R^2 (i.e., the proportion of variation accounted for by fixed and random effects; Nakagawa et al., 2017). Data were plotted and visually inspected to confirm that assumptions of linearity and normality were met. Statistical analyses were conducted using the software R Ver. 4.0.5 (R Core Team, 2022) and the package *lme4* (Bates et al., 2015).

To determine whether concentrations of THg present in *M. temminckii* muscle present a potential risk to the health of humans who consume the species, we compared average THg_{ww} concentrations in muscle samples with dietary consumption limits provided by the USEPA. To curtail the risk of dietary exposure, the USEPA (2022) recommends limiting consumption of food with average THg concentrations between 0.23 and 0.46 μg/g wet weight to one serving a week, and avoiding food with concentrations above this range.

RESULTS

Mean THg_{dw} concentrations were lower in muscle samples (1.16 ± 0.08 μg/g) than in nails (4.21 ± 0.24 μg/g), and both tissue types varied widely between waterbodies (Figure 2). Nail samples exhibited the greatest range in concentrations (Figure 2 and Table 2).

Muscle THg_{dw} concentrations were correlated with nail THg_{dw} concentrations ($R^2 = 0.56$; Figure 2). On average, nail samples contained 3.63 times more THg by concentration than muscle samples. The modeled relationship indicated that on average, every 4.33 μg/g increase in nail THg concentration corresponds to a 1 μg/g increase in muscle THg concentration (Figure 3).

The relationship between carapace length and nail THg_{dw} concentration varied widely by waterbody/sampling site, as did average per-site THg_{dw} concentrations. The model accounting

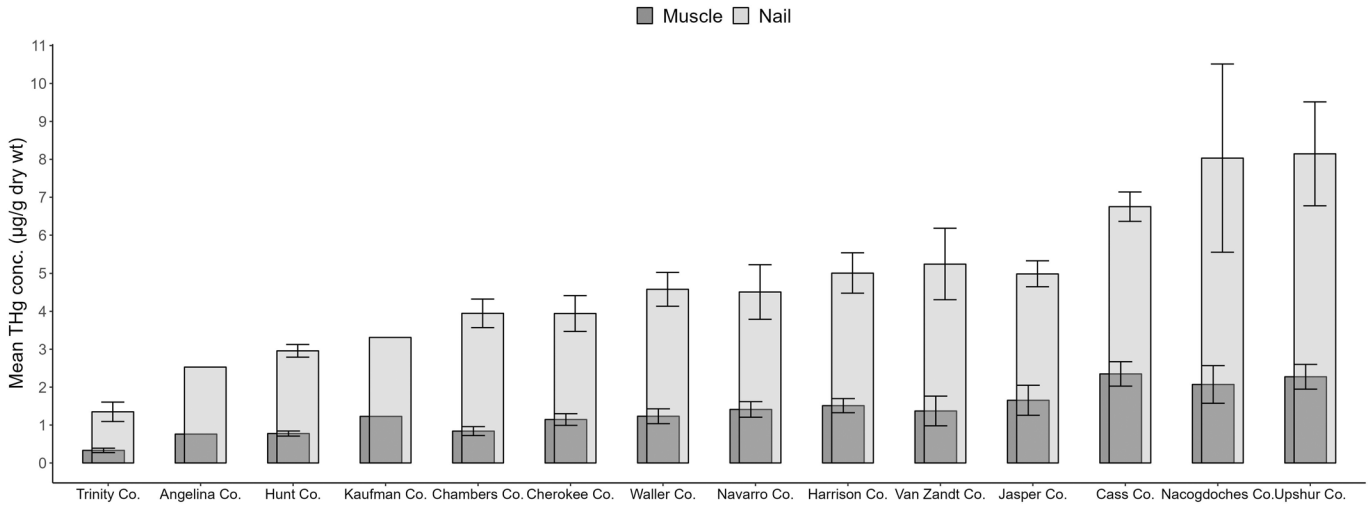

FIGURE 2: Mean dry weight total mercury concentrations (THg) in *Macrochelys temminckii* (alligator snapping turtle) nail and muscle tissue sampled from 14 locations in eastern Texas (USA). Sites listed along the *x*-axis are ordered by increasing mean nail THg concentrations. Error bars depict standard errors of the means, and are not depicted at sites where only one turtle was sampled (Angelina and Kaufman Counties). Nail samples exhibited higher concentrations than muscle and also a wider range in concentrations.

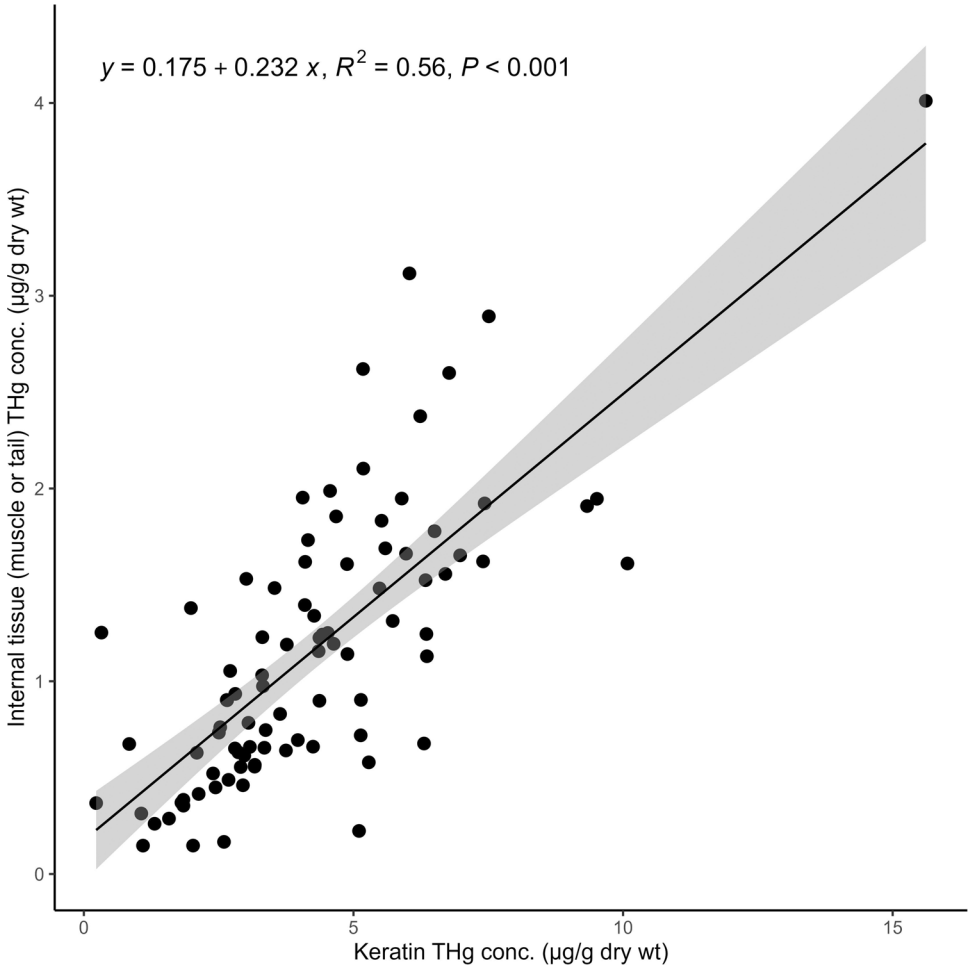

FIGURE 3: Linear regression of nail (i.e., keratin; *x*-axis) total mercury (THg) concentration as a predictor of skeletal muscle and tail clip THg concentration (*y*-axis) of *Macrochelys temminckii* (alligator snapping turtle; $n = 89$ individuals) sampled from eastern Texas (USA) in 2021. The 95% confidence intervals of the intercept and coefficient were −0.0367–0.387 and 0.188–0.275, respectively.

for these sources of variation was the most parsimonious and had the best fit, as determined by AIC (Figure 4 and Table 3). Although the overall relationship between body size and THg concentrations across sites was positive, size alone was a poor predictor of an individual turtle's THg concentration, as indicated by the 95% confidence intervals of the parameter estimate overlapping zero (Table 3). Body size accounted for 8% of the variation in THg concentrations in the best-fit model, whereas body size and random site effects together explained 73% of the variation (conditional R^2; Table 3). The high proportion of variation accounted for by the random site effect indicates that most variation in *M. temminckii* THg concentrations is attributable to factors that vary across sites (e.g., environmental variables) rather than factors that vary across turtles within each site. Body size of *M. temminckii* exhibited a greater positive effect on THg concentrations at sites with higher average THg concentrations, as indicated by the high correlation (95%) between random slopes and intercepts.

The *M. temminckii* mean muscle THg concentration of large individuals (i.e., excluding tail clip samples from small individuals that are unlikely to be consumed by humans) was 0.32 ± 0.02 µg/g, placing the tissue within the range of food concentrations at which the USEPA recommends limiting consumption to one 113 g serving a week (Table 2). People who consume *M. temminckii* at greater rates than this are at risk of deleterious chronic health effects. Nineteen percent of turtles from which muscle samples were analyzed exhibited muscle concentrations greater than 0.46 µg/g wet weight, the average value above which the USEPA (2022) recommends avoiding all consumption to avoid health risk.

DISCUSSION

Because we collected both keratin and muscle samples from individuals, we were able to assess the relationship between THg concentrations in the two tissue types. Sampling keratin is a less invasive procedure than muscle biopsy, and has the potential to serve as a predictor of muscle concentrations of individuals (Day et al., 2005). The results of the present study suggest that inferring concentrations of THg in *M. temminckii* skeletal muscle, and therefore the potential risk of human Hg exposure from consuming it (USEPA, 2022), is

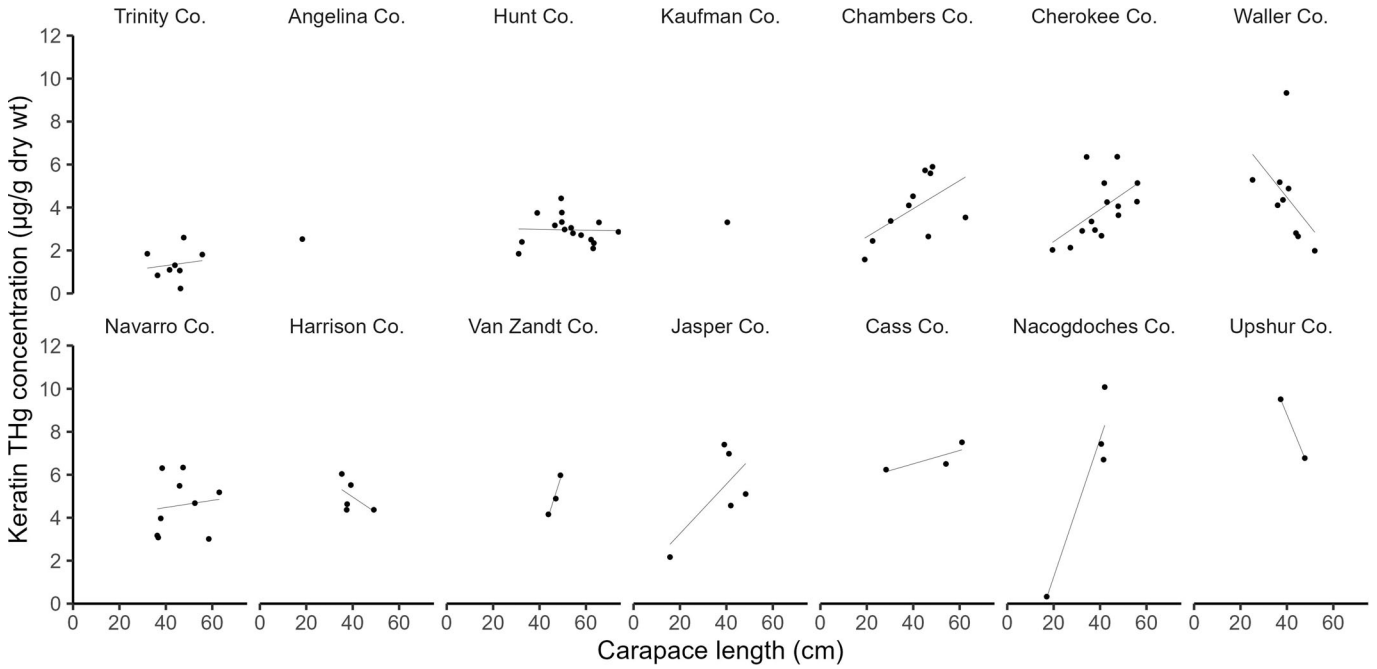

FIGURE 4: Relationships between nail (i.e., keratin) total mercury (THg) concentrations and carapace length (a measure of body size) in *Macrochelys temminckii* (alligator snapping turtles) sampled at 14 aquatic waterbodies (labeled by county) throughout eastern Texas, (USA). Linear regression slopes are depicted for sites where >1 individual was sampled, and sites are ordered by increasing mean THg values. A linear model incorporating random intercepts and slopes by site revealed a high random effects correlation of 0.95, indicating that as average THg concentrations at a site increase, the positive relationship between body size and concentrations also increases. Note that one outlying sample from Nacogdoches County, with a nail concentration of 15.615 µg/g and a carapace length of 45.9 cm, is outside the *y*-axis bounds and is not depicted.

possible using nail samples. However, our results indicated that estimates of *M. temminckii* muscle THg concentrations using nail tissue concentrations would grant a moderate degree of uncertainty. Previous studies have indicated a variation in the nature of the relationship between the two tissue types. For instance, Eggins et al. (2015) reported that muscle THg concentration explained almost all the variation in scute keratin THg concentration in a sample of five *Podocnmenis expansa* (Arrau turtles). Similarly, Hopkins et al. (2013b) reported a high correlation between *C. serpentina* muscle and nail keratin (R^2 values of 0.90 and 0.85, respectively). In *C. serpentina* from various waters throughout New York State (USA), keratin THg concentrations in scute samples accounted for 40% of the variation of concentrations in muscle (Turnquist et al., 2011). Variability in the relationship across studies may result from physiological differences between individual turtles, as well as environmental factors (e.g., differences between sites), laboratory error, and instrument error (Day et al., 2005). Our duplicate samples suggest greater uncertainty in estimated muscle concentrations relative to those in nail, contributing to greater residual error. Given our model, a turtle with a nail THg_{dw} concentration of 3.00 µg/g would be predicted to have a muscle THg_{dw} concentration of 0.870 µg/g, yet the 95% prediction interval ranged from 0 to 1.835 µg/g dry weight. Nevertheless, concentrations in these two types of tissue are positively correlated, and nail concentrations serve as reasonable and effective indicators of average internal THg burdens in the species that could be utilized in studies seeking to minimize stress on live turtles.

Body size alone is insufficient to predict THg concentrations in tissues of *M. temminckii*. Instead, the waterbody of origin is likely important in determining both the species' THg concentrations and the degree to which individuals will accumulate the metal as they grow. Across sites in eastern Texas within areas exhibiting relatively high average *M. temminckii* THg concentrations, larger *M. temminckii* exhibited higher concentrations of THg relative to smaller ones. This indicates that in waters with relatively high levels of bioavailable environmental Hg (i.e., MeHg), the risk of bioaccumulation with size, and potentially age, is greater. These findings are corroborated by studies that have found THg burdens in *C. serpentina* to vary across locations, and that effects of body size are dependent on location and background THg levels (Hopkins et al., 2013b; Turnquist et al., 2011). In a Hg-contaminated stretch of the South River, Virginia (USA), average *C. serpentina* muscle THg concentrations were greater than those from uncontaminated sites (Hopkins et al., 2013b; Landler et al., 2017). Furthermore, there was a significant, positive relationship between body size and muscle THg concentrations at the contaminated site, but in two reference sites, the relationship was not significant (Hopkins et al., 2013b).

Lack of correlation between body size and THg levels has been widely observed in many turtle species. In many regions, body size is not predictive of THg concentrations in *C. serpentina* (Golet & Haines, 2001; Helwig & Hora, 1983; Hopkins et al., 2013b). Similarly, concentrations in six species of turtles in South America were uncorrelated with size (Eggins et al., 2015; Schneider et al., 2010). The methods of Golet & Haines (2001) and Schneider et al. (2010) involved sampling

TABLE 3: Akaike's information criterion (AIC) values for each considered model of *Macrochelys temminckii* nail THg concentrations, with fixed effect parameter estimates and random effect variance estimates

Model	AIC	ΔAIC	Random intercept variance	Residual error	β_0	$\beta_{\text{scaled body size}}$	Random slope variance	Marginal R^2 (%)	Conditional R^2 (%)
Site-specific intercepts; site-specific slopes	376.2	0	4.03	2.16	4.97 (3.73–6.24)	0.81 (–0.29–1.61)	1.27	8.2	73.3
Site-specific intercept; fixed slope	391.2	15	2.81	3.01	4.73 (3.68–5.82)	0.57 (0.16–0.97)		5.3	51.0
Site-specific intercepts	396.5	20.3	2.51	3.35	4.64 (3.62–5.69)			0	42.8

Models are ranked by their relative fit, with differences in AIC (ΔAIC) in secondary models relative to the best-fit one provided. See *Methods* for explanation of each model's structure. The 95% confidence intervals are provided in parentheses for fixed-parameter estimates. The maximal model provided the best relative and absolute fit and minimized residual variance, suggesting that the relationship between body size and THg concentrations in *Macrochelys temminckii* (alligator snapping turtle) depends on local conditions and is not necessarily positive. The low marginal R^2 estimates in all models indicate that fixed effects account for little variation in THg; the high conditional R^2 estimate indicates that most unaccounted-for variation in the data set is between sampled sites rather than within sampled sites. The random intercept variance in the maximal model also indicated high variation in average THg concentrations across sites.

individuals from multiple waters, but did not consider site of origin when assessing effects of body size. Our findings underscore the importance of accounting for variation in concentration across multiple locations, where exposure rates may differ and may cause site-specific effects to confound the influence of size (MacCrimmon et al., 1983).

The inconsistency in the relationship between body size and THg concentrations observed in the present study and other studies may have resulted from variation in growth rate or ontogenetic dietary shifts in *M. temminckii* between sites. Positive relationships between body size and THg concentrations may be caused by the greater total exposure times experienced by older individuals (Kannan et al., 1998), which are often large in size relative to younger individuals. This process would reveal a stronger body size–THg concentration relationship in taxa that continue to grow throughout their lives (e.g., some turtles; Congdon et al., 2013) and therefore would exhibit a strong correlation between size and age. However, individual *M. temminckii* exhibit wide variations in growth rates (Rosenbaum et al., 2023a; Trauth et al., 2016), which could result in size being a poor proxy of age-related bioaccumulation. Additionally, organismal THg concentrations may be influenced by ontogenetic dietary shifts (Brasso et al., 2014), which can plausibly result in nonlinear relationships between THg concentrations and size or age. Larger individuals of some species have a propensity to consume prey from higher trophic levels, and therefore are more prone to bioaccumulation (Mathers & Johansen, 2011). Other species maintain a constant diet regardless of size, and in turn size classes do not vary significantly in THg concentration (Desta et al., 2007). The variation in the body size–THg concentration relationship exhibited by *M. temminckii* across sites in Texas may result from differences in ontogenetic dietary shifts among populations corresponding with variations in local food sources. Future studies should assess ontogenetic dietary shifts and their potential impact on Hg accumulation across multiple populations of *Macrochelys*.

Despite body size alone being a poor predictor of THg concentrations in *M. temminckii*, allowing its relationship with THg concentration to vary by site accounted for approximately 20% of within-site variation (Table 3). Therefore, of the intrinsic features of *M. temminckii* one could measure, body size is a reasonable predictor. Other factors exerting influence on the concentrations of a given individual may be attributable to aspects of the external environment, such as the potential to methylate Hg, available food resources, and land cover within the watershed (Chumchal & Hambright, 2009; Wiener et al., 2003). Subsequent studies that take place across multiple locales and that aim to elucidate factors influencing THg loads of *M. temminckii* should examine environmental features that vary across sampling locations.

In highly contaminated areas, *C. serpentina* accumulates high muscle THg concentrations (e.g., 15.6 μg/g [Landler et al., 2017] and 32.3 μg/g [Hopkins et al., 2013b]) that are negatively associated with fecundity and positively associated with aberrant hatchling behavior (Hopkins et al., 2013a; Landler et al., 2017). None of the *M. temminckii*

sampled in eastern Texas exhibited concentrations as high as those observed in *C. serpentina* that inhabited ecosystems with point sources of Hg. However, assuming that *M. temminckii* Hg accumulation results in deleterious effects at magnitudes similar to those observed in *C. serpentina*, populations of *M. temminckii* exposed to waters contaminated with high levels of Hg may be at risk of deleterious reproductive and developmental effects.

The results of the present study suggest there are health risks to humans who frequently consume *M. temminckii* in eastern Texas. These risks are greatest to children and women of child-bearing age whose diets may expose their offspring in utero (Bose-O'Reilly et al., 2010). As of 2022, Mississippi and Louisiana were the only states in the native range of *M. temminckii* that allow its legal harvest. In Mississippi, a take of one *M. temminckii* a year is permitted (Mississippi Department of Wildlife, Fisheries, and Parks, 2022). Abiding by this regulation should keep consumers below the USEPA-recommended limit, assuming that average concentrations in Mississippi are similar to those observed in Texas. However, in Louisiana, one may legally harvest one turtle a day (Louisiana Department of Wildlife and Fisheries, 2021), and given the concomitant potential for consumption every day, those who regularly supplement their diet within the harvest limits may be exposed to deleterious levels of THg. Illegal harvest for personal subsistence and purchases of *M. temminckii* meat from the black market (Scott & Eliopoulos, 2017), as well as regular subsistence on other aquatic organisms (Chang et al., 2003) provide further opportunities for dietary exposure to THg above the consumption limit recommendations.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5888.

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Data Availability Statement—Data acquired in the study and the R code used for statistical analyses are provided in the Supporting Information.

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