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Mercury Exposure is Associated with Negative Effects on Turtle Reproduction

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ABSTRACT: Mercury (Hg), a ubiquitous and highly toxic bioaccumulative contaminant, can maternally transfer and elicit deleterious effects on adult reproduction and offspring phenotype in fish, amphibians, and birds. However, the effects of Hg on reproduction remain largely unstudied in reptiles. We evaluated the consequences of maternally transferred Hg on a long-lived aquatic omnivore, the common snapping turtle (*Chelydra serpentina*). We collected eggs and tissues from gravid female turtles along a broad Hg contamination gradient in a river in central Virginia. We incubated eggs in the laboratory, quantified embryonic mortality, infertility, and hatching success of each clutch, and assessed all hatchlings and dead embryos for gross morphological malformations. As predicted, Hg concentrations in eggs were strongly and positively correlated with Hg levels in female tissues. We found that Hg in eggs was negatively correlated with hatching success, and this effect was driven by both increased egg infertility and embryonic mortality. In comparison to previous effect-based studies on other amniotes, our findings suggest that *C. serpentina* may be more resilient to Hg exposure and perhaps better suited for long-term monitoring of bioavailability of Hg than as indicators of adverse effects.

INTRODUCTION

Mercury (Hg), a ubiquitous heavy metal contaminant, can be maternally transferred from female to offspring and has received significant attention due to its widespread prevalence, bioaccumulative and biomagnifiying properties, and known toxicity in humans and wildlife.[1−4] The effects of Hg on reproduction and offspring phenotype have recently been documented in several oviparous species.[5−8] For example, female American toads (*Bufo americanus*) collected from a Hg contaminated site transferred approximately 5% of their Hg body burden to their offspring which experienced reduced hatching success.[4,9] Likewise, Hg exposure was associated with reduced female reproductive output and lower fledging success in Tree Swallows (*Tachycineta bicolor*).[8] Along with reductions in offspring viability, maternally transferred Hg has also been associated with a variety of deleterious sublethal effects in offspring. For instance, toad larvae previously exposed to maternally transferred Hg exhibited latent sublethal effects, including reduced body size, impaired larval swimming performance, and increased time to metamorphic climax.[5,6] In fish, Alvarez et al.[10] reported adverse effects on behavioral performance in Atlantic croaker (*Micropogonias undulates*) larvae hatched from eggs laid by Hg-exposed adults. While the effects of maternally transferred Hg have been demonstrated in several vertebrate species, nothing is known about the reproductive effects of Hg in turtles.

Turtles are commonly used to monitor Hg exposure in contaminated areas because of their ecological and life-history attributes.[11−14] Specifically, snapping turtles (*Chelydra serpentina*) serve as excellent model organisms because they are long-lived (exceeding 50 years) apex predators, making them highly susceptible to Hg bioaccumulation and biomagnification.[11,15] Unfortunately, most ecotoxicological studies kill turtles to sample target tissues and therefore lack the ability to relate accumulated toxicant concentrations to the physiological effects that may arise from such exposure. With many turtle populations declining,[16,17] sacrificing adults and their eggs for sampling is neither sustainable nor conservation-minded, and nondestructive methods should be implemented.[18] A study has yet to establish mathematical correlations between less invasive samples in turtles (e.g., nail and blood) to concentrations that can be accumulated in turtle tissues (e.g., muscle and eggs) that are often relevant to overall health, reproductive success, and transgenerational effects attributable to maternal transfer.[18]

In this study, we used common snapping turtles (*C. serpentina*) inhabiting a river historically contaminated with Hg to investigate whether Hg exposure can affect turtle reproduction. A recent study found that muscle Hg concentrations of turtles inhabiting contaminated portions of the same river used in this study were on average 72-fold higher than those of turtles collected from nearby reference sites.[19]

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Based on these findings and the known deleterious reproductive effects of Hg on other wildlife, we hypothesized that exposure to excessive Hg concentrations would negatively correlate with turtle reproduction and development. Specifically, we predicted that Hg exposure would negatively correlate with clutch characteristics (i.e., clutch size, egg mass, clutch mass), and positively correlate with the frequency of malformations, embryonic mortality, and egg infertility. Additionally, we sought to develop nondestructive sampling techniques for assessing bioaccumulation and maternal transfer of Hg in female turtles so that future studies can be performed without sacrificing mature adults or viable eggs.

MATERIALS AND METHODS

Sample Collection Methods. From April-July 2010 and 2011, we collected gravid female snapping turtles at various locations upstream and downstream of a historic Hg point source on the South River, VA, and at a nearby uncontaminated reference river, using baited hoop traps (see Hopkins et al.[19] for additional information). Subsites sampled along the South and Middle Rivers are not independent of one another and we collectively defined them as SR-ref (South River reference), MR-ref (Middle River reference), and SR-cont (South River contaminated). In addition to the relatively small home ranges snapping turtles exhibit,[20] turtle movement between South River reference sites and the contaminated sites was limited by Rife Loth dam located directly upstream from the contamination source during this study. An analysis of surface water and sediment along the South River and the reference sites demonstrated Hg to be the primary contaminant of concern, with other common contaminants such as organochlorine pesticides, polycyclic aromatic hydrocarbons, and other trace metals to be present in very low or undetectable concentrations.[21]

We physically palpated all female turtles for the presence of shelled eggs and transported gravid females back to the field house. We weighed gravid females to the nearest 0.10 kg and placed them separately in egg laying chambers consisting of 378 L Rubbermaid tanks filled with ∼75 L of dechlorinated and deaminated water. We intraperitoneally injected gravid females with 40 mg/kg of oxytocin solution every 24 h for three consecutive days to induce egg laying. We removed deposited eggs within 3 h, weighed each egg to the nearest 0.01 g, measured egg length and width to the nearest 0.1 mm, and marked each egg according to female ID. Because some females may not deposit their entire clutch upon induction with oxytocin, we used radiographs taken at the Wildlife Center of Virginia, Waynesboro, VA to measure the number of withheld shelled eggs in order to determine true clutch size and estimate total clutch mass. We randomly selected three eggs per clutch to be frozen and later homogenized to determine egg Hg concentrations.[22] After oviposition, we measured carapace length, carapace width, and plastron length of each female to the nearest mm using calipers and individually marked turtles by filing three marginal scutes for future identification.[11] We removed 2−4 small (2−3 mm) nail samples from the tips of the left and right hind claws of each turtle using canine nail clippers and took a 1 mL blood sample from the caudal vein. Nail and blood samples were stored separately in 1.5 mL eppendorf tubes at −20 °C prior to Hg analyses. In order to determine accumulated Hg in turtle muscle tissue, we removed a small biopsy from the ventral-lateral portion of the tail following administration of a local anesthetic (Lidocane). We sutured the biopsy site with 2−3 stitches using clear Polydioxanone monofilament suture material ($^3/_8$ cm) and applied a topical antibiotic to reduce risk of infection. Following tissue sample collection, we released turtles at their point of capture.

Egg Incubation. We transported the remaining eggs to Virginia Tech where they were placed in plastic containers with vermiculite (1:1 water to vermiculite, grams), capped with a perforated lid, and set inside styrofoam hovabators (model no. 1602N; G.Q.F. Manufacturing Company, Savannah, GA). Within each incubator we placed Ibutton loggers (DS1923, Embedded Data Systems, KY) so that temperature and humidity levels could be continuously monitored and adjusted in order to achieve a target incubation temperature of 25 °C (producing all-male clutches[23]) and a relative humidity of 85%. Incubation temperature and relative humidity achieved for each incubator averaged 25.3 ± 0.1 °C and 86.0 ± 0.8%, respectively. Approximately every 2 weeks, we candled eggs to assess development. We removed dead embryos from the incubator, and dissected, staged according to Yntema[24] in development as early (1−30 days), middle (31−60 days), and late (61−90 days), and examined them for gross malformations according to Bell et al.[25] Once hatchlings started to pip, we placed a small perforated plastic cup over each egg so that hatchlings could be individually identified after emergence.

Mercury Analysis. We lyophilized and homogenized muscle and eggs and report THg (total mercury) concentrations of these tissues on a dry weight (dwt) basis. Whole blood from each turtle was homogenized using a vortex mixer and we report THg concentrations in blood on a wet weight (wwt) basis. We washed nail clippings by placing them in a sterilized tube with 10 mL solution of 15:1 deionized water to ethanol and sonicated them for 20 min. After sonication we discarded the solution and allowed nails to air-dry on a clean laboratory bench and report THg concentrations on a fresh weight basis (fwt). Percent moisture was 77.2 ± 0.19% (mean ±1 standard error of the mean hereafter) for muscle and 75.5 ± 0.18% for eggs. Muscle, blood, and egg samples were analyzed for THg at the College of William and Mary, Williamsburg, VA, by combustion-amalgamation-cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80, Milestone, Monroe, CT) according to U.S. Environmental Protection Agency (U.S. EPA) method 7473.[26] The equipment was calibrated using DOLT-4 dogfish liver, DORM-3 fish protein (National Research Council of Canada (NRCC), Ottawa, ON. For quality assurance, each group of 20 samples included a replicate, blank, and standard reference material (SRM; DOLT-4 dogfish liver, DORM-3 fish protein). Method detection limits (MDL; 3 times the standard deviation of procedural blanks) for samples averaged 0.0042 mg, and all samples had THg concentrations that exceeded the limit. Average relative percent differences (RPD) between replicate sample analyses were 8.38% ± 1.25% (n = 60). Mean percent recoveries of THg for the DOLT-4 and DORM-3 ranged from 99.77 ± 0.26% to 102.08 ± 0.36%, respectively.

Nail samples were analyzed by the Center for Environmental Sciences and Engineering, University of Connecticut. Samples were digested and analyzed using cold vapor atomic absorption spectrophotometry according to U.S Environmental Protection Agency (U.S. EPA) method 7473.[26] For quality assurance, we used control samples consisting of calibration verifications and blanks, spikes, duplicates, and standard reference material (SRM; DOLT-4 dogfish liver, DORM-3 fish protein). The limit of detection averaged 0.083 ppm (fwt) and all samples had

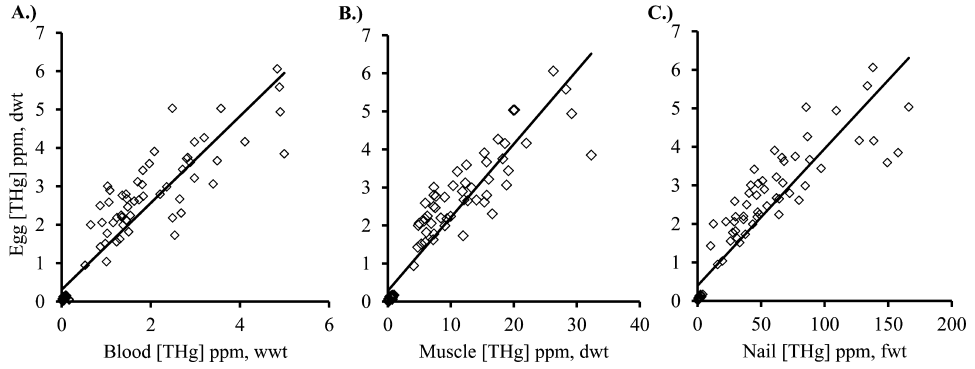

Figure 1. Among-tissue relationships between egg total mercury (THg; dry weight) and A.) blood (wet weight) THg ($y = 1.129x + 0.311$, $r = 0.92$, $p < 0.0001$, $n = 95$), B.) muscle (dry weight) THg ($y = 0.192x + 0.285$, $r = 0.93$, $p < 0.0001$, $n = 92$), and C.) nail (fresh weight) THg ($y = 0.035x + 0.391$, $r = 0.92$, $p < 0.001$, $n = 95$) of gravid female *Chelydra serpentina* collected from the South and Middle Rivers .

THg concentrations that exceeded this limit. Average RPD between replicate sample analyses were 0.5% ± 2.4% ($n = 13$). Mean percent recoveries of THg for the DOLT-4 and DORM-3 were 95.0 ± 1.6% and 94.2 ± 2.3%, respectively. Calibration verification and laboratory control sample recoveries of THg averaged 104.7 ± 0.6% and 103.7 ± 1.2%, respectively. Matrix spike recoveries averaged 108.2 ± 4.4%.

In order to determine the amount of THg that was in the more toxic form of MeHg, we analyzed homogenized egg samples representing 12 individual clutches for MeHg concentrations using liquid chromatography coupled to cold vapor atomic fluorescence spectrometry (Quicksilver Scientific[27]). A combination of blanks (3), SRM's (2: TORT-2, and DOLT-4), laboratory control samples, a matrix spike, and a sample duplicate were used for quality control. The limit of detection was $3.00E^{-7}$ mg kg^{-1} (dwt) for eggs and all samples had Hg concentrations that exceeded this limit. In all three blanks, MeHg and Hg(II) concentrations were $<5.00_E{}^{-5}$ mg kg^{-1}.Average RPD between replicate sample analyses was 9.55 ± 3.65% for Hg II and 3.05 ± 2.85% for MeHg. Percent recovery for HgII/MeHg for TORT-2, DOLT-4, and laboratory control samples were 106.6/109.8%, 96.0/91.3%, and 112.6/111.5%, respectively. Matrix spike recovery of HgII and MeHg was 112.6% and 109.7%, respectively.

Statistical Analysis. We used SAS 9.1 (SAS Institute, Inc., Cary, NC) or Microsoft Excel for all statistical analyses and assessed significance at $\alpha \leq 0.05$. When appropriate, THg concentrations were $\log_{10}$-transformed to improve normality and homoscedasticity. Initial models included all interactions between independent variables and covariates, but non-significant interactions were dropped from final models.

First, we examined relationships between THg concentrations in female blood, muscle, and nail tissues to THg concentrations in eggs using Pearson correlation coefficients. Additionally, we report linear regression equations between female and egg tissues so that future monitoring of female bioaccumulation and maternal transfer can be performed in a nondestructive manner. We examined the relationship between THg and %MeHg in eggs using linear regression. Because Hg can bioaccumulate in muscle tissue over an individuals' lifetime, we examined the relationship between THg concentrations in muscle tissue and body size at each site using analysis of covariance (ANCOVA), with site as the main effect and size (carapace length) as a covariate.

We examined the relationship between THg muscle concentrations and reproductive output using a multivariate generalized linear model for mixed distributions (PROC GLIMMIX), a procedure capable of modeling noncontinuous distributions. The model included clutch size, egg mass, and clutch mass as response variables and body size as a covariate.

Using SAS PROC GLIMMIX, we examined the relationship between egg THg and hatching success, malformation frequency, mortality during development, and infertility using clutch as the experimental unit. This analysis was conducted twice, using all clutches (sites combined) in one model and only individuals from SR-Cont in the other model. However, since these models explained little of the variation in the data set, we also present a more conservative analysis using the same mixed model approach, but with site as the main effect, rather than egg THg. Since all dead eggs were dissected and staged during development, we compared the proportion of embryos that died within each developmental stage (early, middle, and late) among the three sites (MR-ref, SR-ref, and SR-cont) and within a site using a two-way ANOVA on angular transformed data.

RESULTS

In total, we collected 2579 eggs from 92 clutches laid by female snapping turtles from the South and Middle Rivers. Total Hg concentrations in tissues of gravid female snapping turtles ranged from 0.01 to 4.99 ppm (wwt) in blood, 0.05−32.29 ppm (dwt) in muscle, 0.15−161.11 ppm (fwt) in nail, and 0.01−6.61 ppm (dwt) in egg. In all cases, female tissue concentrations of Hg were strongly correlated with egg concentrations (Figure 1; all $p < 0.001$). Methylmercury represented 26−78% of THg within eggs, with %MeHg increasing with egg THg (Figure 2; ($y = 6.98x + 37.52$; $r^2 = 0.52$, $p = 0.008$, $n = 12$).

Body size significantly influenced muscle THg, but this effect was dependent upon site (ANCOVA: site x carapace length: $F_{2, 77} = 4.47$, $p = 0.015$, site: $F_{2, 77} = 0.30$, $p = 0.739$; carapace length: $F_{1, 77} = 1.64$, $p = 0.204$, Figure 3). Specifically, muscle THg concentrations increased with body size for females collected from the contaminated site (SR-Cont; $p < 0.001$) but did not change with size within the two reference sites (MR-ref or SR-ref; in both cases $p \geq 0.16$).

We found no relationship between muscle Hg concentrations and clutch size, clutch mass, or egg mass ($F_{1, 185} = 0.05$, $p = 0.86$). However, body size significantly influenced reproductive output ($F_{1, 185} = 8.08$, $p = 0.005$), with larger females producing larger clutches composed of heavier eggs than smaller females.

Egg THg negatively correlated with hatch success, and this relationship was evident when we compared clutches from all

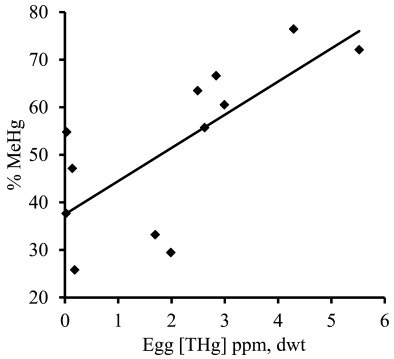

Figure 2. Relationship between % Methylmercury (MeHg) and total mercury (THg; dry weight) in eggs laid by gravid female *Chelydra serpentina* collected from the South and Middle Rivers VA.

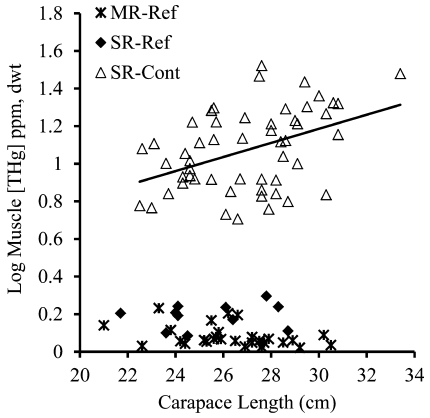

Figure 3. Relationship between carapace length and total mercury (THg) in muscle (dry weight) tissue of gravid *Chelydra serpentina* collected from the two reference sites on Middle River (MR ref; n = 29) and the South River (SR-ref; n =12), and the contaminated portion of the South River (SR-Cont; n = 54), VA. Total Hg in turtle muscle did not increase with increasing body size at the two reference sites ($p \geq 0.16$), but did for individuals collected at the contaminated site (SR-cont; $y = 0.0375x + 0.0616$, $r^2 = 0.18$, $p < 0.001$, $n = 54$).

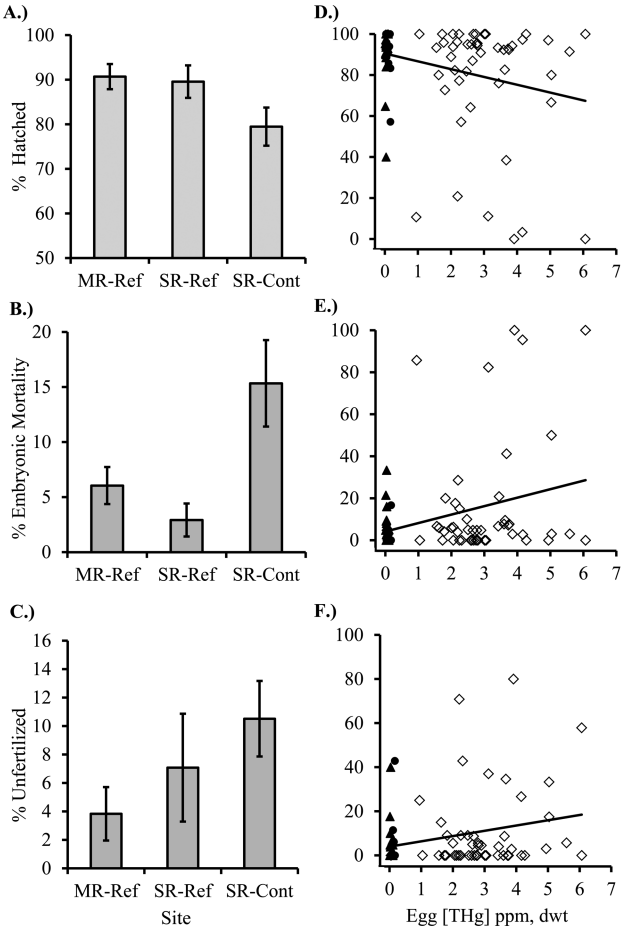

Figure 4. Relationship between mean (±1 SE) (A.) hatching success, (B.) embryonic mortality, and (C.) egg infertility, of clutches (n = 82) laid by female *Chelydra serpentina* from the two reference sites (Middle River [MR-ref] and upstream of the Hg source on the South River [SR-ref]), and the contaminated portion of the South River (SR-Cont), VA. Relationship between egg total mercury (THg, dry weight) and (D.) hatching success, (E.) embryonic mortality, and (F.) egg infertility from clutches collected from turtles inhabiting reference areas along the Middle River (MR-ref: closed triangles) and upstream of the source on the South River (SR-ref: closed circles) and those collected downstream of the Hg contamination source on the South River (Cont: open diamonds). Note, trendlines are representative of the entire data set (reference and contaminated individuals).

sites and when we confined our analysis to clutches downstream of the pollution source (PROC GLIMMIX; all sites: $F_{1, 80} = 91.07$, $p < 0.001$; SR-Cont only: $F_{1, 47} = 9.55$, $p = 0.003$, Figure 4d). Egg THg positively correlated with frequency of embryonic mortality during development ($F_{1, 80} = 27.72$, $p < 0.001$, Figure 4e) and frequency of unfertilized eggs ($F_{1, 80} = 63.17$, $p < 0.001$, Figure 4f). Examining the data by site rather than in relation to egg THg revealed a similar pattern for all reproductive measures (Figure 4a–c). Site significantly influenced hatch success ($F_{2, 79} = 40.22$, $p < 0.001$, Figure 4a), with clutches from females collected from the contaminated site averaging 11–12% lower hatch success than those collected from the two reference sites. Site also influenced the frequency at which embryos died during development ($F_{2, 79} = 15.24$, $p < 0.001$, Figure 4b) and the frequency of unfertilized eggs ($F_{2, 79} = 19.70$, $p < 0.001$, Figure 4c), with clutches from females collected at the contaminated site averaging 154–425% and 49–174% more deaths and unfertilized eggs, respectively, than clutches laid by females collected from the two reference sites.

The proportion of embryos that died at a given developmental stage did not differ among sites ($F_{2, 155} = 0.00$, $p = 0.99$, Table 1), but did differ by stage (stage: $F_{2, 155} = 6.61$, $p = 0.002$, stage x site: $F_{4, 155} = 1.22$, $p = 0.31$, Table 1) with the highest proportion of embryonic mortality occurring during the first week of development. Frequency of malformations was generally low (MR-ref: 3.06 ± 1.51%; SR-ref: 0.48 ± 0.43%; SR-Cont: 1.48 ± 0.50%) and was not influenced by either egg THg ($F_{1, 80} = 2.84$, $p = 0.096$) or site ($F_{2, 79} = 0.80$, $p = 0.452$).

DISCUSSION

Exposure to maternally transferred contaminants can have a profound influence on offspring phenotype and act as a significant source of mortality in wildlife populations.[5,6,28] Our study is the first to describe the maternal transfer of Hg and its associated reproductive effects in a turtle species. Total concentrations of Hg in muscle tissue of female turtles inhabiting the contaminated portions of the South River averaged 33- to 80-fold higher than those of females collected from the two reference sites. Likewise, females from the contaminated sites produced eggs with 30- to 85-fold higher Hg

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Table 1. Overall Proportion of Stage-Specific Mortality of Eggs Collected from Female Snapping Turtles (*Chelydra serpentina*) Collected from the Two Reference Sites on Middle River and the South River (MR-Ref, SR-Ref), and the contaminated portion of the South River (SR-Cont), Virginia[a]

site	total number of clutches collected	number of clutches with embryonic mortality	overall proportion of embryos that died during development	stage in embryonic development (proportion)		
				early (1−30d)	middle (31−60d)	late (61−90d)
MR-ref	23	15	0.07	0.38	0.29	0.33
SR-ref	10	4	0.05	0.75	0.00	0.25
SR-cont	59	33	0.16	0.66	0.15	0.19

[a]The proportion of embryos that died at a given developmental stage did not differ among sites (p = 0.99), but did differ by stage (p = 0.002).

concentrations than those produced by females collected from the two reference sites. Additionally, as THg concentrations in eggs increased so did %MeHg, which is consistent with previous studies on oviparous species.[9,29] Thus, females depositing more THg into their eggs also place their offspring at a greater proportional risk of exposure to the more toxic methylated form of Hg. Here, we provide evidence that Hg exposure negatively correlated with turtle hatching success through increased infertility and embryonic mortality.

Egg THg concentrations were positively correlated with egg infertility, which accounted for a substantial portion of the unhatched eggs in our study. Clutches collected from females inhabiting contaminated portions of the South River had 49−174% higher frequencies of infertility compared to clutches laid by females collected from the two reference sites. Increased frequency of egg infertility could be driven by female or male reproductive impairment through Hg's disruption of essential physiological functions important in gametogenesis and fertilization. A recent review by Tan et al.[30] concluded that many vertebrate species bioaccumulate Hg in their reproductive tissues, resulting in the disruption of important reproductive hormones. For example, in fish, Hg exposure adversely affected reproductive hormones in both females and males causing alterations in egg production, gonadal development, testosterone levels, sperm production, fecundity, and fertility.[7,30,31] However, it remains to be determined whether our observations are driven by maternal, paternal, or combined parental physiological anomalies.

Embryonic mortality also contributed to the observed reduction in hatching success, with mortality significantly correlating with increasing egg THg concentrations. Clutches collected from females inhabiting contaminated portions of the South River had 154−425% higher frequencies of embryonic mortality than those clutches collected from reference females. Mercury is an embryotoxicant that is known to reduce hatchability and cause malformations in multiple vertebrate species.[3] However, our results suggest that maternally transferred Hg is more embryotoxic than teratogenic in snapping turtles, as malformation frequencies were uniformly low across sites and were not correlated with egg THg. Similarly, Bergeron et al.[5] found teratogenicity to be lower than embryotoxicity in American Toad larvae (*Bufo americanus*) exposed to high concentrations of maternal Hg. Embryotoxicity has also been observed in female American kestrels (*F. sparverius*), with eggs laid by females exposed to dietary Hg showing decreased hatching and lower fledging rates than those produced by mothers that were not exposed during nesting.[32] Additionally, availability of lipids, micronutrients, and hormones that are important for embryonic and hatchling quality and development can be affected by Hg exposure.[30,33−36] Thus, it also remains possible that female turtles living in Hg contaminated areas allocated inappropriate quantities of important resources to their eggs, contributing to the reductions in embryonic survival.

Although THg concentrations in eggs produced by females collected from the contaminated portion of the South River exceed those associated with severe reproductive impairment observed in other amniotes (birds),[2,37,38] exposure to Hg does not appear to be as consequential to turtle reproduction as it is in birds. For example, several studies have shown greater reductions in avian egg viability at lower egg Hg concentrations than were observed in turtle eggs sampled for the present study. Jackson et al.[37] reported a 50% reduction in nest success of Carolina wrens (*Thryothorus ludovicianus*) when egg THg concentrations reached ∼2.15 ppm (dwt). In free-living common loons, egg THg concentrations ranging from 2.7 to 3.6 ppm (dwt) were associated with a 28−48% decrease in hatchability mediated through embryotoxicity.[38] In comparison, *C. serpentina* collected from the same area contaminated with Hg produced eggs with average THg concentrations of 3.00 ± 0.183 ppm (dwt), which was associated with a 12% reduction in hatching success compared to the average hatching success at reference sites. From the regression equation generated from the observed egg Hg concentrations, turtles with egg Hg concentrations toward the upper end of the observed distribution (∼5.0 ppm, dwt) are predicted to have a 27% reduction in hatching success. Together these comparisons indicate that turtle THg egg concentrations reported here exceeded those reported in Jackson et al.[37] and Barr[38] but were associated with a less severe effect on embryonic survival, suggesting that sensitivity to egg Hg concentrations can vary considerably among taxa. This is similar to a previous study that reported interspecies variation in embryonic survival among several bird species dosed with the same concentration of Hg.[39] Our findings suggest that *C. serpentina* may be more resilient to the adverse developmental effects of Hg than other amniotes, and perhaps better suited for long-term monitoring of bioavailability and bioaccumulation of Hg than as sensitive indicators of adverse effects. However, studies are needed to determine whether maternal transfer of Hg has latent or long-term effects that influence early survival of turtle hatchlings similar to those observed for other bioaccumulative contaminants.[40]

Although our results suggest that Hg has a negative influence on turtle reproduction, the importance of these effects to turtle populations is still unknown. Turtle population dynamics are highly dependent upon the survival of adults, particularly mature females, whereas egg and hatchling survival are generally thought to have relatively little influence on population dynamics.[41,42] However, population models pro-

duced by Cunningham and Brooks[43] suggest that the importance of annual hatchling survival increases in cases where adult survival is compromised by factors such as road mortality and overharvesting.[16,44] We propose three activities that would aid in maintaining healthy turtle populations in contaminated areas and contribute to our understanding of how Hg might influence turtle population dynamics. First, our results can be used to develop sustainable programs for monitoring exposure of turtles to Hg. We show that Hg concentrations in female tissue strongly correlate with concentrations in eggs, allowing researchers to estimate egg concentrations that would be expected to have adverse effects from relatively easy to collect nondestructive tissues (i.e., adult blood and nail). Second, to accurately assess the influence of impaired reproduction on turtle population viability, factors influencing adult survival must be understood. For example, throughout their range, snapping turtles and their eggs are frequently harvested for human consumption, but the rate and frequency of harvest is largely unknown and in many cases poorly regulated. For turtle populations facing threats of pollution, adult harvest in conjunction with reduced egg viability may influence demographics and ultimately population persistence. Finally, protection and/or construction of nesting habitat may help mitigate reduced reproductive success in contaminated areas. Nest failure due to predation can range from 30 to 100% in turtles, and other factors such as flooding, erosion, suboptimal nest temperatures, and nest destruction can further increase rates of nest failure.[45] If appropriate nesting habitat is readily available and protected from nest predators, the negative reproductive effects elicited by maternally transferred Hg might be offset.

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Notes

The authors declare no competing financial interest.

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