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INFLUENCE OF FEEDING ECOLOGY ON BLOOD MERCURY CONCENTRATIONS IN FOUR SPECIES OF TURTLES

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Abstract—Mercury is a relatively well-studied pollutant because of its global distribution, toxicity, and ability to bioaccumulate and biomagnify in food webs; however, little is known about bioaccumulation and toxicity of Hg in turtles. Total Hg (THg) concentrations in blood were determined for 552 turtles representing four different species (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) from a Hg-contaminated site on the South River (VA, USA) and upstream reference sites. Methylmercury and Se concentrations also were determined in a subset of samples. Because the feeding ecology of these species differs drastically, stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were employed to infer the relationship between relative trophic position and Hg concentrations. Significant differences were found among sites and species, suggesting that blood can be used as a bioindicator of Hg exposure in turtles. We found differences in THg concentrations in turtles from the contaminated site that were consistent with their known feeding ecology: *C. serpentina* ≥ *S. odoratus* > *C. picta* > *P. rubriventris*. This trend was generally supported by the isotope data, which suggested that individual turtles were feeding at more than one trophic level. Methylmercury followed similar spatial patterns as THg and was the predominant Hg species in blood for all turtles. Blood Se concentrations were low in the system, but a marginally positive relationship was found between THg and Se when species were pooled. The blood THg concentrations for the turtles in the present study are some of the highest reported in reptiles, necessitating further studies to investigate potential adverse effects of these high concentrations.

Keywords—Turtle Mercury Selenium Trophic position Stable isotopes

INTRODUCTION

Mercury is a concern for fish, wildlife, and human health because of its toxicity and tendency to bioaccumulate and biomagnify in food webs, especially in its methylated form (methylmercury [MMHg]) [1,2]. Mercury loading in aquatic ecosystems can come from either atmospheric deposition or point-source emissions. The former results in widespread distribution of the metal because of long-range airborne transport [3], and the latter is often associated with high contamination levels at a localized scale (e.g., within riverine or lacustrine systems). We examined the South River, Virginia, USA, which historically has been affected by point-source emissions from E.I. du Pont de Nemours and Company (Waynesboro, VA, USA). Mercuric sulfate was used by the company between 1929 and 1950 as a catalyst while manufacturing acetate fiber [4]. Mercury contamination in the South River was discovered in the 1970s, and today, Hg levels remain high in the river despite the use of Hg being terminated in the 1950s. A large Hg contamination gradient exists in the South River system, ranging from low concentrations, presumably derived from atmospheric deposition and geologic sources (upstream from the point source), to extremely high concentrations downstream from the manufacturing plant. Previous studies determined that Hg concentrations in water and biota increase for several miles before peaking between 10 to 15 miles downstream from the point source [5] (G.W. Murphy, 2004, Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA).

Turtles are potentially excellent model organisms for contaminant studies because of their unique suite of ecological and life-history attributes. Important characteristics include their wide distribution, the variation in the habitat types they occupy, and the range of trophic levels in which they feed [6,7]. In addition, turtles are long-lived, allowing for long-term exposure to contaminants. Because of their ectothermic physiology, turtles often can reach higher biomasses in a system compared with endotherms occupying similar trophic levels [8], and their eggs and young often are important as prey items for other organisms [9,10]. Yet, compared to birds and mammals, turtles have received little attention in terms of Hg pollution [11–13].

Turtle life histories, relative trophic positions, and dietary preferences in aquatic systems are likely important determinants of how much Hg is ingested and bioaccumulated. Four turtle species inhabit the South River: Red-bellied turtles (*Pseudemys rubriventris*), painted turtles (*Chrysemys picta*), stinkpots (*Sternotherus odoratus*), and snapping turtles (*Chelydra serpentina*). These four aquatic species differ drastically in their feeding ecology, providing an opportunity to assess the influence of trophic niche on Hg accumulation within a single turtle assemblage. *Pseudemys rubriventris* are typically associated with large, deep bodies of water [9,10]. Young *P. rubriventris* are omnivorous, but adults are almost entirely herbivorous, primarily consuming aquatic vegetation [10]. *Chrysemys picta* are habitat and dietary generalists. They in-

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habit permanent bodies of water (e.g., ponds, lakes, and rivers) [10] and consume plant and animal material by foraging along the bottom, actively searching in algae clumps, or surface-skimming [9]. *Sternotherus odoratus* occur in many types of aquatic habitats but prefer lentic, soft-bottom areas [9]. They are omnivores but largely scavenge by probing mud and detritus for prey items, such as beetles, crayfish, snails and other mollusks, leeches, larval insects, tadpoles, and dead fish [9,14]. *Chelydra serpentina* also inhabit a variety of aquatic habitats [10]. They consume animal and plant material of many kinds, but fish, amphibians, and aquatic invertebrates comprise a large portion of their diet [9,10]. Young *C. serpentina* actively forage for prey items, but adults ambush prey and consume carrion in the benthos [9].

In the present study, we sampled blood from the four turtle species that occur in the South River. Blood collection and other nonlethal sampling techniques have been recently promoted [15] in studies to understand why reptile populations are declining worldwide [16]. In addition to not harming turtles, blood sampling permits large sample sizes to be collected at multiple sites. Blood Hg levels are often considered an indicator of recent dietary Hg intake. However, correlations have been found between blood Hg concentrations and Hg bioaccumulation in other tissues in turtles [7,17], snakes [18], and alligators [19], suggesting that blood Hg concentrations reflect a combination of recent and longer term exposure [17], especially in reptilian species that undergo long periods of digestive quiescence.

The present study had two objectives. First, we sought to determine whether total Hg (THg) concentrations in turtle blood were elevated in the South River compared to upstream reference sites. Because of the different microhabitat and dietary preferences of each turtle species, we hypothesized that THg concentrations would exhibit the following trend across species: *P. rubriventris* < *C. picta* < *S. odoratus* < *C. serpentina*. Blood stable isotope composition of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) was analyzed to examine the relationship between Hg and the relative trophic position among the turtle species. Within an aquatic ecosystem, stable N isotope ratios (as $\delta^{15}N$ values) can provide information concerning relative trophic position, because δ values typically increase approximately 2 to 5‰ between trophic level as a result of preferential excretion of the lighter ^{14}N caused by amino acid metabolism [20,21]. This technique has been successfully employed using whole-body and muscle tissues in aquatic organisms (see, e.g., [22,23]) and using blood in birds (see, e.g., [24]) to describe relationships between trophic level and Hg concentrations. In a variety of organisms, MMHg and Se concentrations in tissues such as muscle, liver, kidney, and brain are important for interpreting bioaccumulation and toxicity of Hg, both because MMHg is the most toxic species of Hg and because Se has protective effects against Hg toxicity [12]. Of the few studies that have measured blood Hg levels in turtles [7,17,25], none have measured MMHg or Se. Hence, our second objective was to determine what proportion of blood Hg was methylated and whether a relationship existed between Se and Hg in turtle blood.

MATERIALS AND METHODS

Field sampling of turtles

Turtles were collected from multiple sites upstream and downstream of the source of Hg contamination (river mile 0). Sites available to trap turtles were dependent on landowner consent, so we trapped opportunistically along the South River and the Middle River, Virginia, USA. Downstream from the Hg source, turtles were sampled at seven subsites between river miles 2 and 22, hereafter referred as SR 2-22. These subsites in the contaminated portion of the South River could not be treated independently of one another in our spatial comparisons; thus, they are collectively referred to as the contaminated site. The reference sites consisted of an area on the South River (SR Ref) between 1.5 to 5 miles upstream from the E.I. du Pont de Nemours and Company plant (Fig. 1). Turtle movement in the South River between SR Ref and the contaminated site is restricted by Rife Loth dam in Waynesboro, Virginia, USA, upstream from the contamination source. Additional reference sites were sampled on the Middle River (MR Ref), which is northwest of the South River and also joins the South Fork of the Shenandoah River at Port Republic, Virginia, USA (Fig. 1).

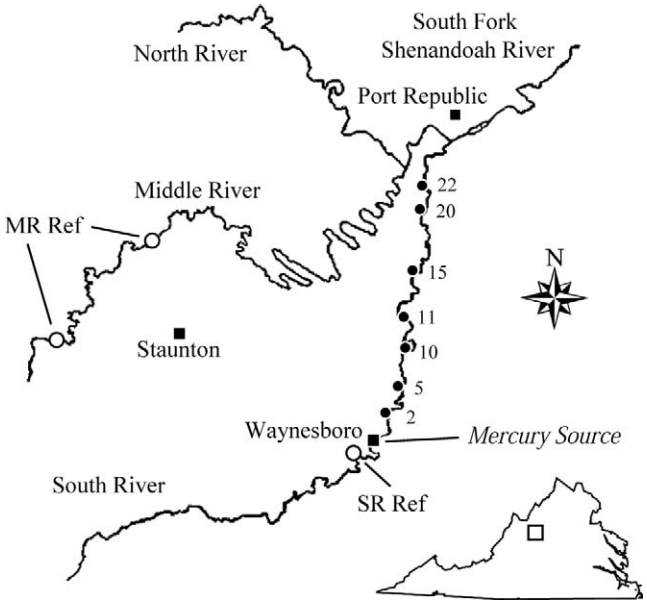

Fig. 1. Sampling locations along the South River (SR) and Middle River (MR) of the Shenandoah Valley (VA, USA). Numbers refer to river miles downstream from contamination source (river mile 0). Open symbols represent reference sites, and closed symbols represent contaminated sites. Note that the river flows from south to north.

Turtles were captured during the spring and summer (May–July) of 2006 by hand, in basking traps, and in baited hoop nets (Memphis Net and Twine, Memphis, TN, USA). Traps were placed in sections of the rivers that matched the microhabitat requirements of target species (e.g., slow-moving water, presence of coarse/woody debris, and structured bank) and then left for one to three nights. Traps were checked daily, and individual traps were moved if not successful after two nights. After the third night, traps were removed from the site or rebaited and moved within a site.

On capture, turtles were measured for carapace length, carapace width, and plastron length to the nearest centimeter and for mass to the nearest 0.5 kg for snapping turtles or the nearest 0.05 kg for the other three species. A 1-ml blood sample was taken for Hg analysis from the cervical sinus or caudal vein of each turtle using a 1-ml heparinized syringe. A second 0.3-ml blood sample was collected for stable isotope analysis from a subset of individuals using nonheparinized syringes. Samples were immediately placed on ice, returned to the laboratory,

Table 1. Individual sample sizes and totals for total mercury (THg) analyses by combustion–amalgamation–cold-vapor atomic absorption spectrophotometry in the four turtle species (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) at the South River (SR) contaminated portion (SR 2-22) and the South River and Middle River reference sites (SR Ref and MR Ref, respectively)[a]

Site	*C. serpentina*	*S. odoratus*	*C. picta*	*P. rubriventris*	Total
SR subsites					
RM 2	30	5	11	13	59
RM 5	8	22	20	0	50
RM 10	14	17	29	2	62
RM 11	24	9	84	11	128
RM 15	9	8	8	4	29
RM 20	4	5	4	2	15
RM 22	10	12	14	4	40
SR 2-22	99	78	170	36	383
SR Ref	14	7	6	0	27
MR Ref	38	4	100	0	142
Total	151	89	276	36	552

[a] All sites in Virginia (USA). RM = river mile.

Table 2. Individual sample sizes and totals for methylmercury (MMHg) and selenium (Se) analyses in the four turtle species (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) at the South River (SR) contaminated portion (SR 2-22) and the South River and Middle River reference sites (SR Ref and MR Ref, respectively)[a]

Site	*C. serpentina*	*S. odoratus*	*C. picta*	*P. rubriventris*	Total
SR subsites					
RM 2	4	4	4	5	17
RM 5	4	4	4	0	12
RM 10	4	4	4	2	14
RM 11	8	7	8	5	28
RM 15	4	4	4	3	15
RM 20	2	4	3	2	11
RM 22	4	4	4	4	16
SR 2-22	30	31	31	21	113
SR Ref	6	5	3	0	14
MR Ref	4	4	3	0	11
Total	40	40	37	21	138

[a] All sites in Virginia (USA). RM = river mile.

and stored frozen until thawed for analyses. Turtles were each given permanent individual marks by notching three marginal scutes of the shell. A Garmin handheld Global Positioning System unit (Garmin International, Olathe, KS, USA) was used to obtain geospatial coordinates for each captured turtle. Turtles were then released at their point of capture.

Sample preparation and analyses

Total mercury analysis. Subsamples (50–200 mg) of whole blood from 552 individual turtles (Table 1) were analyzed for THg content by combustion–amalgamation–cold-vapor atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA) according to U.S. Environmental Protection Agency (EPA) method 7473 [26]. For quality assurance, each group of 10 to 15 samples included a replicate, blank, and standard reference material (SRM; TORT-2 lobster hepatopancreas [National Research Council of Canada, Ottawa, ON] or SRM 966 [Toxic Metals in Bovine Blood Level 2; National Institute of Standards and Technology, Gaithersburg, MD, USA]). The instrument was calibrated using solid SRMs (TORT-2 and DORM-2, dogfish muscle; National Research Council of Canada). Method detection limits (MDLs; threefold the standard deviation of procedural blanks) for blood samples depended on sample mass and were calculated separately for each observation based on the mass of sample analyzed. Method detection limits ranged from 2.83 to 12.17 ng/g wet mass. Most THg concentrations exceeded the detection limits, with the notable exception of 72 observations in MR Ref samples. Average relative percent difference (RPD) between replicate samples analyzed was 7.71% ± 1.20% (n = 57; mean ± standard error throughout). Mean percent recoveries of THg for the SRMs TORT-2 and SRM 966 were 97.6% ± 1.5% (n = 94) and 82.9% ± 3.8% (n = 13), respectively.

Mercury speciation analysis. A subset of blood samples (n = 138) from all sites (MR Ref, SR Ref, and SR 2-22) was analyzed for MMHg and reanalyzed for THg (Table 2). Whole-blood samples (75–300 mg) were digested in sealed, 15-ml polypropylene centrifuge tubes containing 2 to 6 ml of 4.5 M trace metal–grade HNO_3 at 60°C overnight. The resulting digests were centrifuged at 1,000 g for 20 min to remove any insoluble material. Aliquots of the supernatants (25–100 μl) were then analyzed for MMHg content using aqueous-phase ethylation with room-temperature precollection, followed by gas chromatography and cold-vapor atomic fluorescence spectrometry according to the methods described by Liang et al. [27] as modified by Hammerschmidt and Sandheinrich [28]. The SRMs TORT-2 and SRM 966, the blank and duplicate samples, and the samples spiked with standards were processed identically and analyzed simultaneously with the blood samples. Mean recoveries of MMHg for TORT-2 and SRM 966 were 104.8% ± 3.3% (n = 24) and 113.5% ± 34.2% (n = 8), respectively. Whereas TORT-2 has a certified reference value for MMHg, SRM 966 only has a reference value. The estimated MDL for MMHg was 1.54 ng/ml. In general, sample concentrations exceeded the detection limit with the exception of 11 observations largely from the reference sites (n = 8) or from *P. rubriventris* (n = 3). Average RPD between replicate samples was 15.3% ± 2.6% (n = 25). Spike recovery averaged 94.1% ± 2.4% (n = 20).

To determine the percent MMHg (%MMHg) for the subset, THg concentrations in the digestates were determined using an Elan DRC Plus inductively coupled plasma–mass spectrometer (ICP-MS; PerkinElmer, Norwalk, CT, USA) according to U.S. EPA method 6020a [29]. Recoveries of THg in TORT-2 and SRM 966 by this method were 97.4% ± 12.1% (n = 10) and 79.9% ± 14.2% (n = 4), respectively. Average RPD between replicate samples was 5.0% ± 1.5% (n = 5). Estimated MDL depended on sample mass and ranged from 6.60 to 24.89 ng/g wet mass. As with MMHg, sample concentrations generally exceeded the detection limit with the exception of nine observations. Mean spike recovery was 98.4% ± 4.4% (n = 5). The slope of the relationship between THg analyses by the DMA 80 and by ICP-MS was not significantly different from one (t test, p = 0.1).

Selenium analysis. Selenium concentrations in blood were determined for the same subset of turtles (n = 138) analyzed for MMHg from all sites (MR Ref, SR Ref, and SR 2-22) (Table 2). Approximately 250 mg of thawed whole-blood samples were digested in 5 ml of trace metal–grade HNO_3 in flouropolymer digestion vessels using a microwave digestion system (MARS-5; CEM, Matthews, NC, USA) according to

U.S. EPA method 3052 [30]. After digestion, the samples were brought to a final volume of 15 ml with deionized water (>18 MΩ). Analytical method blanks and SRM (TORT-2) were included in each digestion batch. Selenium analysis was performed on diluted samples according to U.S. EPA method 6020a [29] by ICP-MS in standard mode. Calibration was performed using the method of standard addition. Mean recovery of Se for TORT-2 was 113.0% ± 1.6% (n = 9). The estimated MDL for Se was 0.177 ng/ml. Sample concentrations generally exceeded the detection limit with the exception of seven observations from SR 2-22. Average RPD between analytical replicate samples and method replicate samples was 35% ± 9% (n = 7) and 24% ± 4% (n = 3), respectively. Spike recovery averaged 98% ± 4% (n = 7).

Stable isotope analyses. The isotopic composition of N and C were determined on a subset of the samples analyzed for MMHg and Se from river miles 10, 11, 15, and 20 (n_{total} = 67, $n_{C.\ serpentina}$ = 17, $n_{S.\ odoratus}$ = 19, $n_{C.\ picta}$ = 19, $n_{P.\ rubriventris}$ = 12). Lyophilized whole-blood samples of approximately 1.5 mg were weighed to the nearest microgram and placed into precleaned tin capsules. Stable isotope ratios were then determined using an elemental analyzer (NC2500; Carlo Erba, Milan, Italy) coupled to a continuous-flow isotope ratio mass spectrometer (Delta^{plus} XL; Finnigan, San Jose, CA, USA). Stable isotope ratios are reported in per mill units (‰) using δ notation ($\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$), where X = ^{13}C or ^{15}N and R = the ratio of ^{13}C to ^{12}C or of ^{15}N to ^{14}N in a sample or SRM [31]. Values were calibrated to atmospheric nitrogen and Vienna-PeeDee Belemnite through the external working standard, DORM-2 (dogfish muscle), which has an assigned δ^{13}C value of −16.9‰ and an assigned δ^{15}N value of 14.0‰. Isotopic compositions were reproducible to ±0.1‰ (±1 standard deviation; n = 7) for both δ^{13}C and δ^{15}N.

Statistical analyses

We first determined whether body size significantly affected THg levels in blood by regressing log-transformed THg concentration against log body mass (kg) for each species at each site except for *S. odoratus* (SR Ref, n = 4; MR Ref, n = 7) and *C. picta* (SR Ref, n = 6) at reference sites because of small sample sizes. Next, we tested whether sex affected blood THg levels, because some wildlife species exhibit sexual differences in contaminant body burden as a result of sexual dimorphism or female elimination of contaminants to eggs (see, e.g., [32,33]). We removed individuals from the data set that fell below the known threshold body size for sexual maturity in each species [9,10] and tested for a sex effect by comparing THg concentrations in adult males and females within the contaminated site. Total Hg levels did not fit the assumptions of normality, so nonparametric Mann–Whitney U tests [34] were performed for each species. Neither body size nor sex consistently influenced Hg accumulation in turtles (see *Results*), so neither factor was included in subsequent statistical models comparing sites and species.

Total Hg concentrations were not normally distributed, so nonparametric analyses were used to compare species and sites. We performed a Kruskal–Wallis test followed by nonparametric multiple comparisons with Tukey–Kramer adjustments [35] to determine how THg concentrations differed in the four turtle species within the contaminated site. Because no *P. rubriventris* were found at the reference sites, we compared *C. picta*, *S. odoratus*, and *C. serpentina* among sites (MR Ref, SR Ref, and SR 2-22) using the Scheirer–Ray–Hare extension of a second Kruskal–Wallis test [34] that excluded *P. rubriventris*. Almost half the turtles (46%) caught at MR Ref did not have a detectable level of THg, so we assigned each of these half the detection limit for statistical comparisons.

A subset of blood samples from all sites was analyzed for THg, MMHg, and %MMHg; however, small sample sizes and low values (below detection limit [BDL]) precluded statistical comparisons among reference sites and the contaminated site. Instead, Kruskal–Wallis nonparametric tests were performed followed by nonparametric multiple comparisons to examine differences in THg and MMHg among turtle species within the contaminated site. Assumptions of normality for %MMHg were met with an arcsin square root transformation of data, so differences in %MMHg among the four turtle species within the contaminated site were compared using a one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons.

The subset of blood samples analyzed for Se concentrations were log transformed to meet parametric assumptions. Selenium concentrations in the four turtle species in the contaminated site were compared using a one-way ANOVA. As before, additional comparisons among species and sites were performed using two-way ANOVA for all species except *P. rubriventris*, because no individuals of that species were captured at reference sites. All ANOVAs were followed by Tukey's pairwise comparisons. Spearman rank correlation was used to assess relationships between Se and THg or MMHg concentrations.

Finally, we performed a one-way ANOVA followed by Tukey's pairwise comparisons to determine if blood stable isotope compositions (δ^{13}C and δ^{15}N) differed among the four turtle species. Ordinary least-squares linear regression was used to assess relationships between δ^{13}C and δ^{15}N values and between δ^{13}C or δ^{15}N values and THg, MMHg, or Se concentrations. All analyses were performed with SAS® 9.1 (SAS Institute, Cary, NC, USA), and an α value of 0.05 was used to assess statistical significance.

RESULTS

Turtles

During the course of the present study, we trapped, marked, and acquired blood samples from 552 turtles of four different species (*C. serpentina*, *S. odoratus*, *C. picta*, and *P. rubriventris*) in the South and Middle rivers (Table 1). Except for *P. rubriventris*, each species was caught at all sites and subsites along both rivers; however, the relative abundance of species varied greatly among sites. *Pseudemys rubriventris* was not found at the reference sites or in high abundance at the contaminated site. This is not surprising, because the present study represents a significant range expansion for this species, with the nearest previous record being at least one ecoregion to the east [10].

Total mercury concentrations

A large range in blood THg concentrations was found among species and sites (Fig. 2A), with concentrations rising for all species within the contaminated portion of the South River after river mile 2 (Fig. 2B). Although subsites along SR 2-22 could not be treated independently of one another in our statistical comparisons, Figure 2B shows the spatial trends along the contaminated portion of the South River. *Pseudemys*

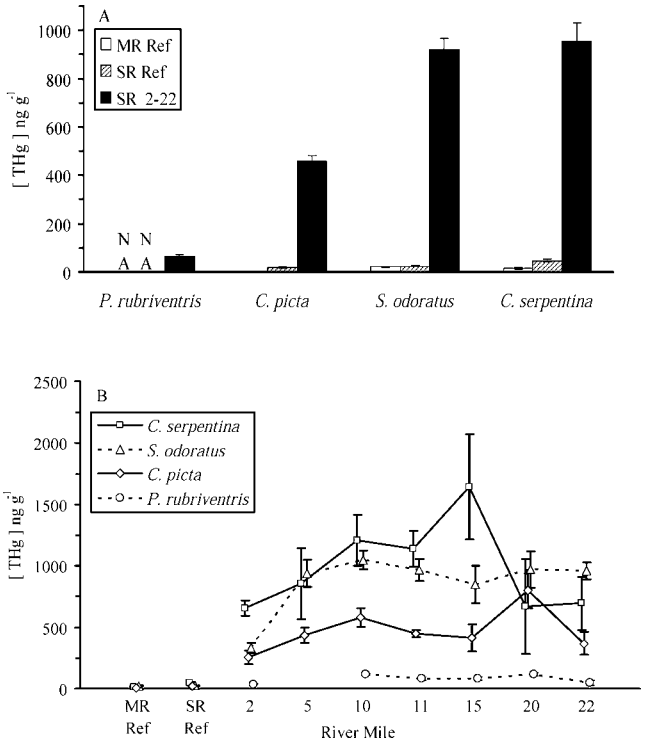

Fig. 2. (**A**) Total mercury (THg) concentrations (ng/g; mean ± standard error [SE]) in blood of four species of turtles (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) from the Middle River (VA, USA) reference site (MR Ref), the South River (VA, USA) reference site (SR Ref), and the contaminated portion of the South River (river miles 2–22 [SR 2-22]). Data for *Pseudemys rubriventris* are not available (NA), because this species was not found at the reference sites. (**B**) Blood THg concentrations (ng/g; mean ± SE) in four species of turtles at the reference sites and the subsites along SR 2-22 downstream from the source of contamination (river mile 0). Lines connecting points are for visual presentation and do not reflect connectivity between means. Symbols for means are staggered for visual presentation.

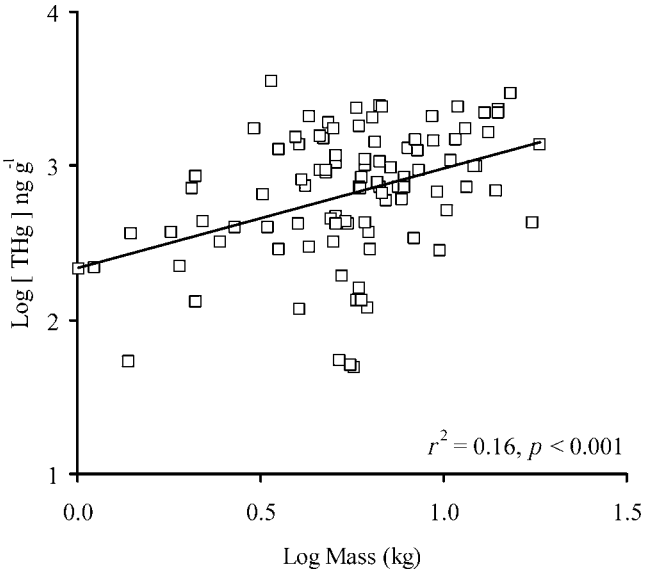

Fig. 3. Relationship between log mass (kg) and log total mercury (THg; ng/g) in *Chelydra serpentina* in the contaminated portion (SR 2-22) of the South River (VA, USA).

rubriventris was not included in the two-factor model comparing species and site, because no individuals were found in the reference sites. This model revealed significant differences among species ($p < 0.05$) for *C. serpentina*, *S. odoratus*, and *C. picta*. Significant site differences ($p < 0.001$) also were found among MR Ref, SR Ref, and the contaminated site. These three species all had THg concentrations that were 20- to 37-fold and 43- to 108-fold higher in the contaminated site than in SR Ref or MR Ref, respectively. No interaction was found between species and site ($p > 0.75$), indicating similar patterns among the species at different sites. The majority of turtles from the reference sites had THg concentrations of less than 50 ng/g, but concentrations generally were higher in the SR Ref than the MR Ref. The THg concentrations were 4.5- and 3.2-fold higher in *C. picta* and *C. serpentina*, respectively, in SR Ref than in MR Ref. This trend was not apparent in *S. odoratus*, likely because of small sample sizes at the reference sites.

Differences were found in THg concentrations among the four species within the contaminated site ($p < 0.001$), which supports the two-factor model for species and site described above. Pairwise comparisons revealed that all species differed from one another ($p < 0.05$) except for *C. serpentina* and *S. odoratus* ($p > 0.05$). *Pseudemys rubriventris* had 85 to 93% lower THg levels compared to all other species, and *C. picta* had THg levels 50 and 52% lower than those in *S. odoratus* and *C. serpentina*, respectively (Fig. 2A).

Body mass did not explain a significant amount of variation in blood THg concentrations for all species at all sites ($r^2 < 0.053$, $p > 0.06$) with one exception: A significant effect of body mass on Hg accumulation was found in *C. serpentina* in the contaminated site ($r^2 = 0.157$, $p < 0.001$) (Fig. 3). Body sizes of *C. serpentina* did not differ among sites ($F_{2,148} = 0.47$, $p = 0.624$; mass [mean ± standard error]: MR Ref, 5.8 ± 0.52 kg; SR Ref, 4.8 ± 0.62 kg; SR 2-22, 5.5 ± 0.36 kg), so any effect of site (Fig. 2A) on Hg concentrations was not confounded by body size differing among sites. No differences were found in THg concentrations between sexually mature male and female *C. picta* ($p = 0.876$), *C. serpentina* ($p = 0.868$), *P. rubriventris* ($p = 0.852$), or *S. odoratus* ($p = 0.488$).

Mercury speciation and selenium

Total Hg concentrations in the subset of samples analyzed for MMHg (Fig. 4) followed the spatial patterns observed in the larger THg data set (Fig. 2B) for the four species. Concentrations of MMHg tracked THg closely along the river, and the %MMHg generally ranged between 70 and 100% for all species and sites. Results from the species comparisons for the subset within the contaminated site ($n = 107$) were similar to statistical comparisons of the complete data set and showed significant differences in blood concentrations for THg and MMHg ($p < 0.001$ for both) in which all species differed from one another ($p < 0.05$) except for *C. serpentina* and *S. odoratus* ($p > 0.05$). Percent MMHg, however, was similar among species in the contaminated site ($F_{3,102} = 0.20$, $p = 0.893$). Twelve samples were BDL for THg and MMHg. Nine of the BDL individuals were from the reference sites, and the remaining three BDL individuals were *P. rubriventris* in the contaminated site.

Mean Se concentrations in the blood of the four turtle species ranged between 260 to 339 ng/g. The two-way ANOVA comparing *C. serpentina*, *S. odoratus*, and *C. picta* among sites (MR Ref, SR Ref, and SR 2-22) was significant for species

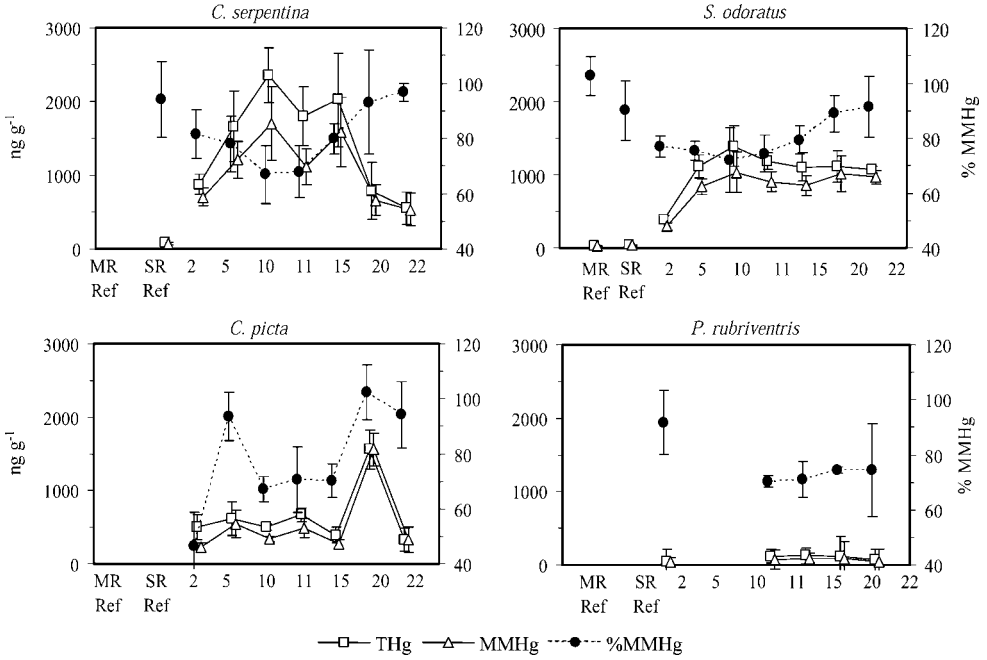

Fig. 4. Total mercury (THg), methylmercury (MMHg), and percent methylmercury (% MMHg) in blood of four species of turtles (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) from the reference sites of the South River (SR Ref) and Middle River (MR Ref; both VA, USA) and the subsites along the contaminated portion of the South River (river miles 2-22 [SR 2-22]). Lines connecting points are for visual presentation and do not reflect connectivity between means. Symbols for means are staggered for visual presentation. Values are presented as the mean ± standard error.

($F_{2,104}$ = 4.55, p = 0.013) but not site ($F_{2,104}$ = 0.76, p = 0.468), and no interaction was found between species and site ($F_{4,104}$ = 1.08, p = 0.373). Species comparisons within the contaminated site also identified significant differences in Se blood concentrations ($F_{3,101}$ = 2.95, p = 0.036). Examination of pairwise species comparisons revealed that only *S. odoratus* differed from *P. rubriventris* (p = 0.026). Seven samples from the contaminated site were BDL for Se (five samples of *P. rubriventris* and one each of *C. serpentina* and *C. picta*).

For data pooled across species, a weak but insignificant trend for Se and Hg to be positively correlated (THg, r = 0.177, p = 0.060; MMHg, r = 0.177, p = 0.056) was observed. Individually, *C. serpentina* (THg, r = 0.307, p = 0.087; MMHg, r = 0.291, p = 0.101) and *C. picta* (THg, r = 0.258, p = 0.169; MMHg, r = 0.322, p = 0.077) had weak positive relationships, but *S. odoratus* (THg, r = −0.026, p = 0.878; MMHg, r = −0.065, p = 0.692) and *P. rubriventris* (THg, r = 0.042, p = 0.897; MMHg, r = 0.064, p = 0.829) did not.

Stable isotope composition

Significant differences were found among species for $\delta^{15}N$ values ($F_{3,63}$ = 46.14, $p < 0.001$). All species were statistically different from one another ($p < 0.001$) with the exception of *P. rubriventris* and *S. odoratus* (p = 0.775). Species differences ($F_{3,63}$ = 4.52, p = 0.006) were also found for $\delta^{13}C$ values. Examination of pairwise comparisons of C isotope signatures revealed that only *C. picta* differed from *S. odoratus* (p = 0.022), whereas *C. picta* and *P. rubriventris* (p = 0.051) were marginally different and *C. serpentina* and *S. odoratus* (p = 0.077) were not significantly different from each other. *Chrysemys picta* did not differ from *C. serpentina* (p = 0.976), and *P. rubriventris* did not differ from either *C. serpentina* (p = 0.134) or *S. odoratus* (p = 1.00). No relationship was found between $\delta^{15}N$ and $\delta^{13}C$ values (p = 0.342). With all species combined, $\delta^{15}N$ showed a significant positive relationship with THg (r^2 = 0.312, $p < 0.001$) (Fig. 5) and MMHg (r^2 = 0.300, $p < 0.001$). Individuals within species did not display relationships between $\delta^{15}N$ and THg (*C. serpentina*, p = 0.096; *S. odoratus*, p = 0.405; *C. picta*, p = 0.069; *P. rubriventris*, p = 0.118) or MMHg (*S. odoratus*, p = 0.465; *C. picta*, p = 0.075; *P. rubriventris*, p = 0.086) with the exception of *C. serpentina* (r^2 = 0.280, p = 0.035). No re-

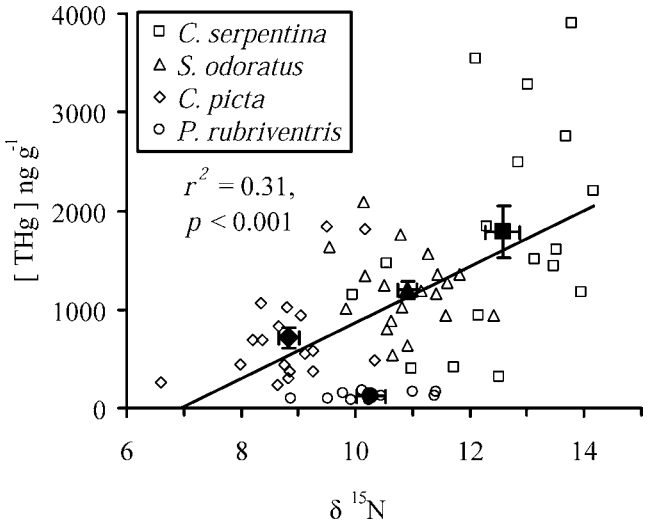

Fig. 5. Relationship between $\delta^{15}N$ values (‰) and total mercury (THg) concentrations (ng/g) in blood of four turtle species (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) from the contaminated portion of the South River (VA, USA; n = 67, river miles 10–20). Open symbols represent values for individual turtles; dark symbols represent the species means (± standard errors). Regression is on data for individual turtles (y = 282.85x − 1,962). The relationship between $\delta^{15}N$ values and methylmercury (MMHg) concentrations (ng/g) was nearly identical to that depicted here (r^2 = 0.300, $p < 0.001$, y = 219.79x − 1,524.5).

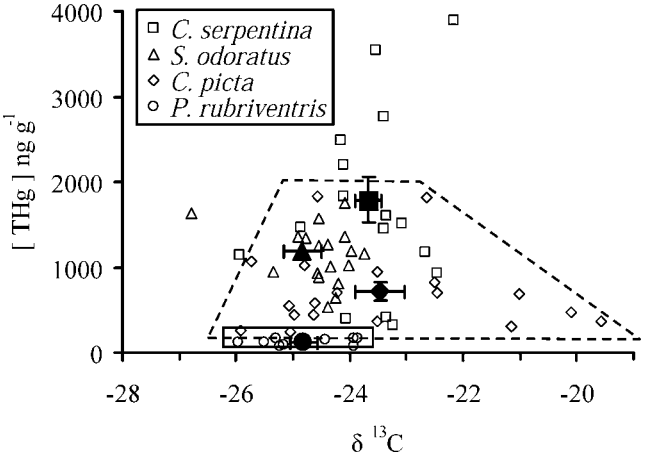

Fig. 6. Relationship between individual $\delta^{13}C$ values (‰) and total mercury (THg) concentrations (ng/g) in blood of four turtle species (*Chelydra serpentina*, *Sternotherus odoratus*, *Chrysemys picta*, and *Pseudemys rubriventris*) from the contaminated portion of the South River (VA, USA; $n = 67$, river miles 10–20). Open symbols represent values for individual turtles; dark symbols represent the species means (± standard errors). The groupings are for visual comparison of a generalist (*C. picta*; large dotted polygon) and a more specialized herbivore (*P. rubriventris*; solid rectangle).

lationship was found between $\delta^{13}C$ values and either THg ($p = 0.946$) (Fig. 6) or MMHg ($p = 0.728$). Selenium was not correlated with either $\delta^{15}N$ ($p = 0.301$) or $\delta^{13}C$ ($p = 0.913$) values.

DISCUSSION

Site differences

We found strong differences in blood THg concentrations among the reference sites and contaminated site (Fig. 2A), suggesting that blood can be used as a bioindicator of Hg exposure for turtles. In spite of the known influence of recent dietary Hg on blood Hg concentrations, studies have shown that blood concentrations correlate with concentrations in muscle, liver, and kidney in turtles, which suggests that blood also can be indicative of bioaccumulation in organs [7,17]. Downstream from the contamination source (SR 2-22), THg (and MMHg) concentrations for all species appeared to peak or plateau between river miles 10 and 15 (Fig. 2B). This phenomenon has been observed previously in water [5], invertebrates, fish (G.W. Murphy, 2004, Master's thesis), and birds (R.A. Brasso, 2007, Master's thesis, College of William and Mary, Williamsburg, VA, USA) in the South River; however, the reason for the gradual increase in Hg contamination downstream from the point-source remains unclear. The leading hypothesis for this phenomenon involves continual loading of Hg into the South River from the floodplain because of erosion, particularly during high water levels (South River Science Team Expert Panel Annual Meeting, Harrisonburg, VA, USA, October 11–12, 2006, unpublished data).

Blood THg concentrations (up to 3,600 ng/g) from some turtles in contaminated reaches of the South River were higher than any previously reported values for turtles, but the present study was the first to focus on known point-source Hg contamination. For example, *C. serpentina* from five small lakes in southeastern Connecticut had blood THg concentrations between 50 and 500 ng/g [7]. *Caretta caretta* (loggerhead sea turtle) from coastal waters between South Carolina (USA) and Florida (USA) had blood THg concentrations from 57 to 141 ng/g [17]. Finally, *Lepidochelys kempii* (Kemp's ridley sea turtle) captured off the coast of Louisiana (USA) and Texas (USA) had blood THg concentrations between 0.50 and 67.3 ng/g [25]. The blood THg concentrations in turtles from the present study are within the range of muscle tissue concentrations (wet wt) in other omnivorous and carnivorous aquatic species in the South River, such as white sucker (*Catostomus commersoni*, 350–1,700 ng/g), sunfish (*Lepomis auritus*, 570–1,300 ng/g), and smallmouth bass (*Micropterus dolomieu*, 500–3,240 ng/g), and their prey, forage fish (67–398 ng/g) or aquatic invertebrates (198–595 ng/g) (G.W. Murphy, 2004, Master's thesis). Many of these fish and invertebrates likely are prey for at least three of the species in the present study (*C. serpentina*, *S. odoratus*, and *C. picta*).

Size and sex differences

Commonly, older (and/or larger) organisms have proportionally higher THg concentrations than younger (and/or smaller) conspecifics. This pattern probably emerges because Hg continually bioaccumulates and/or because animals sometimes exhibit different foraging patterns as they increase in size. For example, THg concentrations often correlate with body mass in fish [36]. Of the turtles we sampled, *C. serpentina* in the contaminated site was the only species with a detectable correlation between blood THg concentration and body mass (Fig. 3). We hypothesize that other turtles also may show similar patterns; however, our sample population for these species was confined to a narrow mass range compared to *C. serpentina*. Whereas *S. odoratus*, *C. picta*, and *P. rubriventris* spanned up to a 45-fold range in mass at the contaminated site, we collected *C. serpentina* that ranged from 0.015 to 17.3 kg (>1,000-fold range). Meyers-Schone et al. [37] also found significant correlations between muscle and kidney Hg and body mass in *C. serpentina* and *Trachemys scripta*, and Kenyon et al. [25] found a positive correlation between blood Hg concentration and body size in *C. caretta*. Conversely, others have not found body size correlations in muscle tissue of *C. serpentina* [7,38].

Some metal concentrations differ with sex, presumably because of their elimination by gravid females to eggs or variation in behavior that leads to differential metal exposure [6]. We found no sex differences in blood THg concentrations between male and female adults. Again, previous studies have reported inconsistent results. Kenyon et al. [25] reported that blood Hg concentrations increased more rapidly with size in females than in males, suggesting that the sexes forage differently. Meyers-Schone et al. [37] found male *T. scripta* generally had higher Hg concentrations in kidney and liver than females, but Albers et al. [39] found no differences in those tissues between the sexes in *C. serpentina*.

Species and trophic positions

In general, our stable isotope data support the known feeding ecology of the four turtle species with the exception of *P. rubriventris* (Fig. 5), which appeared to have higher $\delta^{15}N$ values than expected based on their low Hg concentrations and known dietary preferences. Adult *P. rubriventris* are the only strict herbivores in this turtle assemblage. The literature suggests that *C. serpentina*, *S. odoratus*, and *C. picta* are opportunistic feeders, but *C. serpentina* is by far the most carnivorous of the turtle species, followed by *S. odoratus* and *C. picta* [9,10]. The range in individual $\delta^{15}N$ values (6.6–14.2‰) and mean species $\delta^{15}N$ values (8.8–12.6‰) between the lowest

(*C. picta*) and highest (*C. serpentina*) suggests that the different species are feeding at more than one trophic level within the South River.

With the available data, we can only speculate as to why $\delta^{15}N$ values for *P. rubriventris* were relatively high for such low blood Hg concentrations. Based on the $\delta^{13}C$ values, which can be used to distinguish between different C-based food sources [40], *P. rubriventris* appeared to be feeding narrowly from basal resources ($\delta^{13}C$ standard deviation, 0.75‰) compared to the other species that fed more broadly ($\delta^{13}C$ standard deviation: *C. serpentina*, 0.93‰; *S. odoratus*, 1.48‰; *C. picta*, 1.90‰) (Fig. 6). Thus, *P. rubriventris* may be specializing on a fairly narrow range of resource types compared to the other three species, and these resources may have a very different N signature compared with other portions of the food web. Similarly, Monteiro et al. [41] found that seabird species of the same trophic level had different Hg concentrations when feeding on different prey items (i.e., mesopelagic vs epipelagic). An alternate explanation is that *P. rubriventris* may be more environmentally stressed than the other turtle species in the present study. The occurrence of *P. rubriventris* in the South River represents a significant range expansion for this species. Thus, it is possible that nontoxicological factors associated with this habitat may be more physiologically challenging than conditions within the species' historical range. Stress can cause elevated rates of protein degradation and synthesis, which can result in higher $\delta^{15}N$ values, as seen in toxicant-stressed *Egretta thula* (snowy egret) nestlings [42] and nutritionally stressed female *Chen rossii* (Ross' geese) [43].

Mercury speciation and selenium

Methylmercury is the Hg species of greatest concern because of its toxicity and tendency to biomagnify [12], but to our knowledge, no studies have examined the proportion of MMHg in the blood of turtles. In the present study, Hg in the blood of all turtles was predominantly MMHg, which is consistent with the results of studies examining %MMHg in bird blood (see [33] and references therein). Interestingly, the fraction of THg composed of MMHg differed qualitatively among sites along the contamination gradient (Fig. 4), with the lowest %MMHg occurring in the areas with the highest THg concentrations. This phenomenon is best illustrated with *C. serpentina* and *S. odoratus*, which have samples from both the reference sites and the contaminated site and a large range in Hg concentrations. Similar relationships have been observed in several other biotic and abiotic media, such as water [5], sediment [44], aufwuchs [45], tadpoles [45,46], and fish [47]. Because no differences in %MMHg were found among species and the spatial trends were consistent among species, differences in diet among sites most likely do not explain this relationship. The variation in %MMHg may be related to differences in net methylation rate along the river, where areas with high THg concentrations have exceeded the capacity of the ecosystem to methylate inorganic Hg [44]. Thus, THg increased at a faster rate than MMHg, resulting in the lowest %MMHg in areas where THg peaked.

Selenium concentrations in turtle blood were generally low in the Hg-contaminated site. Whereas Se concentrations differed among species, all Se concentrations (260–339 ng/g) were consistent with background blood concentrations of healthy individuals in other wildlife (100–400 ng/g for birds and 100–500 ng/g for mammals [48]). No difference was found among sites, indicating no influence of urbanized areas, such as Waynesboro (Fig. 1). We found a weak but insignificant relationship between THg and Se and between MMHg and Se in blood when all species were combined. The relationship between Hg and Se is poorly understood in reptiles, but Se is known to have protective effects in other vertebrates, possibly by redistributing Hg to less sensitive tissues (e.g., muscle) or by assisting in sequestration of Hg(II) as HgSe in target organs (e.g., liver and kidney) [49]. Selenium in blood is not necessarily suggestive of protective effects, but it does signify the presence of bioavailable Se in the system and a potential relationship between Hg and Se in target organs [50], where protective effects could occur. The interactions between Se and Hg are complex, but given that Se appears to be bioavailable in this system, future risk assessments at this site should consider both Hg and Se concentrations.

CONCLUSION

More than 50 years after the use of mercuric sulfate was terminated on the South River, Hg concentrations remain high in the system. In the present study, we detected differences in THg concentrations in turtle blood among sites with varying levels of contamination. In addition, THg concentrations in blood differed among the four turtle species consistent with their feeding ecology. Further studies are warranted to investigate adverse effects of this accumulation, such as reproductive impairment. A description of functional mathematical relationships between blood and egg Hg concentrations, as well as the identification of egg concentrations capable of affecting embryonic development, would be of particular value. The large range in Hg concentrations within the South River provides a unique opportunity to quantify threshold concentrations for adverse effects, such as important reproductive outcomes, that will aid in management decisions for similar species at other Hg-contaminated sites around the world.

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