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Non-destructive techniques for biomonitoring of spatial, temporal, and demographic patterns of mercury bioaccumulation and maternal transfer in turtles

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ABSTRACT

Mercury (Hg) is a globally ubiquitous pollutant that has received much attention due to its toxicity to humans and wildlife. The development of non-destructive sampling techniques is a critical step for sustainable monitoring of Hg accumulation. We evaluated the efficacy of non-destructive sampling techniques and assessed spatial, temporal, and demographic factors that influence Hg bioaccumulation in turtles. We collected muscle, blood, nail, and eggs from snapping turtles (*Chelydra serpentina*) inhabiting an Hg contaminated river. As predicted, all Hg tissue concentrations strongly and positively correlated with each other. Additionally, we validated our mathematical models against two additional Hg contaminated locations and found that tissue relationships developed from the validation sites did not significantly differ from those generated from the original sampling site. The models provided herein will be useful for a wide array of systems where biomonitoring of Hg in turtles needs to be accomplished in a conservation-minded fashion.

1. Introduction

The heavy metal mercury (Hg) is an important environmental concern due to its widespread prevalence and deleterious effects on humans and wildlife (Scheuhammer et al., 2007; Schmeltz et al., 2011). Within the United States, Hg is released into the environment by various anthropogenic sources, with coal burning power plants producing the majority of emissions (Schmeltz et al., 2011). Ultimately, Hg is deposited into aquatic ecosystems where sedimentary sulfate-reducing bacteria mediate methylation of Hg into its more toxic form, methylmercury (MeHg) (Lindqvist, 1991; Watras and Bloom, 1992). Methylmercury is problematic because it bioaccumulates over an individual's lifetime and biomagnifies within food webs (Lindqvist, 1991; Watras and Bloom, 1992). Additionally, Hg can be maternally transferred from female to offspring (Bergeron et al., 2010a) and several studies have demonstrated the negative influence of Hg on reproduction in oviparous vertebrates (Barr, 1986; Hammerschmidt et al., 2002; Brasso and Cristol, 2008; Bergeron et al., 2011).

Turtles have been proposed as excellent model organisms for monitoring Hg contamination in aquatic ecosystems because of their ecological and life-history attributes (Golet and Haines, 2001). Many turtle species are long-lived, occupy a wide-range of habitats, occur in high densities in a variety of aquatic habitats, and feed at high trophic levels (Iverson, 1982). Additionally, turtles and their eggs are common food items for predatory wildlife (Mitchell, 1994), and are utilized as a human food source in many regions (Green et al., 2010). Thus, the threat of Hg exposure extends to organisms that eat turtles and describing patterns in tissue concentrations is therefore important for determining safe human consumption limits and potential Hg exposure by predatory wildlife. However, the factors that may influence Hg bioaccumulation patterns in turtles remain unclear. For instance, some studies report Hg concentrations to increase with body size (Kenyon et al., 2001; Bergeron et al., 2007; Turnquist et al., 2011), but others have found no effect (Helwig and Hora, 1983; Golet and Haines, 2001). Additionally, some authors have speculated that females may have lower Hg body burdens since they maternally transfer Hg to their eggs (Meyers-Schöne and Walton, 1994), but reported sex differences have been inconsistent (Albers et al., 1986; Meyers-Schöne et al., 1993; Kenyon et al., 2001; Bergeron et al., 2007). A thorough evaluation of factors that influence Hg exposure and accumulation in turtles requires robust sample sizes across a wide range of Hg concentrations.

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To assess variation in Hg accumulation in turtles, most studies currently collect tissues that require sacrificing adult turtles or their eggs. Because many turtle populations are limited in their capacity to withstand declines in adult survivorship (Congdon et al., 1994) euthanizing adult turtles for monitoring Hg exposure and accumulation has ethical considerations, constrains research questions, and may be unsustainable for populations facing multiple anthropogenic challenges. However, non-destructive sampling techniques have not yet been adequately developed for studying Hg bioaccumulation in turtles. The development of non-destructive sampling methods would facilitate sustainable monitoring of spatial and temporal Hg patterns within turtles inhabiting polluted areas. Non-destructive sampling also enables repeated sampling of the same individual over time, which is critical for understanding temporal changes in Hg accumulation and exposure (Hopkins et al., 2005). Establishing mathematical correlations between tissues that are easy to collect and less invasive to sample (i.e., blood and nail) and tissues that are harder to collect (i.e., muscle and egg) but relevant to the health of turtles, their offspring, and organisms that consume turtles (including humans) may eliminate the need to sacrifice adult turtles or their eggs.

Although previous studies have described correlations between Hg tissue concentrations in turtles (Golet and Haines, 2001; Blanvillain et al., 2007; Turnquist et al., 2011) no study has yet described the relationship between tissues obtained via non-destructive techniques and those relevant to transgenerational effects and consumption risks. Additionally, no previous study has validated their tissue relationships to other Hg contaminated sites, an important step toward understanding whether mathematical relationships developed using data from one site are broadly applicable. Therefore, we sought to address two main objectives. First, we developed and validated non-destructive sampling techniques for assessing Hg bioaccumulation and maternal transfer in turtles so that future monitoring studies can be performed sustainably. Second, we described demographic, spatial, and temporal factors that influence Hg bioaccumulation in turtle tissues along a wide gradient of contamination so that risk assessors could use this information to inform human consumption guidelines for Hg impacted areas.

2. Methods

2.1. Study species

The snapping turtle (*Chelydra serpentina*) is a high trophic level predator widely distributed across freshwater habitats in eastern and central North America. Adult males generally reach larger body size than females, creating a distinct body size dimorphism between the sexes (Gibbons and Lovich, 1990). Female snapping turtles lay a single large clutch (averaging 26–55 eggs) per year (Miller et al., 1989). In Virginia, nesting typically occurs between mid-May and the end of June (Mitchell, 1994).

2.2. Sampling sites

We studied Hg bioaccumulation in snapping turtles inhabiting the South River, located near Waynesboro, VA, USA. From 1929 to 1950, Hg was released into the South River from an industrial plant that manufactured acetate fiber using a mercuric sulfate catalyst (Carter, 1977). An extensive gradient of Hg contamination has been documented along the South River, with water, sediment, and animal tissue concentrations increasing downstream from the contamination source (Southworth et al., 2004; Bergeron et al., 2007, 2010b; Brasso and Cristol, 2008). A previous study found that blood Hg concentrations in turtles downstream of the contamination source were up to 108-fold higher than those collected from nearby reference areas (Bergeron et al., 2007).

From April to July in 2010 and 2011, we collected snapping turtles at various locations upstream and downstream of the industrial plant on the South River and at several sites along the Middle River, a nearby tributary of the South Fork of the Shenandoah River that joins the South River at Port Republic, Virginia, USA (Fig. 1). Some of these sites had been sampled in a previous study on turtles (Bergeron et al., 2007), but we sampled additional sites in this study due to increased accessibility to properties and identification of additional areas of suitable turtle habitat. To encompass the extensive Hg contamination gradient that is present at the river, we sampled a total of eleven sites on the South River located between 2 and 22 (SR-Cont) river miles downstream of the contamination source (SR 0; Fig. 1). We used multiple sites ranging from 1.5 to 4.0 miles upstream of SR 0 as a reference (SR-Ref) along with additional reference sites located on the Middle River (MR-Ref). In addition to the relatively small home ranges snapping turtles exhibit (Obbard and Brooks, 1981), turtle movement between South River reference sites and the contaminated sites was limited by Rife Loth dam, located approximately one mile upstream of SR 0.

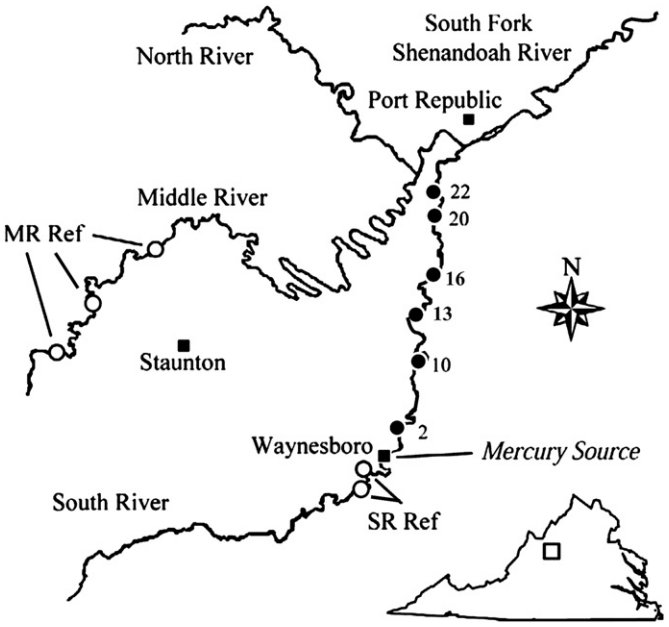

Fig. 1. Sampling locations along the South River (SR) and Middle River (MR), located in central VA, USA. Subsites sampled along the contaminated portion of the South River are represented by closed symbols and labeled by river mile downstream of the contamination source. Open symbols represent reference sites that were sampled upstream of the source along the SR and at additional locations along the MR. Note that the river flows from south to north.

To determine whether our non-destructive tissue models (described below) were applicable to turtles from other sites, we sampled blood and muscle tissues from turtles inhabiting two additional Hg impacted areas located in central and southwestern Virginia. We collected turtles from the South Fork of the Shenandoah River, Shenandoah, VA, and along the North Fork of the Holston River, Saltville, VA. Whereas the areas of the South Fork Shenandoah River have received Hg from downstream movement of contamination from several industrial sources over the years, a single source is responsible for the majority of Hg within the North Fork of the Holston. From 1950 to 1972, a chloralkali plant created a 30-ha disposal pond and filled it approximately 24 m deep with wastes containing Hg. This disposal area has since been identified by the Environmental Protection Agency (EPA) as a superfund site. A previous study in this river showed that snapping turtles have elevated tissue concentrations of Hg downstream from this superfund site (Hopkins et al., 2013). Because sampling at both validation sites occurred after turtle reproduction had ceased, we were unable to sample eggs from these locations.

2.3. Turtle collection and tissue sampling

We collected snapping turtles using baited hoop traps (Memphis Net and Twine, Memphis, TN, USA) set in suitable microhabitats along river banks and baited them with a mixture of sardines, corn, and/or chicken livers. Typically, we set 8–20 traps at a given site depending on habitat and size of the sampling reach. We checked traps daily until a site failed to produce new captures (typically within 3–4 days), at which time traps were removed and reset at a different site. We transported all turtles to the field station where they were measured to the nearest cm (carapace length, carapace width, and plastron length), weighed, sexed by visual examination of cloacal position, and permanently marked along their marginal scutes according to a three scute code, previously used by Bergeron et al. (2007) at this site, for future identification. 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 drew a 1-mL blood sample from either the caudal vein or the cervical sinus. We placed nail and blood samples separately in 1.5 mL Eppendorf tubes and stored them at −20 °C prior

to Hg analyses. To determine Hg concentrations in turtle muscle tissue, we removed a small biopsy from the ventral–lateral aspect of the tail following administration of a local anesthetic (Lidocaine). The biopsy site was then sutured with 2–3 stitches using clear polydioxanone monofilament material (3/8 cm) and a topical antibiotic was applied to reduce risk of infection. After we collected all tissue samples, we released turtles at their point of capture.

2.4. Egg collection

Upon capture, we physically palpated female turtles for the presence of shelled eggs. We injected gravid females intraperitoneally with 40 mg kg^{-1} of oxytocin solution every 24 h for three consecutive days to induce egg laying. In order to determine the correct dosage of oxytocin, we weighed all gravid female turtles prior to induction of egg laying, but to reduce handling stress, we delayed all other procedures until after oviposition. During egg laying, we placed females individually within 378 L Rubbermaid© tanks inside the field house. Each tank was filled with ~75 L of dechloraminated water. We removed all deposited eggs from the tanks within 3 h and marked and measured (length, width, and mass) each egg. Completion of oviposition was confirmed by radiographs taken at the Wildlife Center of Virginia, Waynesboro, VA. Once oviposition was complete, we randomly selected 3 eggs per clutch to be frozen at −20 °C to determine egg Hg concentration. All procedures performed for this study were approved by the animal care and use committee at Virginia Tech.

2.5. Mercury analysis

We lyophilized and homogenized muscle and eggs and report their THg (total mercury) concentrations on a dry weight (dwt) basis. We homogenized whole blood 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). Although intraclutch variation in turtle eggs is small for other contaminants (i.e., PCBs; Bishop et al., 1995), the intraclutch variation of Hg concentrations remains unknown. Therefore, in order to minimize any variation that may be present, a homogenized sample of three randomly selected eggs per clutch was used to determine egg Hg concentration for a given clutch. Percent moisture was 77.3 ± 0.24% (mean ± 1 standard error of the mean hereafter) for muscle and 75.5 ± 0.18% for eggs. Samples were analyzed for THg at the College of William and Mary, Williamsburg, VA, using combustion–amalgamation–cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80, Milestone, Monroe, CT, USA) according to U.S. Environmental Protection Agency (U.S. EPA) method 7473 (USEPA, 1998). 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 (National Research Council of Canada (NRCC), Ottawa, ON)). Method detection limits (MDLs; 3 times the standard deviation of procedural blanks) for samples were 0.0042 mg kg^{-1} (ppm), 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% ($n = 70$) to 102.08 ± 0.36% ($n = 70$), respectively.

We shipped nail clippings to the Center for Environmental Sciences and Engineering, University of Connecticut for analysis. Samples were digested and analyzed using cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80) according to U.S. Environmental Protection Agency (U.S. EPA) method 7473 (USEPA, 1998). 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 mg kg^{-1} and all samples had 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% ($n = 16$) and 94.2 ± 2.3% ($n = 15$), respectively. Calibration verification and laboratory control sample recoveries of THg averaged 104.7 ± 0.6% ($n = 62$) and 103.7 ± 1.2% ($n = 17$), respectively. Matrix spike recovery averaged 108.2 ± 4.4% ($n = 15$).

To understand the proportion of THg that was in the more toxic form of MeHg in tissues, we analyzed a subset of 12 homogenized muscle and egg samples for Hg speciation (MeHg/HgII) at Quicksilver Scientific using high pressure liquid chromatography (Method QS-LC/CVAF-001). Again, we used a homogenized sample comprised of three randomly selected eggs per clutch for egg MeHg analysis. Due to small sample mass, we pooled samples composed of three individual muscle samples (pooled by site, with similar body size and same sex) for muscle MeHg analysis. A combination of blanks (3), SRM's (2:TORT-2, and DOLT-4), laboratory control samples (1), matrix spikes (2) and sample duplicates (2) were used for quality control. The limit of detection for HgII and MeHg was 2.10 E^{-7} mg mL^{-1} for egg and 3.0 E^{-4} mg kg^{-1} for muscle and all samples had Hg concentrations that exceeded these limits. Average RPD between replicate sample analyses ($n = 2$) were 9.55 ± 3.65% for Hg II and 3.05 ± 2.85% for MeHg. Percent recovery for HgII/MeHg for the TORT-2, DOLT-4, and laboratory control samples were 106.6/109.8% ($n = 1$), 96.0/91.3% ($n = 1$), and 112.6/111.5% ($n = 1$) respectively. Average matrix spike recoveries ($n = 2$) of HgII and MeHg were 111.8 ± 0.8% and 107.9 ± 1.9%, respectively.

2.6. Statistical analyses

All analyses were performed using SAS 9.1 (SAS Institute, Inc, Cary, NC, USA) or Microsoft Excel with significance assessed at $\alpha \leq 0.05$. Among comparable demographics (i.e., similar size and same sex), we detected no difference in tissue THg concentrations between 2010 and 2011 captures (analysis of covariance, in all cases $p \geq 0.13$) and therefore these years were pooled for all subsequent analyses. When appropriate, THg concentrations were $\log_{10}$-transformed to improve normality and homoscedasticity of variance. Initial statistical models included all biologically relevant two and three-way interactions between independent variables and covariates, but we removed all interactions where $p > 0.10$ from final models.

We used Pearson correlation coefficients and linear regressions to assess relationships between blood, nail, egg, and muscle tissues from individual turtles collected from the South and Middle Rivers as well as individual turtles from our two validation sites, the North Fork of the Holston River and the South Fork Shenandoah River.

To validate the tissue relationship generated from the South and Middle Rivers to our two validation sites (North Fork Holston and South Fork Shenandoah Rivers), we estimated muscle Hg concentrations from the regression line generated from the South and Middle River dataset and calculated the percent difference between observed and predicted muscle tissue concentrations. In addition, we used an analysis of covariance (ANCOVA) to verify that the slope and y-intercept of the tissue models generated from the South and Middle River were similar at the two validation sites.

Due to potential emigrations and immigrations of individuals, subsites sampled along the Middle River, in addition to those subsites sampled up and downstream of the initial Hg source on the South River, are not independent of one another and are collectively referred to as SR-Ref (South River reference), MR-Ref (Middle River reference), and SR-Cont (South River contaminated) for all statistical comparisons of total Hg in turtle tissues among sites. To understand the spatial and temporal variation in Hg exposure, we evaluated differences in blood THg concentrations between turtles collected in an earlier survey in 2006 (Bergeron et al., 2007) and 2010–11 (this study) using a two-way ANCOVA on rank-transformed data (Conover and Iman, 1982) with year and site as main effects and carapace length as a covariate. Because blood was the only tissue sampled in the previous study (Bergeron et al., 2007), we were only able to test for temporal differences using THg concentrations with this variable. We present mean THg blood values across subsites in Fig. 3 for visual representation of spatial and temporal variation between our study and turtles sampled by Bergeron et al. (2007) in 2006. Additionally, we used a Tukey–Kramer method to determine differences in mean blood THg between years within each of the three sites.

Finally, we sought to understand if bioaccumulation of THg in muscle tissue differed by site and if demographic factors, such as body size and sex, influence Hg accumulation in turtle muscle. We tested for effects of body size and sex on muscle

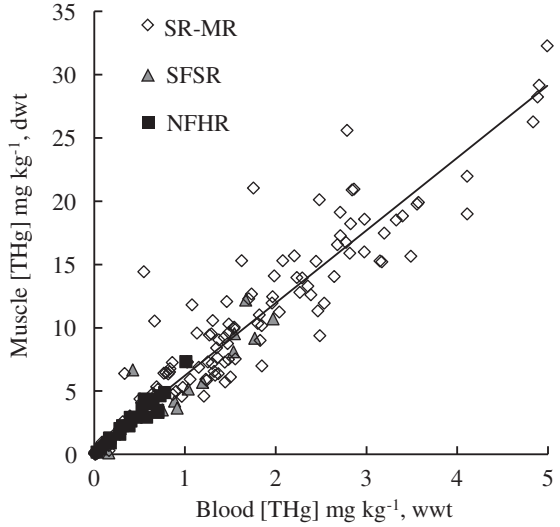

Fig. 2. Comparison between blood (wet weight) total mercury (THg) and muscle (dry weight) THg of *Chelydra serpentina* collected from the South and Middle Rivers (open diamonds) and the two validation sites the North Fork Holston River (closed squares), and the South Fork Holston (closed gray triangles). Note: regression line is representative of entire dataset, $y = 5.737x + 0.490$, $r = 0.95$, $p < 0.001$, $n = 205$.

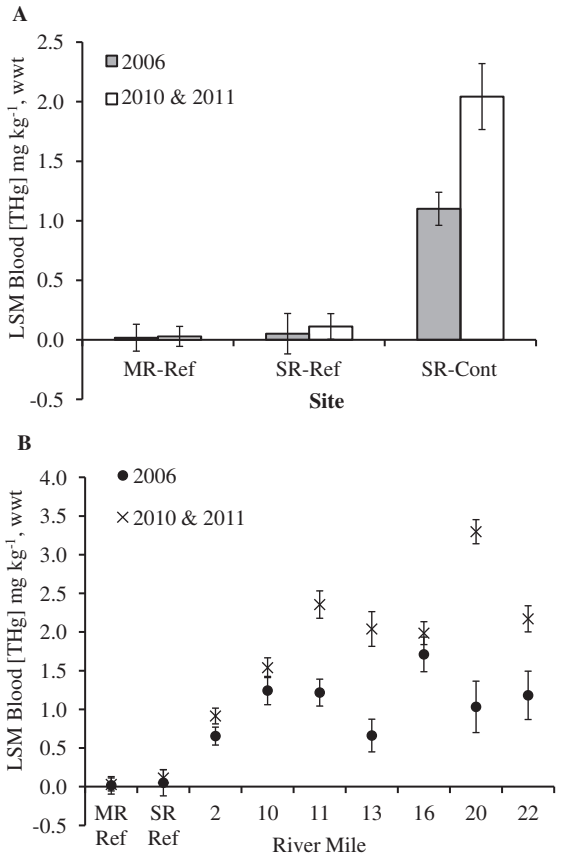

Fig. 3. A.) Average total mercury (THg) concentrations in blood of *Chelydra serpentina* collected from 2006 and 2010/11 sampling periods inhabiting areas of the Middle River reference site (MR-Ref), upstream of the Hg source on the South River (SR-Ref), and downstream of the Hg source on the South River (SR Cont). B.) Spatial representation of average THg concentrations at the two reference sites (MR Ref & SR Ref) and the various subsites downstream of the Hg source (RM 0) along the South River (River Mile 2-22), VA, USA, from the 2006 and the 2010–11 sampling periods. All values shown are least-square means corrected for body size (carapace length) ± 1 S.E.

THg concentrations using a two-way ANCOVA with site and sex as the main effect and carapace length as a covariate. Identifying how these explanatory variables relate to muscle Hg concentrations will assist in determining what factors should be considered when estimating risks of consuming turtles (e.g., size classes with highest Hg concentrations).

3. Results

3.1. Mercury concentrations and non-invasive sampling

Total Hg concentrations in tissues of turtles collected from the MR-Ref site ranged from 0.01 to 0.16 mg kg^{-1} (wwt) in blood, 0.04 to 0.71 mg kg^{-1} (dwt) in muscle, 0.15 to 1.72 mg kg^{-1} (fwt) in nail and 0.01 to 0.11 mg kg^{-1} (dwt) in eggs. Concentrations in tissues collected from turtles trapped just upstream of the source (SR-Ref) ranged from 0.01 to 0.87 mg kg^{-1} (wwt) in blood, 0.11 to 1.47 mg kg^{-1} (dwt) in muscle, 0.27 to 4.15 mg kg^{-1} (fwt) in nail and 0.04 to 0.17 mg kg^{-1} (dwt) in eggs. As expected, we found the highest THg tissue concentrations in individuals collected downstream of the source (SR-Cont), ranging from 0.01 to 4.99 mg kg^{-1} (wwt) in blood, 1.59 to 32.29 mg kg^{-1} (dwt) in muscle, 10.26 to 166.11 mg kg^{-1} (fwt) in nail and 0.95 to 6.06 mg kg^{-1} (dwt) in eggs. A smaller range in Hg concentrations was observed for tissues sampled from turtles collected from the two validation sites. Total Hg concentrations in tissues of turtles collected from the North Fork of the Holston River ranged from 0.03 to 1.01 mg kg^{-1} (wwt) in blood, 0.19 to 7.33 mg kg^{-1} (dwt) in muscle, and 0.81 to 32.33 mg kg^{-1} (fwt) in nail. Turtles collected from the South Fork of the Shenandoah (10–23 miles downstream of the South River confluence) had THg tissue concentrations ranging from 0.16 to 1.77 mg kg^{-1} (wwt) in blood and 0.12 to 12.20 mg kg^{-1} (dwt) in muscle. In all cases, tissues sampled from turtles were strongly and positively correlated with one another (Table 1, Fig. 2; all $p < 0.001$).

In both cases, the regression line formulated from the South and Middle River blood–muscle relationship tended to overestimate muscle Hg concentrations in turtles collected from the two validation sites (Table 1). On average, the regression model generated from the South and Middle River dataset overestimated muscle Hg concentrations by 15.6 ± 4.6% and 9.9 ± 14.2% for the North Fork Holston and the South Fork Shenandoah, respectively. In both cases, however, models describing relationships between tissues generated from turtles sampled from the North Fork of the Holston and the South Fork of the Shenandoah Rivers did not differ significantly from those of the South and Middle Rivers (in both cases $p \geq 0.34$).

Turtles inhabiting our primary study areas had egg and muscle methylmercury concentrations ranging from 25.8 to 77.7% and 86.6 to 96.3%, respectively. As THg concentrations in eggs increased so did the %MeHg (Table 1; $R^2 = 0.52$, $p = 0.007$). However, there was not a significant correlation between THg and %MeHg for muscle tissue sampled (Table 1: $p = 0.13$).

3.2. Spatial and temporal patterns

Blood THg concentrations differed greatly among the Middle River reference, South River reference, and South River contaminated sites (Fig. 3A, site: $F_{2,460} = 48.81$, $p < 0.001$). The contaminated site (SR-Cont) had the highest mean THg concentrations in blood (1.72 ± 0.06 mg kg^{-1}, wwt) followed by South River reference site (0.12 ± 0.15 mg kg^{-1}, wwt) and the Middle River reference site (0.03 ± 0.12 mg kg^{-1}, wwt). In general, blood THg increased from 2006 to 2010–2011 (year: $F_{1,420} = 17.12$, $p < 0.001$) but this effect was dependent upon site (site × year: $F_{2,420} = 3.64$, $p = 0.027$) (Fig. 3A). Post-hoc analysis revealed no differences in mean blood

Table 1
Relationships in total mercury (THg) concentrations among tissues in *Chelydra serpentina* collected from the South River (SR), Middle River (MR), North Fork Holston River (NFHR), and South Fork Shenandoah River (SFSR), VA, USA. All regressions were calculated from raw data reported in mg kg^{-1}. Egg and muscle THg values are reported on a dry weight basis whereas nails and blood are reported on a fresh and wet weight basis, respectively.

Site	Regression	Slope	Intercept	*r*	*p*-value	*n*
SR/MR	THg Blood (*x*) THg Muscle (*y*)	5.723	0.573	0.96	<0.001	170
NFHR	THg Blood (*x*) THg Muscle (*y*)	6.347	0.019	0.96	<0.001	23
SFSR	THg Blood (*x*) THg Muscle (*y*)	5.430	0.293	0.86	<0.001	12
SR/MR	THg Blood (*x*) THg Nail (*y*)	26.503	1.769	0.89	<0.001	131
NFHR	THg Blood (*x*) THg Nail (*y*)	29.384	0.067	0.94	<0.001	23
SR/MR	THg Nail (*x*) THg Muscle (*y*)	0.178	1.113	0.92	<0.001	112
NFHR	THg Nail (*x*) THg Muscle (*y*)	0.201	0.196	0.95	<0.001	23
SR/MR	THg Nail (*x*) THg Egg (*y*)	0.035	0.391	0.92	<0.001	95
SR/MR	THg Blood (*x*) THg Egg (*y*)	1.129	0.311	0.92	<0.001	95
SR/MR	THg Muscle (*x*) %MeHg Muscle (*y*)	0.172	92.241	0.21[a]	0.133	12
SR/MR	THg Egg (*x*) %MeHg Egg (*y*)	6.975	37.517	0.52[a]	0.008	12

[a] Denotes an R^2 value.

THg concentrations between the Middle and South River reference sites (in both cases $p \geq 0.993$) between those published by Bergeron et al. (2007) and current concentrations. In contrast, we detected significant differences between the two sampling time frames within the contaminated site ($p < 0.001$). Within the contaminated site, blood THg concentrations increased by 16.0–219.4% from 2006 to 2010–11, depending on river mile (Fig. 3B).

3.3. Demographic patterns influencing bioaccumulation

Body size (carapace length) significantly influenced THg concentrations in muscle ($F_{1,169} = 9.11$, $p = 0.003$, Fig. 4), but the nature of this effect was dependent upon site (carapace length × site $F_{2,169} = 5.87$, $p = 0.003$). Muscle THg concentrations increased with body size for individuals 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 $p \geq 0.16$). Additionally, muscle THg concentrations differed significantly between sexes after correcting for body size ($F_{1,169} = 12.21$, $p < 0.001$), with females having higher THg in muscle than males collected from a given site (Fig. 5).

4. Discussion

Our results demonstrate that minimally invasive tissues can be used to sustainably monitor Hg concentrations in turtle muscle and those maternally transferred to eggs, which has implications for turtle health, reproduction, and conservation. Consistent with previous studies of both snapping turtles and other herpetofauna (Bergeron et al., 2010a, 2010b; Golet and Haines, 2001; Turnquist et al., 2011; Hopkins et al., 2013), we demonstrated that THg concentrations in all tissues strongly and positively correlated with each other. However, the predictive models presented here are the first to be validated against Hg concentrations from individuals inhabiting other Hg contaminated sites. Finally, our data revealed several important spatial, temporal, and demographic patterns in bioaccumulation that may be important factors to consider when evaluating human consumption risks, and developing monitoring, restoration, and mitigation programs for Hg contaminated systems.

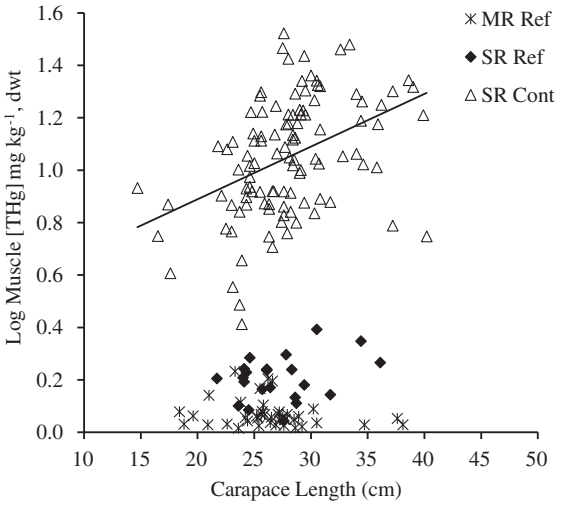

Fig. 4. Relationships between carapace length and total mercury (THg) in muscle (dry weight) tissue of *Chelydra serpentina* collected from two reference sites on Middle River (MR-Ref; $n = 40$) and the South River (SR-Ref; $n = 22$), and the contaminated portion of the South River (SR-Cont; $y = 0.0201x + 0.4869$, $R^2 = 0.17$, $p < 0.001$, $n = 108$), VA, USA. Total Hg in turtle muscle did not increase with increasing body size with the two reference sites ($p \geq 0.16$), but did for individuals collected within the contaminated site ($p < 0.001$).

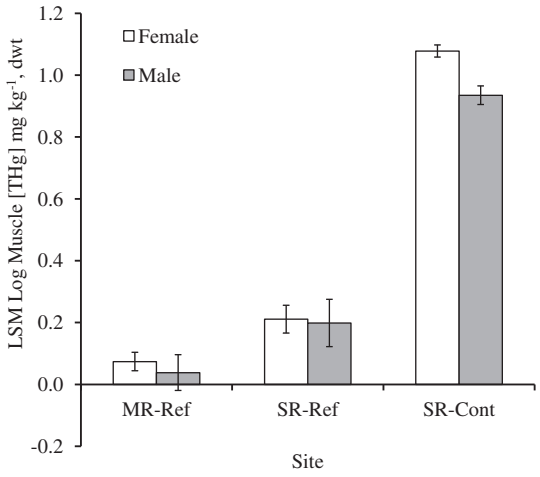

Fig. 5. Total mercury (THg) concentrations in male and female *Chelydra serpentina* muscle tissue (dry weight) collected from the two reference sites along the Middle River (MR-Ref) and upstream of the source on the South River (SR-Ref), and the contaminated site (SR-Cont), located downstream of the Hg source, VA, USA. Values shown are least-squares means (±1 standard error) corrected for body size (carapace length).

Turtles collected from the contaminated portion of the South River had significantly higher THg tissue concentrations than individuals collected from reference sites. Total Hg concentrations in blood, muscle and nail of adult turtles collected from the contaminated portion of the South River are the highest ever reported in turtles and surpass tissue concentrations that are associated with adverse neurological, physiological, and reproductive effects observed in other aquatic animals (Scheuhammer et al., 2007). For example, blood THg concentrations in turtles inhabiting contaminated areas of the South River ranged from 0.01 to 4.99 mg kg^{-1} (wwt). Comparatively, common loons (*Gavia immer*) with blood THg concentrations above 3.0 mg kg^{-1} (wwt) were reported to have reduce fledging success and those exceeding 4.0 mg kg^{-1} (wwt) were associated with reduced reproductive effort, elevated stress hormones, asymmetry in flight feathers, and altered breeding behaviors (Scheuhammer et al., 2007). Muscle THg concentrations in turtles inhabiting contaminated areas of the South River ranged from 0.10 to 32.29 mg kg^{-1} (dry), with 86.6–96.3% being in the more toxic form of MeHg. In fish, muscle THg concentrations reaching ~30 mg kg^{-1} (dwt), which falls within the upper end of the range of THg concentrations reported here in turtle muscle, was associated with reduced growth and survival (Weiner and Spry, 1996).

Our study is the first to rigorously demonstrate that Hg can maternally transfer from female to eggs in turtles (for companion study see Hopkins et al., in press). Maternally transferred THg concentrations in egg were found to range from 0.04 to 6.06 mg kg^{-1} (dwt), with MeHg comprising 29.5–77.7% of the THg within the egg. Although the effects of maternally transferred Hg on turtle reproduction are still unknown, a recent study in Carolina wrens (*Thryothorus ludovicianus*) showed a 50% reduction in nest success when egg THg concentrations reached ~2.15 mg kg^{-1} (dwt) (Jackson et al., 2011a). In free-living common loons, egg THg concentrations of ~2.5 mg kg^{-1} (dwt) were associated with impaired hatchability and embryotoxicity (Barr, 1986). Bergeron et al. (2011) showed that maternally transferred egg THg concentrations of ~1.7 mg kg^{-1} (dwt) were associated with a 25% reduction in hatching success of American toads (*Bufo americanus*). Based on these studies, it is possible that the concentrations of Hg

we documented in turtle eggs may lead to deleterious effects on hatching success or other aspects of reproduction.

We demonstrated that our mathematical models describing the relationships between tissues sampled from turtles inhabiting the South River are applicable to those from other Hg contaminated sites, providing evidence that our tissue models can be used in future sustainable monitoring programs in other Hg contaminated areas. Although the regression model generated from the South and Middle River dataset did not significantly differ from those generated from the validation sites, it tended to overestimate Hg concentrations in turtle tissue at the validation sites. Therefore, in order to capture the variance observed at all three sites that we sampled, we offer the following regression equation, $y = 5.737x + 0.490$, generated by combining blood and muscle Hg concentrations observed in turtles from all sites (Fig. 2). We recommend that future researchers use the combined regression model to predict muscle concentrations from blood concentrations in turtles.

We identified several important spatial and temporal patterns in Hg bioaccumulation within tissues sampled from turtles inhabiting the South and Middle Rivers. Total Hg concentrations in turtle blood slowly increased downstream of the Hg source, peaking ~20 miles downstream and then declining. In addition, THg concentrations in blood collected from contaminated turtles significantly increased since 2006, while Hg concentrations in reference turtles have remained relatively low and unchanged over the same time period. Similar spatial and temporal trends have been reported within other South River biota in recent years (e.g., fish, amphibians, birds, invertebrates) (Southworth et al., 2004; Bergeron et al., 2007, 2010b; Brasso and Cristol, 2008; Jackson et al., 2011b). Mercury has been found to persist within the South River floodplain and can be re-circulated into the river by the combination of flood events, bank erosion, and anthropogenic and agricultural disturbances (Rhoades et al., 2009; Newman et al., 2011), which may explain the temporal changes in Hg tissue concentrations within inhabiting biota. Gradual reintroduction of Hg from large expanses of the floodplain may also help explain why Hg levels peak in turtles ~20 miles downstream of the point source.

We demonstrate that demographic factors, like body size and sex, significantly influenced Hg concentrations. Previous studies have found a similar pattern, with Hg concentrations increasing with respect to body size in turtles and other aquatic species (Kenyon et al., 2001; Bergeron et al., 2007; Turnquist et al., 2011; but see Helwig and Hora, 1983; Golet and Haines, 2001). In snapping turtles, the pattern of increasing Hg concentrations within larger individuals is most likely a consequence of age, as larger turtles are more likely to be older, and therefore have had more time to bioaccumulate Hg than those of smaller size classes. Additionally, we found that female snapping turtles had higher muscle Hg concentrations than males at a given size. This pattern is counter to some studies that suggest females should have lower Hg body burdens since they maternally transfer Hg to their eggs, thus providing females with an additional excretion pathway compared to males (Meyers-Schöne and Walton, 1994). Instead, we believe higher Hg concentrations present in female snapping turtle tissue may be due sexual dimorphism in size and growth rate. Adult female snapping turtles grow at a slower rate and asymptote at a smaller size compared to males (Christiansen and Burken, 1979; Galbraith et al., 1987), therefore, it is likely that many females are older than males of a similar size allowing them to accumulate more Hg than males. Although we cannot eliminate the alternative explanation that the sexes may differ in feeding rates and/or feeding ecology, there is little evidence in the literature to suggest this may be the case.

5. Conclusions

Our study provides a foundation for future studies intending to use snapping turtles to monitor spatial, temporal, and demographic patterns of bioaccumulation and maternal transfer of contaminants. Future research is needed to thoroughly understand the effects that Hg is having on turtles and the risk of exposure that Hg bioaccumulation is posing to wildlife and humans that consume turtles. Although Hg concentrations observed in turtle muscle and egg in some cases exceed those associated with negative effects in other species, our comparisons remain speculative because the actual Hg concentrations associated with adverse effects in turtles remain unknown. Taken together, all previous studies linking Hg exposure to effects demonstrate a large range in Hg sensitivity among different species (Scheuhammer et al., 2007), reinforcing the need for future research to determine the sensitivity of snapping turtles to Hg. Additionally, the rate at which turtles are harvested and consumed by wildlife predators and humans in most systems including the South River remains unknown. To understand the degree of Hg exposure through consuming contaminated turtles, future research is needed to determine human and wildlife consumption patterns of adult turtles and eggs.

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