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MERCURY ACCUMULATION ALONG A CONTAMINATION GRADIENT AND NONDESTRUCTIVE INDICES OF BIOACCUMULATION IN AMPHIBIANS

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Abstract—Mercury (Hg) is an important environmental contaminant due to its global distribution, tendency to bioaccumulate, and toxicity in wildlife. However, Hg has received little attention in amphibians compared to other vertebrates. Amphibians vary widely in life history strategies and feeding ecologies, which could influence Hg exposure and accumulation. To determine whether species and life stage affects Hg bioaccumulation, adults from three species (*Plethodon cinereus, Eurycea bislineata,* and *Bufo americanus*) and larvae from the latter two species were collected along a contamination gradient on the South River (VA, USA). Total Hg (THg) concentrations in the contaminated site were 3.5 to 22 times higher than in the reference site. Differences were found in THg concentrations in amphibians that were consistent with their habitat requirements and feeding preferences. In general, adults ($3{,}453 \pm 196$ ng/g, dry mass) and larvae ($2{,}479 \pm 171$ ng/g) of the most river-associated species, *E. bislineata,* had the highest THg concentrations, followed by *B. americanus* tadpoles ($2{,}132 \pm 602$ ng/g), whereas adults of the more terrestrial *B. americanus* (598 ± 117 ng/g) and *P. cinereus* (583 ± 178 ng/g) had the lowest concentrations. In addition, nondestructive sampling techniques were developed. For the salamander species, THg concentrations in tail tissue were strongly correlated ($r \geq 0.97$) with the remaining carcass. A strong positive correlation ($r = 0.92$) also existed between blood and whole-body THg concentrations in *B. americanus*. These results suggest that amphibians and their terrestrial predators may be at risk of Hg exposure in this system and that nondestructive methods may be a viable sampling alternative that reduces impacts to local populations. Environ. Toxicol. Chem. 2010;29:980–988.
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INTRODUCTION

Mercury (Hg) is an important environmental contaminant due to its global ubiquity, tendency to bioaccumulate, and toxicity in wildlife. Atmospheric deposition results in widespread distribution of Hg due to long-range airborne transport [1]. Mercury tends to accumulate in wetlands where it readily bioaccumulates and can biomagnify in aquatic food webs, especially as (mono)methylmercury (MMHg) [2,3]. Mercury accumulation in wetlands threatens critical breeding and foraging habitats for many fish and wildlife species. The primary route of Hg exposure is through the diet [4,5], and consumption of diets with environmentally realistic concentrations of Hg result in a large range of toxic effects in fish and wildlife, including behavioral, developmental, neurological, hormonal, and reproductive changes [6–9].

Mercury accumulation in amphibians has received little attention compared to fish, birds, and mammals [6,9,10]. Amphibians play a critical role in transferring energy and nutrients through food webs, and therefore are important components of aquatic and terrestrial communities [11,12]. As ectotherms, amphibians are efficient at converting ingested energy into biomass [13,14] and as a result, can often occur in extremely high densities. For example, in Virginia, certain species of amphibians represent the single most abundant vertebrate in many aquatic and terrestrial communities, exceeding 40,000 individuals per hectare [15]. Due to their abundance and high conversion efficiencies, amphibians often serve as vital links for energy and nutrient flow between low and high trophic levels. These characteristics also suggest that amphibians may have high rates of contaminant bioaccumulation compared with other animals of similar trophic positions. In addition, the complex life cycles of many amphibians potentially make them important in transferring contaminants from aquatic to terrestrial food chains [16,17], especially for Hg, because evidence suggests that most Hg accumulated by larvae is retained through metamorphosis [4,18].

Although Hg is ubiquitous due to long-range transport from atmospheric deposition [1], point source emissions can result in high localized Hg concentrations. For example, the South River, VA, USA, was historically (1929–1950) contaminated with mercuric sulfate used by a plant manufacturing acetate fiber in Waynesboro, VA, USA [19]. The use of Hg was terminated in the 1950s, but Hg concentrations currently remain high in the river [20]. This system contains a wide gradient of contamination between locations upstream and downstream from the point source. Previous studies reveal orders of magnitude differences in tissue Hg concentrations from several species between the upstream (uncontaminated reference) and downstream (contaminated) sites (fish [20], G.W. Murphy, 2004, Master's thesis, Virginia Polytechnic Institute and State University, Blackburg, VA, USA; birds [21]; turtles [22]).

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The first objective of the present study was to determine whether Hg accumulation and risk of Hg exposure differs among amphibian species and stages. To accomplish this objective, three species of adult amphibians, *Eurycea bislineata cirrigera*, *Plethodon cinereus*, and *Bufo americanus* were collected, which exhibit different life histories and occupy different ecological niches within the South River system. For *E. bislineata cirrigera,* and *B. americanus*, larvae were also collected, because accumulation of contaminants has been shown to differ among life stages [16]. The second objective was to determine whether spatial patterns of Hg accumulation in these amphibian species were similar to other taxa along the South River. The final objective was to develop nondestructive methods for assessing Hg bioaccumulation in amphibians, which will be critical for future studies of amphibians on the South River, as well as in other localities where Hg contamination is a concern. To meet this objective, Hg concentrations in tissues sampled nonlethally were correlated with carcass Hg concentrations. Blood samples were collected from *B. americanus*, but the small body size of the salamander species precluded this technique. Many salamander species, including *E. bislineata cirrigera,* and *P. cinereus*, exhibit tail autotomy as a natural defensive mechanism to prevent capture by predators [23] so body and tail tissue were collected and analyzed separately for these species.

MATERIALS AND METHODS

Species natural history

Mercury bioaccumulation patterns observed in amphibians should reflect local conditions due to their movement patterns. In general, terrestrial stages of amphibians are not as mobile compared to many other vertebrates, and many exhibit high fidelity to breeding and overwintering habitats [24]. From several studies, Wells [24] summarizes that routine movement during their active season averages less than 10 m for terrestrial salamanders and less than 15 m for anurans. In the present study, *B. americanus* is likely the most mobile species as females have been shown to disperse up to 1 km from their breeding pond [25].

The three amphibians studied in the South River are common, widespread species that exhibit ecological and life history traits encompassing most of the North American amphibian species. The two salamanders are both from the family Plethodontidae, *Eurycea bislineata cirrigera* (hereafter *E. bislineata*; Southern two-lined salamander) and *Plethodon cinereus* (red-backed salamander), and the anuran, *Bufo americanus* (American toad) is from the Bufonidae family. *Eurycea bislineata* inhabit streams from Virginia to the Gulf Coast. Adults can be found beneath rocks and logs along the margins of rocky streams, but also in the forest farther from shore. The larval stage is fully aquatic and lasts one to three years. They reside beneath cover objects until foraging at night. Both adults and larvae consume a variety of small invertebrates [23]. The second salamander species, *Plethodon cinereus*, inhabits the forest litter environment in deciduous, northern conifer, or mixed habitats from North Carolina to the Canadian Maritime Provinces and west to Minnesota. Many individuals remain underground, but when surface active, they remain under cover (i.e., logs, rocks, leaf litter) during the day and emerge at night to forage. *Plethodon cinereus* are top predators within detrital food webs and prey mainly on small invertebrates. *Plethodon cinereus* bypasses an aquatic larval stage and reproduces through direct development [23,26]. *Bufo americanus* are found throughout eastern North America to the edge of the coastal plain. The terrestrial habitat of adult *B. americanus* is diverse, but they require cover objects with moist microhabitats, abundant supply of insects and other small invertebrates as prey, and shallow bodies of water to breed (e.g., ephemeral wetlands, drainage ditches, shallow portions of streams with low flow). *Bufo americanus* tadpoles are omnivorous grazers and will consume algae, suspended organic matter, detritus, and decomposing animal material [26].

Animal collection

Amphibians were collected from multiple sites upstream and downstream of the Hg contamination source (river mile [RM] 0) along the South River (Fig. 1) in all available and accessible habitats for each species. Adult and larval *E. bislineata* were captured by turning rocks, logs, and leaf litter along banks within 3 m of the edge of the water and in shallow margins of the river while using a mesh dip net to collect individuals. *Eurycea bislineata* were sampled at two reference locations, a South River reference site, 5 river miles upstream from the Hg source (SR REF; RM −5), and Coyner Springs Park (Parks and Recreation, Waynesboro, VA, USA; CS REF), an upstream tributary to the South River. The aquatic portion of the reference subsites are separated from contaminated subsites by Rife Loth dam in Waynesboro, VA, USA, upstream from the source of contamination. In the contaminated portion of the South River, eight subsites were sampled downstream from the Hg source

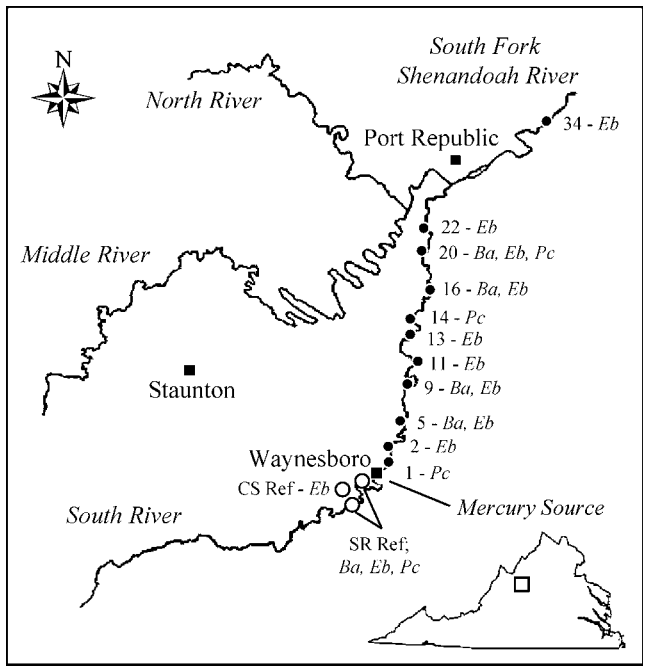

Fig. 1. Sampling locations of the three amphibian species studied (Ba = *Bufo americanus*; Eb = *Eurycea bislineata*; Pc = *Plethodon cinereus*) along the South River (SR), Coyner Springs Park (CS), and South Fork of the Shenandoah River (SFSR) of the Shenandoah Valley (VA, USA). Numbers refer to river miles downstream from contamination source (river mile 0). Open symbols represent reference sites and closed symbols represent contaminated sites. Note that the South River flows south to north.

(RM 2, 5, 9, 11, 13, 16, 20, and 22) and are collectively referred to as the contaminated site. An additional site was sampled approximately 10 miles after the South River joins the South Fork of the Shenandoah River (SFSR; RM 34) (Fig. 1, Table 1).

Adult *P. cinereus* were captured by turning rocks and logs in the riparian zone along the South River. The distribution of *P. cinereus* along the South River was patchy and thus they were only successfully collected at SR REF (RM −5) and three subsites within the contaminated reach (RM 1, 14, and 20) (Fig. 1, Table 1). Collection localities ranged from 5 to 30 m from the edge of the river.

Both species of salamanders were captured over a two-week period in May 2007. Upon capture, salamanders were given a unique identification code and placed in a Ziploc bag with water and/or a wet paper towel. A Garmin (Olathe) hand-held global positioning system (GPS) unit was used to obtain geospatial coordinates for each individual captured. Salamanders were transported to the laboratory and held for 48 h to void their gut contents and then sacrificed using an overdose of buffered tricaine methane sulfonate (MS-222). Mass, total length (TL), and snout–vent length (SVL) were obtained. To standardize sampling for this study, the tail of adult salamanders was removed just posterior to the vent using a sterile blade. In future studies, where salamanders are to be released, tail autonomy could be induced by gently restraining the animal by the tail. Adult bodies and tails and whole-body larvae were frozen separately until analysis.

Breeding pairs of *B. americanus* were captured over 4.5 weeks in March and April 2007 from breeding ponds located in or near the flood plain of the South River. Ponds were located within 30 to 180 m of the river. Toads were collected from the reference site encompassing two subsites on the South River (SR REF; RM −1.7 and −5) and five subsites in the contaminated reach (RM 2, 5, 9, 16, and 20) (Fig. 1, Table 1). Females were given a unique identification code and pairs were placed in individual breeding containers with water. Again, geospatial coordinates for each pair were acquired. The pairs were transported to the laboratory and their eggs were obtained for research on reproductive effects in females (results to be reported elsewhere). After oviposition, the male was released at the site of capture and the female was held for 48 h to void her gut contents. Mass and SVL were recorded. Before sacrificing the female with an overdose of MS-222, approximately 0.6 ml of blood was withdrawn by cardiac puncture (or decapitation after sacrifice) using a 1-ml heparinized syringe. Female toads and blood samples were frozen until analysis.

In May 2007, *B. americanus* tadpoles were collected from the same ponds where breeding pairs were captured. Approximately 20 tadpoles between Gosner stages 28 and 32 (before forelimb emergence) [27] were collected from each subsite. Tadpoles were held for 48 h to void their gut contents, sacrificed with an overdose of MS-222, weighed, and frozen until analysis. Before analysis, four to seven tadpoles were pooled to create three to four composite samples per site (Table 1). When different developmental stages of tadpoles existed within a site, the stages were distributed among composites as equally as possible.

Total mercury analysis

Adult bodies, larval bodies, and salamander tails were lyophilized and homogenized separately and Hg concentrations are reported on a dry weight basis. Whole blood from adult *B. americanus* was homogenized using a vortex mixer and Hg concentrations are reported on a wet weight basis. Whole-body percent moisture, calculated from weights before and after lyophilization, was similar for all species (adult *E. bislineata* $73.9 \pm 0.5\%$; larval *E. bislineata* $76.0 \pm 0.3\%$; *P. cinereus* $75.9 \pm 0.4\%$; *B. americanus* $77.8 \pm 0.4\%$). Subsamples (20 to 150 mg) were analyzed for total Hg (THg) content by combustion–amalgamation–cold vapor atomic absorption

Table 1. Individual sample sizes for total mercury (THg) analyses in the three amphibian species, *Eurycea bislineata* (adult and larvae), *Plethodon cinereus* (adult), and *Bufo americanus* (adult and larvae), at the reference subsites (South River reference [SR REF] and Coyner Springs Park [CS REF]) and contaminated subsites of the South River (SR) and the South Fork of the Shenandoah River (SFSR) VA, USA[a]

Site	*E. bislineata* adult	*E. bislineata* larvae	*P. cinereus* adult	*B. americanus* adult	*B. americanus* larvae[b]
Reference subsites					
SR REF	5	5	6	13	7
CS REF	5	5	NA	NA	NA
Reference (totals)	10	10	6	13	7
Contaminated subsites					
SR RM 1	NA	NA	6	NA	NA
SR RM 2	5	5	NA	1	4
SR RM 5	5	5	NA	12	4
SR RM 9	5	5	NA	12	4
SR RM 11	1	1	NA	NA	NA
SR RM 13	5	5	NA	NA	NA
SR RM 14	NA	NA	6	NA	NA
SR RM 16	5	5	NA	4	4
SR RM 20	5	5	6	6	3
SR RM 22	5	5	NA	NA	NA
South River (totals)	36	36	18	35	19
South Fork Shenandoah River					
SFSR RM 34	5	4	NA	NA	NA
Species Total	51	50	24	48	26

[a] Sites where species were not found are denoted as not applicable (NA).
RM = river mile from contamination source.
[b] Composite samples of four to seven tadpoles each.

spectrophotometry (DMA 80, Milestone) according to U.S. Environmental Protection Agency (U.S. EPA) method 7473 [28]. For quality assurance, each group of 10 to 15 samples included a replicate, blank, and standard reference material (SRM; TORT-2 lobster hepatopancreas, DOLT-2 dogfish liver [National Research Council of Canada, Ottawa, ON] or SRM 966 Toxic Metals in Bovine Blood Level 2 [National Institute of Standards and Technology, Gathersburg, MD, USA]). The instrument was calibrated using solid SRMs (TORT-2 and DORM-2, dogfish muscle [National Research Council of Canada, Ottawa, ON]). Method detection limits (MDLs; three times standard deviation of procedural blanks) for samples depended on sample mass and were calculated separately for each observation based on sample mass. Method detection limits ranged from 5.80 to 48.04 ng/g, and all samples had THg concentrations that exceeded the limit. Average relative percent difference between replicate sample analyses was $3.59 \pm 0.51\%$ ($n = 46$; mean $\pm$ 1 standard error of the mean hereafter). Mean percent recoveries of THg for the standard reference materials TORT-2, DOLT-2, and SRM 966 were $89.66 \pm 0.01\%$ ($n = 46$), $100.99 \pm 0.01\%$ ($n = 8$), and $92.11 \pm 0.04\%$ ($n = 8$), respectively.

Statistical analyses

For spatial comparisons, subsites within the reference and contaminated reaches of the river could not be treated independently of one another and are collectively referred to as the reference site and contaminated site, respectively. Because sampling SFSR occurred 10 miles after the confluence (RM 34, Fig. 1), it was also treated as a separate site. In addition, species could not be compared statistically because each species occurred at different subsites (Table 1) and Hg accumulation patterns may have depended upon which subsites were sampled.

To compare THg concentrations among reference and contaminated sites and stages within each species, the assumptions of analysis of variance (ANOVA) were first tested. Data were $\log_{10}$-transformed to meet ANOVA assumptions or analogous nonparametric tests were employed. For *E. bislineata* a two-way ANOVA was performed to compare sites and stages followed by Tukey's pairwise comparisons. A one-way ANOVA was performed for *P. cinereus* to compare sites. Because transformations failed to meet the assumptions of ANOVA for *B. americanus* data, a Scheirer–Ray–Hare extension of the Kruskal–Wallis test was performed [29] to compare sites and stages. Pearson correlation coefficients were used to assess relationships between salamander bodies and tails as well as between *B. americanus* whole bodies and blood. All analyses were performed with SAS 9.1 (SAS Institute), and an α value of 0.05 was used to assess statistical significance.

RESULTS

Effects of site and stage on mercury accumulation

A large range in THg concentrations was observed among species and stages (Figs. 2–4), with greater concentrations for all species and stages within the South River downstream from the source of contamination (Figs. 2A, 3A, and 4A). Although subsites within the reference and contaminated reaches of the South River could not be treated independently of one another in the statistical comparisons, Figures 2B, 3B, and 4B illustrate

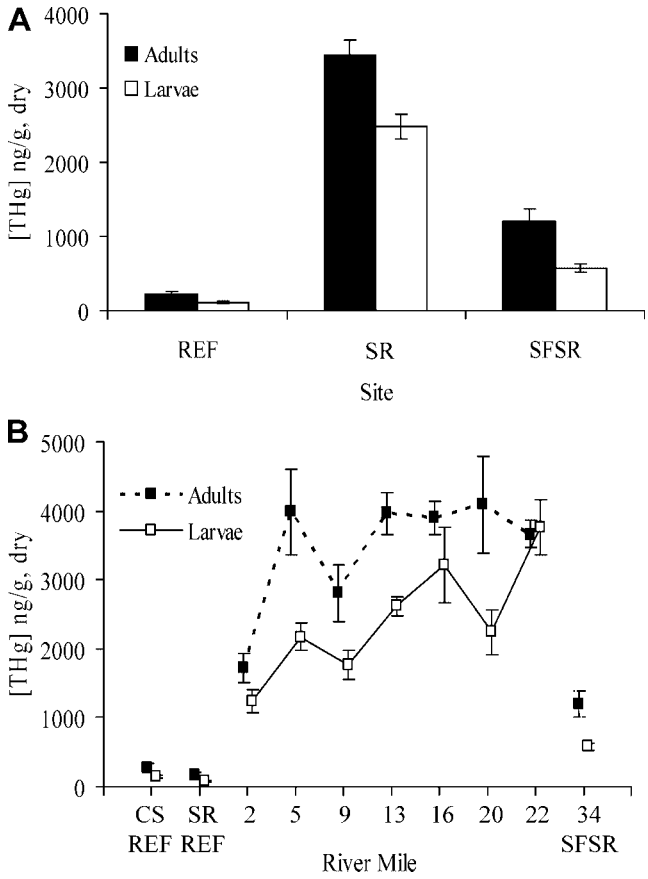

Fig. 2. (**A**) Whole-body total mercury (THg) concentrations (ng/g, dry mass; mean $\pm$ 1 standard error [SE]) in adults and larval *Eurycea bislineata* from the reference (REF) and contaminated portion of the South River (SR) and the South Fork of the Shenandoah River (SFSR). (**B**) Whole-body THg (ng/g, dry mass; mean $\pm$ 1 SE) in adult and larval *E. bislineata* at the reference and contaminated subsites along SR and SFSR. Numbers represent river miles downstream from the source of contamination (river mile 0). Lines connecting points are for visual presentation and do not reflect connectivity among means. Symbols are staggered for clarity.

the spatial trends downstream from the contamination source. In the contaminated site, *E. bislineata* adults and larvae had the highest THg concentrations, followed by *B. americanus* tadpoles, whereas terrestrial adult *B. americanus* and *P. cinereus* had the lowest concentrations.

For *E. bislineata*, THg concentrations were influenced both by site ($F_{2,96} = 23.42$, $p < 0.001$) and stage ($F_{1,96} = 410.89$, $p < 0.001$). No interaction was detected between site and stage ($p = 0.270$), indicating similar patterns between stages at different sites. Indeed, THg concentrations in adult *E. bislineata* were 1.4- to 2.1-fold higher than larval concentrations at each site. Pairwise comparisons revealed significant differences in THg concentrations ($p < 0.001$) between all three sites (Fig. 2A). Total Hg concentrations in adults and larvae at the contaminated site were 15- and 22-fold higher than the reference site and 2.9- and 4.3-fold higher than the SFSR site, respectively. Despite being 34 miles downstream from the original Hg source, THg concentrations in adult and larval *E. bislineata* were 5.2- and fivefold higher in the SFSR than the reference site, respectively.

Total Hg concentrations were 5.4-fold higher in *P. cinereus* from the contaminated site compared to the reference site ($F_{1,22} = 11.83$, $p = 0.0023$) (Fig. 3A). Similarly, THg concentrations were 3.5- and 4.0-fold higher in adult and larval

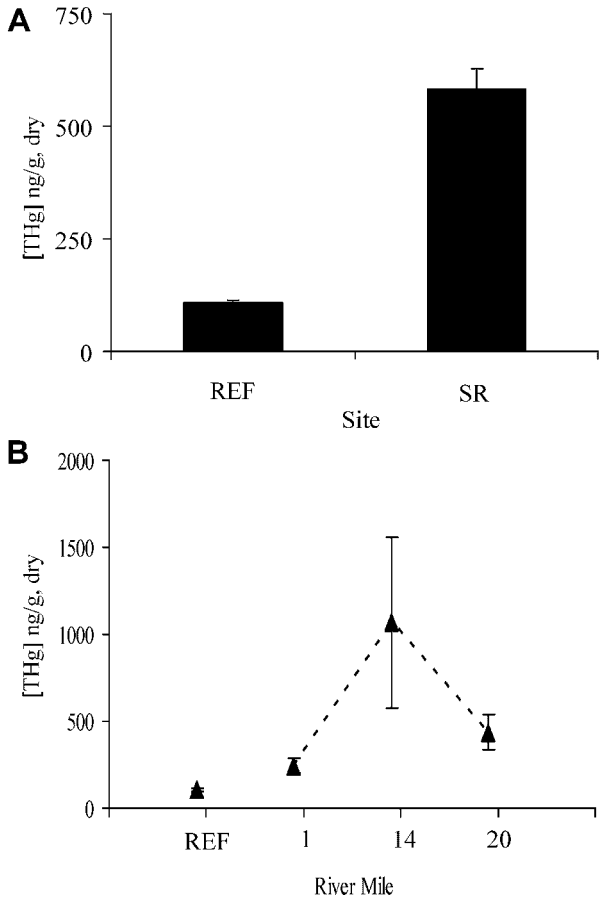

Fig. 3. (**A**) Whole-body total mercury (THg) concentrations (ng/g, dry mass; mean ± 1 standard error [SE]) in adult *Plethodon cinereus* from the reference (REF) and contaminated portion of the South River (SR). (**B**) Whole-body THg (ng/g, dry mass; mean ± 1 SE) in adult *P. cinereus* at the reference and contaminated subsites along SR. Numbers represent river miles downstream from the source of contamination (river mile 0). Lines connecting points are for visual presentation and do not reflect connectivity between means.

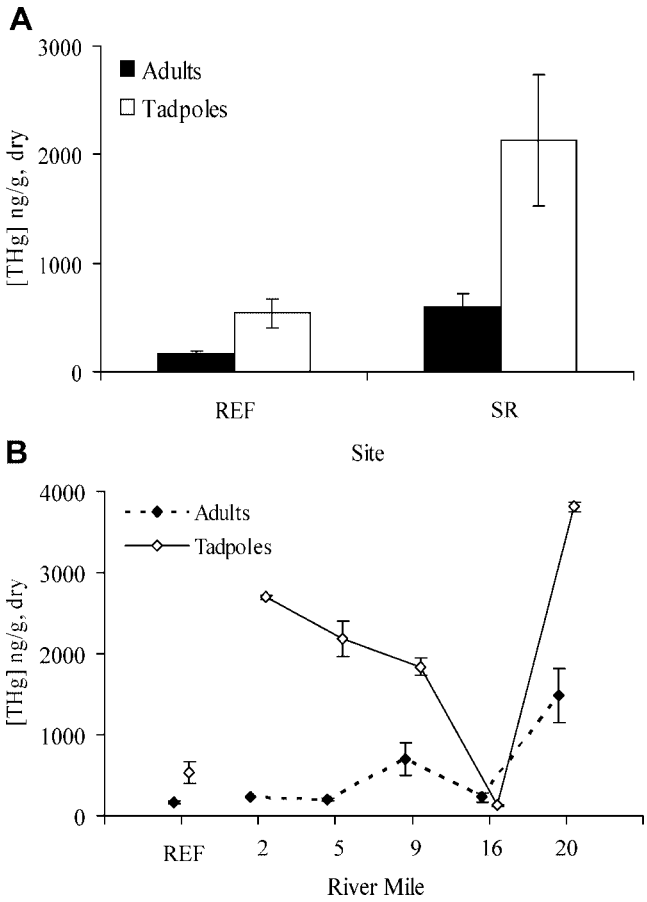

Fig. 4. (**A**) Whole-body total mercury (THg) concentrations (ng/g, dry mass; mean ± 1 standard error [SE]) in adults and larval (tadpoles; composite samples) *Bufo americanus* from the reference (REF) and contaminated portion of the South River (SR). (**B**) Whole-body THg (ng/g; mean ± 1 SE) in adults and tadpoles *B. americanus* at the reference and contaminated subsites along SR. Numbers represent river miles downstream from the source of contamination (river mile 0). Lines connecting points are for visual presentation and do not reflect connectivity among means. Symbols are staggered for clarity.

B. americanus in the contaminated site than the reference site, respectively ($p < 0.005$). Stage also differed ($p < 0.005$) between *B. americanus* adults and larvae (Fig. 4A), but the influence of stage was opposite of that observed in *E. bislineata*. Total Hg concentrations in larval toads were 3.2- and 3.6-fold higher than adults at the reference and contaminated sites, respectively. No interaction was detected between site and stage ($p > 0.975$) for *B. americanus*.

Relationships between bodies and nonlethal samples

There was a strong positive relationship between THg concentrations in salamander bodies and tails for both *E. bislineata* ($r = 0.994$, $p < 0.0001$, $y = 0.9848x - 0.1191$) (Fig. 5A) and *P. cinereus* ($r = 0.973$, $p < 0.0001$, $y = 0.7825x + 0.3413$) (Fig. 5B). The slope of the regression for salamander bodies and tails was significantly steeper for *E. bislineata* than *P. cinereus* (ANCOVA, $p < 0.001$). A strong positive relationship was also found for THg concentrations between adult bodies and blood in *B. americanus* ($r = 0.916$, $p < 0.0001$, $y = 0.9771x - 0.0422$) (Fig. 6). The slopes of all relationships did not differ significantly from 1.0 (t test, $p > 0.1$ for all).

DISCUSSION

Amphibians inhabiting the contaminated portion of the South River had elevated Hg concentrations in their tissues compared to conspecifics from the reference site. Total Hg concentrations were low in the reference site for all species, and THg concentrations for both stages of *E. bislineata* in the reference site were comparable to concentrations of the closely related *E. bislineata bislineata* found in Shenandoah and Acadia National Parks [30]. In contrast, peak whole-body THg concentrations in amphibians from the contaminated site were the highest reported for amphibians in the literature [6,10]. Adult THg concentrations ranged up to 5,785 ng/g in *E. bislineata*, 3,386 ng/g in *P. cinereus*, and 2,350 ng/g in *B. americanus,* and approximately 50% (mean range 46 to 60%) of this was MMHg [31]. Unfortunately, little research has focused on the effects of Hg in amphibians, especially through dietary exposure. However, Unrine et al. [32] found adverse effects on development and decreased metamorphic success in *Rana sphenocephala* at much lower THg tissue concentrations (~240 ng/g) compared to the THg concentrations found in amphibians from the South River. On the other hand, several studies have focused on Hg

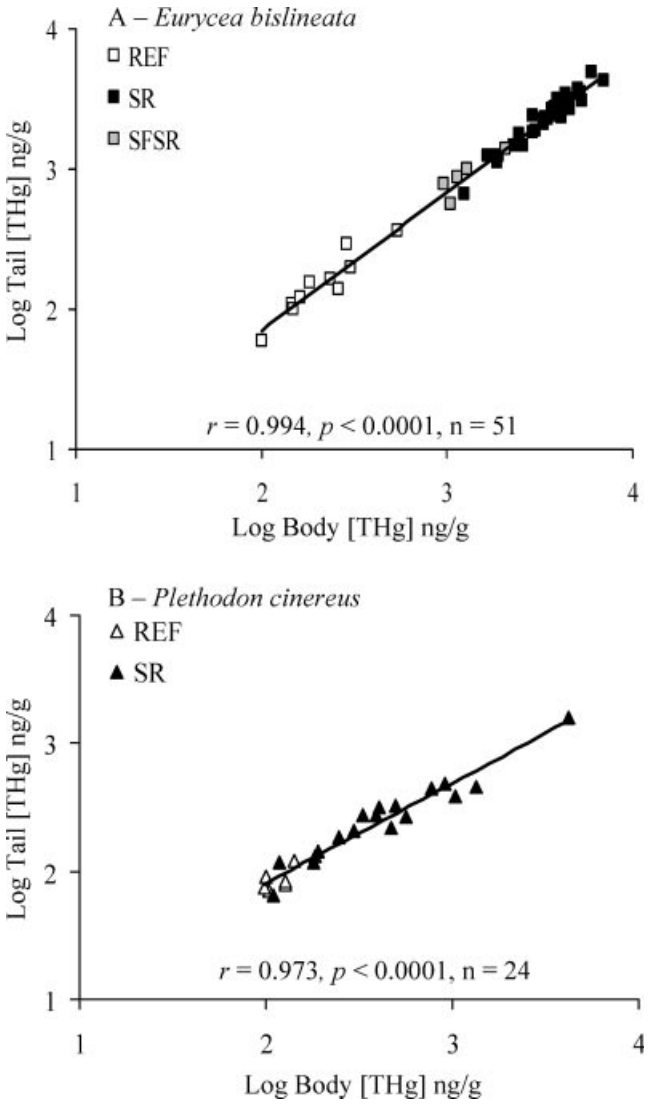

Fig. 5. Relationship between log body total mercury (THg; ng/g, dry mass) concentrations and log tail THg concentrations in (**A**) adult *Eurycea bislineata* and (**B**) adult *Plethodon cinereus* from the reference (REF) and contaminated (SR) portion of the South River, and the South Fork of the Shenandoah River (SFSR) (VA, USA).

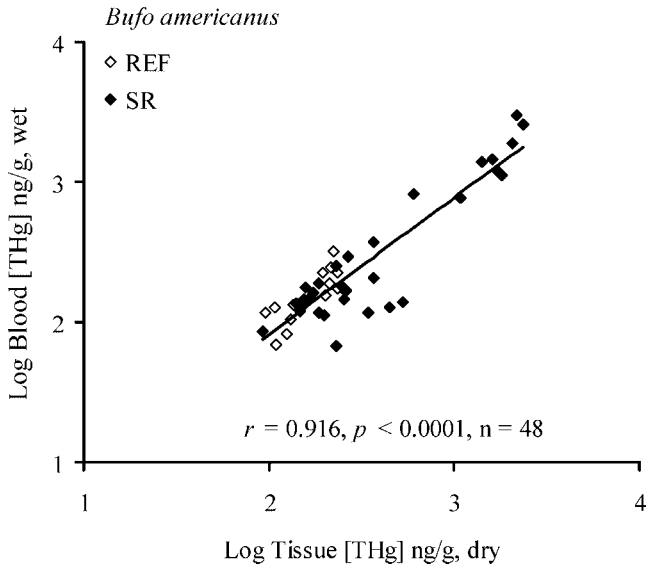

Fig. 6. Relationship between log whole-body total mercury (THg; ng/g, dry mass) concentrations and log blood THg (wet mass) concentrations in adult *Bufo americanus* from the reference (REF) and contaminated (SR) portion of the South River (VA, USA).

concentrations known to affect reproductive success in fish. Beckvar et al. [33] used the data from 10 studies to create a whole-body tissue threshold-effect level of 1,000 ng MMHg/g, dry weight (assuming 80% moisture) for juvenile and adult fish based on sublethal endpoints (growth, reproduction, development, and behavior). In the present study, 91% of *E. bislineata* adults, 11% of *B. americanus* adults, and <1% of *P. cinereus* adults from the contaminated site reached MMHg concentrations above the threshold-effect level for fish [33].

Amphibians are important prey for a variety of organisms along the South River. Many organisms such as raccoons, snakes, screech owls, wading birds, turtles, and predatory fish (e.g., bass and sunfish) are known to eat various lifestages of these amphibian species [23]. Because of their ability to occur in high densities and their high conversion efficiencies, amphibians may serve as critical trophic links for energy, nutrient flow, and Hg to predators. The most well-studied mammalian species in terms of Hg are the mink and otter, which both include amphibians as an important component of their diet [34,35]. Several studies on these species indicate that neurotoxicity and death can occur with the consumption of diets containing Hg (as MMHg) >1,000 ng/g wet weight [36]. In addition, the U.S. EPA estimates that the lowest observable adverse effect level (LOAEL) for mink is 180 ng/g body weight/d or 1,100 ng/g wet weight MMHg in the diet [37]. Several studies have observed reduced reproductive success and juvenile survival in birds with environmentally realistic concentrations of Hg in their diet. The piscivorous common loon (*Gavia immer*), which eats amphibians and fish that eat amphibians [38], had reduced reproductive success with diets that contained Hg (as MMHg) >300 ng/g wet weight [39]. More recently, a study by Burgess and Meyer [40] suggests loon productivity decreases by 50% when fish THg concentrations in the diet were 210 ng/g wet weight and failed when THg concentrations were 410 ng/g wet weight. Bouton et al. [41] fed captive-raised great egret chicks (*Ardea albus*), another piscivorous species, diets of 500 ng/g wet weight MMHg and found the chicks displayed decreased appetite and strength, altered activity and maintenance behavior, and reduced motivation to hunt live prey. The authors suggest that these effects may result in reduced juvenile survival in wild birds. In addition, diets containing MMHg concentrations of 3,000 ng/g dry weight in mallard ducks (*Anas platyrhynchos*) [42] and black ducks (*Anas rubripes*) [43] impaired reproductive success by reducing egg production, hatching success, and embryo and duckling survival. Individual amphibians at the South River with the highest THg concentrations, mainly *E. bislineata*, approach the mink LOAEL and Hg concentrations known to affect reproduction in ducks. However, 80% of adult and 50% of larval *E. bislineata* surpassed the dietary threshold for reproductive success in loons. Thus, the Hg concentrations in the present study support the notion that amphibians, as important components of aquatic and terrestrial communities, potentially transfer Hg within and between these environments and pose significant health risks to their predators.

Species and stage differences

The magnitude of Hg accumulation in amphibians appeared dependent on habitat use and feeding behavior. Within the contaminated portion of the river, both stages of *E. bislineata* were observed to have the highest average THg concentrations in the present study (adults $3{,}453 \pm 196$ ng/g; larvae $2{,}479 \pm 171$ ng/g). *Eurycea bislineata* is the most river-associated species and remains carnivorous throughout its complex life cycle. Like *E. bislineata, P. cinereus* is carnivorous throughout its life, but *P. cinereus* is a direct developer in the terrestrial environment. Both of the more terrestrial species, *P. cinereus* (583 ± 178 ng/g) and *B. americanus* (598 ± 117 ng/g), had the lowest average THg concentrations in the present study. Thus, the results of the present study suggest that individuals of these species encounter reduced amounts of bioavailable Hg because they may be more closely tied to terrestrial than aquatic food webs. Future studies using stable isotopes may disentangle these relationships to better understand ecological risk in relation to amphibian feeding ecology [22].

Interesting ontogenetic changes were found in Hg accumulation in both species with complex life cycles, but the opposite patterns were observed for *E. bislineata* and *B. americanus* even though the larval stages of both species had similar THg concentrations at the contaminated site (larvae: *E. bislineata* $2{,}479 \pm 171$ ng/g and *B. americanus* $2{,}132 \pm 602$ ng/g). For *E. bislineata*, adults had higher THg concentrations than larvae at all sites except RM 22. Because Hg accumulates over time and is not well eliminated from the body, older and larger organisms often have proportionally higher THg concentrations than younger and smaller conspecifics. For example, THg concentrations often correlate with age, body mass, or length in fish [6,8]. Adult *E. bislineata* were significantly larger than the larvae ($p = 0.004$) in both the contaminated (adults: 0.81 ± 0.03 g, larvae: 0.66 ± 0.04 g) and reference (adults: 0.80 ± 0.06 g, larvae: 0.63 ± 0.05 g) site. Because the older and larger adults had higher Hg concentrations than larvae, adults may have simply been exposed to Hg at similar rates but for a longer time. This is plausible because all of the adults sampled were collected within 3 m of the river, where aquatic prey can remain a significant portion of their diet [23]. Alternatively, adults could be ingesting larger prey or prey at higher trophic levels with higher Hg concentrations, or a larger proportion of the more bioavilable Hg form, MMHg. Again, stable isotopes could provide critical insights into whether accumulation differences between stages were attributable to ontogenetic shifts in feeding preferences or exposure duration (e.g., [22]).

The opposite ontogenetic Hg accumulation pattern from *E. bislineata* was observed in *B. americanus,* where the aquatic tadpoles had higher THg concentrations than the terrestrial adults. Although tadpoles are omnivorous grazers, this observation is consistent with the literature that many pond-breeding larvae are excellent accumulators of trace metals because they are closely associated with the benthos [16,44]. Although many metals, including some Hg, may be bound to the gut epithelium in tadpoles [17,18], evidence suggests that Hg becomes mobilized during metamorphic climax and is retained in amphibian tissues after metamorphosis is completed [4,18]. After metamorphosis, *B. americanus* juveniles and adults are highly terrestrial and mobile [25]. Thus, the reduced body concentrations of adults are likely caused by a dilution of the Hg body pool as they consume prey items from the terrestrial environment. Alternatively, because of their mobility, it is possible that some of the adults sampled were immigrants from surrounding uncontaminated habitats that were only recently exposed to Hg.

Spatial differences

Mercury accumulation in amphibians followed the same spatial pattern as observed in birds, fish, and turtles along the South River, which generally increased for several miles downstream from the point source before peaking between miles 10 and 20 and decreasing or remaining high until the confluence with the South Fork of the Shenandoah River ([20,22]; G.W. Murphy, 2004, Master's thesis). Although it is not currently known why Hg concentrations peak downstream from the source, it is theorized that historical deposition in the cobble bed within the river and/or flood plain, coupled with current erosion patterns, may provide a continual and dispersed source of Hg loading to the river. This pattern was the strongest for *E. bislineata,* which is most closely associated with the river. The patchy availability of suitable habitat for *P. cinereus* due to deforestation along the South River precludes descriptions of spatial patterns for this species. A notable exception to the pattern was at RM 16, where *B. americanus* adults and larvae had THg concentrations comparable to the reference site. The breeding pond at RM 16 was 60 m from the South River, but was elevated above the 100-year flood plain. In comparison, the breeding pond at RM 20 had the highest THg concentrations, was 40 m from the river, and within the two-year flood plain. These site differences suggest the risk of Hg exposure in pond-breeding amphibians is strongly influenced by Hg deposition in the South River floodplain from flood events. In addition, these observations suggest that larvae in these breeding ponds may be good bioindicators of localized Hg contamination and bioavailability because they are confined to natal wetlands and integrate Hg from the aquatic environment.

Nondestructive sampling

The present study illustrates the utility of two nondestructive sampling techniques for determining whether amphibians have a history of exposure to Hg. Total Hg concentrations between bodies and tails of both salamanders and between blood and whole body in *B. americanus* were highly correlated. Importantly, the large range in Hg contamination at the South River allows inferences to be drawn at other Hg-contaminated sites that fall within this broad range.

A variety of nondestructive sampling techniques have been developed for reptiles, but not for amphibians. For example, concentrations of metals and metalloids in skin, tails, and/or blood are predictive of whole-body or target tissue concentrations in snakes and lizards [45–47]. In contrast, amphibians are typically sacrificed for whole-body contaminant analysis because nondestructive techniques have not been developed. This practice is not conservation minded, nor is it convenient for continued sampling at sites over protracted time scales because of the impact it could have on local populations. Cardiac

puncture is a recommended method for blood collection in anurans [48], and can be used repeatedly on the same individuals [49]. Although tail loss in salamander species can decrease fitness through several routes such as reduction in courtship success, locomotive performance, growth rate, or reproductive success [50], the tail is regenerated over time. Thus, the impact of tail sampling on local populations is minimized compared to the lethal alternative. Improved analytical techniques may allow smaller quantities of tail tissue to be analyzed and decrease the impact it has on the individuals sampled.

Although the correlations with nondestructive tissues provide a predictive index of Hg concentrations at the South River, they should be validated at other Hg contaminated sites for these three species and closely related species. For example, the slopes of the correlations between body and tail differed between the two salamander species indicating one relationship is not necessarily predictive for all salamander species. In addition, blood is often correlated with contaminant concentrations in whole-body or target organs, and is commonly used as a nondestructive sampling technique for other vertebrates (e.g., [45,46,51]). However, blood THg concentrations may reflect recent dietary intake while muscle and other tissues represent a more consistent integration of Hg intake over time [52].

CONCLUSION

A common theory regarding Hg in wildlife is that piscivores and other top predators feeding in the aquatic food chain have the greatest risk of Hg exposure and toxicity [6,7]. The present study demonstrated that amphibians occupying both aquatic and terrestrial habitats in a contaminated site accumulated Hg in concentrations that exceed those of the reference site by up to an order of magnitude. In some cases, Hg concentrations in amphibians exceeded threshold concentrations for adverse effects in juvenile and adult fish, indicating amphibians may be at risk of Hg exposure and toxicity. In addition, Hg concentrations in amphibians exceeded dietary levels known to cause adverse effects in some mammals and birds, indicating that predators of amphibians may also be at risk. Because the South River has a large range in Hg concentrations, it affords the opportunity to quantify threshold concentrations for adverse effects in the field. The development of thresholds at this model system may assist in risk assessments and management decisions for other Hg-contaminated sites with concentrations within this broad range. In addition, the nondestructive sampling techniques developed for the present study will be most useful if they can ultimately be used to predict health risks to individuals, such as adverse reproductive effects.

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