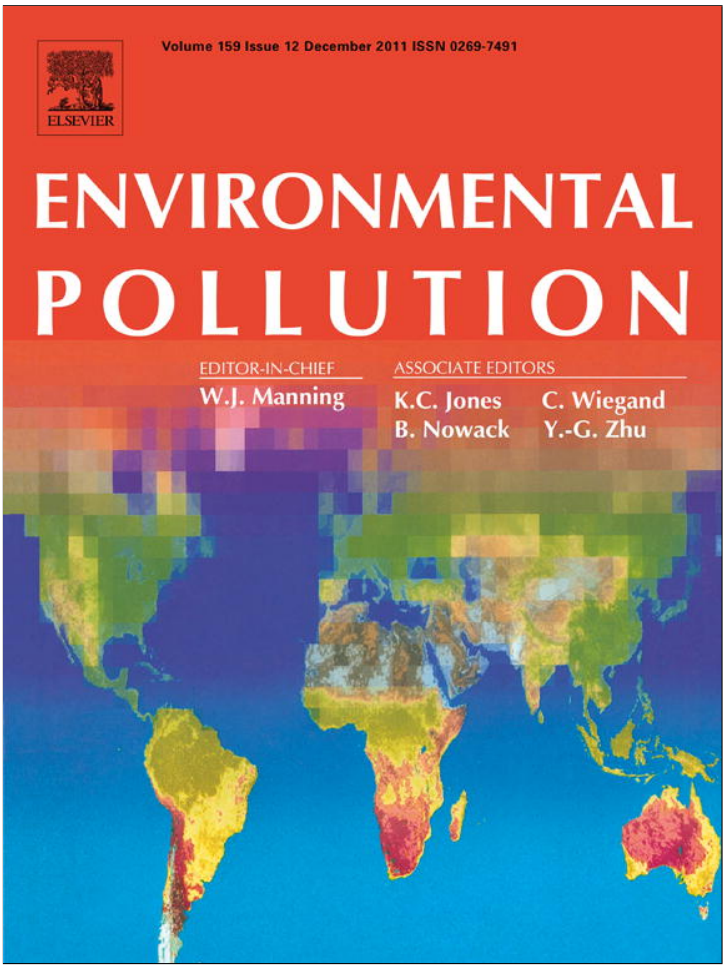

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Mercury exposure in terrestrial birds far downstream of an historical point source

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ABSTRACT

Mercury (Hg) is a persistent environmental contaminant found in many freshwater and marine ecosystems. Historical Hg contamination in rivers can impact the surrounding terrestrial ecosystem, but there is little known about how far downstream this contamination persists. In 2009, we sampled terrestrial forest songbirds at five floodplain sites up to 137 km downstream of an historical source of Hg along the South and South Fork Shenandoah Rivers (Virginia, USA). We found that blood total Hg concentrations remained elevated over the entire sampling area and there was little evidence of decline with distance. While it is well known that Hg is a pervasive and long-lasting aquatic contaminant, it has only been recently recognized that it also biomagnifies effectively in floodplain forest food webs. This study extends the area of concern for terrestrial habitats near contaminated rivers for more than 100 km downstream from a waterborne Hg point source.

1. Introduction

Mercury (Hg) is a well-studied environmental contaminant, with wide-ranging impacts on the health of various taxa (Scheuhammer et al., 2007). Because Hg can be methylated – and therefore become bioavailable – in aquatic systems, it has historically been considered a problem for species directly associated with aquatic ecosystems, such as piscivorous birds (Wolfe et al., 1998). In particular, rivers have been contaminated by Hg used historically for industrial processes such as chlor-alkali production and gold mining. A recent study of three of North America's great rivers, the Missouri, Mississippi, and Ohio, suggests that along approximately half of the lengths of these rivers, piscivorous belted kingfishers (*Megaceryle alcyon*) would exceed daily Hg intakes associated with adverse effects (Walters et al., 2010). River floodplains may be prime locations for mercury methylation, for the same reasons that methylation occurs in other wetlands (Wiener et al., 2003). Recent research has shown that Hg is not only a problem for piscivorous and aquatic wildlife inhabiting contaminated rivers, but also for terrestrial songbirds (Cristol et al., 2008) and amphibians (Bergeron et al., 2010) in the floodplain.

Various effects of exposure to riverine Hg have been documented in songbirds, including: reduced survival (Hallinger et al., 2011), impaired reproduction (Hallinger and Cristol, 2011), depressed immune competence (Hawley et al., 2009), altered behavior (Hallinger et al., 2010) and disrupted endocrine function (Franceschini et al., 2009; Wada et al., 2009). Because of this risk of adverse effects, and the fact that there are thousands of rivers and other waterbodies contaminated with Hg in the USA (Environmental Protection Agency National Listing of Fish Advisories, 2008), it is important to understand the potential spatial extent of Hg exposure to wildlife from waterborne Hg point sources.

Between 1929 and 1950, the South River, Virginia, USA was contaminated by a point source of mercury 39 km upstream of its confluence with the South Fork Shenandoah River. Mercury entered the river as mercuric sulfate, which was used as a catalyst in an acetate production process at a factory in Waynesboro (Carter, 1977). While the exact amount of mercury released is unknown, Hg is still a concern in the South River more than a half-century later. There is a fish consumption advisory for human anglers (Virginia Department of Health Fish Consumption Advisories, 2009) as well as strikingly elevated tissue Hg concentrations in reptiles, birds, amphibians and mammals (Bergeron et al., 2007, 2010; Cristol et al., 2008; Sleeman et al., 2010; Wada et al., 2010). These findings come from research focused primarily on the South River, the site of the historical Hg point source, and relatively little is known about sites farther downstream. The South Fork Shenandoah River begins

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at the confluence of the South and North Rivers and continues approximately 156 km before it merges with the North Fork Shenandoah River to become the even larger Shenandoah River. In other river systems, Hg in fish and birds tied directly to the aquatic ecosystem remains elevated far from the original source of contamination (Dye and Benton, 2001; Ullrich et al., 2007), but few studies have looked at species not directly tied to the aquatic environment. We hypothesized that the Hg contamination documented in the terrestrial floodplain biota of the South River might continue downstream along the South Fork Shenandoah River, despite dilution in the larger river. Previous studies that have considered the downstream extent of Hg contamination have generally been complicated by multiple point sources (Hothem et al., 2008) or dam/reservoir complexes that change the movement and methylation of Hg (Campbell et al., 1998; Custer et al., 2007). The present study measured blood total Hg concentrations in breeding songbirds found in similar habitats within the floodplain for approximately 100 km downstream of the South River, to identify whether and how far downstream there is a risk of Hg contamination to floodplain biota from this legacy Hg source.

2. Materials and methods

2.1. Study sites

Songbirds were captured at two reference sites on the South River upstream from the point source of Hg (Waynesboro, VA) and five sites downstream of the contamination source (Fig. 1, Table 1). One site (GRCP) was located on the South River, while the remaining four were located after the confluence of the North and South Rivers, on the South Fork Shenandoah River. We are not aware of any significant point sources of Hg along the entire 137 km downstream of the waterborne point source in Waynesboro. Although we included no reference sites along the North River in this study, previous work has shown no significant Hg contamination along the North River upstream of its confluence with the South River (Brasso and Cristol, 2008; Cristol et al., 2008; Hallinger and Cristol, 2011). While distance between sites was similar, exact placement was opportunistic, depending on landowner permission, accessibility, and presence of riparian woodland extensive enough to support bird populations. The riparian woodlands at each site were dominated by sycamore (*Platanus occidentalis*), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), silver maple (*Acer saccharinum*), and birch (*Betula* spp.). The understory character varied by site, but spicebush (*Lindera benzoin*) predominated. The two reference sites also consisted of riparian woodland and were selected based on the presence of the same bird species as the downstream sampling sites.

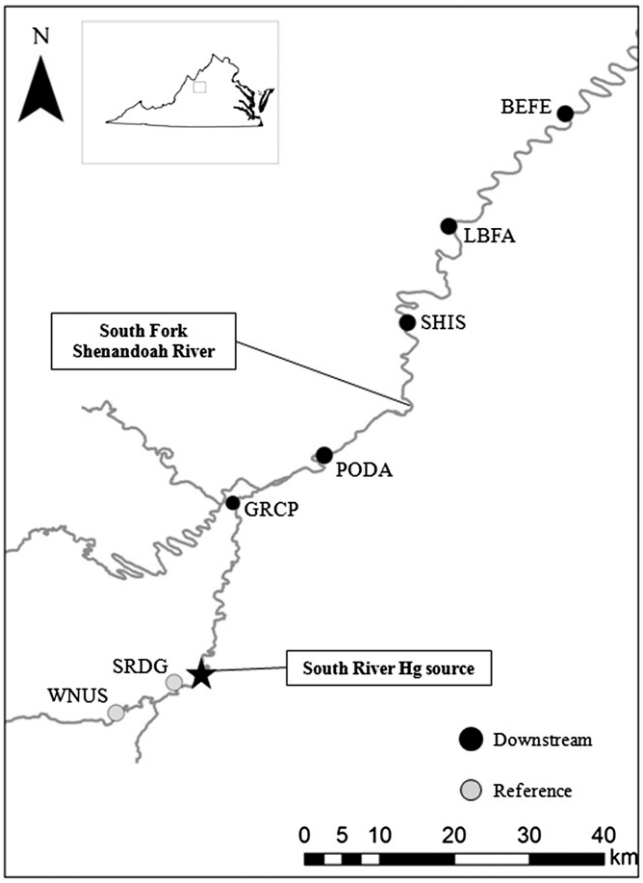

Fig. 1. Study sites where terrestrial songbirds were captured along the South and South Fork Shenandoah Rivers (Virginia, USA).

Table 1
Sampling sites along the South and South Fork Shenandoah Rivers, Virginia. Km is the river channel distance from the contamination source in Waynesboro. Sampling dates are the ranges during 2009 in which blood was sampled.

Location	Site	Km	Sampling dates
Reference	Waynesboro Nursery (WNUS)	−10	6/1–7/7
	Ridgeview Park (SRDG)	−2	6/19–7/9
South River	Grottoes City Park (GRCP)	35	5/25–7/6
South Fork	Power Dam (PODA)	51	6/6–6/10
Shenandoah River	Shuller's Island (SHIS)	79	6/5–6/16
	Long Bend Farm (LBFA)	107	6/24–7/2
	Bealer's Ferry (BEFE)	137	6/18–6/30

2.2. Choice of bird species

The biomagnification of methylmercury in birds is likely influenced by age, trophic position, foraging habitat, and duration of annual exposure (Evers et al., 2005; Condon and Cristol, 2009; Eagles-Smith et al., 2009). Therefore, we sampled only adult birds (at least one year of age) and used a two-by-two design for trophic position (i.e., foraging guild: omnivore or invertivore) and duration at site (i.e., migratory strategy: resident or migrant) to group all sampled species into four exposure assemblages (Table 1). Foraging guild was assigned based on De Graaf et al. (1985) and migratory strategy was based on individual species accounts in Birds of North America (Poole, 2005). We relied on author expertise for the field sparrow (*Spizella pusilla*) and eastern towhee (*Pipilo erythrophthalmus*), where the literature was ambiguous on their migration pattern in Virginia.

To evaluate whether variable species composition caused differences in Hg levels between sites, we also analyzed Hg trends for the individual species with the largest sample size ($N > 40$) distributed across all study sites. We considered the following to be a representative of each assemblage: Carolina wren (*Thryothorus ludovicianus*, invertivore/resident), red-eyed vireo (*Vireo olivaceus*, invertivore/migrant), song sparrow (*Melospiza melodia*, omnivore/resident), and indigo bunting (*Passerina cyanea*, omnivore/migrant). The Carolina wren is a highly territorial year-round resident and eats primarily insects and spiders during the breeding season (Haggerty and Morton, 1995). Carolina wrens have among the highest blood Hg concentrations of all birds sampled on the South River, potentially because of their reliance on higher trophic level prey items, such as spiders (Cristol et al., 2008). The red-eyed vireo eats primarily insects during the breeding season, and overwinters in the Amazon Basin of South America (Cimprich et al., 2000). Song sparrows are resident birds, but their diet is more omnivorous, including insects, seeds, and fruit (Arcese et al., 2002). The indigo bunting is similarly omnivorous, eating insects, berries and seeds during the breeding season and then overwintering in Central America (Payne, 2006).

2.3. Bird sampling

We sampled birds between 25 May and 9 July, 2009, using 10–13, 12-m, 36-mm mesh mist nets at each site. Birds were captured within the floodplain, which varied in extent among sites. At each site, most of the mist nets were placed within 50 m of the riverbank, and all were within 100 m. Nets were situated mainly in forested areas and along forest edges, with the exact placement chosen according to vegetation structure. We used playback recordings to attract the target species and checked the nets every 20–40 min. We extracted each captured bird and placed it in a cotton holding bag until it could be processed, within 40 min but usually sooner, whereupon all birds were released unharmed. We banded all captured birds with a U.S. Geological Survey aluminum band before release and determined age and sex by external characteristics (Pyle, 1997).

We collected no more than 1% of the bird's body weight in blood, using 26–28 gauge needles to puncture the cutaneous ulnar vein in the wing. Blood was collected in heparinized capillary tubes, sealed at both ends with Crito-caps® and placed in a labeled 10 ml plastic vacutainer (BD, Franklin Lakes, NJ, USA). We kept all samples on ice for 3–6 h, after which they were frozen at −25 °C until analysis.

Blood is an ideal tissue for evaluating recent dietary uptake and there is ample evidence that adult blood Hg levels reflect prey Hg levels in the breeding territory (Kahle and Becker, 1999; Burgess and Meyer, 2008; Evers et al., 2008). Because blood transports nutrients, waste and contaminants to internal organs, the Hg load in blood can be considered to be in dynamic equilibrium with other body tissues. Blood Hg concentrations are closely correlated with Hg in internal tissues, but have the advantage that they can be sampled non-lethally (Eagles-Smith et al., 2008). Over 95% of the Hg in blood is in the methyl form at this and other sites, so we measured only the more convenient total Hg values (Rimmer et al., 2005; Wada et al., 2010).

2.4. Hg analysis

Samples were analyzed for total Hg between 8 July and 6 November, 2009 using atomic absorbance spectroscopy on a DMA-80 (Milestone, Shelton, CT). Blood samples were thawed and blown directly onto a balance before being transferred to the Hg analyzer; values are reported on a wet-weight basis. The DMA-80 was calibrated using homogenized fish standard reference materials (hereafter SRMs, DORM-3 and DOLT-4; National Research Institute, Canada) according to machine specifications prior to the analysis and approximately monthly thereafter, or more often when necessary to keep SRM values within 7.5% of certified values. A blank, an empty sample container, a duplicate and two aliquots of each SRM were run with every 20 samples. Two separate capillary tubes of blood from the same collection date of the same bird run on the same day were considered duplicate blood samples. The factory calibrated minimum detection limit for the analyzer is 0.005 ng Hg. Method detection limit was between 0.0023 and 0.0064 μg/g Hg during the period of analysis. Recovery of standard reference materials was 102.1 ± 11.9% for DORM-3 ($n = 172$) and 98.7 ± 11.5% for DOLT-4 ($n = 159$). During the period of this analysis we spiked chopped domestic bird feather expected to have low Hg concentration with DOLT-4 ($n = 10$) and recovered 100.0 ± 1.5%. The mean relative percent difference between pairs of blood samples run as duplicates was 9.8 ± 12.5% ($n = 54$ pairs of blood samples).

2.5. Statistical analysis

We analyzed the data using both Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and JMP Version 9.0.0 (SAS Institute, Cary, NC, USA). The blood mercury levels from each target species from each assemblage are presented. For analysis, all blood Hg values were $\log_{10}$ transformed to improve normality and stabilize variances for analysis. We designed a mixed-effects model (ANCOVA) with categorical main effects of assemblage, site, an assemblage*site interaction, and a continuous main effect of Julian date. Because sample size for individual species varied greatly and we were interested in overall assemblage-level differences in blood mercury, we included species (nested within guild) as a random effect. To simplify interpretation, we first ran this mixed model on the birds captured at the two reference sites, to determine if grouping these two sites was reasonable. Because we found no effect of site in this preliminary analysis, we grouped these two sites in future analyses. We then ran a mixed model with all the sampled species. Because the assemblage*site interaction was significant, we then ran a separate analysis of each assemblage to determine differences between sites for each assemblage. We present back-transformed least squares means and standard error for each assemblage graphically.

3. Results

We sampled 440 adult songbirds of 38 species on seven study sites (Table 2). We sampled four species within the invertivore/resident assemblage ($n = 90$), 19 invertivore/migrants ($n = 98$), four omnivore/residents ($n = 103$), and 11 omnivore/migrants ($n = 149$; Table 2).

3.1. Blood Hg levels

The blood Hg concentrations for the best-sampled species in each assemblage were, on average, higher than reference at all downstream sampling sites (Table 3). There did appear to be a large amount of variation between species in different assemblages. For example, considering only the South River site (GRCP), there is an order of magnitude difference between the invertivore/resident Carolina wren average (2.77 μg/g ± 1.14) and the omnivore/migrant indigo bunting average (0.27 μg/g ± 0.14).

3.2. Reference site comparison

Blood Hg values collected on reference sites revealed no significant effect of site, assemblage, or the site*assemblage interaction (ANOVA: site $F_{1,101.2} = 1.75$, $P = 0.19$, assemblage: $F_{3,17.7} = 2.87$, $P = 0.07$, site*assemblage: $F_{3,61.2} = 1.25$, $P = 0.30$). Thus, we pooled the two reference sites together in further analyses.

Table 2
Songbird species in the four exposure assemblages.

Assemblage (N)[a]	Common name	Scientific name	N
Invertivore/Resident ($N = 90$)	Carolina chickadee	*Poecile carolinensis*	22
	Carolina wren	*Thryothorus ludovicianus*	49
	Tufted titmouse	*Baeolophus bicolor*	14
	White-breasted nuthatch	*Sitta carolinensis*	5
Invertivore/Migrant ($N = 98$)	Acadian flycatcher	*Empidonax virescens*	2
	American redstart	*Setophaga ruticilla*	2
	Black and white warbler	*Mniotilta varia*	1
	Blue-gray gnatcatcher	*Polioptila caerulea*	2
	Common yellowthroat	*Geothlypis trichas*	1
	Eastern phoebe	*Sayornis phoebe*	2
	Eastern wood-pewee	*Contopus virens*	4
	Great-crested flycatcher	*Myiarchus crinitus*	6
	House wren	*Troglodytes aedon*	3
	Louisiana waterthrush	*Parkesia motacilla*	2
	Northern parula	*Parula americana*	1
	Northern waterthrush	*Parkesia noveboracensis*	3
	Orchard oriole	*Icterus spurious*	1
	Ovenbird	*Seiurus aurocapilla*	3
	Red-eyed vireo	*Vireo olivaceus*	50
	Warbling vireo	*Vireo gilvus*	7
	Willow flycatcher	*Empidonax traillii*	1
	Worm-eating warbler	*Helmitheros vermivorum*	6
	Yellow-billed cuckoo	*Coccyzus americanus*	1
Omnivore/Resident ($N = 103$)	Blue jay	*Cyanocitta cristata*	2
	Field sparrow	*Spizella pusilla*	5
	Northern cardinal	*Cardinalis cardinalis*	33
	Song sparrow	*Melospiza melodia*	63
Omnivore/Migrant ($N = 149$)	American goldfinch	*Spinus tristis*	10
	American robin	*Turdus migratorius*	1
	Baltimore oriole	*Icterus galbula*	4
	Brown thrasher	*Toxostoma rufum*	4
	Brown-headed cowbird	*Molothrus ater*	2
	Common grackle	*Quiscalus quiscula*	10
	Eastern towhee	*Pipilo erythrophthalmus*	4
	Gray catbird	*Dumetella carolinensis*	54
	Indigo bunting	*Passerina cyanea*	51
	Veery	*Catharus fuscescens*	1
	Wood thrush	*Hylocichla mustelina*	8

[a] N is the number of individuals sampled.

3.3. Mixed-effects model

We included all adult songbird blood Hg samples in a mixed-effects model, with site, assemblage, site*assemblage and date as fixed effects, and species (nested within guild) as a random effect. We found significant effects of site (ANOVA: $F_{5,402.5} = 71.16$, $P < 0.0001$), assemblage (ANOVA: $F_{3,32.4} = 3.57$, $P = 0.02$), and site*assemblage (ANOVA: $F_{115,399.1} = 3.09$, $P < 0.0001$) but no significant effect of date (ANOVA: $F_{3,407.6} = 1.93$, $P = 0.17$). Because the interaction of site and guild was significant, and we were most interested in differences between sites, we ran a similar mixed-effects model for each assemblage separately.

Within the invertivore/resident assemblage, we found no significant effect of date (ANOVA: $F_{1,80.3} = 0.71$, $P = 0.40$) but a significant effect of site on blood mercury concentration (ANOVA: $F_{5,80.5} = 29.40$, $P < 0.0001$). Using Tukey's HSD test, we found that blood mercury in this assemblage remained elevated above reference at all downstream sites, but sites along the South Fork Shenandoah River were significantly lower than the site on the South River (Fig. 2A). For the invertivore/migrant assemblage, we similarly found no significant effect of date (ANOVA: $F_{1,88.35} = 0.10$, $P = 0.75$) and a significant effect of site (ANOVA: $F_{5,81.76} = 12.70$, $P < 0.0001$). Tukey's HSD test indicated that, for the invertivore/migrant assemblage, all downstream contaminated sites had blood

Table 3
Site-specific average blood mercury (Hg) concentrations (μg/g, ww) along with minimum and maximum values for best-sampled species from each exposure assemblage at each site. *N* is the number of individuals sampled and SD is the standard deviation.

Site	Carolina wren					Red-eyed vireo					Song sparrow					Indigo bunting				
	Hg	*n*	SD	Max	Min	Hg	*n*	SD	Max	Min	Hg	*n*	SD	Max	Min	Hg	*n*	SD	Max	Min
REF	0.33	12	0.13	0.70	0.23	0.24	2	0.01	0.25	0.23	0.05	16	0.02	0.08	0.02	0.02	7	0.01	0.04	0.00
GRCP	2.77	10	1.14	5.62	1.59	1.47	9	0.62	2.47	0.59	1.63	1				0.27	17	0.14	0.57	0.12
PODA	1.18	8	0.65	2.29	0.21	1.31	10	0.38	1.80	0.67	0.68	17	0.38	1.35	0.17	0.16	8	0.11	0.38	0.05
SHIS	0.75	5	0.25	0.93	0.37	1.17	6	0.25	1.52	0.88	0.42	16	0.27	0.95	0.10	0.07	8	0.05	0.18	0.03
LBFA	1.09	8	0.42	1.65	0.39	1.22	7	0.39	1.83	0.57	0.82	6	0.45	1.34	0.10	0.26	6	0.13	0.45	0.09
BEFE	0.90	6	0.30	1.41	0.54	1.52	16	0.67	2.88	0.57	0.98	7	0.26	1.35	0.61	0.30	5	0.24	0.64	0.08

Hg concentrations that were significantly higher than reference levels, with no variation between South River and South Fork Shenandoah River sites (Fig. 2B).

Omnivore/residents had similar patterns to the invertivore/migrants, with the mixed model indicating no significant effect of date (ANOVA: $F_{1,93.19} = 0.09$, $P = 0.76$) and a significant effect of site (ANOVA: $F_{5,93.27} = 34.92$, $P < 0.0001$); all downstream sites (South River and South Fork Shenandoah River) were significantly elevated above reference (Fig. 2C). Omnivore/migrants also revealed no significant effect of date (ANOVA: $F_{1,134.5} = 2.25$, $P = 0.14$) and a significant effect of site (ANOVA: $F_{5,136.6} = 15.35$, $P < 0.0001$). Although all downstream sites were elevated above reference, the furthest downstream site (BEFE, river kilometer 137) was significant higher than SHIS, located 79 km downstream of the historical contamination source (Fig. 2D).

4. Discussion

Songbird blood Hg concentrations remained elevated above reference for at least 137 km downstream of an historic point source of Hg, which is approximately 100 km farther than previously documented for this site (Cristol et al., 2008). While it is not surprising that mercury discharged into a river moved downstream and entered the floodplain food web, it is a novel finding that the mercury remained bioavailable to terrestrial wildlife 137 km downstream and that there was no indication of substantial decline over this distance. Other studies have reported unexpected trends in distribution of riverine mercury – albeit over a smaller spatial scale – citing Hg bioaccumulation in fish that subsequently move into new areas (Southworth et al., 2000), complex hydrodynamics (Campbell et al., 1998), or the effects of dams and reservoirs (Haines et al., 2003) as explanations. These factors are unlikely to have influenced the terrestrial bird community along the South Fork Shenandoah River system. We hypothesize that three factors may have combined to overwhelm the forces of dilution and sequestration that might be expected to create a gradual reduction in mercury bioavailability with distance from the source: 1) persistent historical Hg in the riverbed sediment may be consistently redeposited into the floodplain with each seasonal flood and 2) habitat features at downstream sites may cause increased

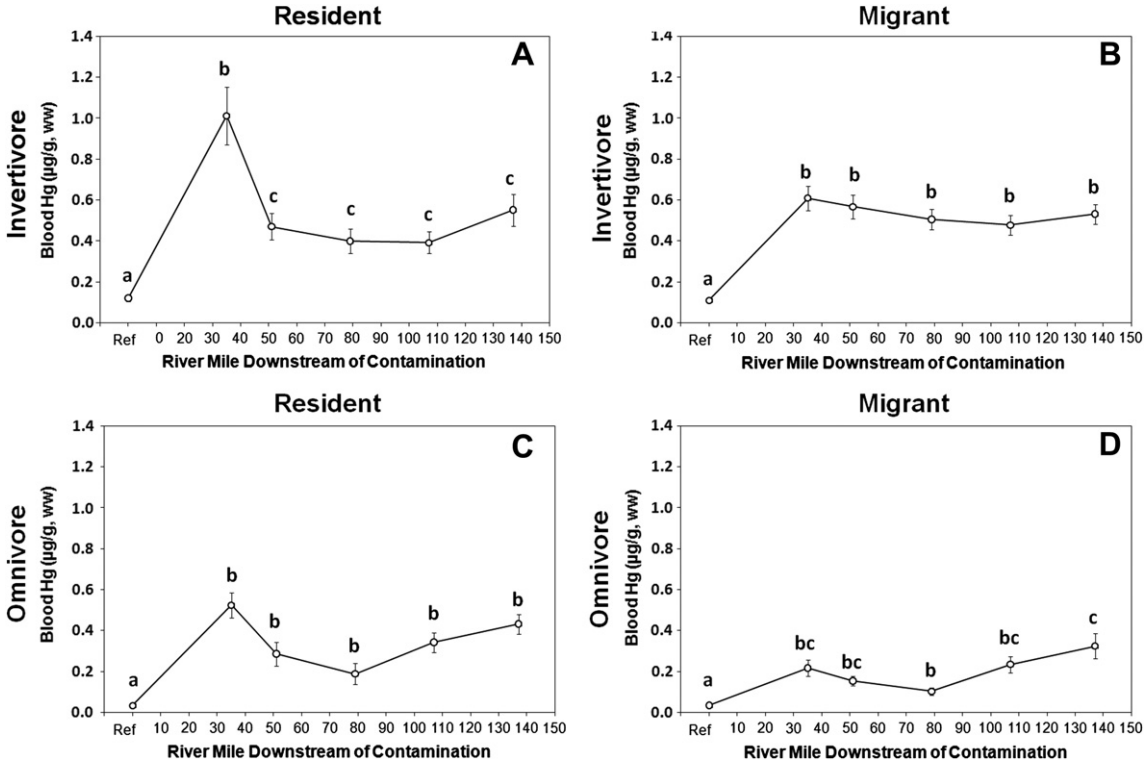

Fig. 2. Back-transformed least squares mean blood mercury at each study site (calculated from the assemblage-specific mixed-effects model) for A) invertivore/residents, B) invertivore/migrants, C) omnivore/residents and D) omnivore/migrants. Letters indicate significant differences based on Tukey's HSD. Error bars represent back-transformed standard error. Ref includes the two reference sites upstream of the contamination and river kilometer = 0 indicates the location of the legacy point source.

opportunities for methylation and 3) variation in prevalence of emergent aquatic insects within different stretches of the river may amplify transfer of Hg from the river into the terrestrial ecosystem.

4.1. Movement of sediment Hg

Aquatic Hg tends to adsorb to fine-grained sediments where it may have a long residence time (Eisler, 1987). Fine-grained sediments in the South River/South Fork Shenandoah system may experience long-range transport in high flow situations, and thus Hg may be transported downstream where it can be sequestered in the riverbed sediment. Because the South Fork Shenandoah River experiences annual flooding, as well as catastrophic events associated with hurricanes and tropical storms, the mercury sequestered in this sediment can be deposited on the floodplain, thereby contaminating soils (U.S. Geological Survey Water Supply Paper 2375). Although the South River was contaminated by a point source in Waynesboro, eroding floodplain soils now appear to be important non-point sources of Hg to the river (Eggleston, 2009). Movement of this legacy Hg may now occur on a fairly small scale within the downstream stretches of the river, with Hg cycling between riverbed sediment and floodplain soils but little dilution occurring.

In the South River, the majority of the mercury in riverbed sediments is within the first 14 km downstream of the historic point source (Flanders et al., 2010). At another river with mercury contamination from a legacy point source, in Kazakhstan, mercury was detected in fish 125 km downstream (Ullrich et al., 2007), although the majority of mercury in riverbed silt was sequestered in the first 25 km (Heaven et al., 2000). The limited downstream extent of sediment contamination in the Kazakhstan case appears to be the result of flooding events that transported mercury-rich sediment over the river banks into the floodplain, where it might be available for terrestrial wildlife. There has been no information published on mercury from floodplain biota in that case, but our results, from an older point source, suggest that flooding events may have distributed mercury into the floodplain much farther downstream, where it is still incorporated into songbird food chains.

4.2. Site-specific methylation

For most assemblages of songbirds, blood Hg levels remained consistent at all downstream sites despite increasing distance from the Hg source. We hypothesize that, though inorganic Hg likely decreases due to downstream dilution, increased methylation at downstream sites may lead to consistent biological-availability of methylmercury within the entire study area. Because Hg must be methylated before it can easily bioaccumulate in birds, this would be consistent with our results. Variation in methylation may occur, in part, because of the hydrology of the river, which varies across the South Fork Shenandoah River; immediately after the confluence of the South and North Rivers the river widens and flows quickly, but at the most distal site (BEFE) it slows considerably and meanders, potentially creating a more favorable environment for methylation or biotic transport out of the river during floods. Other studies have shown an increase in Hg on low-flow stretches of a river, even far downstream of the source of contamination (Hothem et al., 2008). Although our study revealed little spatial change in concentration of Hg in birds, this could be the result of lower Hg levels downstream – combined with increased opportunity for methylation – resulting in consistent levels across all sites despite dilution.

4.3. Variation in prevalence of insect emergences

Emergent aquatic insects may provide a subsidy of Hg from the aquatic to terrestrial ecosystem (Walters et al., 2008). Because emergent insects are more prevalent in shallow areas, the morphology of the river may also contribute to an increased subsidy in the downstream stretches of the river. This, combined with the previous two hypotheses, may help explain why we detected no decrease in terrestrial songbird blood Hg in the downstream portions of the SFSR, but further mechanistic studies are required.

Invertivores generally had higher blood Hg concentrations than omnivores, consistent with previous findings that feeding at higher trophic levels increases the individual body burden of Hg (Evers et al., 2005; Scheuhammer et al., 2007). Within each feeding guild, residents tended to have higher mercury than migrants, presumably because of their longer annual exposure to mercury at this site. Because we sampled blood, which reflects recent dietary intake, and all birds had been eating at the site for at least a few weeks before sampling, the difference between residents and migrants that might have been detectable in tissues that reflect longer-term bioaccumulation was not found. Patterns for the target species, which were sampled consistently across all sites, were similar to those for each exposure assemblage, suggesting that our finding that mercury exposure is a risk for riparian songbirds for at least 137 km downstream of a legacy point source is robust and not an artifact of changing distributions of species sampled.

Although we have demonstrated that songbird blood Hg concentrations remained elevated above reference far downstream of the point source, we have not yet addressed what these concentrations mean in terms of the general health of songbirds along the South Fork Shenandoah River. It is difficult to assess what level of blood Hg has a negative effect on avian health, as there is likely species-specific variation in sensitivity to Hg (Heinz et al., 2009). There is some evidence that songbirds may be more sensitive to Hg exposure than other species, but very little is known about adverse effect thresholds for Hg in free-living birds (Seewagen, 2010). For the long-lived, piscivorous common loon, a blood Hg concentration above 3.0 μg/g (ww) translates into significant reproductive impairment (Evers et al., 2008). Swallows on the South River exhibited an array of sub-lethal fitness effects with blood Hg levels ranging between approximately 2.0 and 3.5 μg/g (ww) over three breeding seasons (Hallinger et al., 2011). It should be noted that these are demonstrated effects levels from the field, rather than lowest observed adverse effects concentrations measured under controlled conditions. It is possible that effects will be detected at lower blood Hg concentrations in the future, because few species have been studied and isolating subtle effects of contaminants in field studies is notoriously difficult. While variance was high in the present study and some individuals of many species were above these effects levels, the average blood Hg levels of birds on the SFSR were below currently demonstrated effects levels. Further work will be necessary on birds in the field to determine the actual adverse effects level for Hg in songbirds and their respective diets. While we cannot definitively determine whether downstream Hg levels detected in this study affect songbird fitness, what is remarkable about our results is the great reach of Hg from a legacy aquatic point source into terrestrial food webs.

Although this study sampled the avian community exclusively, it has implications for other terrestrial or semi-aquatic species that also live in the floodplain. Amphibians, for example, show similar trends in terms of Hg bioaccumulation based on habitat preferences and feeding strategy (Bergeron et al., 2010). In the Cache Creek (California, USA) watershed, spatial variation in Hg exposure was correlated between birds (cliff swallows, *Petrochelidon*

pyrrhonota) and frogs (*Rana catesbeiana* and *R. boylii*) collected at the same sites (Hothem et al., 2008). This indicates that downstream amphibians in the South Fork Shenandoah River floodplain may also be at risk for Hg accumulation. Although we know very little about the effect Hg has on amphibian populations (Burke et al., 2010), future risk assessments of Hg in aquatic systems must consider the possibility that this contaminant can travel far downstream and impact many different taxa of wildlife, both aquatic and terrestrial, that feed at relatively low trophic levels. We have demonstrated that blood Hg concentrations remain elevated in both omnivorous (low trophic level) and insectivorous (higher trophic level) songbirds, which has significant implications for top-level predators, such as hawks, owls, and snakes, that were not sampled for this study. Further study looking at Hg levels and effects in these apex predators is warranted.

5. Conclusions

Mercury from a half-century old industrial point source has traveled in a river system to such an extent that floodplain birds 137 km downstream had elevated blood mercury concentrations. In fact, there was no clear decline in avian mercury level with distance downstream, suggesting that floodplain food webs are probably contaminated even farther downstream than demonstrated here. Insectivorous birds feeding higher on the food web, and non-migratory species that remain at this site year-round, had higher mercury than omnivores and migrants, as expected. This study, in conjunction with recent results indicating that mercury leaves aquatic ecosystems and enters terrestrial food webs (Cristol et al., 2008), as far as several hundred meters from the river shoreline (M. Howie, unpublished data), greatly expands the scale of concern for those tasked with managing risk to wildlife near mercury-contaminated rivers.

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