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INFLUENCE OF A CHLOR-ALKALI SUPERFUND SITE ON MERCURY BIOACCUMULATION IN PERIPHYTON AND LOW-TROPHIC LEVEL FAUNA

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Abstract

In Berlin, NH, the Androscoggin River flows adjacent to a former chlor-alkali facility that is a US EPA Superfund site and source of mercury (Hg) to the river. A study was conducted to determine the fate and bioaccumulation of methylmercury (MeHg) to lower trophic-level taxa in the river. Surface sediment directly adjacent to the source showed significantly elevated MeHg (10–40x increase, mean±sd: 20.1±24.8 ng g⁻¹ DW) and total mercury (THg, 10–30x increase, mean±sd: 2045±2669 ng g⁻¹ DW) compared to all other reaches, with sediment THg and MeHg from downstream reaches elevated (3–7x on average) relative to the reference (THg mean±sd: 33.5±9.33 ng g⁻¹ DW; MeHg mean±sd: 0.52±0.21 ng g⁻¹ DW). Water column THg concentrations adjacent to the point source for both particulate (0.23 ng L⁻¹) and dissolved (0.76 ng L⁻¹) fractions were 5-fold higher than at the reference sites, and 2–5-fold higher than downstream. Methylmercury production potential (MPP) of periphyton material was highest (2–9 ng g⁻¹ d⁻¹ DW) adjacent to the Superfund site; other reaches were close to or below reporting limits (0.1 ng g⁻¹ d⁻¹ DW). Total Hg and MeHg bioaccumulation in fauna was variable across sites and taxa, with no clear spatial patterns downstream of the contamination source. Crayfish, mayflies and shiners showed a weak positive relationship with porewater MeHg concentration.

Keywords

Mercury; Bioaccumulation; Periphyton; Mayflies; Low-trophic level

INTRODUCTION

Mercury (Hg) pollution is a pervasive global problem, affecting human and wildlife health mainly through the consumption of contaminated fish [1, 2]. Aquatic environments are conducive to the conversion of inorganic divalent Hg (Hg(II)); the form typically released

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into the environment) to the more toxic organic form, methylmercury (MeHg). Methylmercury exposure can have negative cardiovascular, neurological, developmental, and behavioral effects in both humans [3, 4] and wildlife [5–7]. Bioaccumulation of MeHg in lotic systems is partially controlled by local conditions such as sediment organic content, dissolved organic carbon (DOC) concentration, or pH [8]. The releases, transfer, and fate of Hg in watersheds have raised serious ecological and human health concerns; consequently, the need to better understand Hg availability and transfer is important on both global and local scales.

Hg can enter waterways through either atmospheric deposition (non-point source) or known sources pathways (largely from mining areas and industrial sites). Chlor-alkali facilities, which have used Hg in the production process, can contribute to Hg pollution in aquatic systems through atmospheric emissions, wastewater discharge, and solid waste disposal at active sites, or by the leaching, erosion, and transport of contaminated soils around legacy sites [9, 10]. Previous studies associated with chlor-alkali facilities have found that in river sediment, Hg concentrations are highest either directly adjacent to the facility or within the nearest downstream reservoir [11–13], although Hg bioaccumulation in invertebrates and upper trophic level fish can occur far downstream of the point source [12–14]. However, patterns of Hg bioaccumulation associated with such facilities are often complicated, with both increasing and decreasing fish tissue Hg levels with distance being reported [12, 13]. In addition, elevated Hg in hair samples from humans who regularly consumed fish from a reservoir located downstream of an active chlor-alkali facility has been documented [11]. Together, these findings indicate that the effects of known-source Hg contamination in river systems may occur at a broader scale than just localized near the source of Hg.

In freshwater systems, the sediment-water interface has been considered the dominant location for Hg(II)-methylation, which is facilitated mainly by sulfate and iron reducing bacteria [15–18]. In fast-moving streams where little sediment accumulates, periphyton may play a significant role in both production and uptake (e.g. entry into the food web) of MeHg. Periphyton consists of a mixed community of algae, fungi, and prokaryotes typically associated with abiotic substrates (e.g. rocks, detritus, or sediment) [19, 20]. Periphyton matrices have been shown to be areas of active Hg(II)-methylation [21–26]. Substrate composition, as well as local watershed characteristics, can influence periphyton growth and the associated MeHg production and bioaccumulation [27–30]. Further, Hg(II) and/or MeHg from the water column can accumulate within the periphyton matrix through diffusion, particle settling or adhering to the exopolysaccharide matrix and resident cells [31, 32]. Periphyton is an important food source for many aquatic fauna including insects such as mayflies and small fish such as dace [29, 30], although other organisms may rely on food webs with a different trophic base (e.g. phytoplankton or detritus). Thus, the role of periphyton in MeHg trophic transfer may vary between and within river systems depending on food web structure.

The objectives of the present study were to examine the influence of the chlor-alkali Superfund site (Berlin, NH) on the Androscoggin River ecosystem in terms of: 1) the extent of Hg contamination in surface sediment, water, and biota within a series of river reaches adjacent to and downstream from the former chlor-alkali facility, 2) testing the hypothesis

that MeHg production and bioaccumulation increases downstream from the point source as has been reported at other Hg contaminated sites (e.g. Carrasco et al.[12]), and 3) the role of periphyton in the production and biological transfer of MeHg. These objectives were addressed using Hg speciation and methylation potential analyses of field-collected samples.

MATERIALS AND METHODS

Study site

The Berlin, NH Chlor-alkali Superfund site was added to the National Priorities List in 2005, and consists of a former chlor-alkali cell house complex located within a larger abandoned pulp and paper mill facility that was originally established in the 1800's. The cell house facility occupied approximately 4.6 acres on the east bank of the Androscoggin River and was active from the 1920s until the 1960s, producing chlorine for bleaching paper [33]. Hg was used as part of the chlorine production process, and although it is unclear exactly in what capacity Hg was utilized over the lifespan of the chlor-alkali plant, Chalmers et al.[34] and others indicate that it was a part of the electrolytic cells used to separate chlorine from a brine solution. Mercury from the cell house complex was disposed of throughout the property and was also likely transported back and forth across the river for use at a kyanization (anti-decay wood treatment) facility on the west bank of the river (across from the cell house complex) to treat and preserve wood with mercuric chloride. The remaining buildings at the chlor-alkali plant were demolished and the site partially capped in 1999, but despite this and other preventative measures, elemental Hg continues to seep into the river through fractures in the bedrock [33–35 and references contained within], and the river sediment remains contaminated. Geologic setting and generalized flow characteristics for the studied section of the river were previously described by Degnan et al.[35].

Field sampling

Field samples were collected during August 2010 (sediment and porewater) and August 2011 (surface water, periphyton, invertebrates and fish) in the reaches above Sawmill Dam, Riverside Dam, Smith Hydro Dam, CrossPower Dam, Gorham Brown Dam, Gorham Dam, Shelburne Dam, and in Wheeler Bay (Figure 1). Wheeler Bay and Sawmill are both upstream of the Superfund site, while the remaining five sampling sites are downstream. Fish, crayfish, surface water, and sediment samples were collected in the “reservoir” area, just upstream of the lower dam within the reach, while periphyton and co-located mayfly samples were collected throughout the whole reach (between the upper and lower dams). All samples were collected using “clean hands, dirty hands” technique [36] to minimize sample contamination.

Surface sediment (top 0–2 cm interval) samples were collected during August 2010 using 6 cm diameter push cores. Eight replicates per sample site were collected, except for Riverside where n=5. Only Wheeler Bay, one of the two reference sites, was sampled for sediment. Sediments were kept cool until porewaters were extracted (within 48 h of sample collection). Sediments were transferred to centrifuge tubes in an anaerobic hood, capped and centrifuged, and returned to the anaerobic hood. The supernatant was filtered through pre-combusted 0.7 µm quartz fiber filters under anaerobic conditions [37]. Porewaters were

transferred to septa vials, spiked with enriched Hg isotopes (for quantitation by isotope dilution), then capped with no headspace and refrigerated until analysis (< 1 wk).

Surface water samples were collected by hand using clean technique [36] during August 2011. Within 24 h of collection, water samples were filtered through a 0.7µm quartz fiber filter for both dissolved and particulate analysis; filters were frozen and filtrate was acidified to 0.4% HCl for preservation until analysis.

Periphyton samples were also collected in 2011 from streambed rocks (a minimum of 12 per site) by scrubbing with a stiff brush and rinsing the material with a minimum amount of river water into a collection bin, allowing the solids to settle out, and decanting excess water. The condensed periphyton slurry was then transferred to a pre-weighed 250ml PETG bottle, and stored in the dark on ice until further analysis. The exact surface area of processed rock (0.15–0.34 m² per site) was measured using the technique described by Bell and Scudder[31].

Macrofauna collections included mayflies (Ephemoptera, species not determined), crayfish (*Orconectes* sp.), shiners (*Notropis* sp.) and juvenile smallmouth bass (*Micropterus dolomieu*). Invertebrates and juvenile/small fish were collected using a combination of seine and kick nets, baited minnow traps, and hand collection. All field collections were carried out in compliance with Dartmouth College Institutional Animal Care and Use Committee protocols and all relevant permits were obtained from NH Fish and Game. At least three replicate samples of similar size crayfish (4.7±2.2cm) and fish (shiners: 3.4±0.3cm; juv. smallmouth bass: 4.3±0.4cm, all sites) were analyzed for each taxa at each sampling site when possible. Mayflies were analyzed as composites of multiple individuals per sample. Whole body samples of invertebrates and small fish were freeze-dried and homogenized prior to analysis.

Analytical methods

Mercury speciation—Hg speciation analyses were conducted at the Dartmouth Trace Element Analysis Core facility. For sediment MeHg analysis, sediment was first extracted with KBr/H₂SO₄/CuSO₄ according to Bloom et al [38], then analyzed by isotope dilution GC-ICP-MS. Quality control and assurance for these and subsequent analyses were assessed by the analysis of duplicates, spiked samples and standard reference materials (Supplemental Data, Table S1). For THg analysis, sediment samples were digested in 9:1 HNO₃: HCl in a closed vessel microwave, and analyzed by isotope dilution ICP-MS (7500 Agilent). Sediment percent fines (% fines) were calculated as the dry weight of the sediment <63µm divided by the total weight of all fractions. Sediment percent loss on ignition (%LOI) was determined by combusting samples at 550°C for 4 h [39].

Surface water and sediment porewater were analyzed for MeHg by isotope dilution GC-ICP-MS [40], using an Element 2 magnetic sector mass spectrometer (Thermo Finnigan). For THg determination, water samples were treated with 0.5% BrCl overnight, then reduced with SnCl₂ and analyzed by purge and trap cold vapor atomic fluorescence spectroscopy using a MERX-T automated system (Brooks Rand Labs). Surface water and porewater were analyzed for DOC using a Teledyne Dohrmann Apollo 9000 (Teledyne Tekmar). Partition

coefficients (log K_d) were calculated as the log of sediment Hg concentrations divided by porewater Hg concentrations.

Biological samples were extracted with 4M HNO_3 and analyzed for MeHg and inorganic Hg by species-specific isotope dilution using an automated MeHg system MERX-M (Brooks Rand Labs) interfaced with an Agilent 7500cx ICP-MS (Agilent) using EPA method 1630 [41, 42]. Total mercury was calculated as the sum of MeHg and Hg(II).

Periphyton methylation potential—Within 12 h after field collection, the periphyton slurry was sub-sampled into 13 cm^3 serum bottles, all subsamples were collected volumetrically and then weighed to calculate the exact slurry density (g ml^{-1}) for each site. Additionally, chlorophyll *a* (*chl a*) samples were processed by filtering 3.0 ml of homogenized slurry thru a pre-combusted glass fiber filter (GF/F, 47 mm) [43]. Filters were extracted in 90% acetone for 24 h and quantified via spectrophotometric analysis [44].

Periphyton methylmercury production potential (MPP) rates are used as a relative measure of the ability of the periphyton to convert available reactive Hg(II) to MeHg. MPP rates were measured on three periphyton slurry subsamples per site using a stable isotope incubation approach [45] initiated within 12 h of field collection. An isotopically enriched solution of inorganic Hg ($^{200}\text{HgCl}_2$) was injected (0.1 ml; final amendment concentration of 13.8–16.2 ng of $^{200}\text{Hg(II)}$ per g of slurry, wet weight), and gently hand shaken for 1 min. One sample per set was immediately flash frozen in a bath of dry ice and ethanol; representing the killed control. The remaining two samples per set were incubated in the dark for 4.0–4.3 h at the average water field temperature ($\pm 1^\circ\text{C}$) for all sites sampled during a given sampling event (incubation range for all sampling sites: 21.0–24.6 $^\circ\text{C}$). Afterwards the incubated samples were also flash frozen in dry ice and ethanol and stored frozen until further processing. Subsequently, samples were thawed in the USGS Menlo Park laboratory and the entire contents transferred to pre-weighed 50ml centrifuge tube by rinsing with 4.0ml of 0.5% trace clean hydrochloric acid (weight recorded), and subsequently neutralized (33 μl of 45% potassium hydroxide), freeze dried, and re-weighed. A sub-sample of freeze-dried material was transferred to a 15ml centrifuge tube (0.24–0.47g) and extracted with 25% KOH in methanol, and quantified for Me^{200}Hg via isotope dilution ICP-MS [45]. MPP rates were then calculated from the stable isotope derived rate constant (k_{meth}) and the sample-specific isotope amendment concentration on a periphyton dry weight basis (range = 46–488 ng g^{-1} dry wt). The detection limit for this assay was 0.004 d^{-1} (0.4% d^{-1}) for k_{meth} and the reporting limit was 0.1 $\text{ng g}^{-1} \text{d}^{-1}$.

Statistical analysis

Statistical analyses were conducted in JMP 10 (SAS 2012). When necessary, data were transformed prior to ANOVA analysis to meet assumptions of homoscedasticity (assessed by Levene's test) and normality (assessed with normal quantile plot). Sediment and porewater THg and MeHg concentrations were $\log_{10}$ transformed. Periphyton THg (area normalized), periphyton MPP rates, and biota THg and MeHg data were natural log transformed. Data for periphyton MeHg concentration (area normalized) and *chl a* (area normalized) were not transformed. Surface water MeHg and THg concentrations were not

analyzed for statistical differences between sites as there were only two samples per site. For all other data, an ANOVA approach was used to examine species-specific spatial differences among sites for individual analytical parameters. When the null hypothesis was rejected ($\alpha = 0.05$), a post-hoc multiple comparisons Tukey's HSD was used to determine which sites differed. At least one replicate MPP measurement was below reporting limits for all sites except Riverside. Despite this, the ANOVA approach was used, acknowledging the increased uncertainty in the low MPP rate estimates. Log K_d did not meet assumptions of homoscedasticity, and was analyzed using non-parametric Kruskal-Wallis test. Linear regressions were utilized to analyze relationships between sediment Hg concentrations as well as between %LOI, %fines, DOC and log K_d, as previous studies [34, 46] have indicated an effect of these parameters on Hg partitioning. Linear regressions were also used to determine if particulate, porewater or sediment MeHg concentrations were predictive of biota tissue MeHg concentrations, as would be anticipated if either MeHg flux from the sediment or particulate loading was driving bioaccumulation. All figures are presented as non-transformed data. Mean, standard deviation and sample sizes for each parameter are reported in the Supplemental Data (Table S2–S4).

RESULTS AND DISCUSSION

Influence of mercury from the Superfund site on abiotic compartments

Surface sediment collected in 2010 showed significantly elevated MeHg and THg directly adjacent to the point source in comparison to all other sites ($p < 0.0001$, Figure 2, and Supplemental Data, Table S2). Downstream sites were elevated relative to the Wheeler Bay reference site, but were still significantly lower than Riverside. This pattern was maintained when normalized to organic content (as percent loss on ignition; %LOI) as %LOI did not differ significantly between sites (Table S2). There was a significant positive relationship between THg and MeHg in the sediment across all sites (Table 1). As seen with sediment Hg values, porewater had significantly higher THg at Riverside than at any other site ($p < 0.0001$, Figure 2, and Table S2).

Spatial patterns of Hg in surface sediment and porewater are consistent with previous studies that have found the highest THg sediment concentrations levels near an active chlor-alkali plant [12, 13]. These findings also support the hypothesis that the Superfund site is a source of Hg to downstream reaches of the Androscoggin River, a conclusion consistent with that from a recent USGS study of the Androscoggin conducted to inform the Superfund site impact assessment [34]. Concentration ranges of MeHg and THg in sediment in the current study were comparable to those found in Chalmers et al., [34], although the previous study did not analyze samples from Riverside, adjacent to the Superfund site. Concentrations of MeHg in porewater were comparable between the two studies, whereas porewater THg concentrations in the present study were approximately four times higher than reported in Chalmers et al., [34], likely due to differences in the collection techniques (centrifugation of top 2cm [37] in the current study versus in situ push-point sampling from 7–15cm depth [47] in Chalmers) resulting in the collection of different operationally defined (e.g. dissolved, exchangeable) fractions. Both porewater and sediment MeHg and THg were significantly higher in downstream reaches in the present study, with variability between sites suggesting

a heterogeneous distribution of contamination. These results differed from the Chalmers et al. study [34], which found porewater MeHg, but not THg, to be significantly elevated in downstream regions, again likely due to different sample horizons and techniques between the two studies.

Sediment THg and MeHg and porewater THg measurements were not only higher, but also more variable at Riverside compared to the other sampling sites, indicative of the spatial variability of sediment deposition and Hg(II)-methylation within this river reach. There are few depositional areas within the Riverside site, as a result, sediment sampling at Riverside occurred over a larger total area of riverbed compared to the other sites in order to achieve comparable replicate sample numbers, which may partially explain the high degree of sediment THg and MeHg variability at the point-source site. In addition, the input of elemental Hg from the Superfund site and sediment transport downstream may be episodic rather than steady; with proximity to the source resulting in little time/space for mixing of these episodic inputs, leading to a high degree of variability in Hg content of the sediment at Riverside. This within site spatial variability is transferred to benthic organisms such as mayflies, which also exhibited higher variability in THg at Riverside than other sites, and has also been observed in spot-tail shiners in the St. Lawrence River [48], suggesting that within site variability of sediment contamination is transferred to fauna with high site fidelity.

Past studies have shown that the fate of Hg from contaminated sites is complex in riverine systems, particularly those with impoundments. Wiener and Shields [49] found that reservoirs associated with the Nyanza Superfund site on the Sudbury River, MA were depositional sinks for THg, with sediment concentrations decreasing over time due to burial after industrial operations ceased, though the Nyanza site continued to be major source of Hg to surficial sediments. Analysis of sediment cores from a contaminated site by Delongchamp et al. [50] indicate subsurface peaks of Hg that were subsequently buried by less contaminated sediment after regulations decreasing Hg emissions were enacted, but that MeHg is highest in surface sediments. Therefore, though Hg emissions may have been higher historically, current inputs as reflected by surface sediments are what drive exposure. Though deposition was not directly measured in the present study, higher depositional rates due to reduced flow in the lower gradient downstream areas of the Androscoggin River would affect Hg inputs, distribution, and concentrations in sediment throughout the reach, as would controlled releases of water from the impoundments above the dams. Contaminated sediment from the point-source both settles out in impoundments and becomes more effectively buried or “diluted” by the input of comparatively clean sediment from particulates transported from above Riverside or from incoming tributaries downstream of Riverside, leading to the relatively lower sediment Hg within downstream reaches. The effect of both large and small dams on sediment transport has been well studied (e.g.[51–53]), with fewer studies specifically assessing the role of dams in trapping and or releasing contaminants [54–57]. On the Androscoggin, the presence of numerous dams within a relatively short span of river invariably affects transportation of Hg from the Superfund site.

Elevated particulate and dissolved THg were observed in the water column adjacent to the Superfund site (Figure 2 and Supporting Data, Table S3), providing further evidence of the

point source's impact on the adjacent river. Downstream particulate THg remains slightly elevated relative to the reference sites. Porewater MeHg concentrations are consistently higher than those of the overlying water, thus diffusive flux from sediments to the water column would be expected. However, dissolved and particulate MeHg levels in the surface water were not markedly elevated adjacent to or downstream from the contamination point source, suggesting that controls on methylation and flux to the surface water are not governed solely by sediment THg concentrations. In a study of eight pristine watersheds, river bed-sediment MeHg production was concluded to be only a minor input to the riverine water column, with drainage from the upland catchment considered the prominent source of MeHg in streams [58]. Waldron et al.[59] also found that MeHg concentration in surface water downstream from the Nyanza site was more dependent upon season and proximity to riparian wetlands than on proximity to the contamination point source. However, the Nyanza site directly impacted THg in surface water. Similar mechanisms are likely at work in the Androscoggin River, where surface water THg is greatly elevated adjacent to the Superfund site due to the direct delivery of inorganic and elemental Hg to the river and decreases in downstream reaches, while MeHg shows less clear spatial trends. The patterns of surface water THg and MeHg concentrations could be due to factors discussed earlier, such as flow regulation by the series of dams impacting delivery of Hg to downstream reaches. In addition, more local characteristics such as the amount of adjacent riparian wetlands (optimal methylation habitats) or upland drainage inputs could differentially influence MeHg production and bioaccumulation in each reach.

Hg bioavailability and MeHg production

In the current study, sediment partitioning coefficients (log K_d for MeHg and THg) are significantly but weakly correlated with log %fines and log %LOI (Table 1), meaning that finer and/or higher organic content sediment binds more Hg, making it potentially less available for methylation or uptake. Unlike the across stream comparison in Marvin-Dispasquale et al.[46], there were no significant relationships within the Androscoggin R. between K_d and porewater DOC or K_d and DOC/%fines. The highest log K_d (MeHg) was at Riverside, with the lowest log K_d (MeHg) at Wheeler Bay, Gorham, and Cross Power, though these differences were not statistically significant (Supplemental Data, Table S2). In contrast, log K_d (THg) is highest at Wheeler Bay and Gorham Brown, suggesting that the sediment composition at these sites is such that THg is less bioavailable for methylation, while the lowest K_d (THg) was at Riverside.

In freshwater ecosystems with relatively low THg contamination, MeHg production in sediment is related to sediment THg concentration, but in highly contaminated systems, THg concentration often plays only a minor role in net MeHg production with factors such as wetland density or organic carbon content exhibiting more control over MeHg production than THg loading [60]. Across a wide range of lotic systems (including the Androscoggin River), porewater-sediment partitioning of MeHg and THg has been correlated to the combined effect of porewater DOC and sediment grain-size [34, 46]. Factors that affect the partitioning coefficient (K_d) may also affect relative bioavailability of Hg(II) for methylation or MeHg for uptake into biota. Low K_d values can be interpreted as

comparatively more available Hg (more Hg in the porewater, where it can flux to surface water) whereas higher K_d indicates comparatively more Hg is bound in the sediment.

Relatively higher K_d (MeHg) does not necessarily translate to lower exposures for resident fauna, as evidenced by the conditions at Riverside. Here, despite a comparatively higher log K_d (MeHg) (thus presumably lower bioavailability of MeHg), the actual concentrations of MeHg in abiotic compartments are higher than in other regions of the Androscoggin, and some resident fauna show similarly elevated tissue concentrations, though these concentrations are not always significantly different from other sites (Figures 2,3,4, and Supporting Data, Table S4). Given the short distances between the sampled sites along the Androscoggin, a small and fast moving river, the influence of sediment Hg concentrations and partitioning is likely small and difficult to decipher, particularly on pelagic-feeding species due to rapid turnover of surface water. While K_d can provide a good estimate of availability of Hg under similar sediment contaminant loads, a more meaningful estimate of sediment-based exposure might be the relationship between sediment or porewater concentrations and faunal concentrations. In the current data set, MeHg tissue concentration is significantly but weakly correlated with mean sediment and porewater MeHg concentration in only crayfish and mayflies, (Table 1). However, these relationships are driven by the significantly elevated values at Riverside, and when the Riverside data are excluded the only relationship that is found is between mean porewater MeHg and shiner MeHg (Table 1). Variability in particulate MeHg is too low to exhibit a relationship with biota MeHg concentrations. Together, these findings indicate possible MeHg sources within the Androscoggin other than methylation within the sediment and subsequent flux to surface waters.

Periphyton communities are known to methylate Hg(II) [21, 25], and could be a source of MeHg to the water column, particularly in the fast-moving sections of rivers where there is little sediment present. In the Androscoggin River, periphyton MPP rates were highest adjacent to the chlor-alkali plant ($p=0.008$, Figure 3, and Supplemental Data, Table S4). While downstream periphyton MPP rates were not statistically different from either the upstream reference site (Sawmill) or the point source area (Riverside), multiple replicates at these sites were at or near reporting limits, making determination of low-level differences less certain. The MPP rates at Riverside (Range: $2.0\text{--}8.5\text{ ng g}^{-1}\text{ d}^{-1}$ dry wt., $n=3$) were much higher than those reported by Zizek et al. [26] in a highly Hg impacted river ($\sim 4\text{ pg g}^{-1}\text{ d}^{-1}$ dry wt.) and comparable to those found in boreal lakes ($2\text{--}41\text{ ng g}^{-1}\text{ d}^{-1}$ dry wt. [61]). The elevated surface water particulate MeHg concentrations and periphyton MPP rates at Riverside indicate that some of the Hg(II) at Riverside is being methylated by periphyton and being taken up by suspended particles.

MeHg production associated with periphyton represents a previously unrecognized source of MeHg to the Androscoggin River and may function as an important entry point for MeHg into the food web. Point-source Hg contamination from the Superfund site at Riverside appears to exacerbate this phenomena in two potential ways. First, the microbial activity (k_{meth}) associated with the periphyton slurry incubations were highest at this site, potentially indicating a microbial community that is adapted to relatively high Hg concentrations and able to rapidly cycle Hg(II) via the methylation pathway. Second, the dry weight mass of

periphyton per unit area sampled was lowest at Riverside (26–65 g m⁻², n=3), resulting in the lowest slurry density (28–34 mg g⁻¹, dw/ww, n=3) and the highest slurry ²⁰⁰Hg(II) isotope amendment concentration (379–488 ng g⁻¹ periphyton dry wt., n=3) among all sites sampled. If periphyton at all sites were able to methylate Hg(II) at equal rates, the above isotope amendment conditions would predict that k_{meth} would be lowest at Riverside, instead of highest. Although the low periphyton density at Riverside cannot be explained by our data alone, we hypothesize that the high gradient environment in this reach limits periphyton colonization and stabilization on rock surfaces to some degree, relative to low gradient (low energy) locations.

The combined effects of high k_{meth} and low periphyton density resulted in comparatively high MPP rates at the point-source sampling location, indicating that periphyton may play a significant role as a zone of MeHg production in portions of the Androscoggin River. Further, a comparison of k_{meth} values for periphyton measured in the present study (range: < 0.0035 – 0.20 d⁻¹, median = 0.013, mean = 0.045, n=13) with those reported for Androscoggin R. 0–10 cm surface sediment by Chalmers et al.[34] (range: < 0.0012 – 0.044 d⁻¹, median = 0.0094, mean = 0.012, n=15) show somewhat higher microbial activities associated with the periphyton matrix. While sediment methylation and upland drainage likely remain the dominant sources of MeHg in the Androscoggin River, the input of MeHg from periphyton methylation may be relatively more important in high gradient reaches where sediment makes up only a small proportion of the total surface area.

Fauna Hg bioaccumulation

Periphyton can contribute MeHg to higher organisms through direct consumption or by releasing MeHg into the water column for subsequent uptake by other primary producers [21, 24]. Cremona et al.[62] found that the proportion of epiphytes in macroinvertebrate diets correlated with percent MeHg in the tissue. If the food web within the Androscoggin River were based solely on periphyton primary production, it would be expected that consumer MeHg bioaccumulation would parallel the spatial pattern in periphyton MeHg concentration. Periphyton THg concentration spatial patterns appeared qualitatively similar to those of water column THg, but high variability in Sawmill and Riverside measurements resulted in no significant differences among sites. While periphyton MeHg concentration on a dry weight basis did not vary significantly among the study sites, MeHg concentrations normalized on an aerial basis (per m²) significantly increased from upstream to downstream (p=0.001, Figure 3, and Table S4). This may be related to delivery of Hg to sites downstream of the point source combined with decreased periphyton mass per m² at Riverside and elevated mass per m² downstream (Sawmill is intermediate). In pelagic phytoplankton, the effect of biodilution predicts lower MeHg concentrations with higher densities, and consequently higher mass per area, of plankton [63], yet despite decreased biomass (g m⁻²) at Riverside, *chl a* concentrations (ug m⁻²) are similar to Gorham where MeHg (ug m⁻²) is highest (p=0.01, Figure 3, Table S3 and S4.) indicating similar amounts of algal growth between the two sites. This suggests that the composition (amount of dead material, types of microbial and algal species, etc.) as well as the incorporation of settling particulates into the periphyton matrix is different between reaches, resulting in differences in both methylation and uptake of MeHg within the periphyton community. Similar to

periphyton MPP rates, this could be a function of riverine energetics that decrease from the upstream higher grade areas to the downstream low grade areas and affect particulate settling and availability of suitable substrate for periphyton growth.

Total mercury in biota was variable across sites and taxa (Figure 4, and Supplemental Data, Table S4). Statistically significant differences in THg tissue concentrations among sites were found for mayflies ($p=0.0013$), shiners ($p<0.0001$), and juvenile smallmouth bass ($p=0.0224$), though the spatial patterns were not consistent across taxa. We hypothesized that an increase in MeHg bioaccumulation would be evident in downstream reaches, particularly in the areas just above Gorham and Shelburne dams where the grade of the river decreases, as this presumably favors particulate deposition and more extensive depositional zones for Hg(II)-methylation. This hypothesis was supported by results for adult smallmouth bass in the USGS report [34], and for area-normalized periphyton (present study), but not for the invertebrates and small fish sampled in the current work. In fact, the influence of a known source of Hg to the river was most apparent directly adjacent to the contamination site in the increased Hg(II) levels in abiotic compartments and lowest trophic levels. Total Hg in mayflies was over five times higher at Riverside than any other site, and while MeHg was higher, it was not significantly so, resulting in approximately 15% MeHg in mayflies at Riverside compared to an average of 38% MeHg in mayflies at all other sites. Additionally, both periphyton and mayfly THg concentrations were higher at Riverside than in higher trophic level taxa (Figure 4), though as expected, percent MeHg was lowest in mayflies and increased with presumed trophic level across all sites. These data indicate that in these primary consumers, Hg(II) was ingested or adhered to the exoskeleton of the mayflies, but that MeHg was not bioaccumulating at levels significantly higher than at other sites despite higher MeHg concentrations in sediment and particulates. Inorganic Hg was also elevated in sediment and suspended particulates at Riverside, which likely contributes to the high inorganic Hg in mayflies as they scavenge organic detrital material and may also be entrained within the periphyton community matrix.

While the mayflies appear more closely linked to sediment and water column Hg concentrations (particularly with regards to THg), this relationship is not transferred consistently through trophic levels, due partially to feeding preferences (omnivores like crayfish that feed at different trophic levels often do not exhibit clear patterns of bioaccumulation) and other confounding factors discussed. Ridal et al. [64] attributed significant differences in THg concentrations observed for perch but not for benthic invertebrates within contaminated zones of the Cornwall Area of Concern to prey selection, zone-specific Hg sources, and/or heterogeneity of habitat influencing Hg dynamics. For most taxa, differences between sites in biota MeHg concentrations were non-significant, with the exception of shiners ($p<0.001$) and juvenile smallmouth bass ($p=0.0193$) (both highest at CrossPower and Gorham on an among-site basis (Figure 4, and Table S4)). There was a weak but significant relationship between crayfish size and MeHg concentration (Table 1), but not for shiners or juvenile smallmouth bass. When adjusted for size by normalizing to 6cm, differences between sites in crayfish MeHg concentration still were not significant. The downstream increase previously seen in adult smallmouth bass THg, but not currently in other macrofauna did not appear to be directly related to water or sediment MeHg concentrations, and is more likely to result from differences in size and feeding

strategies used by fish within each site or increased MeHg available through periphyton. Additionally, adult fish have been integrating MeHg uptake over a longer time span and are likely better indicators of exposure than taxa with shorter life spans.

Given that there is a known source of Hg to the river, we were surprised that MeHg concentrations in downstream biota were not higher, especially considering that the samples were collected in reservoirs, which are generally associated with relatively high Hg concentrations in resident biota due to elevated organic content of sediment, periodic wetting and drying, and common associations with industry [1, 65–67]. Reservoirs may also contribute to downstream MeHg loading and bioaccumulation through discharge of particulate and dissolved Hg [65, 68]. However, THg tissue concentrations in the present study are within the range of or lower than those found in other studies: e.g., for crayfish in New England [69] as well as those in a study associated with a Spanish chlor-alkali plant [14, 70]; for mayflies in Maryland [71] and at the Spanish chlor-alkali facility [70]; and for similar types of small fish in environments ranging from Maryland streams to California mine-impacted areas [71–74].

CONCLUSIONS

Major findings

- 1) MeHg production associated with periphyton in the Androscoggin River represents a previously unrecognized source of MeHg in this system, and may function as an important entry point for MeHg into the Androscoggin food web. MPP rates were highest at the point-source sampling location as a result of high microbial activity and low periphyton density. Methylmercury concentrations normalized on an aerial basis (per m²) significantly increased from upstream to downstream. Both the increase in periphyton MeHg concentration on an aerial basis and the decrease in periphyton MPP rates can be attributed to riverine energetics and periphyton community composition.
- 2) Contrary to expectations, spatial patterns of bioaccumulation previously published for large adult fish were not apparent in the lower trophic level fauna examined in the current study; however, currently measured MeHg concentrations in periphyton on an areal basis were reflective of the patterns observed prior for large fish.
- 3) The Superfund site is directly impacting Hg concentrations in both abiotic and biotic compartments within the adjacent river. Further study is necessary to determine controls on fate of Hg, which may be more localized than originally hypothesized due to the influence of dams and river grade on delivery of Hg to downstream sites, combined with potential proximity to wetlands and upland drainage.

Future directions

The present study clearly demonstrates the impact of the chlor-alkali Superfund site on Hg dynamics within the studied section of the Androscoggin River, but future studies should further investigate the influence of individual site characteristics such as wetland presence, upland drainage, river grade, and depositional area on methylation and bioaccumulation. An even greater potential may exist for Hg bioaccumulation downstream of Shelburne Dam,

where the river broadens and slows even further to create a lower energy deposition zone. Additionally, the presence of dams within this system likely impose restrictions on transport of Hg to downstream reaches, affecting bioaccumulation, and the implications of dam and/or sediment removal on transport of legacy pollutants within river systems (e.g. [54, 64]) is an important avenue of continuing research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Map of Androscoggin River with sampling sites indicated by stars. Inset shows elevation and distance (from Google Earth) between Sawmill and Shelburne Dams. Wheeler Bay is ~16km upstream of Sawmill Dam. Base map modified from design by Avanru Environmental. Inset modified from Chalmers et al., 2013 [34].

Figure 2.

Surface water and sediment THg (bottom panel) and MeHg (top panel) concentration by river reach (mean $\pm$ standard error). Particulate (open triangle) and dissolved (x) surface water fractions (ng L⁻¹) and sediment porewater (open circle, ng L⁻¹) and whole sediment (closed circles, ng g⁻¹) are depicted. Sediment and porewater MeHg and THg concentrations were significantly different among sites. Statistics associated with among site differences are presented as Supplemental Data (Tables S2 and S3).

Figure 3.
Mean $\pm$ standard error of periphyton methylation potential and area normalized MeHg and Chl a concentrations across sites.

Figure 4.

Mean $\pm$ standard error of THg (bottom panel) and MeHg (top panel) concentrations for biotic samples across sites. Mayflies (closed circle), crayfish (open circle), shiner (x), and juvenile smallmouth bass (open triangle) are depicted. Statistics associated with among site differences are presented as (Supplemental Data Table S4).

Table 1

Significant linear relationships as assessed by a bivariate fit of X vs. Y

X	Y	slope	intercept	R ²	p value	N
log [sediment THg]	log [sediment MeHg]	0.72	-1.38	0.67	<0.0001	52
log [sediment %LOI]	log [Kd MeHg]	0.98	2.52	0.20	0.0045 ^a	38 ^b
log [sediment %LOI]	log [THg Kd]	1.03	3.17	0.28	0.0006 ^a	39 ^b
log [sediment %fines]	log [Kd THg]	0.95	2.54	0.24	0.0028 ^a	35
log [sediment %fines]	log [Kd MeHg]	0.84	1.93	0.29	0.001 ^a	34
crayfish length (cm)	crayfish MeHg (ng/g)	20.54	124.27	0.20	0.0059	37
mean site sediment MeHg (ng/g)	crayfish MeHg (ng/g)	5.91	194.97	0.17	0.0161 ^c	33
mean site sediment MeHg (ng/g)	mayfly MeHg (ng/g)	2.07	70.40	0.32	0.0333 ^{c*}	14
mean site pore water MeHg (ng/L)	crayfish MeHg (ng/g)	62.58	139.03	0.13	0.0431 ^{c*}	33
mean site porewater MeHg (ng/L)	mayfly MeHg (ng/g)	24.45	50.93	0.34	0.0272 ^{c*}	14
mean site porewater MeHg (ng/L)	shiner MeHg (ng/g)	95.45	207.82	0.29	0.0368 ^{c*}	15 (Riverside excluded)

^a Multiple regressions were run to determine significant relationships between sediment parameters (%fines, %LOI, porewater DOC, and DOC/%fines) and Kd (THg) and Kd (MeHg). A Bonferroni correction yielded alpha=0.0125, all reported regressions predicting Kd are significant with this alpha.

^b One outlier was excluded from analysis. Outliers were evaluated using an outlier box plot generated by JMP software.

^c Bonferroni corrections for predicting biota MeHg concentrations from sediment or porewater MeHg yielded a conservative alpha=0.025. Regressions that are not significant at this level are marked with “*”.