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Environmental Toxicology

NET METHYLMERCURY PRODUCTION IN 2 CONTRASTING STREAM SEDIMENTS AND ASSOCIATED ACCUMULATION AND TOXICITY TO PERIPHYTON

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Abstract

Periphyton uptake of bioaccumulative methylmercury (MeHg) may be an important entryway into the food web of many stream ecosystems where periphyton can be dominant primary producers. The net production of MeHg in stream sediment, its bioaccumulation in periphyton, and the potential toxicity of divalent Hg (Hg[II]) and MeHg in sediment to periphyton were investigated with a 67-d in situ incubation experiment using chemical exposure substrates containing either a fine-grained, organic-rich or a sandy, low-organic sediment, each amended with varying concentrations of mercuric chloride. Methylmercury was produced in sediment, and concentrations increased with greater amounts of added Hg(II); however, the net production of

MeHg was inhibited in the highest Hg(II) treatments of both sediments. The range of total Hg concentrations that inhibited MeHg production was between approximately 80 000 ng Hg and 350 000 ng Hg per gram of organic matter for both sediments. Periphyton colonizing substrates accumulated MeHg in proportion to the concentration in sediment, but periphyton exposed to the sandy sediment accumulated approximately 20-fold more than those exposed to the organic-rich sediment relative to sediment MeHg concentrations. Toxicity of either Hg(II) or MeHg to periphyton was not observed with either periphyton organic content, net primary production, or respiration as endpoints. These results suggest that in situ production and bioaccumulation of MeHg in stream ecosystems can vary as a function of sediment characteristics and Hg(II) loadings to the sediment.

Keywords: Mercury, Methylmercury, Bioavailability, Sediment chemistry

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INTRODUCTION

Mercury (Hg) is a pervasive environmental contaminant that enters stream ecosystems through weathering of natural deposits, discharge from point sources, and atmospheric deposition from natural and anthropogenic emission sources. Within stream ecosystems, complexes of divalent Hg (Hg[II]) can be transformed to methylmercury (MeHg), which bioaccumulates and biomagnifies within aquatic food webs [1,2] to levels that may be harmful to some organisms, particularly piscivorous wildlife [3,4] and humans who eat fish [5]. Methylation of Hg(II) to MeHg can occur abiotically [6], but most MeHg production is thought to occur

biologically [7], with the mechanism recently attributed to proteins associated with the *hgcAB* gene cluster [8] that is present in a variety of anaerobic microorganisms [9,10]. Periphyton (i.e., a complex community of algae, bacteria, microinvertebrates, and detritus attached to submerged surfaces) are known to be capable of Hg methylation [11–14], and this organic-rich material can either scavenge or bioaccumulate MeHg from water [15]. Periphyton uptake of MeHg, whether from in situ production or other sources in the watershed, may be an important entryway into the food web of many stream ecosystems where periphyton can be dominant primary producers [16,17].

Multiple studies have examined Hg methylation by a variety of periphyton communities, but very few studies have examined the potential for toxicity of either total Hg or MeHg to periphytic communities. Periphyton are sensitive to environmental stressors such as either enrichment or deficit of nutrients and metals [18] and pesticides [19] and are useful for assessing the ecological health of aquatic systems [16,20]. Many controlled chemical exposure studies have been conducted on both native and cultured periphyton, but it has been suggested that the best method to assess contaminant effects on periphyton is by use of in situ chemical exposure substrates under natural conditions in stream environments [21].

For the present study we conducted an in situ experiment with chemical exposure substrates to investigate 1) net production of MeHg in 2 geochemically dissimilar stream sediments in southwest Ohio, 2) accumulation of MeHg and total Hg in periphyton naturally colonizing the substrates, and 3) the potential toxicity of MeHg and total Hg effluxing from sediment to periphyton. This was done by serially adding $HgCl_2$ to 2 contrasting sediment types, 1 that was fine-grained and organic-rich and another that was sandy with low organic content. The sediment samples were incubated for 67 d in chemical exposure substrates in a southwest

Ohio stream with fritted glass lids that allowed for solute exchange and colonization by natural stream periphyton [21]. At the end of the incubation, we quantified MeHg and total Hg concentrations in sediment and periphyton colonizing the chemical exposure substrates as well as measured periphyton organic content and metabolic activity (net primary production and respiration) as endpoints of toxicity from the exposures.

MATERIALS AND METHODS

Experimental design

The exposure design of the present study followed that of Costello and colleagues [21], which was adapted from prior investigations using nutrient-diffusing substrates [22,23]. Sandy, low-organic (1% loss on ignition, acid volatile sulfide [AVS] = 0.4 µmol/g dry wt) sediment was collected from Sugar Creek (Sugarcreek Township, OH, USA) and fine-grained, organic-rich (17% loss on ignition; AVS = 4.8 µmol/g dry wt) sediment from Warden Ditch (Fairborn, OH, USA). Four liters of sediment from both streams were transported to Wright State University, where they were homogenized with a stainless-steel mixer and each sediment was divided into 5 high-density polyethylene 500-mL bottles. Ambient concentrations of total Hg averaged 7.3 ± 1.2 ng/g dry weight in Sugar Creek sediment and 87 ± 5 ng/g in Warden Ditch, and ambient MeHg concentrations averaged 0.2 ± 0.1 ng/g dry weight in Sugar Creek and 0.7 ± 0.2 ng/g in Warden Ditch. Sediment in the 500-mL bottles (excluding untreated reference sediment from both streams) was amended with $HgCl_2$ dissolved in a small volume of stream water to produce sediment treatments having nominal total Hg concentrations of approximately 150 ng/g, 800 ng/g, 3000 ng/g, and 20 000 ng/g dry weight for Sugar Creek and 500 ng/g, 2000 ng/g, 16 000 ng/g, and 60 000 ng/g for Warden Ditch. These concentrations were selected to span a range from those typical of most freshwater sediments (<500 ng/g) to Hg concentrations in sediment

contaminated by industrial and mining effluent [24,25]. Amounts of Hg(II) added to Warden Ditch sediment were greater than those added to Sugar Creek because we presumed that Hg(II) would be less bioavailable in the organic-rich sediment [26]. After Hg addition, sediment in each bottle was homogenized with a mixer for 30 s, allowed to equilibrate for 16 h at room temperature, and mixed again for 30 s prior to loading into vials of the chemical exposure substrates. The 16-h equilibration period should have been sufficient for added Hg(II) to bind with natural ligands; 15 min was sufficient for 99% of added Hg(II) to adsorb to sandy (2% loss on ignition) Long Island Sound sediment [26].

Chemical exposure substrates, consisting of a vial of sediment covered with a fritted glass disk, were prepared for each sediment type and Hg(II) treatment and incubated in Warden Ditch (Supplemental Data, Figure S1). Hinge-capped vials made of black high-density polyethylene (30-mL Poly-Cons®; 38 mm height, 38 mm diameter) were prepared for use as chemical exposure substrates by boring a 22-mm-diameter hole into the vial cap, and each vial was etched with a unique identification number. The vials were filled with approximately 30 mL of sediment and covered with a 25-mm-diameter (4 mm thick) fritted glass disk secured directly to the sediment surface inside the chemical exposure substrates with the hinged cap. Fritted glass is a preferred attachment substrate for combined autotrophic and heterotrophic periphytic communities because of the durability, consistency, and cost-efficiency of fritted glass [21,23]. Ten replicate chemical exposure substrates were prepared for each sediment type and Hg treatment and attached with plastic cable ties to a plastic *L*-shaped bar (1 bar/treatment) secured to the bottom of Warden Ditch with steel stakes. The chemical exposure substrates were incubated with the fritted glass surface facing upward for 67 d, from 28 June to 3 September 2013, to allow for Hg methylation and MeHg demethylation processes to reach a quasi–steady-

state condition and for periphyton to colonize. Peres and colleagues [27] found that 34 d were sufficient for periphyton colonization of artificial substrates and that colonization continued to increase up to 71 d. The chemical exposure substrates were arranged in the stream so that Hg(II) concentrations in sediment increased downstream, with approximately 0.3 m between each *L*-bar, to minimize potential cross-contamination among treatments. Warden Ditch was selected because it is a fen-fed, minimally disturbed headwater stream with weak and relatively consistent water flow and high water quality. These conditions allowed for the chemical exposure substrates to be deployed for an extended period during mid-summer without risk of either emergence as a result of drought or loss during extreme rain events.

Chemical exposure substrates were removed from the stream to determine MeHg and total Hg concentrations in sediment and periphyton as well as to measure periphyton organic content and metabolic activity. For each treatment, 5 of the 10 chemical exposure substrates were randomly selected a priori for analysis of MeHg and total Hg in sediment and periphyton, and the other 5 were used for analysis of periphyton metabolic activity and organic content. Periphyton for Hg analysis was scraped from each fritted disk with plastic spoons into plastic tubes, and sediment from each vial was transferred to plastic tubes for preservation. Samples were not composited. Periphyton and sediments were stored on ice in the field and frozen (−20 °C) on return to Wright State University on the day of sampling.

Periphyton metabolic activity

Periphyton metabolism and organic content were determined from the 5 chemical exposure substrates in each treatment not destructively sampled for Hg analysis. Net primary production and respiration of periphyton were used as parameters to assess potential Hg toxicity by measuring changes of dissolved O_2 [28] during light-bottle and dark-bottle incubations of

fritted glass disks colonized with periphyton. Immediately after removal from the stream, individual fritted glass disks were transferred to translucent 30-mL polypropylene screw-cap jars filled with stream water. Dissolved O_2 and temperature were measured with a calibrated, handheld optode (YSI ProODO), and then the jars were capped and incubated under in situ temperature conditions, 10 cm below the water surface attached to a wire shelf (Supplemental Data, Figure S2). The jars were removed from the stream after 3.6 h to 4.3 h of incubation and opened, and dissolved O_2 was measured again to quantify net primary production ([final dissolved O_2 – initial O_2]/time). Subsequently, respiration was quantified by wrapping the jars with Al foil, placing the jars back in the stream, and measuring dissolved O_2 after an additional 2.0 h to 2.2 h of incubation in the dark ([initial dissolved O_2 – final O_2]/time). Gross primary production was not determined because net primary production and respiration could not be quantified simultaneously for the same disk. For comparison of rates of net primary production and respiration among the 2 sediment types and Hg treatments, rates were normalized to the organic content of periphyton colonizing fritted disks (i.e., nanomoles of O_2 per milligram of organic material per hour), which was determined as described in the following section: *Periphyton organic content*.<!--<query>*ET&C* style states that sections must be named. Is *Periphyton organic content* the correct section referred to by "the following section"?</query>-->

Periphyton organic content

The mass of periphytic organic matter attached to the fritted glass disks was used as an endpoint to examine the toxicity of Hg. After the final dark-bottle measurements of dissolved O_2, fritted glass disks were removed from the jars containing stream water and transported on ice to Wright State University, where they were stored frozen until lyophilization. Freeze-dried fritted

disks and associated periphyton were burned at 550 °C for 4 h, with the mass difference before and after burning being a proxy for organic periphyton biomass, expressed as milligrams of organic matter per square centimeter of exposed surface area of disk.

Hg determinations

Total Hg and MeHg were measured in lyophilized sediment. Dried sediment (0.5–1.0 g) was accurately weighed into 60-mL digestion vials, to which was added 10 mL of 16 M HNO_3 and 0.5 mL of BrCl solution [29], and then digested for 6 h in a hot block at 95 °C. Digestates were diluted with reagent-grade water (nominal resistivity >18 MΩ-cm), and total Hg was determined by either inductively coupled plasma mass spectrometry (ICP-MS) with a Perkin Elmer ELAN 9000 [30] or, for reference sediments, by dual-Au amalgamation cold vapor atomic fluorescence spectrometry (AFS) [31,32]. Methylmercury was extracted from separate aliquots of dried sediment (0.25–0.50 g) by aqueous distillation [33] and quantified by gas-chromatographic cold vapor AFS after derivatization with sodium tetraethylborate [34,35].

Freeze-dried samples of periphyton were digested with dilute HNO_3 for measurement of MeHg and total Hg [36]. Briefly, periphyton (0.03–0.25 g) were digested with 7 mL of 4.6 N HNO_3 in a 60 °C water bath for 12 h prior to MeHg determination by gas-chromatographic cold vapor AFS. For measurement of total Hg in periphyton, 1-mL aliquots of the digestates were transferred to different tubes and oxidized with 0.3 mL of BrCl solution for 12 h, and total Hg was quantified by dual-Au amalgamation cold vapor AFS.

Quality control

Trace-metal clean techniques were used for all sampling and analytical procedures. Experimental and analytical plasticware were cleaned with 10% HCl and rinsed with reagent-grade water. Total Hg and MeHg analyses were calibrated with standard solutions traceable to

the US National Institute of Standards and Technology. Certified reference material TORT-2, lobster hepatopancreas (National Research Council of Canada), was digested and analyzed to assess the accuracy of analyses of MeHg and total Hg in periphyton. Sediment total Hg analyses included National Research Council of Canada reference material MESS-3 (marine estuarine sediment). Each digestion and distillation batch contained procedural blanks and replicate samples to assess precision and identify potential contamination.

Determinations of total Hg and MeHg in periphyton and sediment were accurate. Measured total Hg concentrations in MESS-3 were within their certified ranges and demonstrated no procedural bias (mean measured total Hg = 94 ± 10 ng/g, n = 4; certified range = 91 ± 9 ng/g). Procedural reproducibility of total Hg determinations among triplicate digestates of sediment averaged 9.2% relative standard deviation (SD; n = 2 triplicate sets) for analysis by ICP-MS and 9.5% relative SD for 1 triplicate set analyzed by cold vapor AFS. Procedural reproducibility of sediment MeHg determinations averaged 8.0% relative SD among 16 triplicate sets of sample distillates. Measured Hg concentrations in TORT-2 also were within their certified ranges, averaging 269 ± 11 ng/g (certified range = 270 ± 60 ng/g) for total Hg and 156 ± 7 ng/g (certified range = 152 ± 13 ng/g) for MeHg. Analytical precision of MeHg and total Hg measurements in periphyton digestates averaged 12% (n = 8) and 4.4% (n = 5) relative difference, respectively; procedural reproducibility of periphyton Hg determinations was not assessed because of the small sample sizes.

Statistical analysis

Relationships between paired variables (i.e., MeHg, total Hg, %MeHg, net primary production, respiration) were examined by least-squares linear regression analysis. Differences between Sugar Creek and Warden Ditch sediments were evaluated either by t test or by Mann-

Whitney rank sum test depending on whether the data were normally distributed. Differences among Hg treatments within each sediment type were evaluated with one-way analyses of variance (ANOVA) and Tukey's post hoc pairwise comparisons. All statistical analyses were conducted with SigmaPlot Ver 12.

RESULTS AND DISCUSSION

Total Hg in sediment

Measured concentrations of total Hg in sediment at the end of the 67-d incubation were comparable with intended nominal concentrations. Mean (± SD) measured concentrations of total Hg in Sugar Creek sediment, from the reference to the highest Hg(II) treatment, were 8 ± 1 ng/g, 170 ± 30 ng/g, 810 ± 140 ng/g, 3100 ± 890 ng/g, and 22 000 ± 3400 ng/g dry weight. Total Hg concentrations in Warden Ditch sediment averaged 90 ± 5 ng/g, 450 ± 60 ng/g, 1700 ± 240 ng/g, 15 000 ± 4700 ng/g, and 59 000 ± 27 000 ng/g dry weight, from reference to the highest treatment after the incubation. Total Hg concentrations in the highest Hg(II) treatment of Sugar Creek and the 2 highest treatments of Warden Ditch sediment are comparable with those in the most Hg-polluted aquatic systems contaminated by wastes from mining and chlor-alkali plants [24,25].

MeHg in sediment

Methylmercury was produced in sediment, and concentrations increased with greater amounts of added Hg(II). At the end of the incubation period, mean (± SD) concentrations of MeHg in Sugar Creek sediment were 0.2 ± 0.1 ng/g, 3.2 ± 0.2 ng/g, 7.6 ± 1.0 ng/g, 21.9 ± 6.6 ng/g, and 28.5 ± 4.6 ng/g dry weight, from reference to the highest Hg(II) treatment. Similarly, MeHg concentrations in Warden Ditch sediment increased from reference to the highest Hg(II) treatment: 0.7 ± 0.2 ng/g, 4.1 ± 0.5 ng/g, 14.5 ± 2.7 ng/g, 41.3 ± 10.1 ng/g, and 101 ± 29 ng/g.

Concentrations of MeHg in the reference of both sediment types at the end of the incubation were not significantly different from those at the start of the test (*t* tests, $p \geq 0.2$). The fraction of total Hg as MeHg (i.e., %MeHg) among all treatments of Sugar Creek sediment (1.3 ± 1.1%) was significantly greater than that in Warden Ditch sediment (0.6 ± 0.3%, $p = 0.05$). Divalent Hg has a high affinity for dissolved and solid-phase organic matter, which can inhibit its bioavailability to methylating organisms and production of MeHg in sediment [26,37,38]. A greater fraction of total Hg as MeHg in Sugar Creek compared to Warden Ditch sediment suggests a greater net rate of Hg methylation in Sugar Creek sediment [39], which would be consistent with increased availability of Hg(II) substrate to methylating bacteria as a result of the lower organic content of Sugar Creek (1% loss on ignition) sediment compared with those from Warden Ditch (17% loss on ignition). Gross rates of Hg methylation in marine sediment have been observed to vary as a function of organic content, with lower organic content sediment having greater Hg(II) availability and in situ methylation potentials [26,40].

Although concentrations of MeHg increased with total Hg among treatments of each sediment type, there was not a linear dose response between Hg(II) added and MeHg produced: the fraction of total Hg as MeHg was significantly different among Hg treatments of Warden Ditch and Sugar Creek sediment ($p < 0.001$; Figure 1). The fraction of total Hg as MeHg in the highest Hg(II) treatment of Warden Ditch sediment (total Hg = 59 000 ng/g) was significantly less than that in the reference and 2 lowest Hg(II) treatments ($p < 0.05$). Likewise, %MeHg in the highest Hg treatment of Sugar Creek sediment (total Hg = 22 000 ng/g) also was significantly less than that in the reference and lowest Hg treatment ($p < 0.05$). Moreover, the second highest Hg(II) treatment of Sugar Creek sediment (total Hg = 3100 ng/g) also contained significantly lower %MeHg than the reference ($p < 0.05$). This suggests that total Hg concentrations between

15 000 ng/g and 59 000 ng/g in organic-rich sediment (Warden Ditch) and between 810 ng/g and 3100 ng/g in sandy sediment (Sugar Creek) inhibit net MeHg production. When total Hg concentrations are normalized to sediment organic content, the concentration ranges are remarkably similar between the 2 sediment types, corresponding to 88 000 ng/g to 350 000 ng of total Hg per gram of organic matter for Warden Ditch and 81 000 ng/g to 310 000 ng/g of organic material for Sugar Creek sediment. Gross potential rates of Hg methylation and MeHg demethylation are known to be rapid in sediment [26,39,41], and steady-state concentrations should have been achieved within a 67-d incubation period. Accordingly, a possible explanation for decreased %MeHg in the highest treatments of both sediments is that increased Hg(II) concentrations either were inhibitory to methylating bacteria or induced microbial transcription of *mer* operon genes that exacerbated demethylation [11,41], particularly the *merB* gene that encodes for an organomercurial lyase protein that can demethylate MeHg. Either a slower rate of Hg methylation or a greater rate of MeHg demethylation could decrease the net production of MeHg (i.e., %MeHg) in sediments.

Hg in periphyton

Methylmercury and total Hg were mobilized from Sugar Creek and Warden Ditch sediment to overlying periphyton but to different degrees between the 2 sediment types. At the end of the incubation period, MeHg concentrations in periphyton were much less than, but strongly related to, concentrations in both sediment types (Figure 2). Methylmercury concentrations in periphyton exposed to Sugar Creek sediment treatments ranged from 0.8 ng/g to 15 ng/g dry weight and were considerably greater than those in periphyton colonizing frits above Warden Ditch sediment, which had periphyton MeHg concentrations ranging from 0.3 ng/g to 3.4 ng/g dry weight. Periphyton exposed to Sugar Creek sediment accumulated

approximately 20-fold more MeHg than those exposed to Warden Ditch sediment, based on comparison of linear regression slopes relative to the sediment MeHg concentration (Figure 2). The difference of MeHg accumulation in periphyton between sediment types may be explained by differences in the affinity of MeHg for solid-phase ligands in sediment. Partitioning coefficients (K_D, liters per kilogram) of MeHg between sediments and pore fluids, which can range from approximately $10^{1.5}$ to $10^{3.5}$, increase with sediment organic content [38,40,42]. Partitioning coefficients for MeHg can be much greater in oxic surface water [43]. Although sediment–water partitioning of MeHg was not examined in the present study, a greater organic content of Warden Ditch (17% loss on ignition) compared with Sugar Creek (1% loss on ignition) sediment likely resulted in MeHg being less mobile and bioavailable to periphyton overlying Warden Ditch compared with Sugar Creek sediment. Concentrations of MeHg in stream periphyton in the present study were low relative to concentrations observed in Boreal Shield lakes, which ranged from 3 ng/g to 55 ng/g dry weight [14].

Concentrations of MeHg and total Hg were strongly correlated within periphyton (Figure 3); however, the ratio of MeHg to total Hg in periphyton differed between Sugar Creek and Warden Ditch sediment exposures. The mean (± SD) fraction of total Hg as MeHg in periphyton overlying Sugar Creek sediment was 2.3 ± 1.5% and significantly greater than that in periphyton above Warden Ditch sediment (0.9 ± 0.5%; Mann-Whitney *t* test, $p = 0.003$). Differences of mean %MeHg in periphyton between Sugar Creek and Warden Ditch sediment would not be expected if either uptake from stream water or in situ microbial methylation within periphyton biomass were the primary source of MeHg in the periphyton because both Sugar Creek and Warden Ditch chemical exposure substrates were exposed to the same stream water and presumably colonized by the same periphytic organisms. A strong relationship between

%MeHg in periphyton and %MeHg in both Sugar Creek and Warden Ditch sediment suggests that sediments were the primary source of MeHg accumulated by periphyton (Figure 4), rather than in situ microbial production within periphyton biomass. The slope value of the linear regression in Figure 4 is greater than unity and may result from MeHg being more readily mobilized to periphyton than Hg(II) from underlying sediment. Alternatively, if the flux of MeHg and Hg(II) from sediment were in proportion to their ratio in sediment, a greater %MeHg in periphyton compared with sediment could result from periphyton preferentially bioconcentrating MeHg relative to Hg(II), as has been observed for seston and plankton [2,36,44].

Periphyton organic content

The organic biomass of periphyton was examined to investigate the potential toxicity of either Hg(II) or MeHg to periphytic colonies exposed to Hg(II)-amended sediments (Supplemental Data, Figure S3). Among all Hg(II) treatments, the organic biomass of periphyton colonizing Warden Ditch chemical exposure substrates (12.5 ± 4.1 mg/cm^2) was greater than the amount of periphyton on Sugar Creek chemical exposure substrates (10.2 ± 1.9 mg/cm^2; Mann-Whitney, p = 0.03). The modestly greater amount of periphytic biomass colonizing disks above Warden Ditch sediment may have resulted from greater organic matter respiration and nutrient efflux from the sediment. The organic and, by extension, fixed nutrient content of Warden Ditch sediment was much greater than that of Sugar Creek deposits, so enhanced organic matter remineralization and efflux of nutrients from Warden ditch sediment may have promoted periphyton growth.

Organic biomass of periphyton colonizing chemical exposure substrates did not differ significantly among Hg(II) treatments of either Sugar Creek sediment (ANOVA, p = 0.7) or

Warden Ditch sediment (ANOVA, $p = 0.1$; Supplemental Data, Figure S3). A study using artificial substrates for periphyton growth found that 500 ng/L of MeHg had a pronounced negative effect on diatom density and species composition [27]. Moreover, exposure of marine algae to either 5000 ng/L of Hg(II) [45] or 100 ng/L of MeHg [46] resulted in a reduction of growth. In the present study, the highest Hg treatments had mean measured MeHg concentrations of 29 ng/g and 101 ng/g dry weight and total Hg concentrations of 22 000 ng/g and 59 000 ng/g in Sugar Creek and Warden Ditch sediments, respectively. We estimated porewater concentrations of both MeHg and Hg(II) based on empirical relationships between sediment–water partitioning coefficients of MeHg and Hg(II) and the organic content of sediment [42]. The maximum concentration of MeHg in lyophilized bulk sediment in the present study corresponded to estimated porewater MeHg concentrations of approximately 20 ng/L for Warden Ditch (17% loss on ignition) and 600 ng/L for Sugar Creek (1% loss on ignition) and Hg(II) concentrations of approximately 120 ng/L for Warden Ditch and 12 000 ng/L in Sugar Creek pore fluids, for which there was no detectable impact on periphyton biomass. Because some of the sediments were amended with Hg(II) to concentrations far above natural levels, periphyton may have developed a resistance to Hg toxicity or, more likely, only communities highly tolerant of Hg(II) were able to survive on the contaminated sediment in these tests.

Periphyton metabolic activity

Net primary production and respiration were measured to examine potential toxicity of Hg to periphyton metabolism. Many members of stream periphyton communities are primary producers and therefore photosynthesize in addition to respire. Accordingly, Hg toxicity could be expressed as inhibition of either net primary production (net O_2 production in light bottle), respiration (O_2 consumption in dark bottle), or both. Rates of periphyton respiration were

directly proportional to those of net primary production, and the slope of the linear regression between the 2 was unity (Supplemental Data, Figure S4), which suggests that gross primary production was nearly double respiration, although dark-bottle and light-bottle tests could not be conducted simultaneously with the same periphyton-colonized disk. That the community was mostly photoautotrophic is consistent with our microscopic analysis of diatoms being the dominant periphytic microorganisms colonizing the disks.

On an organic mass–normalized basis (i.e., nmol O_2/mg/h), neither rates of net primary production nor respiration by periphyton colonizing fritted disks differed significantly between all combined Hg treatments of Sugar Creek versus all treatments of Warden Ditch sediment (*t* tests, $p \geq 0.4$). This suggests that metabolism of the periphyton was similar between the 2 sediment types. Sediment Hg treatments also had little or no discernable effect on periphyton metabolism (Supplemental Data, Figure S5); there were no Hg(II)-treatment effects for either net primary production by periphyton exposed to Warden Ditch sediment (ANOVA, $p = 0.12$) or respiration by periphyton exposed to either sediment (ANOVA, $p > 0.05$). Significant differences among Hg treatments were observed only for net primary production by periphyton exposed to Sugar Creek sediments (ANOVA, $p = 0.01$), for which a Tukey post hoc comparison indicated the lowest Hg treatment (170 ng Hg/g dry wt) was significantly greater than the reference sediment (8 ng/g), with no significant differences among other pairwise comparisons. We interpret the difference in primary production between the low sediment and reference sediment to be an anomaly and conclude that sediment Hg(II) exposures used in the present study had no discernable effect on either net primary production or respiration by periphyton under the conditions and methods used in our test. The absence of Hg(II)-treatment effects on either net primary production or respiration may be attributed to periphyton developing a resistance to Hg

toxicity or the fact that only communities highly tolerant of Hg(II) were able to survive on the chemical exposure substrates, which should be a focus of future research.

Implications

We observed that differences in the organic content of sediment had implications for the net production of MeHg in sediment and its availability for accumulation by stream periphyton, the dominant primary producers in many stream ecosystems [16]. Proportionately more Hg(II) was transformed to MeHg in a sandy, low-organic sediment than in a fine-grained, organic-rich sediment. Moreover, and relative to the concentration of MeHg in sediment, periphyton accumulated approximately 20-fold more MeHg from the low-organic as opposed to the organic-rich sediment. Although toxicity of either Hg(II) or MeHg to periphyton was not observed with either of our endpoints of toxicity (periphyton organic content, net primary production, respiration), MeHg accumulated in periphyton in proportion to its concentration in sediment. Accumulation in periphyton increases the susceptibility for MeHg to be transferred to higher trophic levels in stream ecosystems because nearly all of the MeHg accumulated by higher aquatic organisms is from their diet [47,48]. The present study's results suggest that in situ production and bioaccumulation of MeHg in stream ecosystems can vary as a function of sediment characteristics and Hg loadings to the sediments.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3324.

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Disclaimer—The authors report no conflict of interest. The authors alone are responsible for the content and writing of the present study.

Data Availability—All data, associated metadata, and calculation tools are available on request from the authors (chad.hammerschmidt@wright.edu).

References

1. Chumchal MM, Rainwater TR, Osborn SC, Roberts AP, Abel MT, Cobb GP, Smith PN, Bailey FC. 2011. Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem. *Environ Toxicol Chem* 30:1153–1162.

2. Mason RP, Reinfelder JR, Morel FMM. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ Sci Technol* 30:1835–1845.

3. Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12–18.

4. Depew DC, Basu N, Burgess NM, Campbell LM, Devlin EW, Drevnick PE, Hammerschmidt CR, Murphy CA, Sandheinrich MB, Wiener JG. 2012. Toxicity of dietary methylmercury to fish: Derivation of ecologically meaningful threshold concentrations. *Environ Toxicol Chem* 31:1536–1547.

5. Mergler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, Stern AH. 2007. Methylmercury exposure and health effects in humans: A worldwide concern. *Ambio* 36:3–11.

6. Celo V, Lean DRS, Scott SL. 2006. Abiotic methylation of mercury in the aquatic environment. *Sci Total Environ* 368:126–137.

7. Benoit JM, Gilmour CC, Heyes A, Mason RP, Miller CL. 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In Cai Y, Braids OC, eds, *Biogeochemistry of Environmentally Important Trace Elements*, Vol 835—ACS Symposium Series. American Chemical Society, Washington, DC, pp 262–297.

8. Parks JM, Johs A, Podar M, Bridou R, Hurt RA Jr, Smith SD, Tomanicek SJ, Qian Y, Brown SD, Brandt CC, Palumbo AV, Smith JC, Wall JD, Elias DA, Liang L. 2013. The genetic basis for bacterial mercury methylation. *Science* 339:1332–1335.

9. Gilmour CC, Podar M, Bullock AL, Graham AM, Brown SD, Somenahally AC, Johs A, Hurt Jr RA, Bailey KL, Elias DA. 2013. Mercury methylation by novel microorganisms from new environments. *Environ Sci Technol* 47:11810–11820.

10. Podar M, Gilmour CC, Brandt CC, Soren A, Brown SD, Crable BR, Palumbo AV, Somenahally AC, Elias DA. 2015. Global prevalence and distribution of genes and microorganisms involved in mercury methylation. *Sci Adv* 1:e1500675.

11. Barkay T, Wagner-Dobler I. 2005. Microbial transformations of mercury: Potentials, challenges, and achievements in controlling mercury toxicity in the environment. *Adv Appl Microbiol* 57:1–52.

12. Mauro JBN, Guimaraes JRD, Hintelmann H, Watras CJ, Haack EA, Coelho-Souza SA. 2002. Mercury methylation in macrophytes, periphyton, and water—Comparative studies with stable and radio-mercury additions. *Anal Bioanal Chem* 374:983–989.

13. Cleckner LB, Gilmour CC, Hurley JP, Krabbenhoft DP. 1999. Mercury methylation in periphyton of the Florida Everglades. *Limnol Oceanogr* 44:1815–1825.

14. Buckman KL, Marvin-DiPasquale M, Taylor VF, Chalmers A, Broadley HJ, Agee J, Jackson BP, Chen CY. 2015. Influence of a chlor-alkali Superfund site on mercury bioaccumulation in periphyton and low-trophic level fauna. *Environ Toxicol Chem* 34:1649–1658.

15. Desrosiers M, Planas D, Mucci A. 2006. Total mercury and methylmercury accumulation in periphyton of Boreal Shield lakes: Influence of watershed physiographic characteristics. *Sci Total Environ* 355:247–258.

16. Sabater S, Guasch H, Ricart M, Romani A, Vidal G, Klunder C, Schmitt-Jansen M. 2007. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. *Anal Bioanal Chem* 387:1425–1434.

17. Walters DM, Raikow DF, Hammerschmidt CR, Mehling MG, Kovach A, Oris JT. 2015. Methylmercury bioaccumulation in stream food webs declines with increasing primary production. *Environ Sci Technol* 49:7762–7769.

18. Ivorra N, Hettelaar J, Kraak MHS, Sabater S, Admiraal W. 2002. Responses of biofilms to combined nutrient and metal exposure. *Environ Toxicol Chem* 21:626–632.

19. DeLorenzo ME, Scott GI, Ross PE. 2001. Toxicity of pesticides to aquatic microorganisms: A review. *Environ Toxicol Chem* 20:84–98.

20. McCormick PV, Stevenson JR. 1998. Periphyton as a tool for ecological assessment and management in Florida Everglades. *J Phycol* 34:726–733.

21. Costello DM, Rosi-Marshall EJ, Shaw LE, Grace MR, Kelly JJ. 2015. A novel method to assess effects of chemical stressors on natural biofilm structure and function. *Freshw Biol*, in press. DOI: 10.1111/fwb.12641.<!--<query>Reference 21: Please update with volume and page numbers if possible.</query>-->

22. Fairchild GW, Lowe RL, Richardson WB. 1985. Algal periphyton growth on nutrient-diffusing substrates: An in situ bioassay. *Ecology* 66:465–472.

23. Capps KA, Booth MT, Collins SM, Davison MA, Moslemi JM, El-Sabaawi RW, Simonis JL, Flecker AS. 2011. Nutrient diffusing substrata: A field comparison of commonly used methods to assess nutrient limitation. *Journal of the North American Benthological Society* 30:522–532.

24. Covelli S, Faganeli J, Horvat M, Brambati A. 2001. Mercury contamination of coastal sediments as the result of long-term cinnabar mining activity (Gulf of Trieste, northern Adriatic sea). *Appl Geochem* 16:541–558.

25. Ullrich SM, Ilyushchenko MA, Kamberov IM, Tanton TW. 2007. Mercury contamination in the vicinity of a derelict chlor-alkali plant. Part I: Sediment and water contamination of Lake Balkyldak and the River Irtysh. *Sci Total Environ* 381:1–16.

26. Hammerschmidt CR, Fitzgerald WF. 2004. Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ Sci Technol* 38:1487–1495.

27. Peres F, Coste M, Ribeyre F, Ricard M, Boudou A. 1997. Effects of methylmercury and inorganic mercury on periphytic diatom communities in freshwater indoor microcosms. *J Appl Phycol* 9:215–227.

28. Bott TL, Brock JT, Baattrup-Pedersen A, Chambers PA, Dodds WK, Himbeault KT, Lawrence JR, Planas D, Snyder E, Wolfaardt GM. 1997. An evaluation of techniques for measuring periphyton metabolism in chambers. *Can J Fish Aquat Sci* 54:715–725.

29. Bloom NS, Crecelius EA. 1983. Determination of mercury in seawater at sub-nanogram per liter levels. *Mar Chem* 14:49–59.

30. Gamby RL, Hammerschmidt CR, Costello DM, Lamborg CH, Runkle JR. 2015. Deforestation and cultivation mobilize mercury from topsoil. *Sci Total Environ* 532:467–473.

31. Fitzgerald WF, Gill GA. 1979. Subnanogram determination of mercury by two-stage gold amalgamation applied to atmospheric analysis. *Anal Chem* 51:1714–1720.

32. Bloom NS, Fitzgerald WF. 1988. Determination of volatile mercury species at the picogram level by low-temperature gas chromatography with cold-vapor atomic fluorescence detection. *Anal Chim Acta* 208:151–161.

33. Horvat M, Bloom NS, Liang L. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low-level environmental samples. 1. Sediments. *Anal Chim Acta* 298:135–152.

34. Bloom NS. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Can J Fish Aquat Sci* 46:1131–1140.

35. Tseng C-M, Hammerschmidt CR, Fitzgerald WF. 2004. Determination of methylmercury in environmental matrixes by on-line flow injection and atomic fluorescence spectrometry. *Anal Chem* 76:7131–7136.

36. Hammerschmidt CR, Fitzgerald WF. 2006. Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. *Environ Contam Toxicol* 51:416–424.

37. Lamborg CH, Tseng C, Fitzgerald WF, Balcom PH, Hammerschmidt CR. 2003. Determination of the mercury complexation characteristics of dissolved organic matter in natural waters with “reducible Hg” titrations. *Environ Sci Technol* 37:3316–3322.

38. Hammerschmidt CR, Fitzgerald WF, Balcom PH, Visscher PT. 2008. Organic matter and sulfide inhibit methylmercury production in sediments of New York/New Jersey Harbor. *Mar Chem* 109:165–182.

39. Heyes A, Mason RP, Kim E-H, Sunderland E. 2006. Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. *Mar Chem* 102:134–147.

40. Ogrinc N, Monperrus M, Kotnik J, Fajon V, Vidimova K, Amouroux D, Kocman D, Tessier E, Žižek S, Horvat M. 2007. Distribution of mercury and methylmercury in deep-sea surficial sediments of the Mediterranean Sea. *Mar Chem* 107:31–48.

41. Marvin-DiPasquale M, Agee J, McGowan C, Oremland RS, Thomas M, Krabbenhoft D, Gilmour CC. 2000. Methyl-mercury degradation pathways: A comparison among three mercury-impacted ecosystems. *Environ Sci Technol* 34:4908–4916.

42. Hammerschmidt CR, Fitzgerald WF. 2006. Methylmercury cycling in sediments on the continental shelf of southern New England. *Geochim Cosmochim Acta* 70:918–930.

43. Balcom PH, Hammerschmidt CR, Fitzgerald WF, Lamborg CH, O'Connor JS. 2008. Seasonal distributions and cycling of mercury and methylmercury in the waters of New York/New Jersey Harbor estuary. *Mar Chem* 109:1–17.

44. Hammerschmidt CR, Finiguerra MB, Weller RL, Fitzgerald WF. 2013. Methylmercury accumulation in plankton on the continental margin of the northwest Atlantic Ocean. *Environ Sci Technol* 47:3671–3677.

45. Kuiper J. 1980. Fate and effects of mercury in marine plankton communities in experimental enclosures. *Ecotoxicol Environ Saf* 5:106–134.

46. Harriss RC, White DB, MacFarlane RB. 1970. Mercury compounds reduce photosynthesis by plankton. *Science* 170:736–737.

47. Hall BD, Bodaly RA, Fudge RJP, Rudd JWM, Rosenberg DM. 1997. Food as the dominant pathway of methylmercury uptake by fish. *Water Air Soil Pollut* 100:13–24.

48. Tsui MTK, Wang W-X. 2004. Uptake and elimination routes of inorganic mercury in *Daphnia magna*. *Environ Sci Technol* 38:808–816.

Figure 1. Variation of the fraction of total mercury (Hg) as methylmercury (%MeHg) in Hg-amended sediments from Sugar Creek and Warden Ditch after incubating in Warden Ditch for 67 d. Error bars are ± 1 standard deviation.

Figure 2. Methylmercury (MeHg) concentrations in periphyton colonizing chemical exposure substrates filled with Hg-amended sediment from both Sugar Creek ($p < 0.0001$, $r^2 = 0.85$) and Warden Ditch ($p < 0.0001$, $r^2 = 0.64$) relative to the concentration of MeHg in sediment. Slopes of regression lines are 0.34 ± 0.03 for Sugar Creek and 0.015 ± 0.003 for Warden Ditch.

Figure 3. Relationship between periphyton methylmercury (MeHg) and total Hg concentrations for Hg-amended sediments from Sugar Creek ($p < 0.001$, $r^2 = 0.87$) and Warden Ditch ($p < 0.001$, $r^2 = 0.85$).

Figure 4. Linear-regression analysis (solid line) of the fraction of total Hg as methylmercury (%MeHg) in periphyton versus that in underlying Hg-amended sediments from both Sugar Creek and Warden Ditch ($p < 0.001$, $r^2 = 0.69$). The dashed line is the 1:1 reference line for comparison to the regression line.