Mercury Bioaccumulation in a Stream Network

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Received May 25, 2009. Revised manuscript received July 21, 2009. Accepted July 23, 2009.

Mercury (Hg) contamination is common in stream and river ecosystems, but factors mediating Hg cycling in the flowing waters are much less understood than in the lakes and wetlands. In this study, we examined the spatial patterns of methylmercury (MeHg) concentrations in the dominant groups of aquatic insect larvae across a network of streams (drainage area ranging from 0.5 to 150 km2) in northern California during summer baseflow conditions. We found that, with the exception of water striders, all invertebrate groups showed significant (p < 0.05) increases in MeHg concentrations with drainage area. The largest stream in our study watershed, the South Fork Eel River, had the highest aqueous MeHg concentration (unfiltered: 0.13–0.17 ng L−1) while most of the upstream tributaries had aqueous MeHg concentrations close to or below the established detection limits (0.02 ng L−1). A filamentous alga abundant in South Fork Eel River (Cladophora glomerata) had an exceptionally high fraction of total-Hg as MeHg (i.e., %MeHg from 50–100%). Since other potential hotspots of in-stream Hg methylation (e.g., surface sediment and deep pools) had %MeHg lower than or similar to surface water (~14%), we hypothesize that Cladophora and possibly other autotrophs may serve as hotspots of in-stream MeHg production in this bedrock-dominated stream. Recent studies in other regions concluded that wetland abundance in the watershed is the predominant factor in governing Hg concentrations of stream biota. However, our results show that, in the absence of wetlands, substantial spatial variation of Hg bioaccumulation can arise in stream networks due to the influence of in-stream processes.

Introduction

Mercury (Hg) contamination of aquatic ecosystems is widespread globally due primarily to atmospheric deposition (1). The majority of atmospherically deposited Hg is in the inorganic form which can be converted to highly bioavailable methylmercury (MeHg) through natural processes in water bodies and watersheds (2). While there are regional relationships between average Hg concentrations of aquatic biota (e.g., mosquitoes and fish) and annual rate of Hg atmospheric deposition (3, 4), Hg concentrations in the same aquatic taxa often vary widely among water bodies within confined geographic areas with similar atmospheric Hg deposition (e.g., refs 5–7). For example, Wiener et al. (7) demonstrated that Hg concentrations in 1 year old yellow perch differed by more than 5 fold among 17 lakes in semiremote Voyageurs National Park in Minnesota (total area of 882 km2); the authors attributed the spatial pattern to variation in the percentage of each lake’s watershed covered by wetlands. This and many other investigations suggest that local processes may play a more important role in mediating Hg bioavailability (i.e., production of MeHg) than atmospheric deposition. Over the past three decades, numerous studies have identified both in-lake methylation and adjacent wetlands as important sources of MeHg to lake food webs (8).

Contamination of Hg is widespread in stream and river ecosystems (e.g., ref 9) but in contrast to lakes, the underlying controls on Hg bioavailability and bioaccumulation have been examined much less frequently and are thus comparatively poorly known. A recent study examined environmental factors governing Hg cycling in stream ecosystems across a geographic gradient in the United States. As observed in lakes, this study showed a positive relationship between the percentage of land cover by wetlands and aqueous MeHg concentrations, whereas the annual rate of Hg atmospheric deposition played a minor role in determining aqueous MeHg concentrations (10). This same research group identified that Hg bioaccumulation in the stream food web was directly proportional to the dissolved MeHg concentrations, further suggesting the importance of upstream wetlands in mediating Hg bioaccumulation in stream ecosystems (11).

While wetlands are an important landscape feature in some regions, there are many other watersheds that have elevated and/or variable Hg bioaccumulation yet lack extensive wetland cover or point sources of Hg (e.g., ref 9, 12). This implies that factors besides wetlands may also be important to Hg bioaccumulation in streams. In lakes, MeHg can be generated through internal production, representing an alternate source of highly bioavailable Hg to lake organisms (8); the potential for internal processes within streams to affect Hg accumulation has been little studied, however (13, 14). There are a number of reasons why in-stream processes might be important in mediating Hg bioavailability and bioaccumulation. For example, streams are rich in organic carbon (C) and are often sites of intense biogeochemical transformations (15, 16), features that may be also conducive to methylation processes (2, 13). Moreover, growth rates of primary producers and consumers may vary widely in drainage networks as a result of differences in environmental conditions and nutrient limitation, potentially affecting MeHg concentration in food webs through processes such as growth biodilution (17).

In this study, we examined variation in MeHg concentrations in stream food webs across a network of sites that spanned gradients of stream size and productivity typical of temperate forested watersheds. The study watershed has steep slopes with no wetlands; lack of rainfall in the summer months means that streamflow is driven entirely by groundwater. The site is thus suitable for examining in-stream cycling of elements in the absence of strong watershed heterogeneity. We focused this study on aquatic insect larvae that are the dominant prey for higher trophic levels, especially because stream invertebrates have short life cycles so that their tissue Hg contents derive almost entirely from growth during the productive summer baseflow months. By examining changes in MeHg contents in stream invertebrates across a typical habitat gradient, we aimed to analyze the potential
for stream ecosystems to mediate Hg bioaccumulation through internal processes.

**Experimental Section**

**Site Description.** Our study sites were within or near the Angelo Coast Range Reserve of the University of California Natural Reserve System in Mendocino County, California (39° 44′ N, 123° 39′ W). The region has a Mediterranean climate in which most precipitation falls between October and April; after winter rains, discharge declines steadily starting in May to stable summer baseflows when peak river productivity occurs (Supporting Information (SI) Figure S1; see also ref 18). In late June 2007, we surveyed a total of 12 sites in 10 streams in the forested headwaters of the South Fork Eel River, and we revisited 6 of these sites in late July 2008 (SI Figure S2). The drainage area for each sampling point spanned 3 orders of magnitude, ranging from 0.5 to 150 km². The upstream tributaries have step-pool to riffle-pool sequences and the South Fork Eel River is an entrenched channel consisting of shallow runs, long riffles, and a number of deep pools (>3 m). The substrata within the streams are coarse particles ranging from sand (<2 mm) to boulders (>256 mm) (16). As the channel widens, canopy cover decreases, and sunlight penetration increases leading to increasing water temperature and algal productivity (SI Figure S3 and Table S1). Due to the strong association between drainage area and stream productivity during the study period, we used drainage area as proxies of stream size and productivity (see also ref 19–22).

**Field Collection.** All labware for sample collection and storage were cleaned appropriately to minimize sample contamination (SI part I). At each sampling point, we collected surface water and dominant functional feeding groups (FFGs) of insect larvae at late instars (i.e., scrapers, shredders, filter-feeders, collectors, and predators) in both riffles and pools. In 2008, we focused on scrapers, filter-feeders, collectors and predators in riffles only. We also collected periphyton and dominant macroalgae (*Nostoc pruniforme* and *Cladophora glomerata*) in the most productive site (South Fork Eel 1) (SI Figure S2 and Table S1). At each site, we sampled riffle and pool habitats; within each habitat, we pooled individual taxa into a composite sample consisting of 2–30 individuals. In 2007, filter-feeding caddisflies, predatory stoneflies and water striders were collected at all 12 sites; other groups of insect larvae were collected from 5 to 11 sites, depending on availability (see details in SI Table S2). In 2007, surface water was collected directly into an acid-cleaned 1 L Teflon bottle at each stream, and samples were transported on ice to the University of Minnesota for further processing within 36 h (23). In 2008, we resampled surface water at five streams and performed MeHg analysis with a larger sample volume (~90 mL) than used in 2007 (~45 mL) in order to lower the method detection limit (i.e., 0.02 ng L⁻¹) (see below).

All biological samples were collected by clean stainless-steel forceps, transferred into clean plastic vials and transported in a cooler (at 10–15 °C) to the field station laboratory within 2–6 h. All animal samples were dissected to remove gut contents (19). Gut clearance is particularly important to minimize the influences of dietary Hg on tissue Hg concentrations (24). Algal samples were rinsed thoroughly with filtered streamwater and inspected to remove microinvertebrates and detritus. All biological samples were immediately frozen at −20 °C after processing in the laboratory.

**Sample Preparation and Analyses.** In 2007, water samples were processed in the analytical laboratory, samples for dissolved Hg were filtered through an acid-leached disposable filter unit (pore size = 0.45 μm, nylon membrane) and placed into 125 mL Teflon bottles. Samples for unfiltered Hg were placed directly into 125 mL Teflon bottles. Aqueous samples for THg were digested with an acid-permanganate mixture at 95 °C for >2 h (25), whereas aqueous samples for MeHg were acidified with 0.4% trace-metal grade HCl (23) and subsequently distilled to remove matrix interferences. In 2008, surface water samples were collected with an acid-cleaned 1 L Teflon bottle and samples were filtered immediately in the field with a clean disposable filter unit (0.45 μm), subsamples (unfiltered or filtered) were placed into clean 125 mL Teflon bottles. Samples were spiked with 0.75% BrCl for THg analysis or 0.4% for MeHg analysis, and stored at 4 °C in the dark prior to analyses.

For both years, processed biological samples were freeze-dried, weighed (1.0–20.0 mg) into acid-cleaned 15 mL polypropylene tubes, and digested by 4.6 M HNO₃ at 60 °C for 12 h to quantitatively release MeHg (3). Following MeHg analysis, the remaining solution in the digestion tube was spiked with KMnO₄ and K₂SO₃ (2:1 v/v) in 2007 and further digested at 60 °C for 12–16 h, or spiked with BrCl in 2008 and digested overnight at room temperature for subsequent THg analysis (3). THg was determined by double amalgamation cold vapor atomic fluorescence spectrometry (CVAFS) (SI part II). MeHg was quantified by CVAFS following gas chromatography separation and pyrolysis (SI part III) and with slight modification in analytical procedures for all water samples in 2008 (SI part IV).

Invertebrate samples collected in 2007 were also analyzed for stable C (δ¹³C) to assess diet sources (26). Briefly, samples were ground into fine particles, weighed into tin capsules (Costech, Valencia, CA) with an automated ultramicrobalance (±0.1 μg), and analyzed by a Thermo Electron gas isotope-ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (Flagstaff, AZ). The average standard deviation for 20 samples run in duplicate was 0.16‰ for δ¹³C. In aquatic ecosystems, stable C isotopic information can provide information about the contribution of terrestrial and algal C sources to consumers (26). Terrestrial plant δ¹³C values are essentially constant across our study sites with a mean value of −27‰, but algal δ¹³C values increase from small to larger streams (19, 20). Therefore, increasing consumer δ¹³C with drainage area is consistent with close tracking of algal resources, while values around −27‰ indicate reliance on terrestrial organic matter except for a few sites where algal and terrestrial δ¹³C values overlapped (19, 20).

**Statistical Analysis.** Drainage area of streams was log₁₀-transformed to minimize skewness of the data for linear regression analyses. At each individual site, averaged values from multiple samples (if collected) were used for all regression analyses. All regression analyses were performed using SigmaPlot 7.0 after passing normality test (Point Richmond, CA), and α value was set at 0.05.

**Results**

**Environmental Characteristics.** Although our study sites are located in the California coast range where geologic Hg sources are widespread (27) our aqueous data demonstrated no point sources of Hg contamination in the study watershed (SI Table S3). Overall, the study watershed represents a suitable system to examine the influences of in-stream processes on Hg bioavailability to stream food webs because external supplies of MeHg such as terrestrial runoff (28) and upstream wetlands (10) were absent in the watershed during the study period. Summer baseflow in the watershed is predominantly driven by groundwater flow (29), as evidenced by a stable summer hydrograph (SI Figure S1) as well as similarly water stable oxygen isotope signatures (δ¹⁸O) among sites (−7.46 ± 0.07‰, n = 6; mean ± SE) (Finlay JC, unpublished data). Several biologically active elements varied longitudinally due to the increasing primary productivity and metabolism (21) (SI Table S1). For example, dissolved organic C (DOC) significantly increased with drainage area (r² = 0.737;
solved N (TDN) increased significantly with drainage area inputs (Finlay JC, unpublished data). Moreover, total dissolved suspended solids (TSS) in these streams (i.e., 0.05 ng L\(^{-1}\)) which is mainly attributed to the very low levels of total dissolved N (TDN) increased significantly with drainage area (\(r^2 = 0.687; \ p = 0.0009\)), which is likely driven by increasing in situ N fixing activities along the drainage gradient (30).

In both years, aqueous total-Hg (THg) levels were consistently low among sites (i.e., 0.3–1.2 ng L\(^{-1}\)) (SI Table S3) as is typical of streams in undisturbed watersheds (31). On average, 79 ± 15% of THg was in the dissolved phase, which is mainly attributed to the very low levels of total suspended solids (TSS) in these streams (i.e., 0.05–1.15 mg L\(^{-1}\) in 2007, SI Table S1). Only 3 of 11 samples had unfiltered MeHg concentrations above the established method detection limit (MDL; i.e., 0.04 ng L\(^{-1}\)) in 2007, whereas only one filtered samples had MeHg levels above the MDL (SI Table S1). In July 2008, we resampled five sites and analyzed for MeHg in both THg and MeHg with a lower MDL for MeHg (i.e., 0.02 ng L\(^{-1}\)). Aqueous THg was similar across sites but aqueous MeHg concentrations somewhat increased with drainage area, as observed in the previous year (SI Table S3). Therefore, while our data are limited by sampling frequency and many aqueous samples below the MDL for MeHg, it appears that aqueous THg levels were similar across sites but aqueous MeHg levels increased downstream, and especially in the largest stream (South Fork Eel River), both in the particulate (>0.45 μm) and dissolved phases.

**Stream Insect Larvae.** Along the habitat gradient we sampled, several dominant groups of insect larvae had different ranges and spatial patterns of MeHg concentrations, but most showed a pattern of increasing MeHg with stream size (Figure 1). Armored algal scrapers (Glossosoma spp., Neophylax spp., and Dicosmoecus gilvipes) had relatively low MeHg concentrations (7–88 ng g\(^{-1}\) dry wt.) compared to most other FFGs but their MeHg concentrations showed a marginally significant relationship with drainage area (\(r^2 = 0.148; \ p = 0.027\)) (Figure 1A), despite the high variability of the data. Collectors (heptageniid mayflies) had relatively high MeHg concentrations (63–265 ng g\(^{-1}\)) which were not significantly related to drainage area when data were combined for both years (\(r^2 = 0.242; \ p = 0.063\)) but, in contrast to all other groups, was unrelated to MeHg concentrations in collectors and drainage area (\(r^2 = 0.587; \ p = 0.010\)) (Figure 1B). Filter-feeders (hydropsychid caddisflies) had intermediate MeHg concentrations (34–157 ng g\(^{-1}\)) and showed a strong relationship between MeHg concentrations and drainage area (\(r^2 = 0.624; \ p = 0.0001\)) over both years (Figure 1C). Invertebrate predators (perlid stoneflies) had relatively high MeHg concentrations (51–271 ng g\(^{-1}\)) which were significantly related to drainage area over both years (\(r^2 = 0.427; \ p = 0.005\)) (Figure 1D). In pools, we sampled two groups of shredders (Psychoglypha spp. and Lepidostoma spp.) as well as water striders. We found that burger concentrations of both shredders were relatively low (2–42 ng g\(^{-1}\)) but increased significantly with drainage area (\(r^2 = 0.321; \ p = 0.012\)) (Figure 1E). Concentrations of MeHg in water striders were in general high and similar to perlid stoneflies (43–222 ng g\(^{-1}\)) but, in contrast to all other groups, was unrelated to drainage area gradient (\(r^2 = 0.081; \ p = 0.370\)) (Figure 1F).

Data on stable C isotopes were used to assess the degree to which changes in MeHg concentrations in common stream invertebrates were related to changes in diet sources. Measurement of \(\delta^{13}C\) revealed that several groups of insect larvae showed enrichment in \(\delta^{13}C\) as drainage area increased (SI Figure S4), which suggests their complete or partial
reliance on algal C along the drainage area gradient (20). Armored algal scrapers showed the largest range in δ13C (from −42 to −20 ‰) with a significant enrichment with drainage area (p = 0.0002), consistent with their diets strongly linked to algae (22). Collectors, filter-feeders, and invertebrate predators showed positive but nonsignificant increases in δ13C toward larger streams (p > 0.05) suggesting mixed diets of both terrestrial and algal origins. For shredders, δ13C of Psychoglypha increased somewhat with drainage area (p = 0.164) while δ13C of Lepidostoma was relatively constant across the watershed and slightly enriched relative to the previously defined δ13C of terrestrial particulate matter (20). These patterns reflect a transition from a terrestrial to algal diet source for Psychoglypha as stream size increased, and close reliance on terrestrial C by Lepidostoma. The δ13C range of water streams was very narrow and did not vary significantly over the drainage basin (p > 0.05).

**Bioaccumulation in Invertebrates.** Concentrations of MeHg in invertebrates are mainly governed by uptake, efflux, and animal growth rate. Since direct aqueous uptake is generally negligible (32), we focus on potential effects of diet, efflux and animal growth in mediating MeHg bioaccumulation. In the study watershed, diet sources for aquatic invertebrates varied spatially with contributions of algal C sources increasing with stream size for several FFGs due to increases in algal productivity (20). Therefore, the dietary contribution of MeHg could change with shift of diets (e.g., from terrestrial to algal foods) if these diets have contrasting MeHg levels. Territorial organic materials had significantly lower MeHg concentrations compared to periphyton collected at South Fork Eel River (0.38 ± 0.24 vs 3.4 ± 0.90 ng g−1; p = 0.011), suggesting that the relative diet contribution of these organic materials may influence MeHg accumulation in consumers. However, patterns in MeHg concentrations in consumers cannot be completely explained by dietary source (terrestrial vs algal) because the two groups with the strongest affiliation for the terrestrial and algal resources (i.e., shredders and scrapers, respectively) had the lowest MeHg concentrations among all groups of animal consumers.

For each type of organic matter, we expect increasing MeHg concentrations with stream size since aqueous MeHg concentrations were higher in larger streams (SI Table S3). Basal resources (e.g., periphyton) have MeHg uptake proportional to aqueous and sedimentary MeHg concentrations (33). Efflux of MeHg is not a commonly measured parameter, but previous laboratory experiments showed that efflux of MeHg by invertebrates (e.g., zooplankter, Daphnia magna) did not vary substantially under different ambient conditions (e.g., food concentrations and quality, water temperature) and tissue MeHg concentrations (17, 32, 34, 35), suggesting similar efflux rate of MeHg by the same group of invertebrates along stream size in the study stream network. Because water temperature and food quality generally increase along this drainage area gradient (SI Table S1), growth rate of the invertebrates may be higher in larger streams (36). If this is true, increasing growth would be expected to reduce tissue concentrations of MeHg in downstream invertebrates (17) but this is inconsistent with our observation of increasing MeHg bioaccumulation with stream size. Taken together, the observed spatial patterns of increasing MeHg bioaccumulation with stream size in this stream network is consistent with either a dietary shift toward foods with higher MeHg contents or simply increased MeHg concentrations in the diets, or both.

In this study, we observed that MeHg concentrations differed substantially among different FFGs in the stream network. Specifically, the FFGs with the closest affinity for terrestrial detritus (shredders) and epilithic algae (scrapers) (20, 22) had the lowest MeHg concentrations, and also showed weak increases with stream size (Figure 1). These patterns indicate that MeHg concentrations of their diets (i.e., terrestrial detritus, and periphyton) did not change considerably along the stream size gradient we sampled.

**TABLE 1. Concentrations of THg, MeHg, and Fraction of THg as MeHg (i.e., %MeHg) of Three Groups of Algae Collected at South Fork Eel 1 during Summer Baseflows in Late June 2007 and Late July 2008. Data are Means ± SD**

<table>
<thead>
<tr>
<th>algae</th>
<th>status</th>
<th>year</th>
<th>THg (ng g−1)</th>
<th>MeHg (ng g−1)</th>
<th>%MeHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periphyton</td>
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<td>2007</td>
<td>39.8 ± 5.7</td>
<td>3.4 ± 1.6</td>
<td>8</td>
</tr>
<tr>
<td>Nostoc</td>
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<td>2007</td>
<td>15.1 ± 1.7</td>
<td>6.6 ± 0.1</td>
<td>44</td>
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<tr>
<td>Cladophora</td>
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<td>2008</td>
<td>48.7 ± 20.9</td>
<td>15.2 ± 10.9</td>
<td>29</td>
</tr>
<tr>
<td>Cladophora</td>
<td>green</td>
<td>2007</td>
<td>41.2 ± 3.5</td>
<td>34.1 ± 2.9</td>
<td>84</td>
</tr>
<tr>
<td>Cladophora</td>
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<td>2008</td>
<td>209 ± 9.0</td>
<td>211 ± 24.1</td>
<td>102</td>
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<tr>
<td>Cladophora</td>
<td>yellowish-green</td>
<td>2008</td>
<td>81.2 ± 35.4</td>
<td>42.2 ± 24.1</td>
<td>50</td>
</tr>
</tbody>
</table>

**Discussion**

Previous research has emphasized the role of terrestrial runoff and upstream wetlands as sources of MeHg to stream ecosystems (10, 11, 28). However, streams have other ecosystem characteristics that may promote in-stream Hg methylation, including abundant organic matter as well as dynamic biogeochemical cycling. Despite the potential to actively transform Hg, streams have been little investigated as compared to other landscape compartments within watershed in mediating Hg cycling. In a watershed with no apparent external MeHg sources, we observed significant increases in MeHg bioaccumulation with stream size, and variation in MeHg bioaccumulation that may also be linked to shifts in diet sources. Although the exact source of observed MeHg in the water and biota is not completely clear, these spatial contrasts are best explained by increases in dietary MeHg concentrations potentially driven by increased production of MeHg within the stream channel and routed to specific fractions of organic resources for consumers, as discussed below.
In contrast, filter-feeders and collectors had considerably higher MeHg concentrations and showed stronger downstream increases in MeHg concentrations than shredders and scrapers. The increase of MeHg bioaccumulation in invertebrate predators with drainage area implies that their prey includes members of the filter feeder or collector FFG. These two FFGs relied on mixed algal and terrestrial diets throughout the watershed as indicated by their δ13C values consistently being intermediate between algal scrapers and terrestrial detritus (20). Patterns of increasing MeHg bioaccumulation with stream size thus cannot be accounted solely by a shift from terrestrial to algal diets while these findings also suggest that increases of dietary MeHg concentrations in specific food sources are very immediate of mediating the spatial patterns of MeHg concentrations of animal consumers in the stream network. Given that the two nonpredatory groups with the strongest downstream increase in MeHg relied on fine particles (i.e., filter feeders and collectors), we suggest that detrital processing plays a key role in mediating Hg bioaccumulation in stream food webs which warrants future investigation. In contrast to these FFGs, water striders showed no relationship between their tissue MeHg concentrations and drainage area which implies that these invertebrates may derive MeHg from terrestrial sources (37).

**Mediation of Hg Cycling in Streams.** The study watershed has no wetlands and terrestrial surface runoff is essentially absent during the baseflow conditions. The streamwater had both very low DOC (mean = 0.45 mg L⁻¹) and total suspended solids (TSS; mean = 0.36 mg L⁻¹), both of which are often positively associated with THg/MeHg in the dissolved and particulate phases in streamwater, respectively (10, 25, 26). Our water data revealed that only the three most productive streams (South Fork Eel River, Elder Creek, Jack of Hearts Creek) had detectable MeHg levels (in 2008) compared to all upstream tributaries (SI Table S3), suggesting a potential of in-stream production of MeHg. Recently, Marvin-DiPasquale et al. (14) concluded that in-stream Hg methylation was unimportant compared to the watershed sources of MeHg, which were primarily derived from wetlands. One possible explanation for the deviation of our conclusion from that of Marvin-DiPasquale et al. (14) is that their study streams had several to 10-folds higher dissolved MeHg concentrations (e.g., St. Mary’s River in Florida; range: 0.04–1.03 ng L⁻¹) than our study streams (<0.02–0.12 ng L⁻¹), and therefore, any MeHg produced in situ would appear to be a small fraction in their study. In contrast, MeHg produced in stream channels at our study watershed would be substantial relative to upstream sources which were all below detection limit (i.e., 0.02 ng L⁻¹).

Previous studies have established that anaerobic sulfate reduction is an important pathway for converting inorganic Hg to MeHg (2). Methylation of Hg mediated by sulfate reduction is common in sediments where oxic and anoxic interfaces meet (38). We examined potential sedimentary sources of MeHg in the most productive stream (South Fork Eel River) by sampling porewaters from the sediment surface at two riffles and a 4 m deep pool in July 2008 (SI part V). For the porewaters, we found that dissolved THg concentrations were similar over depths (surface to 8 cm: 1.0–1.7 ng L⁻¹) but %MeHg decreased significantly with depth (SI Figure S5A; p < 0.05). These results suggested that these substrata are not hotspots of MeHg production because these compartments should have a higher than ambient (i.e., surface water) %MeHg or MeHg concentrations if they serve as source of MeHg (39). Instead, our data suggest that these sites may serve as a net sink of MeHg which can be due to in situ demethylation or microbial uptake of MeHg, and the surface water appears to be the source of MeHg to these subsurface compartments. For the deep pool surveyed, low dissolved oxygen (4–5 mg L⁻¹) was observed in bottom waters during summer daytime, but we found that %MeHg slightly decreased with depth (p > 0.05). Therefore the bottom part of the pool was also apparently not a hotspot of MeHg production, with surface water again acting as a source of MeHg (SI Figure S5B). Moreover, we found that the bottom sediment is composed mostly of organic matter (e.g., remains of previously decomposed algae) and the %MeHg of these organic materials was 11.6% (n = 1) which is similar to %MeHg of surface and pool waters.

In contrast to these above compartments, the filamentous algae, *Cladophora glomerata*, had exceptionally high %MeHg even though the values varied between years of sampling and condition of the algae. Comparatively, in many aquatic food webs %MeHg is often the lowest for algae (~10–20%) and highest for predatory animals (~90–100%) (5, 12). MeHg measured in these algal mats could represent direct uptake from the dissolved phase or in situ production of MeHg by microbes associated with the filamentous algae which greatly enhance the surface areas for the attachment of epiphytes and microbes. For example, Power et al. (30) estimated that 6–8 m long branched *Cladophora* turfs can increase functional surface area by up to 200 000 times in South Fork Eel River, which highly enhance microbial transformations in the water column. Production of MeHg by *Cladophora* has not been previously studied but *Cladophora* biomass collected from lakeshore of Lake Michigan has been shown to harbor high density of sulfate reducing bacteria (40). Therefore this alga may also accommodate a high level of methylating bacteria in South Fork Eel River, and elsewhere. Interestingly, the epiphytized, or “yellowish-green”, *Cladophora* had much lower THg and MeHg concentrations and %MeHg compared to green *Cladophora* collected at the same location and date (Table 1), suggesting a role of epiphytes (e.g., *Cocconeis* and gomphonemoid diatoms; ref 30) in mediating the overall Hg content and speciation of the algal mats, which clearly needs further study. Across the study watershed, the importance of algal metabolism and biomass to stream cycling of C (29), N (30) and trace elements (e.g., Fe, Mn; Tsui and Finlay, manuscript in preparation) becomes progressively greater with stream size, which are parallel to the increasing Hg bioavailability (i.e., aqueous MeHg) and bioaccumulation as observed in this study. Therefore, a common mechanism (e.g., algal proliferations, warmer water, lower nighttime dissolved oxygen, etc.) may operate for governing the biogeochemical cycles of multiple elements.

In summary, our study is the first to demonstrate that Hg bioaccumulation can vary spatially in stream network. Spatial patterns of MeHg accumulation potentially arose due to variation in in-stream processes that mediate Hg biotransformation (i.e., production of MeHg), dietary sources and dietary MeHg concentrations. These findings show that factors beyond the presence of wetlands in watersheds (10, 11, 14) are also important in influencing Hg bioavailability and bioaccumulation in streams, though the influence of external inputs is most often studied (e.g., refs 6, 10, 11, 14, 28). Although Hg concentrations in stream biota have been shown to be positively related to dissolved MeHg concentrations (11), our results also suggest that shifts in dietary sources and changes in dietary MeHg concentrations could play important roles in mediating MeHg concentrations in stream biota. Therefore, more quantitative studies on Hg cycling in diverse types of streams and rivers are necessary in order to better understand the underlying factors (both within stream and
drainage basin) influencing Hg bioaccumulation in stream ecosystems.

Acknowledgments
We thank Carrie Booth, Sandy Brovold, Hannah Grun, Luke Gumke, Becky Stark, and Jill Welter for assistance in different parts of this project, and the University of California’s Angelo Coast Range Reserve for access and logistics support. We also thank Steve Balogh and Yabing Nollet for assisting in methylmercury analysis of water samples in 2008. We appreciate the helpful comments on earlier drafts of the manuscript from Dan Engstrom, Bruce Monson, Mary Power, Ed Swain, Wen-Xiong Wang, and three anonymous reviewers. This study was supported by SETAC/ICA Chris Lee Awards for Metals Research and Thesis Research Grant of the University of Minnesota Graduate School to M.T.K.T., and by the DEB program of NSF (0543363), STC program of NSF via the NCED (EAR-0120914), and the Grant-In-Aid Program of the University of Minnesota Graduate School to J.C.F.

Supporting Information Available
SI I: Labware cleaning; SI II: Total-mercury analysis; SI III: Methylmercury analysis; SI IV: Analysis of streamwater collected in July 2008; SI V: Collection of porewaters and pool waters in July 2008; Table S1: Environmental characteristics of the study streams; Table S2: Sites where specific animal consumers were collected; Table S3: Aqueous Hg concentrations in sites of different drainage areas; Figure S1: Discharge, rainfall and water temperature of selected study streams; Figure S2: Map showing site locations; Figure S3: Pictures of three sites of contrasting drainage areas; Figure S4: Stable carbon isotope of different animal consumers along the drainage area; Figure S5: %MeHg in filtered porewaters in substrata and filtered water samples from a deep pool in South Fork Eel River. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


