Variation in Terrestrial and Aquatic Sources of Methylmercury in Stream Predators as Revealed by Stable Mercury Isotopes

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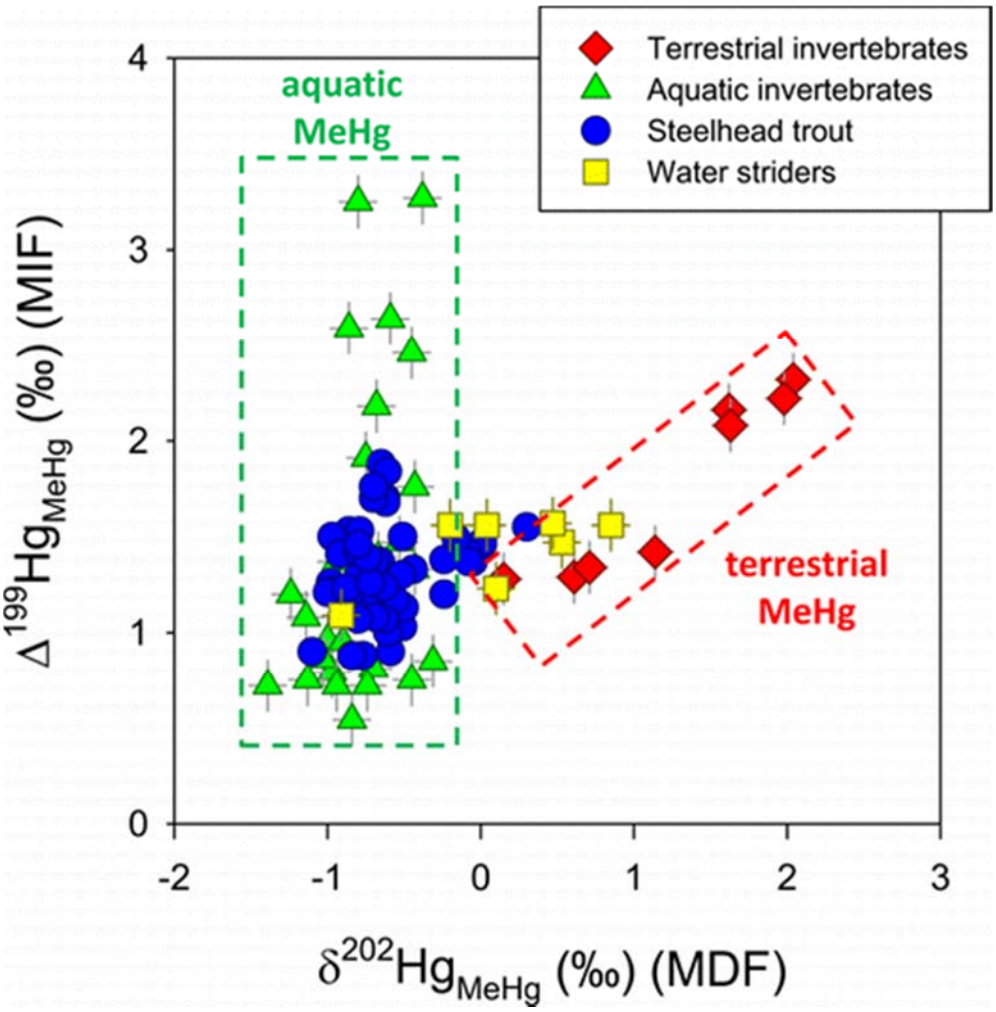
Abstract:

Mercury (Hg) is widely distributed in the environment, and its organic form, methylmercury (MeHg), can extensively bioaccumulate and biomagnify in aquatic and terrestrial food webs. Concentrations of MeHg in organisms are highly variable, and the sources in natural food webs are often not well understood. This study examined stable isotope ratios of MeHg (mass-dependent fractionation, as $\delta^{202}Hg_{MeHg}$; and mass-independent fractionation, as $\Delta^{199}Hg_{MeHg}$) in benthic invertebrates, juvenile steelhead trout (*Oncorhynchus mykiss*), and water striders (*Gerris remigis*) along a stream productivity gradient, as well as carnivorous terrestrial invertebrates, in a forested watershed at the headwater of South Fork Eel River in northern California. Throughout the sampling sites, $\delta^{202}Hg_{MeHg}$ (after correction due to the effect of MeHg photodegradation) was significantly different between benthic (median = −1.40‰; range, −2.34 to −0.78‰; total number of samples = 29) and terrestrial invertebrates (median = +0.51‰; range, −0.37 to +1.40‰; total number of samples = 9), but no major difference between these two groups was found for $\Delta^{199}Hg_{MeHg}$. Steelhead trout (52 individual fishes) have MeHg of predominantly aquatic origins, with a few exceptions at the upstream locations (e.g., 1 fish collected in a tributary had a purely terrestrial MeHg source and 4 fishes had mixed aquatic and terrestrial MeHg sources). Water striders (seven pooled samples) derive MeHg largely from terrestrial sources throughout headwater sections. These data suggest that direct terrestrial subsidy (e.g., terrestrial invertebrates falling into water) can be important for some stream predators in headwater streams and could represent an important means of transfer of terrestrially derived MeHg (e.g., in situ methylation within forests, atmospheric sources) to aquatic ecosystems.

Moreover, these findings show that terrestrial subsidies can enhance MeHg bioaccumulation of consumers in headwater streams where aqueous MeHg levels are very low.



Abstract Image

Keywords: Mercury | methylmercury | bioaccumulation | Steelhead trout | water striders

Article:

Introduction

Mercury (Hg) is an atmospheric pollutant that can be carried long distances and deposited in watersheds remote from point sources.[1] Atmospherically deposited Hg is predominantly inorganic, but a small fraction can be microbially methylated into highly bioavailable methylmercury (MeHg).[2] Wetlands are widely regarded as “hotspots” of Hg methylation that contribute MeHg to aquatic ecosystems including lakes and streams.[1] However, aquatic systems that lack adjacent wetlands (e.g., mountainous streams) can also have MeHg mainly derived

from in situ production.[3] Similarly, MeHg is ubiquitous in forest ecosystems,[4, 5] but its ultimate sources and biogeochemical cycling remain elusive.

Studies of Hg cycling and bioaccumulation have often focused on patterns of total Hg and MeHg concentrations in different environmental pools. These studies have attempted to understand variations in ecosystem processes affecting Hg transformations and/or how food web complexity influences trophic transfer of MeHg. For example, studies examining MeHg transfer in food webs have often analyzed stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to infer dietary sources and trophic positions, respectively.[6] These light isotope tracers provide some insights on MeHg (dietary) sources and trophic relationships in food webs, but this approach does not allow direct tracing of specific MeHg sources, nor does it provide information on the biogeochemical processes affecting MeHg in the ecosystems (e.g., MeHg photodegradation, Hg methylation).

Stable Hg isotope analysis provides new information on Hg cycling because it represents a direct tracer of Hg sources and transport in the natural environment. There are two types of isotopic fractionation associated with stable Hg isotopes: mass-dependent fractionation (MDF) and mass-independent fractionation (MIF).[7] MDF can be caused by many redox reactions, whereas large-magnitude MIF is known to be caused only by photochemical reactions such as the photodegradation of MeHg.[8] Previous studies examining stable Hg isotopes in biota have demonstrated their usefulness in revealing specific sources of MeHg in food webs and biogeochemical processes affecting MeHg (e.g., photodegradation) in the environment.[5, 9, 10]

Our recent study in a semi-remote forested watershed in northern California showed that MDF of MeHg in food webs differs between a river (the South Fork (SF) Eel River) and an adjacent upland forest.[5] We further demonstrated that two riparian spiders (of the tetragnathids and lycosids families) had MDF of MeHg midway between stream and forest food webs. We used a two-source mixing model and estimated that both spider families derived about 50% of their MeHg body burden from aquatic and 50% from terrestrial sources.[5] Because isotopic fractionation of MeHg during trophic transfer is negligible,[11] we can directly compare the isotopic signatures of MeHg in organisms across trophic levels (e.g., fish vs invertebrates) and habitats (e.g., upstream vs downstream sites) in the SF Eel River ecosystems to understand their trophic transfer pathways for MeHg.

In headwater streams, direct terrestrial subsidies (e.g., forest invertebrates falling into or landing on water) may be important to stream predators[12] because algal productivity is often very low due to light limitation.[13] Common stream predators such as steelhead trout and salamanders are known to receive some terrestrial subsidy in unproductive streams.[14] Due to the lower rates of Hg methylation on land,[15] terrestrial resources (e.g., detritus) are likely to accumulate less MeHg than aquatic resources (e.g., algae). Therefore, direct terrestrial subsidies, where large, could potentially dilute MeHg levels in stream predators in unproductive streams.[16]

In this study, we report stable Hg isotope ratios (both MDF and MIF) in two types of common stream consumers (i.e., juvenile steelhead trout and water striders) in a semi-remote forested watershed in northern California. We use previously published data on stable Hg isotope ratios in benthic invertebrates of four functional feeding groups along a drainage gradient and terrestrial predatory invertebrates in the same watershed,[5, 17] with the addition of some new data to define the isotopic limits of aquatic versus terrestrial MeHg pools. Also, the sites in the stream network have been previously studied for bioaccumulation and in situ production of MeHg.[3, 18] Here we examine how MDF of MeHg in juvenile steelhead trout and water striders changes longitudinally, and we investigate the relative contributions of aquatic and terrestrial MeHg sources (as reflected by stable Hg isotope ratios measured in respective invertebrate groups) to these two stream predators and how they vary with their stream network positions.

Experimental Section

Study Sites and Sample Collection

Sampling sites were within or near the Angelo Coast Range Reserve (39′44″ N, 123′39″ W) in Mendocino County, California (USA), in the forested headwaters of the South Fork (SF) Eel River. As the channel widens, overhead canopy cover decreases and sunlight penetration increases, leading to generally increasing algal productivity downstream.[19] Throughout this paper, we used drainage area (DA) as a proxy for stream size and productivity, following previous work in this watershed.[17-19] Stream predators for this study were collected in the summer dry season of 2008 (fish) and 2012 (water striders). Benthic invertebrates were collected in 2011, and analyses of these have been reported previously,[5, 17] but additional new samples were obtained in the summer of 2012 and are reported here for the first time. The complete sampling history can be found in the Supporting Information (SI), Table S1. A total of 14 stream sites with DA ranging from 2.0 to 346 km^2 were sampled for juvenile steelhead trout by a field crew using a backpack electroshocker. Fish samples were filleted in the laboratory to obtain samples of muscle tissue. We analyzed fillets of 52 individuals after freeze-drying and homogenization for natural abundance stable Hg isotope ratios (see below). Fishes had total body lengths ranging from 82 to 220 mm (median = 130 mm).

Moreover, we sampled water striders (*Gerris remigis*, Gerridae) at seven sites with DA from 0.5 to 150 km^2 in the summer of 2012 to examine the stable isotope ratios of their MeHg throughout the stream network (SI Table S1). For the benthic invertebrates, a total of 10 sites in the stream network (including 2 sites farther downstream with DAs of 642 and 1212 km^2) were sampled for four different functional feeding groups (i.e., scrapers *Glossosoma* and *Neophylax*; collectors*Heptagenia* and *Nixe*; filterers *Hydropsyche*; and predators *Hesperoperla* and *Calineuria*). Results for MeHg concentration and isotope ratios (MIF only) in stream invertebrates from the summer of 2011 have been previously reported in Tsui et al.[17] and are used here to constrain isotope values of in-stream MeHg sources along the productivity gradient. In the summer of 2012, we sampled benthic invertebrates (*Hydropsyche*,

and *Hesperoperla*, and/or *Calineuria*) in two additional sites in the headwater section of the watershed. For the terrestrial biota, we used the published stable Hg isotope data from Tsui et al.[5] for different groups of terrestrial predatory invertebrates (i.e., ground beetles, scorpions, and centipedes) collected in the summer of 2011 in an upland forest (Chaparral) within the Angelo Coast Range Reserve. In the summer of 2012, we sampled additional terrestrial predatory invertebrates in several locations within the Angelo Coast Range Reserve to examine the differences in stable isotope ratios of MeHg between years and sampling sites. These additional forest sites in the summer of 2012 are both upland (Black Oat Mountain and Madrone Forest) and near a stream (a forest within 5 m of two tributaries, McKinley Creek of DA = 0.5 km^2 and Fox Creek of DA = 2.7 km^2) to see if there are any differences in isotopic signatures in terrestrial invertebrates there compared to those collected in upland forests, but obvious differences in isotopic contents of MeHg were not observed.

As all of these food webs (especially the aquatic food webs) experience a relatively recurrent Mediterranean seasonal climate pattern, we assumed that stable isotope data for MeHg in these organisms would be comparable across years, and our Hg isotope data in this study spanning five years (2008–2012) on the stream food webs have largely validated this assumption. However, we found a much larger variation in isotopic composition of MeHg between years and locations in terrestrial food webs in the study watershed, and this may suggest more variability in the biogeochemical processes acting on MeHg in the terrestrial setting.

Sample Processing and Hg Analyses

All biota samples were analyzed for total Hg and MeHg concentrations using cold vapor atomic fluorescence spectrometry (CVAFS) (see SI Part I) and for stable Hg isotope ratios using multicollector–inductively coupled plasma–mass spectrometry (MC-ICP-MS) (see SI Part II). As demonstrated in previous studies, inorganic Hg (e.g., in sediment) and MeHg (e.g., in fish) often have different isotopic compositions (both MDF and MIF) within the same ecosystem;[5, 8-10, 20] thus, we cannot simply compare stable Hg isotope ratios among food web members of different trophic levels because of the mixing of inorganic Hg and MeHg in their tissues.[5] Due to the variability of the fraction of total Hg as MeHg (i.e., f_{MeHg}) in many invertebrate samples analyzed here, we estimated end member MeHg isotopic compositions in MDF as $\delta^{202}Hg_{MeHg}$ and MIF of odd-mass isotopes as $\Delta^{199}Hg_{MeHg}$ for each pooled sample (see SI Part III) by extrapolating data with variable f_{MeHg} to a pure MeHg end member value using an approach we developed previously.[5, 17] It should be noted that MDF of MeHg isotopes can be caused by many redox reactions (e.g., microbial methylation, dark "microbial" MeHg degradation), whereas significant MIF of odd-mass MeHg isotopes (e.g., > +0.4‰) is believed to be exclusively caused by photodegradation of MeHg.[7]

We used habitat-specific mean isotopic values of inorganic Hg from stream or forest food webs in the study watershed (see derived values[5]) to estimate the isotopic composition of MeHg in invertebrate samples from each habitat. Estimates of the isotope ratios of MeHg in invertebrates

could then be directly compared with each other and to those in fish because Hg in fish fillets is predominantly MeHg in this study (median f_{MeHg} = 1.06; range, 0.91–1.21; n = 52).

Statistical Analyses

Linear regression analyses were performed using SigmaPlot 7.0 (Systat), and the significance level was set at $p < 0.05$.

Results and Discussion

Spatial Variation of MeHg Bioaccumulation

There were general increases of MeHg in several functional feeding groups of benthic invertebrates with increasing DA over a range of 0.5–150 km^2 (Figure 1a–d; SI Table S2). The increasing MeHg bioaccumulation is due mainly to increasing levels of aqueous MeHg with stream size (e.g., <20 pg L^{-1} in upstream tributaries to >100 pg L^{-1} in downstream channels[3, 18]), which is attributed to in situ MeHg production in larger stream channels associated with extensive filamentous algal communities.(3) However, MeHg bioaccumulation in benthic invertebrates decreased at sites farther downstream (DAs of 642 and 1212 km^2), a pattern we attributed to stronger MeHg photodegradation due to the absence of riparian canopy shading (or essentially zero canopy cover) at larger and wider river reaches.(17) From the current sampling, we observed no significant increase ($p > 0.05$) in MeHg bioaccumulation in water striders with DA (Figure 1e; SITable S3). In steelhead trout, we normalized the MeHg concentrations to a standard size (i.e., 130 mm), and we found moderate but significant ($p = 0.04$) increases of MeHg with DA (Figure 1f; SITable S4). Therefore, the relationships between MeHg bioaccumulation and stream size (DA) were different between these two groups of stream predators, with longitudinal patterns of fish MeHg being closer to those of benthic invertebrates.

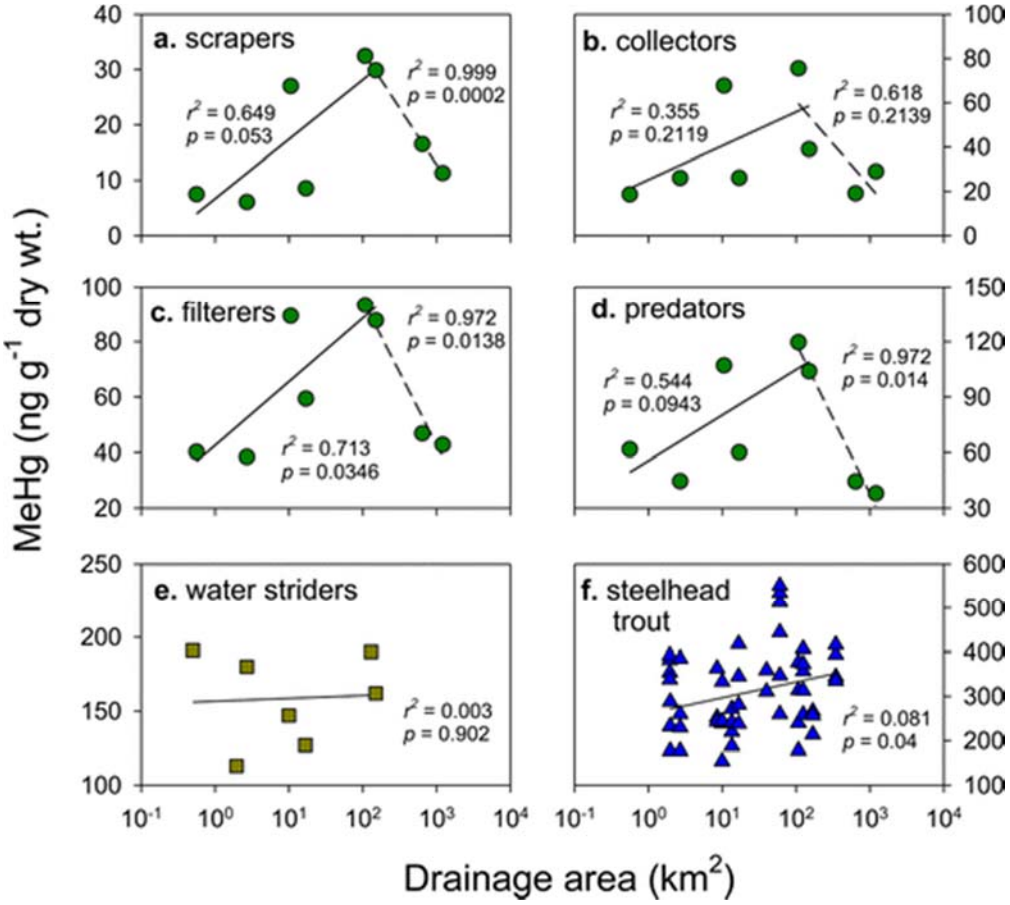


Figure 1. Spatial variation of MeHg concentrations in four functional feeding groups of benthic invertebrates: (a) scrapers (armored caddisflies *Glossosoma*and *Neophylax*); (b) gatherer–collectors (flathead mayflies *Heptagenia* and*Nixe*); (c) particle filterers (net-spinning caddisflies *Hydropsyche*); and (d) invertebrate predators (perlidae stoneflies *Calineuria* and *Hesperoperla*). Two-phase regression analyses were carried out for the benthic invertebrates: solid lines for streams with drainage area from 0.56 to 150 km^2 (rising limb); dashed lines for streams with drainage area from 108 to 1212 km^2 (declining limb). Concentrations of MeHg in (e) water striders and (f) steelhead trout are normalized to a standard total length of 130 mm. Invertebrate data are obtained from Tsui et al.[17]

At different forest locations in the study watershed, we collected three groups of carnivorous invertebrates (ground beetles, centipedes, and scorpions) and found that MeHg concentrations in ground beetles (60–73 ng g^{-1}, on a dry weight basis) were much lower than those in centipedes (182–220 ng g^{-1}) and scorpions (153–252 ng g^{-1}) (SI Table S5). These differences in MeHg were, however, unrelated to their trophic positions as revealed by δ^{15}N measurements in their tissues (i.e., mean δ^{15}N, +4.9‰ in beetles, +4.6‰ in centipedes, and +5.3‰ in scorpions; Tsui et al., unpublished data).

Spatial Variation of Stable Hg Isotope Ratios in Biota

In Figure 2, we plot MDF versus MIF estimated for MeHg for all samples collected in the study watershed. We observe a very large isotopic variation of MeHg across all biota samples and sampling sites with a total range of ~3.3‰ for $\delta^{202}Hg_{MeHg}$ (MDF) and ~2.8‰ for $\Delta^{199}Hg_{MeHg}$(MIF) (see also SI Tables S2–S5). Interestingly, benthic invertebrates showed only a relatively small range of $^{202}Hg_{MeHg}$ (~1.1‰) despite being collected over a wide range of stream size (DA from 0.5 to 1212 km^2). Benthic invertebrates had a much larger range of $\Delta^{199}Hg_{MeHg}$ (~2.8‰) that changed along gradients of increasing stream size and decreasing canopy cover[17] due to variable amounts of MeHg photodegradation (prior to bioaccumulation) that causes MIF.[8]

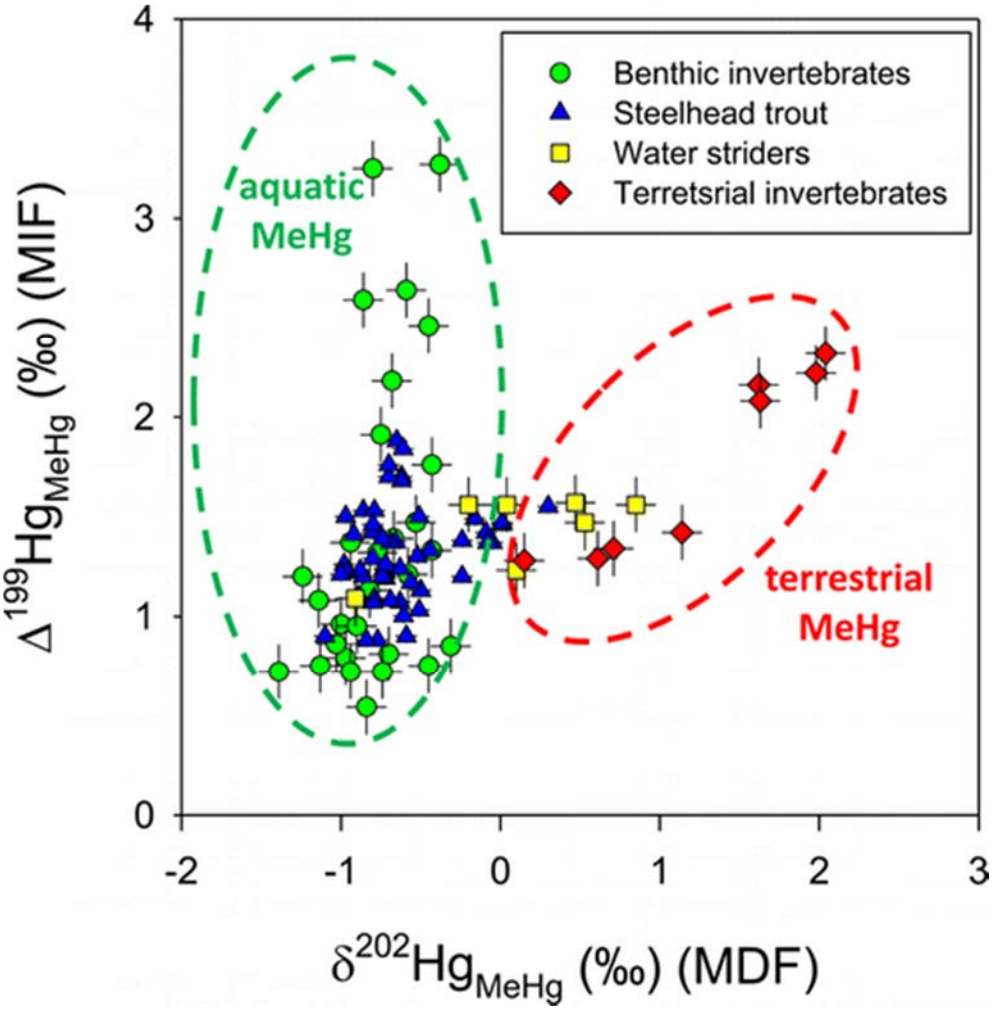


Figure 2. Plot showing mass-dependent fractionation (MDF, as $\delta^{202}Hg_{MeHg}$) and mass-independent fractionation (MIF, as $\Delta^{199}Hg_{MeHg}$) of MeHg in benthic and terrestrial invertebrates, steelhead trout, and water striders in the study watershed. Invertebrate MIF data are obtained from Tsui et al.[17] Error bars associated with symbols are analytical reproducibility (2SD).

More importantly, whereas $\Delta^{199}Hg_{MeHg}$ (MIF) values overlap between benthic and terrestrial invertebrates, there are distinct and significant differences in $\delta^{202}Hg_{MeHg}$ (MDF) values between

these two groups of invertebrates throughout the watershed (Figure 2). Therefore, as indicated in the ovals outlined by dashed lines on Figure 2, there are distinct, non-overlapping, isotopic signatures of MeHg between benthic and terrestrial invertebrates in this watershed, a finding we previously observed only for a single stream reach (DA at 150 km^2) at SF Eel River.[5]

The majority of MeHg isotope data for steelhead trout falls within the green oval of benthic invertebrates, whereas data on a few individuals are on the margin or outside the oval (mainly from headwater streams), implying that these individuals may acquire MeHg through feeding partially on terrestrial invertebrates (i.e., terrestrial subsidy) (see below). It is clear that isotope data for water striders have a large range of MDF but a very small range of MIF, and their isotopic compositions cover the isotopic range in both aquatic and terrestrial invertebrates (Figure 2).

Because MeHg photodegradation can imprint both MDF and MIF in the remaining, nondegraded MeHg in the environment,[8] to compare the MDF of MeHg isotope data between sites and groups of organisms we need to "correct" the fraction of MDF caused by MeHg photodegradation, and we can do this on the basis of the amount of MIF measured in individual samples. For example, Gehrke et al.[9] used this approach to "correct" MDF of MeHg in forage fish in San Francisco Bay and then to relate the source of MeHg in fish to that in bay sediment, using an experimental relationship between MDF and MIF from a previous laboratory study.[8] We use the experimental relationship at dissolved organic carbon (DOC) of 1 mg L^{-1} (with $\Delta^{199}Hg/\delta^{202}Hg$ slope of 2.43, based on Bergquist and Blum[8]) due to the low DOC levels throughout the SF Eel River during summer baseflow.[19] After the correction of MDF values, we find that the total range of $\delta^{202}Hg_{MeHg}$ increases from ∼1.1‰ (before) to ∼1.6‰ (after) due to the adjustment of the photodegradation effect especially in downstream samples with large MIF.[17] By plotting "corrected" $\delta^{202}Hg_{MeHg}$ versus DA, we find that "corrected" MDF for both benthic invertebrates and steelhead trout significantly decreases downstream ($p < 0.0001$), whereas "corrected" $\delta^{202}Hg_{MeHg}$ values for water striders show a decreasing but nonsignificant trend with DA (Figure 3a). There is no overlap (after considering the external analytical reproducibility of each data point) in the "corrected" $\delta^{202}Hg_{MeHg}$ between terrestrial invertebrates and benthic invertebrates throughout the watershed (Figure 3a). If we assume that benthic invertebrates obtain their MeHg solely from in-stream sources, whereas terrestrial invertebrates obtain their MeHg solely from forest sources, then we can infer that any stream predators would have mixed MeHg sources from streams and forests if their "corrected" $\delta^{202}Hg_{MeHg}$ values show signals intermediate between aquatic and terrestrial end members. Specifically, there are different degrees of overlap in the MDF values of steelhead trout and water striders with both aquatic and terrestrial sources of MeHg (Figure 3a), which will be discussed further below.

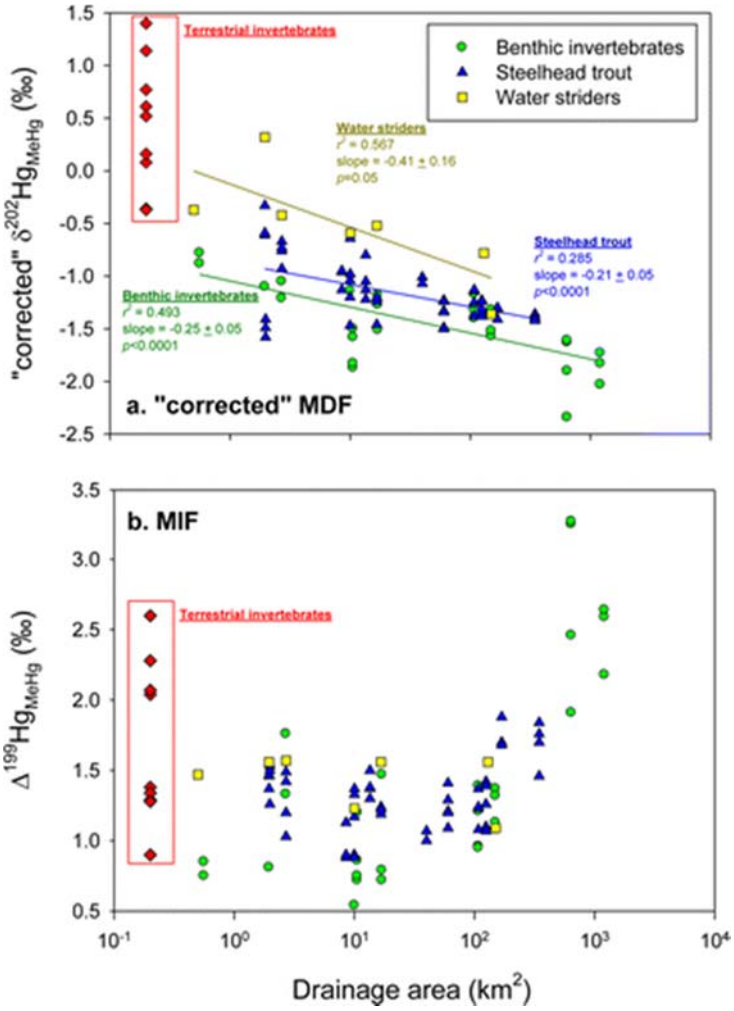


Figure 3. Relationship between drainage area and (a) "corrected" MDF of MeHg ($\delta^{202}Hg_{MeHg}$) in different stream consumers and terrestrial consumers and (b) MIF ($\Delta^{199}Hg_{MeHg}$) of MeHg in different stream consumers and terrestrial consumers. Values for slope are the mean ± standard error.

In contrast to MDF, we observed generally increasing MIF of MeHg in both benthic invertebrates and steelhead trout with DA (Figure 3b). We observe variable $\Delta^{199}Hg_{MeHg}$ associated with MeHg found in terrestrial invertebrates, and the range of $\Delta^{199}Hg_{MeHg}$ in terrestrial invertebrates essentially overlaps that of all aquatic organisms in sites with highly variable DAs (Figure 3b). Values of MIF, $\Delta^{199}Hg_{MeHg}$, in aquatic organisms vary from +0.5 to +1.7‰ in sites with DA ranging from 0.5 to 150 km^2, but beyond that range in DAs $\Delta^{199}Hg_{MeHg}$ increases drastically downstream, which can be attributed to decreases in canopy cover and increases in sunlight availability in stream channels.[17] However, for sites with DA < 100 km^2 MIF signatures are indistinguishable between aquatic and terrestrial invertebrates and overlap those of steelhead trout and water striders (Figure 3b), and it thus appears that in this

watershed MIF is not useful compared to MDF in distinguishing between aquatic and terrestrial sources of MeHg.

Implications for MeHg Sources and Cycling

The "corrected" MDF of aquatic MeHg (based on the isotope ratios of MeHg in benthic invertebrates and the majority of fish) decreased similarly (with similar slopes) and significantly with DA (Figure 3a). The pattern of "corrected" MDF versus DA for water striders was different, with a significantly larger slope than those for both benthic invertebrates and steelhead trout (Figure 3a). This suggests that MeHg in water striders from upstream sites may be more influenced by different sources of MeHg such as those from terrestrial prey (see below).

On the basis of our current knowledge, we propose two possible explanations for the shift of "corrected" MDF of MeHg with DA. First, lighter isotopes of inorganic Hg (i.e., substrate for $MeHg$[2]) are preferentially removed from solution and bound to inorganic particle surfaces (e.g., goethite[21]) and thiol-rich organic matter.[22] This process may be enhanced toward downstream channels as the abundance of these Hg-binding ligands should increase downstream, for example, through precipitation of iron oxyhydroxides and enhanced algal exudation of organic matter. We speculate that this pool of ligand-bound inorganic Hg may be preferentially deposited in sediment and methylated by Hg-methylating microbes[23] in downstream reaches.[3] Second, it is possible that there are different sources of inorganic Hg with different δ^{202}Hg values along the drainage gradient in the watershed, such as different atmospheric and/or geologic pools of Hg. However, the gradual decline of δ^{202}Hg with stream sizes suggests that this explanation is less likely as it requires multiple pools of inorganic Hg of different (or gradually decreasing) δ^{202}Hg values along the drainage gradient. Given the site-specific nature of these processes (e.g., types of inorganic particles and organic matter and specific Hg-methylating microbes), it would be difficult to use the isotopic fractionation factors derived from these laboratory experiments[21, 22, 24] to directly evaluate our stable Hg isotope data from the field. Clearly, future research is warranted to resolve such biogeochemical complexity at the watershed scale.

Methylmercury, once formed, can undergo extensive photodegradation in the environment, which can imprint positive MIF in the remaining, nondegraded MeHg that can be taken up by food webs.[8] Despite the similar MIF signatures for different groups of organisms at sites with DA < 100 km^2(Figures 2 and 3b), we observed subtle differences in the relationship between Δ^{199}Hg and Δ^{201}Hg. Specifically, Bergquist and Blum[8] experimentally measured $\Delta^{199}Hg/\Delta^{201}Hg$ slopes of 1.36 ± 0.02 for MeHg undergoing photodegradation and 1.00 ± 0.02 for inorganic Hg undergoing photoreduction. We found that $\Delta^{199}Hg/\Delta^{201}Hg$ slopes are similar for all samples of benthic invertebrates (1.20 ± 0.05; mean and standard error) and steelhead trout (1.24 ± 0.05) in our study (Figure 4). These slopes are comparable to those found in previous studies of fish (mostly MeHg in tissues) from diverse aquatic systems including San Francisco Bay (i.e., 1.26),[9] the Pacific Ocean (i.e., 1.20),[10] and Florida lakes (i.e., 1.30).[20]

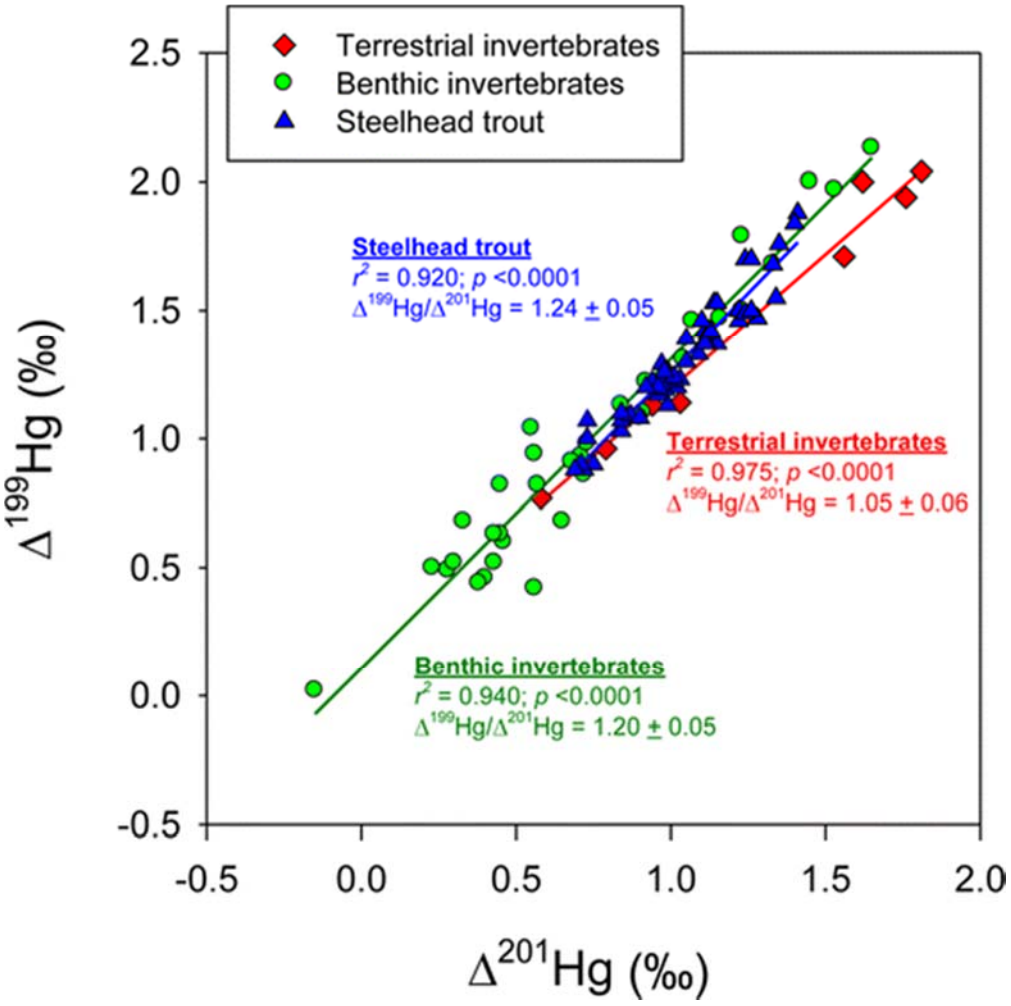


Figure 4. Relationship between Δ^{199}Hg and Δ^{201}Hg of two stream consumer types and terrestrial invertebrates. Water striders data are not included due to the small sample size. All invertebrate samples have $f_{MeHg} > 0.6$. Values for slope are the mean ± standard error.

In contrast to our analyses of aquatic organisms, our samples of terrestrial predatory invertebrates ($f_{MeHg} > 0.6$ in tissues) show a different Δ^{199}Hg/Δ^{201}Hg slope (1.05 ± 0.06) from their aquatic counterparts (1.20 ± 0.05) (Figure 4), and this value is close to the slope observed for photoreduction of inorganic Hg in a controlled experiment.(8) Our results may imply that the mechanism causing MIF of Hg isotopes is somewhat different between terrestrial and aquatic ecosystems. For example, this slope could be interpreted to suggest that the substrate for terrestrial MeHg, that is, Hg(II), is first extensively photoreduced before the non-photoreduced Hg(II) is methylated in the terrestrial and/or atmospheric environment. Terrestrial Hg(II) associated with vegetation and soil has slightly negative MIF,(25) that is, Δ^{199}Hg, and thus a certain amount of Hg(II) photoreduction is required to make this pool acquire less negative or even positive MIF before methylation occurs. Therefore, this specific isotopic relationship (i.e., Δ^{199}Hg/Δ^{201}Hg slope) may be diagnostic of different pools and/or intermediate steps prior to the production of MeHg in natural ecosystems in addition to their measured MDF and MIF values.

Tracing Sources of MeHg in Stream Predators

On the basis of the entire range of "corrected" $\delta^{202}Hg_{MeHg}$ values in aquatic and terrestrial invertebrates (Figure 3a) and their associated measurement variability (i.e., external analytical reproducibility (2SD) of 0.14‰, denoted as error bars), we derived the isotopic limits of "corrected" $\delta^{202}Hg_{MeHg}$ for MeHg (see details in SI Part IV) in the aquatic (−0.64‰) and terrestrial pools (−0.51‰) in the study watershed (see horizontal dashed lines in Figure 5). We believe that it is a fairly conservative estimate as there is a large range of variation of "corrected" $\delta^{202}Hg_{MeHg}$ values within each group of invertebrates throughout the study watershed. We expect that the estimated isotopic limits (or the differences between these two thresholds) could change if we increase our sample size and/or the spatial extent of our sample collection (especially for terrestrial invertebrates). Here, we assume that any organisms with "corrected" $\delta^{202}Hg_{MeHg}$ values between these two thresholds (i.e., between −0.51 and −0.64‰) derive MeHg from mixed aquatic and terrestrial sources. The "corrected" $\delta^{202}Hg_{MeHg}$ values of the majority of the steelhead trout (47 of 52 samples) are on or below the aquatic limits of "corrected" $\delta^{202}Hg_{MeHg}$ value (i.e., −0.64‰), indicating that these individuals obtain their MeHg mainly through feeding on aquatic prey.

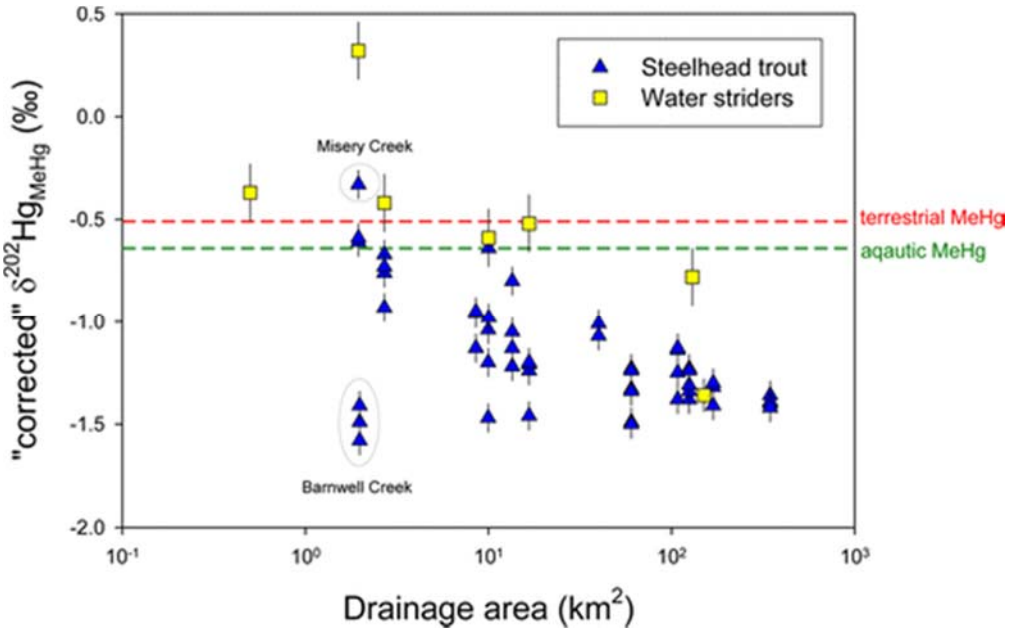


Figure 5. Relationship between "corrected" MDF of MeHg ($\delta^{202}Hg_{MeHg}$) and drainage area in steelhead trout and water striders. Error bars associated with symbols are analytical reproducibility (2SD). Dashed lines are isotopic limits derived for aquatic (red) and terrestrial (green) sources of MeHg.

However, in a tributary (Misery Creek, DA = 2.0 km^2) the "corrected" $\delta^{202}Hg_{MeHg}$ value for one steelhead trout was entirely in the terrestrial range, and four more individuals in different headwater streams had "corrected" $\delta^{202}Hg_{MeHg}$ values between the aquatic and terrestrial

thresholds (i.e., suggesting mixed sources) (Figure 5). In contrast, another small tributary (Barnwell Creek, DA = 2.0 km^2) had predominantly aquatic MeHg in fish (see circle in Figure 5), and we speculate that this is due to the close proximity of the site (<500 m) to the confluence of the main stem the SF Eel River, which could allow movement of fish recently to the tributary from the more productive main channel of the SF Eel River. In contrast, the Misery Creek site is very far (~4.6 km) from the confluence of the main stem of the SF Eel River, and thus movement to/from the main stem is unlikely during a short period of time. Thus, fish may maintain high reliance on terrestrial diets throughout most of their freshwater residence.

For water striders, except for samples from two sites in SF Eel River having "corrected" $\delta^{202}Hg_{MeHg}$ values below the aquatic isotopic limit, the other five pooled samples had $\delta^{202}Hg_{MeHg}$values either between aquatic and terrestrial isotopic limits or completely within the range of $\delta^{202}Hg_{MeHg}$ of terrestrial invertebrates (Figure 5), suggesting that their MeHg sources are partially or entirely derived from feeding on terrestrial prey. These data are consistent with the known feeding preferences of water striders from other stream ecosystems,[26] further supporting the application of stable Hg isotopes in deciphering aquatic versus terrestrial sources of MeHg through dietary uptake. Therefore, different stream predators (e.g., steelhead trout vs water striders) would derive a different proportion of their MeHg body burden from aquatic versus terrestrial sources throughout the stream network.

Terrestrial Subsidy of MeHg to Stream Predators

It is increasingly recognized that natural forest ecosystems distant from ponds, streams, or other aquatic ecosystems can also be contaminated by MeHg. For example, terrestrial songbirds[4] and carnivorous invertebrates[5] in upland temperate forests remote from point sources can accumulate MeHg levels similar to organisms of comparable trophic positions in aquatic habitats, suggesting that the notion of low MeHg levels in forest food webs needs to be re-examined.[15] The sources of MeHg in natural forest ecosystems remain elusive, however. Our previous study suggested that atmospheric sources of MeHg could be important to forest food webs[5] because precipitation including rain and fog is consistently shown to contain measurable amounts of MeHg.[27, 28] Regardless of the ultimate sources, the present study suggests that terrestrial pools of MeHg can be important to bioaccumulation in some stream predators in headwater sections of stream networks, which is consistent with studies of food web ecology in headwater streams,[29] especially when in-stream productivity is low (i.e., less in-stream prey availability).

Distinguishing between aquatic and terrestrial sources of organic matter in aquatic food web accumulation of MeHg is of considerable current interest. Recently, Jardine et al.[26] and Ward et al.[16] measured $\delta^{13}C$ and found that brook trout (*Salvelinus fontinalis*) consuming allochthonous carbon sources in streams accumulated less MeHg than coexisting fish [Atlantic salmon (*Salmo salar*) and blacknose dace (*Rhinichthys atratulus*)] utilizing autochthonous carbon sources, because terrestrial detritus (e.g., fresh leaf litter) often contains very low levels of MeHg (e.g.,

0.04–0.29 ng g^{-1} dry wt[30]). In fact, aquatic ecosystems are often assumed to be hotspots of Hg methylation, whereas terrestrial upland forests are assumed less important,[15] leading to the general assumption that terrestrial subsidies, when present, would simply "dilute" MeHg concentrations in aquatic food webs.[16] We found, however, that concentrations of MeHg in predatory terrestrial invertebrates (e.g., 60–252 ng g^{-1} dry wt in ground beetles, scorpions, and centipedes) were comparable to, or even higher than, those in predatory benthic invertebrates (e.g., 38–120 ng g^{-1} dry wt in perlid stoneflies) in the study watershed.

Our current study shows that MeHg levels in water striders in upstream tributaries in the study watershed did not differ significantly from their counterparts collected in downstream channels (Figure 1e) despite significant increases in aqueous MeHg levels with DA during summer.[17, 18] These results suggest that the terrestrial subsidy might actually enhance MeHg accumulation in these stream predators in the small tributaries, instead of "diluting" their tissue concentrations of MeHg. Thus, the role of terrestrial subsidies in determining MeHg bioaccumulation in stream consumers would mainly depend on the relative levels of MeHg between the two habitats, but there are currently very limited data on MeHg bioaccumulation in terrestrial invertebrates in the literature.

Overall, our findings suggest that where stream predators consume terrestrial prey (e.g., due to low aquatic resource availability in streams), MeHg bioaccumulation in stream predators can be linked to MeHg sources and biogeochemical transformations in the terrestrial environment. The extent of such terrestrial subsidy largely depends on the relative productivity and food quality (e.g., C:N ratios) in streams compared to adjacent forests.[31] More importantly, direct terrestrial subsidies may enhance MeHg concentrations of stream predators in headwater streams if aqueous MeHg levels are very low in these habitats. This study shows that stable Hg isotopes can provide unique insight into the sources of MeHg in stream predators and biogeochemical pathways of MeHg in stream ecosystems.

Supporting Information (http://pubs.acs.org/doi/suppl/10.1021/es500517s)

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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