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Letter

Mercury Isotope Values in Shoreline Spiders Reveal the Transfer of Aquatic Mercury Sources to Terrestrial Food Webs

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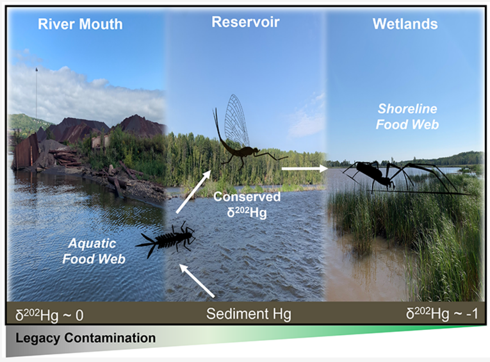
ABSTRACT: The transfer of aquatic contaminants, including mercury (Hg), to terrestrial food webs is an often-overlooked exposure pathway to terrestrial animals. While research has implemented the use of shoreline spiders to assess aquatic to terrestrial Hg transfer, it is unclear whether Hg sources, estimated from isotope ratios, can be successfully resolved to inform site assessments and remedy effectiveness. To examine aquatic to terrestrial Hg transfer, we collected shoreline spiders (*Tetragnatha* spp.) and aquatic insect larvae (suborder Anisoptera) across a mosaic of aquatic and shoreline habitats in the St. Louis River and Bad River, tributaries to Lake Superior. The fraction of industrial Hg in sediments was reflected in the δ^{202}Hg values of aquatic dragonfly larvae and predatory fish, connecting benthic Hg sources to the aquatic food web. Shoreline spiders mirrored these aquatic Hg source signatures with highly positive correlations in δ^{202}Hg between tetragnathids and dragonfly larvae (r^2 = 0.90). Further assessment of different spider taxa (i.e., araneids and pisaurids) revealed that differences in prey consumption and foraging strategies resulted in isotope differences, highlighting the importance of spider taxa selection for Hg monitoring efforts.



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■ INTRODUCTION

Adult aquatic insects provide subsidies to linked terrestrial ecosystems, supporting consumers such as birds, bats, amphibians, and spiders.[1,2] However, contaminants that are accumulated by aquatic insect larvae and retained through metamorphosis can result in insect-mediated contaminant transfer (i.e., flux) to terrestrial biota.[3−6] The transfer of contaminants from aquatic to terrestrial ecosystems represents an often overlooked exposure pathway that can elevate health risks to wildlife,[4,7,8] highlighting the need for monitoring recipient terrestrial animals that eat aquatic insects emerging from contaminated waters. Shoreline spiders (e.g., tetragnathids, pisaurids, and araneids) are increasingly recognized as effective biosentinels (i.e., organisms that accumulate contaminants in their tissues without adverse effects to their health) for measuring the transfer of chemicals from aquatic to terrestrial biota.[9,10] Because they are ubiquitous, are relatively sedentary, and feed predominantly on adult aquatic insects, spiders have the potential to inform environmental injury and remediation effectiveness across diverse environments.[4−8]

A common approach for tracing the transfer of contaminants through food webs, including across linked aquatic and terrestrial habitats, is the use of paired, light stable isotope ratios (e.g., δ^{13}C and δ^{15}N values). However, assessments of light stable isotope ratios in aquatic insects through metamorphosis have shown increases in δ^{15}N values caused by protein catabolism and nitrogen excretion, resulting in difficulties in assessing the risk of exposure to terrestrial biota.[11−14] Thus, additional approaches are needed to constrain energy and contaminant transfer pathways between aquatic and terrestrial food webs, including novel application of different isotope systems.[15,16] Mercury (Hg) stable isotope ratios are increasingly used to study Hg cycling, ascertain Hg sources, and serve as an ecological tracer for animal movement and energy transfer within aquatic food webs.[16,17] Similar to the challenges inherent to light stable isotopes, fractionation of Hg isotopes, recorded by δ^{202}Hg, has previously been observed during biotransformation (e.g., demethylation),[18−20] internal partitioning,[21] and habitat specific processes (e.g., photochemical demethylation),[22,23] but the role of these processes as they relate to Hg isotope preservation in insects has not been studied. Apart from smaller scale field studies,[17,24] Hg stable isotopes have had limited application in tracking Hg sources and energy flow between aquatic and terrestrial environments, particularly at larger scales. Given the uncertainty in the degree

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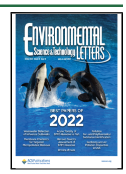

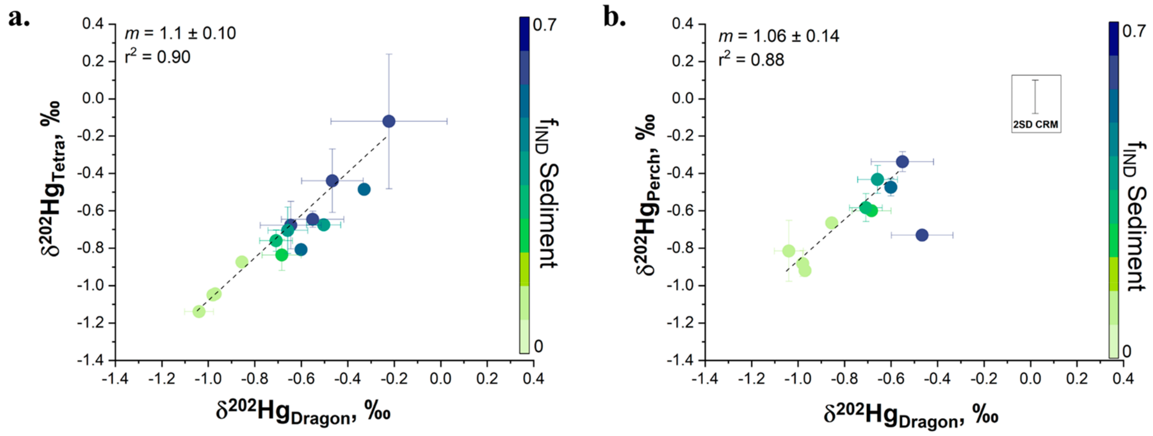

Figure 1. Relationship between site-averaged δ^{202}Hg in dragonflies (abbreviated-dragon) and (a) tetragnathids (abbreviated-tetra) and (b) yellow perch from the St. Louis River and Bad River. Color ramping represents the estimated amount of legacy Hg present in sediments (f_{IND}) from the collection sites.[27] Previously reported yellow perch data[27] were not available at all 14 habitat locations where spiders and dragonflies were collected. Error bars represent one standard deviation (SD) of samples collected from a site (e.g., site variability), and symbols without error bars represent a single composite sample collected at a site. The dashed lines are regression slopes. The error for δ^{202}Hg is represented as two SDs of the certified reference material (CRM) IAEA 407. Individual data and site designations are denoted in Figure S4.

of fractionation that Hg isotopes may undergo as Hg is transferred from aquatic to terrestrial environments, more targeted field-based research is needed.

In this study, we use Hg stable isotope ratios to assess if shoreline spiders reflect a gradient of aquatic Hg sources (e.g., industrial, precipitation, and terrestrially derived Hg) across a mosaic of aquatic and terrestrial habitats in the St. Louis River and Bad River, tributaries to Lake Superior. We tested the following hypotheses. (1) Hg isotope ratios in shoreline spiders will mirror those observed in aquatic dragonfly nymphs and insectivorous fish due to the dietary dependence of spiders on adult aquatic insects. (2) Variation in shoreline spider Hg isotope ratios will be dictated by aquatic Hg sources in sediments over a diversity of freshwater habitats due to a large gradient of Hg sources. (3) Hg isotope ratios vary among different, co-occurring shoreline spider taxa due to dissimilar feeding ecologies. Mercury stable isotopes are increasingly being utilized for site assessments[25] and evaluation of global scale Hg mitigation efforts;[26] therefore, it is imperative to assess the efficacy of Hg isotopes in candidate biosentinel species, particularly for those that uniquely reflect the transfer of Hg from aquatic to terrestrial environments.

METHODS

Site Information and Sample Collection. Biological samples [spiders, dragonfly larvae, and yellow perch (*Perca flavescens*)] and sediments were collected within the St. Louis River and the Bad River from 2017 through 2021. Sites in the lower estuary of the St. Louis River were collected as part of an intensive sampling effort to assess the connection between contaminated sediments and aquatic biota.[27] Collections in 2021 were performed to supplement this work, extending to upstream reservoirs in the St. Louis River (Scanlon, Thomson), main river wetland regions [Loon's Foot Landing (LF) and Clough Island (CL)], and remedial zones [Pickle Pond (PP) and Erie Pier Ponds (EPP)] (Figure S1) to assess different habitat types within the river not captured in the original sampling. In total, 14 different zones within the rivers were sampled, encompassing 41 sampling sites and four distinct aquatic habitat types, including riverine zones, river—estuarine transition zones, embayments along the Lake Superior shoreline, and upstream flow-controlled reservoirs (Figure S1). At each site, long-jawed spiders (*Tetragnathidae* spp.) were collected from emergent and riparian vegetation using previously established methods.[28] We also sampled two additional spider taxa [*Pisauridae* sp. (fishing spiders, *Dolomedes*) and *Araneidae* spp. (orb-weaver spiders)] at four sites (PP, EPP, CL, and LF) to assess differences in Hg isotope values.

Mercury Concentration and Mercury Stable Isotope Analyses. Total mercury (HgT) and methylmercury (MeHg) analyses were performed by the U.S. Geological Survey (USGS) Contaminant Ecology Research Laboratory (CERL, Corvallis, OR). Briefly, the MeHg concentration was determined via weak nitric acid extractions coupled to ethylation, gas chromatography, and fluorescence detection.[29,30] HgT was analyzed via direct combustion coupled to atomic absorption spectroscopy[31] or, in the case of limited mass samples, via bromination of the MeHg extract[27] followed by HgT analysis.[32] Quality control and assurance protocols are outlined in the Supporting Information.

Mercury stable isotope analyses were performed by the USGS Mercury Research Laboratory (MRL, Madison, WI). Samples were prepared via hot nitric acid digestion with 10% (v/v) bromine monochloride additions. Stable isotope measurements were performed using a multicollector inductively coupled mass spectrometer following previously established protocols.[33] Quality control and assurance during analysis were monitored via certified reference materials (IAEA 407) and secondary check standards (NIST RM 8610, UM Almaden) (Table S1). Hg isotope data for sediment and yellow perch (*P. flavescens*) were supplied by previous work on the St. Louis River and Bad River.[27] Hg isotope data for spiders can also be located in the corresponding data releases.[34,35]

RESULTS AND DISCUSSION

Transfer of Aquatic Mercury to Tetragnathids from Emergent Insects. The examination of Hg isotope values in spider tissues has been limited[24] despite the utility of spiders,

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primarily tetragnathids, for assessing aquatic to terrestrial Hg transfer.[3,9] Within our study of the St. Louis River and Bad River, we found that δ^{202}Hg ranged from −1.16‰ to 0.29‰ and Δ^{199}Hg ranged from −0.06‰ to 0.39‰ (Figure S2), exceeding the previous δ^{202}Hg range measured in spider tissues in a stream-riparian system.[24] Given that this study design encompassed a gradient of aquatic (e.g., reservoirs, estuarine, and embayment) and riparian (e.g., forested, armored banks) habitats, it was unclear if the range in δ^{202}Hg and Δ^{199}Hg truly represented the bioaccumulation of different aquatic Hg sources or if process-driven changes, such as biotransformation or photochemical fractionation, also altered Hg isotope values.

To test the hypothesis that tetragnathid δ^{202}Hg values were capable of tracking aquatic Hg sources, we compared spider tissues to co-located dragonfly nymphs (aquatic insects). Dragonflies had a range (−1.11‰ to −0.07‰) similar to that of tetragnathid δ^{202}Hg and showed a strong, positive correlation for δ^{202}Hg (m = 1.10 ± 0.10; r^2 = 0.90) between organisms (Figure 1a). To determine if there was a difference between shoreline and aquatic predators, we examined the linear slope associated with dragonfly to yellow perch δ^{202}Hg [m = 1.06 ± 0.14; r^2 = 0.88 (Figure 1b)], which was similar to the tetragnathid-dragonfly slope. This strong 1:1 relationship between dragonflies and tetragnathids and the similarities to aquatic predators, like yellow perch, suggests that tetragnathids predominantly accumulate aquatic-derived Hg sources.[3,36,37]

To assess if the strong association between tetragnathid and aquatic organism δ^{202}Hg values was a function of source or if a processing offset was present, we examined the MeHg content and Δ^{199}Hg values within spider tissues. Tetragnathids collected across all habitats had variable MeHg contents (78 ± 8%; n = 41) within their tissues, ranging from 64% (n = 4) for the upstream St. Louis River reservoir sites to 86% (n = 7) within the Bad River sites (Table S2). None of the apparent variance in tetragnathid δ^{202}Hg values could be explained by the %MeHg (Figure S3), indicating that varying %MeHg values, potentially related to internal detoxification or dietary differences, did not alter source bioaccumulation in tetragnathids. Photochemical degradation is another process that could induce isotope fractionation of both Δ^{199}Hg and δ^{202}Hg, skewing δ^{202}Hg patterns related to Hg source bioaccumulation.[23] We expect positive Δ^{199}Hg in these samples to be predominantly attributed to water column photoprocessing because fractionation of Δ^{199}Hg is not expected to occur during internal transport or detoxification.[21,38] Values of Δ^{199}Hg were on average 0.17 ± 0.11‰ (n = 41), with the highest values associated with upstream reservoirs and the urbanized estuary of the St. Louis River (Figure S4); these values are substantially lower than measurements taken in prey and game fish collected from the lower St. Louis River estuary[27] and Lake Superior.[22] To assess how photochemical processing impacted δ^{202}Hg values, we applied a photochemical correction to tetragnathid data (SI Methods)[39] and observed that the largest shift to δ^{202}Hg would be approximately 0.02‰ for the site with the highest Δ^{199}Hg value (Table S2), indicating little overall change to δ^{202}Hg. Hence, we conclude that the range of δ^{202}Hg observed in tetragnathids in this study is attributed to differences in dietary intake and not internal fractionation processes or photochemical degradation driven by the habitat type.

Given the connection between aquatic insects and tetragnathids within these two systems, it is likely that the δ^{202}Hg range corresponds to a gradient of Hg sources distributed across the systems. Mercury sources have previously been established within sediments of the St. Louis River and Bad River, and the results revealed a complex mixing of Hg from legacy contamination, terrestrially derived watershed runoff, and precipitation.[27] Previous isotope assessments in sediments spanned from highly negative δ^{202}Hg values, indicative of terrestrial runoff,[40,41] to values closer to zero, associated with legacy Hg contamination.[25] When this sediment source gradient, denoted by fraction industrial (f_{IND}), is overlaid with tetragnathid data, we see a pattern between isotope values in spiders and the proportion of the industrial Hg source such that spiders with higher δ^{202}Hg values are from sites with higher fractions of industrial Hg in the sediment and vice versa (Figure 1a). This observation also holds true for dragonflies and yellow perch tissues (Figure 1b). These findings demonstrate that Hg isotopes in tetragnathids can be used to assess the contributions of aquatic Hg sources to terrestrial food webs.

The Foraging Strategy Influences the Sources of Mercury for Co-occurring Spiders. Tetragnathids are horizontal web builders that rely on adult aquatic insects for prey, whereas other shoreline spider species vary in aquatic versus terrestrial invertebrate prey consumption (e.g., araneids) and hunting strategy (e.g., pisaurids hunt on land−water surfaces).[9] To determine if there were Hg source differences between co-located taxa, we evaluated Hg isotope data from tetragnathids, pisaurids, and araneids from the same zones (n = 9) (Figure 2). We observed large variation in δ^{202}Hg (−0.68‰ to 0.29‰) and Δ^{199}Hg (−0.004‰ to 0.54‰) values among different co-occurring taxa. In general, δ^{202}Hg was lower in tetragnathids than in araneids (mean difference of −0.18 ± 0.11‰ in δ^{202}Hg), whereas more variability was observed between pisaurid and tetragnathid δ^{202}Hg values (Table S3). At some sites (e.g., CL-05, PP-01, and PP-03), the differences in δ^{202}Hg values measured in non-tetragnathid spider taxa suggested exposure to different Hg sources or processing.[27] We attribute the inconsistent offset in δ^{202}Hg values between tetragnathids and pisaurids to differences in hunting strategies

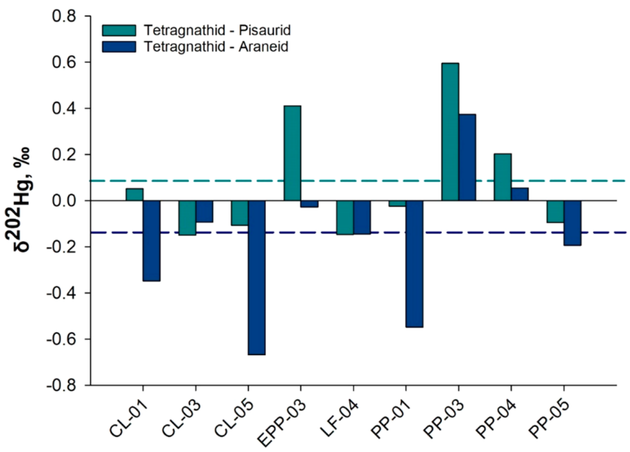

Figure 2. Relative differences in δ^{202}Hg in tetragnathids compared to pisaurids and araneids. Values greater than zero indicate relatively higher tetragnathid values, and values less than zero indicate relatively lower tetragnathid values compared to those of pisaurids or araneids. The dashed lines indicate the mean difference in Hg isotope observations across sites for tetragnathid vs pisaurid (green) and tetragnathid vs araneid (blue).

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and prey consumption. Specifically, pisaurids actively hunt on aquatic and terrestrial surfaces, in contrast to horizontal web building by tetragnathids that target emergent flying insects.[9] Differences between araneids and tetragnathids are more difficult to resolve but may be attributed to variation in aquatic prey consumed. Dietary differences may also result in differences in %MeHg between araneids (70 ± 9%) and tetragnathids (81 ± 12%) (Table S3). Comparison of Δ^{199}Hg values among taxa showed that except for one site (CL-03), Δ^{199}Hg values for tetragnathids were lower than for pisaurids [mean difference of −0.16 ± 0.04‰ (standard error) Δ^{199}Hg], whereas araneids on average were similar to tetragnathids [mean difference of −0.02 ± 0.03‰ (standard error) Δ^{199}Hg] (Figure S5), which we again attribute to species-specific differences in hunting strategy and prey resource utilization. Future assessments using a multi-isotope approach (e.g., Hg, C, N, and H) may be beneficial in further deciphering feeding habits across different spider taxa.

Our results showed differences in δ^{202}Hg and Δ^{199}Hg among spider taxa, indicating varying reliance on different aquatic and terrestrial prey, in contrast to the other existing case study that assessed Hg isotopes.[24] Differences may be more apparent among spider taxa in this study due to high spatial variation in the availability of different prey resources across habitat types, including terrestrial invertebrates. Habitat differences and prey utilization may also drive variability in total Hg concentrations observed in tetragnathids (399 ± 301 ng g^{-1}), pisaurids (239 ± 156 ng g^{-1}), and araneids (246 ± 148 ng g^{-1}) across sites. Similar studies examining PCBs[4] and metals[42] have attributed taxon level differences to the relative reliance on emergent aquatic insects versus terrestrial invertebrates,[36] biological traits such as age and size,[43] and seasonal differences in contaminant exposure.[44] Our results suggest that researchers using spiders to track Hg isotopes need to account for taxon-specific differences and that compositing different spider taxa when collecting samples may result in inaccurate interpretations of Hg sources or processing. Nevertheless, differences in Hg isotope signatures among taxa also provide opportunities to use δ^{202}Hg and Δ^{199}Hg as tracers to better understand aquatic−terrestrial energy flow and trophic connections.

Implications for Effectiveness Evaluation. There has been a concerted effort to compile and assess the usefulness of Hg stable isotope ratios for contaminated site remediation[25] and global mitigation efforts related to the Minamata Convention on Mercury,[26] but to effectively compare biological Hg isotope ratios, the research community would benefit by selecting appropriate biosentinels and developing robust sampling guidelines. Here, we present the use of tetragnathids, a widespread and common shoreline species, as indicators to connect aquatic Hg pollution to terrestrial ecosystem burden. The connection between spider tissues and aquatic invertebrates was robustly observed across a variety of ecosystems within this study, including freshwater wetlands, reservoir shorelines, and urbanized shorelines. Through this data set, we were also able to perform cross-taxon comparisons, which revealed Hg isotopes in spider tissues could vary as a function of habitat usage and prey selection, highlighting the need to define sentinel species for Hg isotope applications.

Critically, this study indicates that Hg source signatures observed in sediments are transferred and highly conserved across a series of aquatic trophic linkages and into terrestrial consumers. The assessment of Hg isotopes in spiders serves as an important ecological indicator of terrestrial Hg burdens, which are often more difficult to assess in birds due to larger foraging ranges[45] and internal detoxification processes.[19,20] The future application of Hg isotopes in spider tissues could be implemented to assess environmental injury within terrestrial environments and provide further insight into contaminant and energy transfer mechanisms in shoreline environments. In summary, shoreline spiders have the potential to be utilized as effective biosentinels to monitor the transfer of Hg from aquatic to terrestrial environments and to identify the source of mercury (e.g., industrial, precipitation, and terrestrially derived Hg) to the environment, which is key for implementing monitoring efforts for Hg-sensitive regions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.3c00450.

Additional details about site and sampling descriptions, mercury isotope notation, quality control and assurance metrics, and ancillary data sources; sampling map (Figure S1); supplementary Hg isotope plots for dragonflies and tetragnathids (Figures S2−S4); comparison of Δ^{199}Hg between spider species (Figure S5); summary of Hg isotope quality control standards (Table S1); and summary tables of Hg stable isotope data in spider tissues (Tables S2 and S3) (PDF)

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Notes

The authors declare no competing financial interest.

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