Supporting Information for "Mercury Isotope Values in Shoreline Spiders Reveal Transfer of Aquatic Mercury Sources to Terrestrial Food Webs"

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Contains: Supporting Text, **4** data tables, and **5** figures

Supporting Methods

S1. Site Descriptions and Sampling Description

The St. Louis River is listed as an Area of Concern (AOC) within the Great Lakes due to severe environmental degradation within the estuary and upstream reservoirs. The lower estuary consists of two zones within this study, the St. Louis Bay (SLB), an estuarine habitat, and Superior Bay (SB), an embayment adjacent to Lake Superior. Both regions have urbanized shorelines and are subject to legacy chemical contamination (e.g., heavy metals and organic contaminants) [1] as well as contemporary wastewater effluents (e.g., higher nutrient loading). [2] Numerous remedial sites are also located within this region including Erie Pier Ponds (EPP) and Pickle Pond (PP). Spirit Lake (SL) and Boy Scout Landing (BSFS) are upstream of the lower estuary and represent lotic riverine habitats within the St. Louis River. [3] These zones have extensive shoreline wetland coverage and mixed mercury (Hg) sources related to upstream runoff and legacy Hg contamination. Scanlon reservoir (SRES) and Thomson reservoir (TRES) are upstream portions of the AOC and are characterized as lentic habitat surrounded by forest land cover. These reservoirs differ in size with SRES being approximately 40 acres whereas TRES is a 339-acre reservoir.

Two zones were also sampled outside the St. Louis River and include Allouez Bay (AB) and the Bad River (BR). AB is another embayment adjacent to Lake Superior and directly east of SB. AB is described as a coastal wetland with little known historic Hg contamination. The Bad River (BR) is situated on tribal land of the Bad River Band of the Lake Superior Tribe of the Chippewa Indians and is 106 km east of the St. Louis River. There is no known Hg contamination within BR, but it does have similar estuarine and riverine habitats when compared to the St. Louis River. [3]

Spider and dragonfly samples were co-located, limiting the collection of shoreline spiders to within 50-m of dragonfly collection sites. Dragonfly (suborder Anisoptera) larvae were collected using a kick net in shoreline regions at all sites. Although shoreline spiders eat a diverse community of aquatic insect adults, we used dragonflies as a model aquatic biosentinel because they are ubiquitous across the diverse aquatic habitats sampled, they occur in sufficient biomass for chemical analyses, and we have observed all three spider taxa actively feeding on dragonflies while conducting field studies.

S2. Mercury Isotope Notation

Mercury isotope values are expressed in delta notation and calculated as follows: [4]

$$\delta^{XXX}Hg\ (‰) = \left[\frac{\left(\frac{^{202}Hg}{^{198}Hg}\right)sample}{\left(\frac{^{202}Hg}{^{198}Hg}\right)standard} - 1\right] \times 1000$$

where xxx is the isotope of interest (199, 200, 201, 202, 204); $^{xxx}Hg/^{198}Hg_{sample}$ represents the ratio of the sample and $^{xxx}Hg/^{198}Hg_{standard}$ represents the ratio of National Institute of Standards and Technology [NIST] 3133 bracketing standards. Mass independent fractionation (MIF) was denoted as $\Delta^{xxx}Hg$ and is calculated as follows: [4]

$$\Delta^{XXX}Hg\ (‰) = \delta^{XXX}Hg - \delta^{202}Hg\ (\beta)$$

where xxx is the isotope of interest (199, 200, 201, 204) and β is the mass scaling factor. Photochemically corrected values[5,6] were calculated for biological tissues following the equation below:

$$\delta^{202}Hg_{COR} = \delta^{202}Hg - (\Delta^{199}Hg/4.79)$$

In this equation $\delta^{202}Hg_{COR}$ is the $\delta^{202}Hg$ corrected value post photochemical demethylation, $\delta^{202}Hg$ and $\Delta^{199}Hg$ are measured values, and 4.79 is the slope ($\Delta^{199}Hg / \delta^{202}Hg$) associated with photodemethylation.[7] Dissolved organic carbon in both the St. Louis and Bad Rivers exceed 5 mg L^{-1}, making the selection of the 4.79 slope an appropriate choice. [3,8]

S3. Quality Control and Assurance for Mercury (Hg) Concentration and Isotope Analyses

Quality-assurance measures for total Hg (HgT) included analysis of two certified reference materials (either dogfish muscle tissue [DORM-4; National Research Council of Canada, Ottawa, Canada], or dogfish liver [DOLT-3; National Research Council of Canada, Ottawa, Canada]), two instrument and method blanks, and two duplicates-per-batch of 30 samples. Recoveries (± standard error [SE]) averaged 100.4 ± 0.8 percent (n = 20) and 96.8 ± 1.1 percent (n = 33) for certified reference materials and calibration checks, respectively. Relative percent difference for all duplicates averaged 2.4 ± 0.7 percent. Quality assurance measures for methylmercury (MeHg) analysis included two independently derived liquid calibration standards, two certified reference materials (either scallop tissue [IAEA-452; International Atomic Energy Agency, Vienna, Austria], dogfish muscle [DORM-4; National Research Council of Canada, Ottawa, Canada], or lobster hepatopancreas [TORT-3; National Research Council of Canada, Ottawa, Canada]), reagent blanks, a matrix spike, and sample duplicates with every run of 72 samples. Average percent recoveries (±SE) were 101.2 ± 1.1% and 98.2 ± 0.9% for liquid standards (n = 20), and certified reference materials (n = 48), respectively. Matrix spike recoveries for MeHg averaged 101.2 ± 2.1% (n = 12) and the relative percent difference for all duplicates was 4.6 ± 0.8% (n = 20).

Quality control and assurance checks for Hg stable isotope analyses included the measurement of a secondary standard (NIST RM8610) every five samples and certified reference material (International Atomic Energy Agency [IAEA] 407) every 12 samples. Acid digestions of IAEA 407 for isotope analysis on average showed 99.8% concentration recovery (229.6 ± 7.8 ng g^{-1}, n = 6). Secondary standards and reference materials, reported in Table S1, were in agreement with Hg stable isotope values established in the literature.[9,10]

S4. Ancillary Data Sources

Additional Hg stable isotope data for fish tissues and sediment are available through the U.S. Geological Survey ScienceBase (https://doi.org/10.5066/P9EOTIR3).[11] Data regarding tributary water chemistry for the St. Louis and Bad Rivers are also publicly available at https://doi.org/10.5066/P9W6I5EK.[8] Data collected from this study can be located in the corresponding data release (https://doi.org/10.5066/P96HIBA4).[12]

Supporting Figures

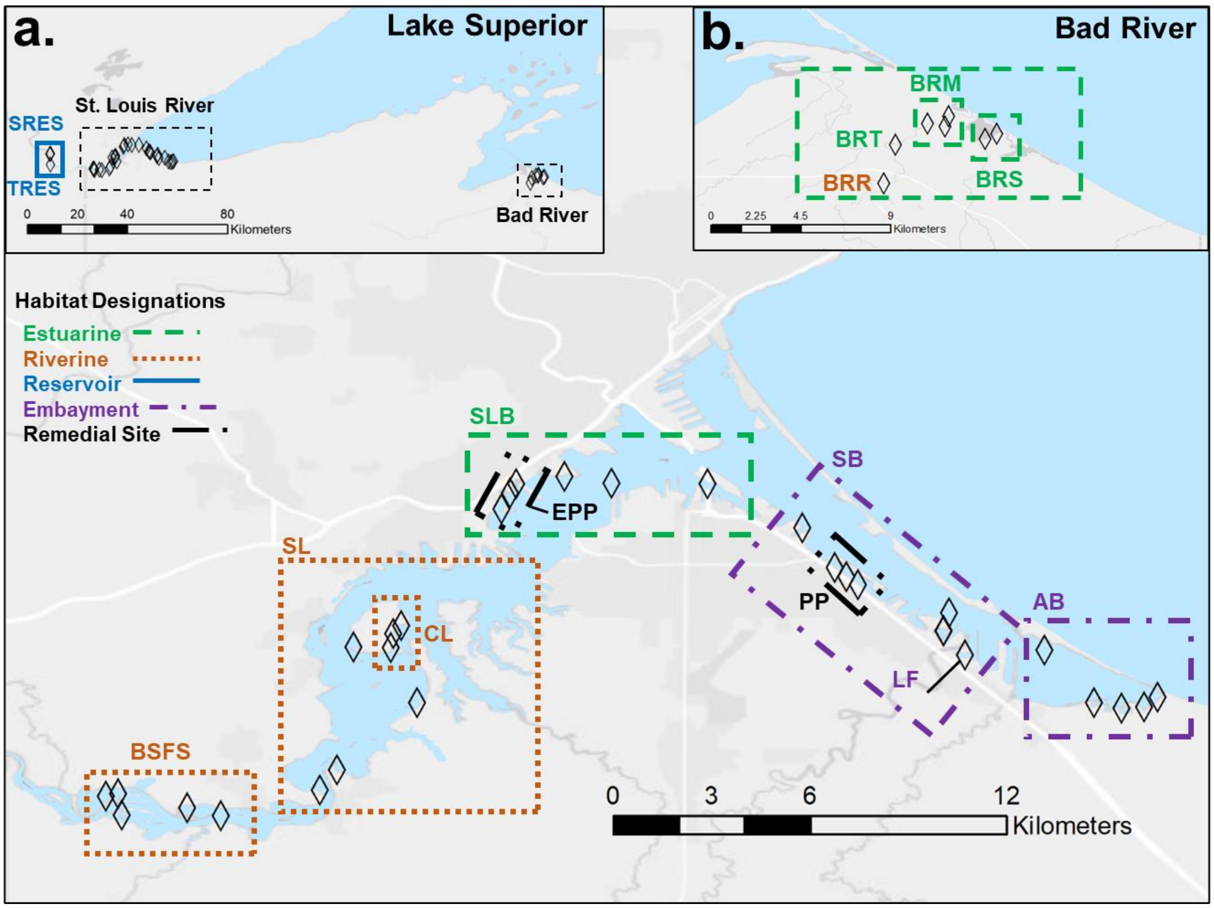

Figure S1: Sampling locations within the Saint Louis River, inset (a) displays a spatial zoom out and (b) displays Bad River sites. Site abbreviations are as follows: Allouez Bay (AB), Bad River Mixed (BRM), Bad River Riverine (BRR), Bad River Slough (BRS), Bad River Transition (BRT), Boy Scout Landing (BSFS), Clough Island (CL), Loon's Foot Landing (LF), Pickle Pond (PP), Erie Pier Ponds (EPP), Superior Bay (SB), Spirit Lake (SL), Saint Louis Bay (SLB), Scanlon Reservoir (SRES), and Thomson Reservoir (TRES). Two types of regions were sampled including larger sampling zones (outlined in dashed boxes) and specific wetland (e.g., LF, CL) or industrially contaminated sites (e.g., PP, EPP).

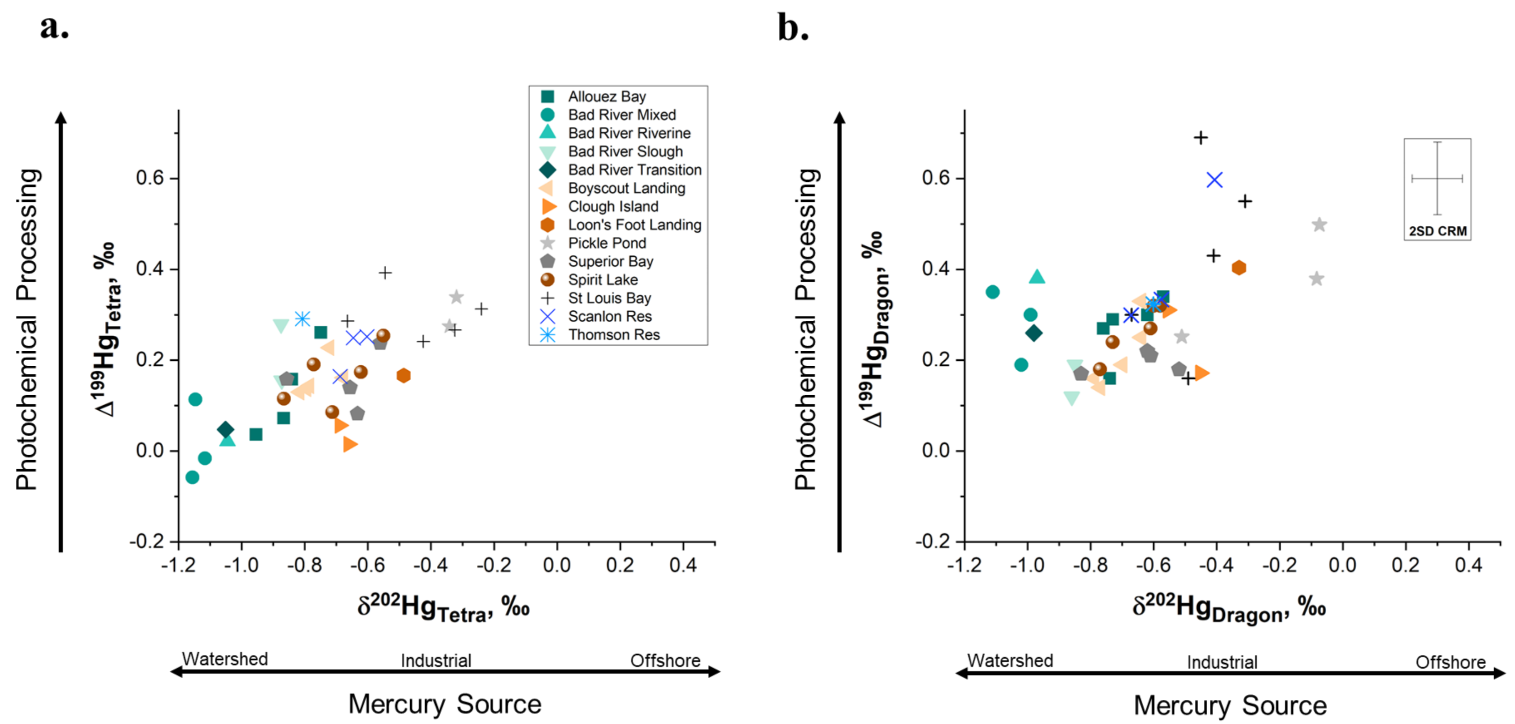

Figure S2: Isotope plots of Δ^{199}Hg and δ^{202}Hg for (a) tetragnathids (abbreviated-tetra) and (b) dragonflies (abbreviated-dragon). Error for δ^{202}Hg and Δ^{199}Hg is represented as the 2-standard deviation (SD) of the certified reference material (CRM) IAEA 407.

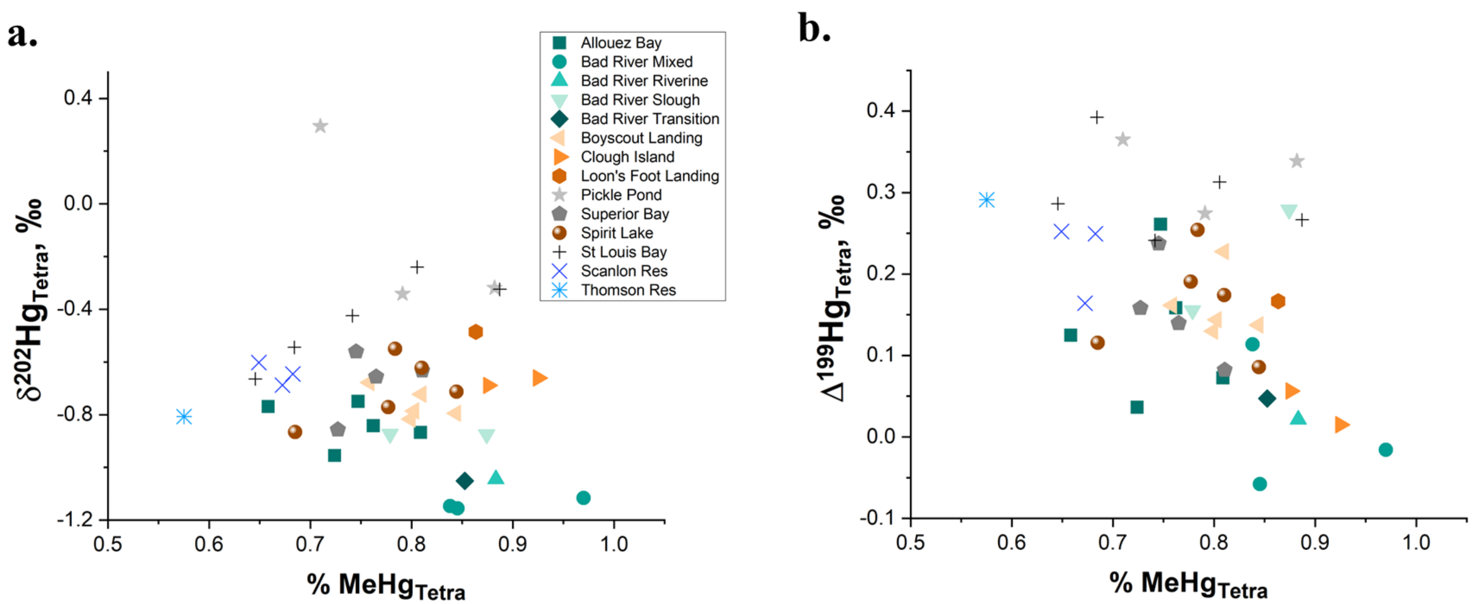

Figure S3: Relation between % MeHg, (a) δ^{202}Hg and (b) Δ^{199}Hg in tetragnathids (abbreviated-tetra). No significant correlation was observed between % MeHg and δ^{202}Hg ($R^2 < 0.10$) or % MeHg and Δ^{199}Hg ($R^2 < 0.20$).

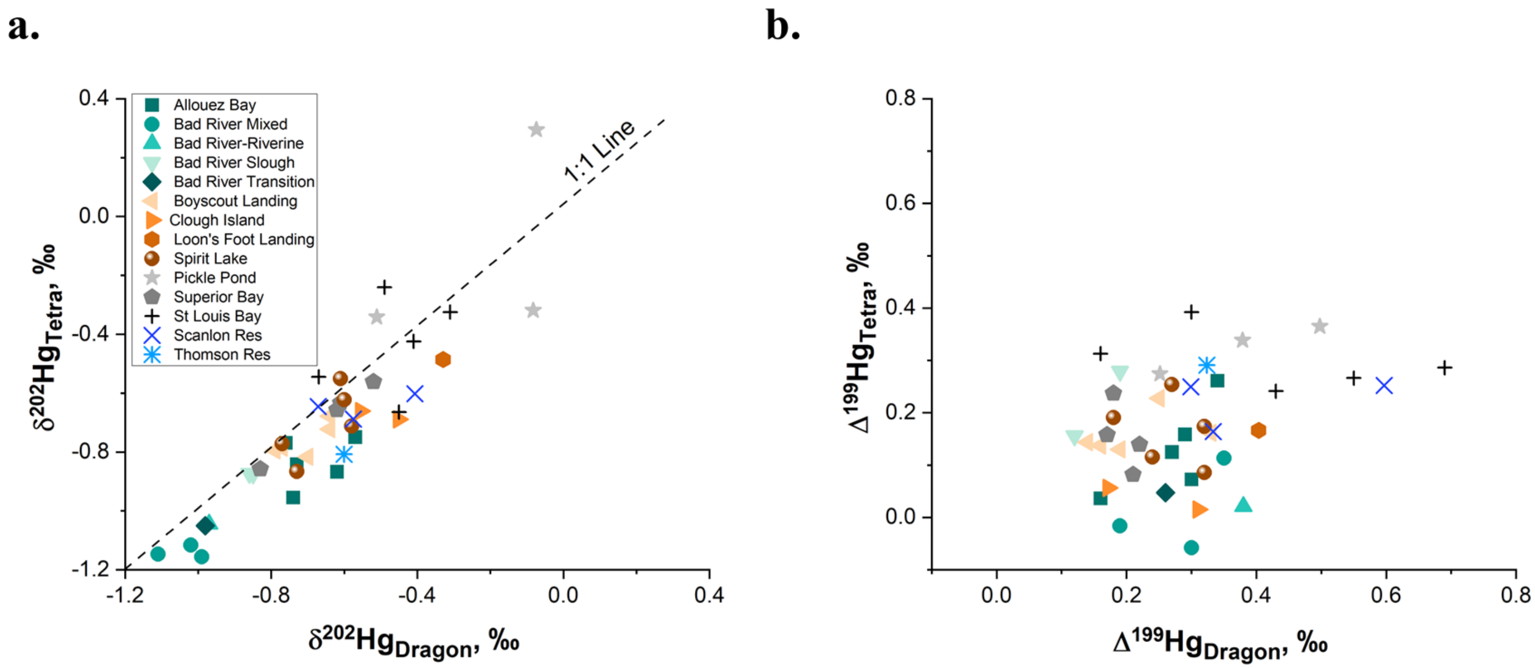

Figure S4: Comparison of (a) δ^{202}Hg and (b) Δ^{199}Hg between tetragnathids (abbreviated-tetra) and dragonflies (abbreviated-dragon) across sites.

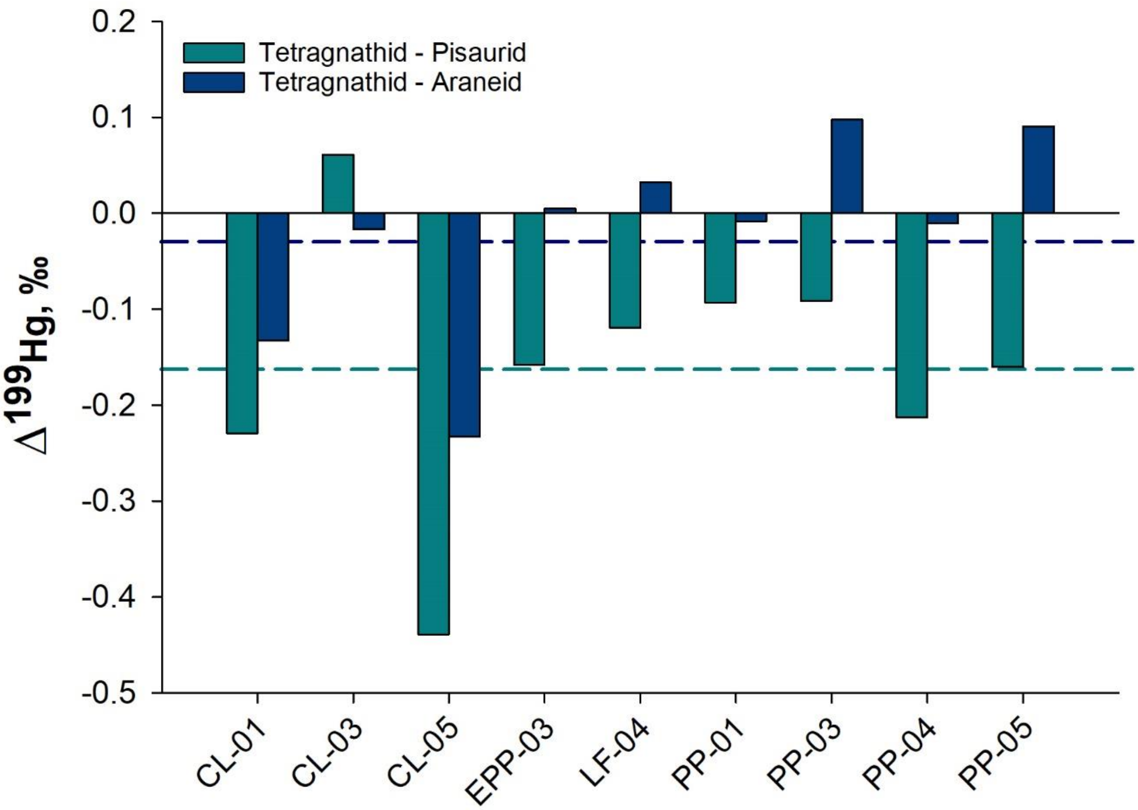

Fig S5: Relative differences in Δ^{199}Hg between tetragnathids to pisaurids and araneids, respectively. Values greater than zero indicate relatively higher tetragnathid values, and values less than zero indicate relatively lower tetragnathid values compared to pisaurids or araneids. The dashed lines indicate the mean difference in Hg isotope observation across sites for tetragnathid-pisaurid (green) and tetragnathid-araneid (blue).

Supporting Tables:

Table S1. Average values and 2 standard deviations (SD) for Hg isotope quality control standards compared to literature values. “Measured” denotes the Hg isotope values measured in this study. Isotope values not reported for the reference data are denoted as NR.

Quality Control Sample	$\delta^{202}Hg_{AVE}$, ‰	$\delta^{202}Hg_{2SD}$, ‰	$\Delta^{199}Hg_{AVE}$, ‰	$\Delta^{199}Hg_{2SD}$, ‰	$\Delta^{200}Hg_{AVE}$, ‰	$\Delta^{200}Hg_{2SD}$, ‰	$\Delta^{201}Hg_{AVE}$, ‰	$\Delta^{201}Hg_{2SD}$, ‰	$\Delta^{204}Hg_{AVE}$, ‰	$\Delta^{204}Hg_{2SD}$, ‰	n
IAEA 407- *Measured*	0.65	0.08	1.09	0.09	0.03	0.06	0.87	0.09	-0.03	0.09	6
IAEA 407- *Lepak et al. 2018* [10]	0.67	0.09	1.05	0.04	0.04	0.03	NR	NR	NR	NR	13
NIST RM8610-*Measured*	-0.56	0.07	-0.02	0.05	0.00	0.03	-0.03	0.05	0.01	0.12	12
NIST RM8610-*Certified* [9]	-0.56	0.03	-0.03	0.02	0.00	0.01	-0.04	0.01	0.00	0.02	NR

Table S2. Summary of methylmercury (MeHg), total mercury (HgT), % percentage of total mercury present as methylmercury in tissue (%MeHg), and Hg stable isotope values in paired dragonflies and tetragnathids. Site abbreviations are as follows: Allouez Bay (AB), Bad River Mixed (BRM), Bad River Riverine (BRR), Bad River Slough (BRS), Bad River Transition (BRT), BSFS (Boy Scout Landing), CL (Clough Island), LF (Loon’s Foot Landing), PP (Pickle Pond), SB (Superior Bay), SL (Spirit Lake), SLB (Saint Louis Bay), Scanlon Reservoir (SRES), and TR (Thomson Reservoir).[13]

			Damselflies						Tetragnathid					
Site	Latitude	Longitude	MeHg, ng g^{-1}	HgT, ng g^{-1}	%MeHg	$\delta^{202}Hg$, ‰	$\Delta^{199}Hg$, ‰	$\delta^{202}Hg_{COR}$, ‰	MeHg, ng g^{-1}	HgT, ng g^{-1}	%MeHg	$\delta^{202}Hg$, ‰	$\Delta^{199}Hg$, ‰	$\delta^{202}Hg_{COR}$, ‰
AB-01	46.681	-91.984	26.1	27.4	95%	-0.76	0.27	-0.77	227.0	344.8	66%	-0.77	0.12	-0.77
AB-02	46.684	-91.974	35.9	37.4	96%	-0.62	0.30	-0.63	397.0	490.9	81%	-0.87	0.07	-0.87
AB-03	46.683	-91.992	27.2	30.6	89%	-0.57	0.34	-0.58	216.0	289.1	75%	-0.75	0.26	-0.76
AB-04	46.682	-91.978	50.8	53.2	95%	-0.74	0.16	-0.75	254.0	350.8	72%	-0.95	0.04	-0.96
AB-05	46.697	-92.005	22.3	23.4	95%	-0.73	0.29	-0.74	189.3	248.4	76%	-0.84	0.16	-0.85
BRM-01	46.633	-90.672	43.7	48.7	90%	-1.11	0.35	-1.12	352.0	420.0	84%	-1.15	0.11	-1.15
BRM-02	46.632	-90.664	40.1	45.5	88%	-0.99	0.30	-1.00	831.0	983.0	85%	-1.16	-0.06	-1.15
BRM-04	46.636	-90.663	43.3	46.7	93%	-1.02	0.19	-1.03	894.0	921.7	97%	-1.12	-0.02	-1.12
BRR-01	46.606	-90.692	32.8	34.2	96%	-0.97	0.38	-0.98	532.5	602.8	88%	-1.04	0.02	-1.05
BRS-01	46.626	-90.647	17.6	19.0	93%	-0.86	0.12	-0.86	300.0	385.2	78%	-0.87	0.16	-0.88
BRS-02	46.629	-90.641	17.7	18.8	94%	-0.85	0.19	-0.86	244.0	279.1	87%	-0.87	0.28	-0.89
BRT-01	46.624	-90.687	39.0	41.5	94%	-0.98	0.26	-0.99	620.5	727.8	85%	-1.05	0.05	-1.05
BSFS-01	46.657	-92.263	35.5	36.5	97%	-0.70	0.19	-0.71	387.5	485.0	80%	-0.82	0.13	-0.82
BSFS-02	46.652	-92.259	39.9	39.5	101%	-0.77	0.14	-0.78	420.0	523.4	80%	-0.79	0.14	-0.79
BSFS-03	46.654	-92.241	21.8	24.2	90%	-0.64	0.33	-0.65	590.0	777.9	76%	-0.68	0.16	-0.68
BSFS-04	46.652	-92.232	33.2	35.8	93%	-0.64	0.25	-0.65	368.0	454.5	81%	-0.72	0.23	-0.73
BSFS-05	46.658	-92.260	48.4	47.3	102%	-0.79	0.16	-0.80	418.0	495.3	84%	-0.79	0.14	-0.80
CL-03	46.702	-92.184	311.0	282.0	110%	-0.45	0.17	-0.46	381.0	435.0	88%	-0.69	0.06	-0.69
CL-05	46.698	-92.185	205.0	193.0	106%	-0.56	0.31	-0.57	962.0	1040.0	93%	-0.66	0.02	-0.66
LF-04	46.702	-92.033	200.0	182.3	110%	-0.33	0.40	-0.34	428.0	495.7	86%	-0.49	0.17	-0.49
PP-01	46.720	-92.063	198.0	196.0	101%	-0.08	0.38	-0.10	254.0	288.0	88%	-0.32	0.34	-0.33
PP-03	46.717	-92.060	103.0	90.3	114%	-0.07	0.50	-0.09	137.0	193.0	71%	0.29	0.36	0.28
PP-05	46.715	-92.057	213.0	203.0	105%	-0.51	0.25	-0.52	125.0	158.0	79%	-0.34	0.27	-0.35
SB-02	46.696	-92.027	25.0	28.2	88%	-0.61	0.21	-0.62	586.0	723.0	81%	-0.63	0.08	-0.64
SB-03	46.702	-92.033	28.8	30.9	93%	-0.62	0.22	-0.63	323.5	422.9	77%	-0.66	0.14	-0.66
SB-04	46.707	-92.032	50.0	53.9	93%	-0.52	0.18	-0.53	287.7	386.0	75%	-0.56	0.24	-0.57
SB-05	46.731	-92.072	37.3	40.0	93%	-0.83	0.17	-0.84	532.0	731.6	73%	-0.86	0.16	-0.86
SL-01	46.659	-92.204	46.4	47.0	99%	-0.73	0.24	-0.74	472.0	689.1	68%	-0.87	0.12	-0.87
SL-02	46.704	-92.182	21.2	24.5	87%	-0.60	0.32	-0.61	355.0	438.2	81%	-0.62	0.17	-0.63
SL-03	46.698	-92.195	26.9	30.8	87%	-0.61	0.27	-0.62	517.7	660.5	78%	-0.55	0.25	-0.56
SL-04	46.664	-92.200	46.7	46.5	100%	-0.77	0.18	-0.78	308.0	396.4	78%	-0.77	0.19	-0.78
SL-05	46.683	-92.178	30.8	36.7	84%	-0.58	0.32	-0.59	560.0	663.3	84%	-0.71	0.09	-0.72
SLB-01	46.736	-92.155	40.8	40.3	101%	-0.67	0.30	-0.68	180.3	263.6	68%	-0.54	0.39	-0.56
SLB-02	46.745	-92.137	30.6	35.3	87%	-0.49	0.16	-0.50	313.3	389.0	81%	-0.24	0.31	-0.25
SLB-03	46.740	-92.152	20.7	24.9	83%	-0.31	0.55	-0.33	428.7	483.2	89%	-0.32	0.27	-0.33
SLB-04	46.743	-92.098	13.7	14.9	92%	-0.45	0.69	-0.48	158.7	245.8	65%	-0.66	0.29	-0.68
SLB-05	46.743	-92.124	16.9	17.9	94%	-0.41	0.43	-0.43	274.7	370.4	74%	-0.42	0.24	-0.43
SRES-01	46.712	-92.416	185.0	187.0	99%	-0.58	0.33	-0.59	188.3	280.1	67%	-0.69	0.16	-0.69
SRES-02	46.710	-92.418	130.0	144.0	90%	-0.41	0.60	-0.43	194.3	299.3	65%	-0.60	0.25	-0.61
SRES-03	46.711	-92.417	167.0	173.0	97%	-0.67	0.30	-0.68	146.0	213.9	68%	-0.65	0.25	-0.66
TR-01	46.672	-92.416	284.0	285.0	100%	-0.60	0.32	-0.61	171.0	297.3	58%	-0.81	0.29	-0.82

Table S3. Mercury isotope and concentration values for paired spider taxa from Clough Island (CL), Ponds behind Erie Pier (EPP), Loon's Foot Landing (LF), and Pickle Pond (PP).[13]

	Araneidae spp.					Pisauridae sp.					Tetragnathidae spp.				
Site	**MeHg, ng g^{-1}**	**HgT, ng g^{-1}**	**%MeHg**	**δ^{202}Hg, ‰**	**Δ^{199}Hg, ‰**	**MeHg, ng g^{-1}**	**HgT, ng g^{-1}**	**%MeHg**	**δ^{202}Hg, ‰**	**Δ^{199}Hg, ‰**	**MeHg, ng g^{-1}**	**HgT, ng g^{-1}**	**%MeHg**	**δ^{202}Hg, ‰**	**Δ^{199}Hg, ‰**
CL-01	240	287	84%	-0.29	0.39	152	148	103%	-0.69	0.48	381	435	88%	-0.63	0.25
CL-03	427	609	70%	-0.60	0.07	594	587	101%	-0.54	0.00	962	1040	93%	-0.69	0.06
CL-05	147	188	78%	0.01	0.25	194	199	97%	-0.55	0.45	646	676	96%	-0.66	0.02
EPP-03	94	154	61%	-0.19	0.38	54	68	80%	-0.63	0.54	88	141	62%	-0.22	0.38
LF-04	242	298	81%	-0.34	0.13	369	395	94%	-0.34	0.29	428	496	84%	-0.49	0.17
PP-01	97	130	74%	0.23	0.35	205	211	97%	-0.29	0.43	254	288	88%	-0.32	0.34
PP-03	97	161	60%	-0.08	0.27	122	212	58%	-0.30	0.46	137	193	71%	0.29	0.36
PP-04	146	230	63%	-0.32	0.31	141	149	95%	-0.47	0.51	117	171	68%	-0.27	0.30
PP-05	98	159	62%	-0.15	0.18	178	190	94%	-0.25	0.43	125	158	79%	-0.34	0.27

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