Supporting Information for "Chemical and Physical Controls on Mercury Source Signatures in Stream Fish from the Northeastern United States"

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Site Classification Criteria

Sites classified as forested-rural (n=7) had < 15% urban-developed land cover (total of open-, low-, medium-, and high-developed classes), 0% industrial land-use (industrial/military class), and < 15 U.S. Environmental Protection Agency (EPA) Federal Registry System (FRS) locations per 100km^2 in either the watershed or lower basin (the latter defined by the portion of the watershed contained within a circle having radius of 5km and centered on the sampling site). Sites classified as residential (n=4) had > 30% urban-developed land cover in either the watershed or lower basin, < 1% industrial land-use in both the watershed and lower basin, and < 15 FRS locations per 100km^2 in both the watershed and lower basin. Industrial-urban sites (n=12) had $\geq$ 30% urban-developed land cover and either > 2% industrial land-use or $\geq$15 FRS facilities per 100km^2 in either the watershed or lower basin. Tallies of industrial locations and potential point sources from the EPA FRS (https://www.epa.gov/enviro/facility-registry-service-frs) did not include retail, educational, and medical facilities.

Fish Selection Criteria and Processing

Small invertivorous species, or small individuals of larger species (e.g., sunfish [*Lepomis* spp.]) were collected from each site and processed as single-species composites of 2 to 16 (median 10) similarly-sized whole specimens; these are hereafter termed 'prey fish'. Targeted prey fishes were dace (Blacknose dace [*Rhinichthyes atratulus*], Longnose dace [*R. cataractae*]), Tessellated darter (*Etheostoma olmstedi*), and small-sized sunfish. Game fish species were also collected from each site, and processed as individual skinless fillets. Multiple samples of prey and game fish species were collected from some sites. Targeted game fishes were black bass (Largemouth bass [*Micropterus salmoides*], Smalmouth bass [*M. dolomieu*]), salmonids (Brook trout [*Savelinus fontinalis*], Brown trout [*Salmo trutta*], Rainbow trout [*Oncorhynchus mykiss*]), and larger-sized sunfish. Non-migratory species considered top predators at each site were targeted as game fish, except for the collection of a single large Creek Chub (*Semotilus atromaculatus*) at one site (Hallocks, NY) lacking typical game fish species.

Water Sample Collection and Analysis

Water samples were collected by grab sampling using trace-metal clean techniques[1] during three weekly site visits spanning July 18 and August 5 (2016). Unfiltered water for total (HgT) and methylmercury (MeHg) analyses were immediately acidified with hydrochloric acid (0.12 M) upon collection and sent to the Mercury Research Lab (MRL, Middleton, WI). Total mercury analysis was conducted by EPA method 1631 using a Brooks Rand Total Mercury “manual” system.[2] Samples were initially treated by bromine chloride oxidation, followed by reduction using sequential additions of hydroxylamine hydrochloride and then stannous chloride. Reduced gaseous elemental mercury was purged from the sample, captured onto gold-coated quartz beads, thermally desorbed, and detected by atomic fluorescence spectrometry (LTDL = 0.040 ng L^{-1}). Methylmercury analysis was conducted using a modified version of EPA method 1630.[3] A sample aliquot was first amended with an addition of a single methylmercury isotope (as $Me^{199}Hg$) and distilled to reduce matrix interference. Sodium tetraethylborate is added to the distillate, resulting in ethylation of the oxidized mercury species (Hg^{++} and $MeHg^{+}$). The volatile ethylated mercury is purged from the distillate, retained onto Tenex traps, thermally desorbed, and speciated with a gas chromatography column. The resulting gaseous mercury is introduced to an inductively coupled plasma mass spectrometer (Brooks Rand Merx-M coupled to a Thermo Electron iCAP RQ) for detection. Quantification of methylmercury concentrations are calculated by isotope dilution (long-term detection lmit (LTDL) = 0.020 ng L^{-1}). Detailed descriptions of the analytical methods, as well as quality assurance and control criteria, are fully described in the MRL standard operating procedures (available at https://wi.water.usgs.gov/mercury-lab/research/analysis-methods.html) .

Several ancillary parameters in surface waters were also measured. A filtered aliquot (0.45 µm) of the raw water sample was analyzed for DOC and ultraviolet absorbance at 254 nm (UVA_{254}) by EPA method 415.3.[4] Specific ultraviolet absorbance at 254 nm (SUVA) was calculated as UVA_{254} divided by DOC. Measurements of pH were made and samples for chloride, TN, and TP also were collected during each weekly visit; collection and field-processing are detailed elsewhere.[5,6] Chloride and nutrients were analyzed at the USGS National Water Quality Laboratory as described elsewhere.[7-9] Median water chemistry data are found in Table S2 and all raw are publically available on the USGS National Water Information System (NWIS) and locatable by USGS station identifier in Table S1 (https://waterdata.usgs.gov/nwis).

Mercury Isotope Analysis

Measurements were made on a Neptune Plus MC-ICP-MS located at the Wisconsin State Lab of Hygiene. Mercury was introduced continuously to the instrument (0.95 ml min^{-1}) as Hg(II) solutions and mixed in line with stannous chloride (3% SnCl2 in 10% HCl), the mixture was then introduced to a custom frosted glass gas liquid separator where Hg(0) was released from the solution using a counter stream of Ar (0.18-0.20 L min^{-1}). A dry Tl aerosol was also introduced to the gas liquid separator (0.80-0.85 L min-1) using an Apex-Q desolvating nebulizer in free flow mode equipped with a 20 µL PFA nebulizer. The Tl standard (NIST SRM 997, 40 ng mL^{-1} in 3% HCl) was used as an internal standard for mass bias correction, and was mixed with Hg(0) in the gas-liquid separator prior to introduction into the plasma. The instrument was fitted with nickel X skimmer and jet sample cones. The MC-ICP-MS was set up to monitor all 9 equipped faraday cups with associated 10^{-11} amplifiers for ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg, ^{203}Tl, ^{205}Tl, and ^{206}Pb with ^{202}Hg in the center cup and an extended clip to allow for the simultaneous measurement of ^{204}Hg. No signals above 10^{-4} V were measured for isobaric interreferences of Pt isotopes (^{196}Pt and ^{198}Pt) or ^{204}Pb, though Pb was specifically monitored throughout the runs to ensure no interferences with ^{204}Hg. Instrument parameters (gases, lenses, stage position) were optimized for maximum voltage for ^{202}Hg and signal stability which was typically 1V per 1 ng mL^{-1}.

Calculations for Photochemical Corrections

The general calculations for δ^{202}Hg and Δ^{199}Hg follow conventions set forth in the literature[10] and outlined in the main methods section. Photochemical corrections of δ^{202}Hg were performed as set forth in Gehrke et al.[11], Sherman et al.[12], and Blum et al.[13] using a slope determined from bench top experiments for photochemical demethylation.[14] A slope of 2.43 was utilized for consistency since the average dissolved organic carbon (DOC) across sites was below 5 mg L-1. Corrections were performed as:

$$\delta^{202}Hg_{COR} = \delta^{202}Hg_{fish} - (\Delta^{199}Hg_{fish}/2.43) \qquad \text{(Equation 1)}$$

where $\delta^{202}Hg_{COR}$ = δ^{202}Hg corrected for the photodemethylation effect, and 2.43 is the slope (Δ^{199}Hg /δ^{202}Hg) associated with photodemethylation at a DOC concentration of 1 mg L^{-1}.[14] Given that some sites exceeded the average median DOC specifically the Charles (6.24 mg L^{-1}), Ipswich (8.09 mg L^{-1}), Allen (7.2 mg L^{-1}), Hallocks (6.61 mg L^{-1}), Muscoot (6.11 mg L^{-1}), and Scriba (9.21 mg L^{-1}) sites (Table **S3**), the slope of 4.8 which relates to a 10 mg L^{-1} condition for photodemethylation[14] was also calculated. The maximum difference between fish values using the two slopes was 0.11 ‰, (shown in parentheses in Table **S2**) with values calculated using the 4.8 slope being slightly more enriched. The small differences between calculated values did not alter any isotopic patterns or statistical tests, thus all $\delta^{202}Hg_{cor}$ values are reported using the 2.43 slope for consistency.

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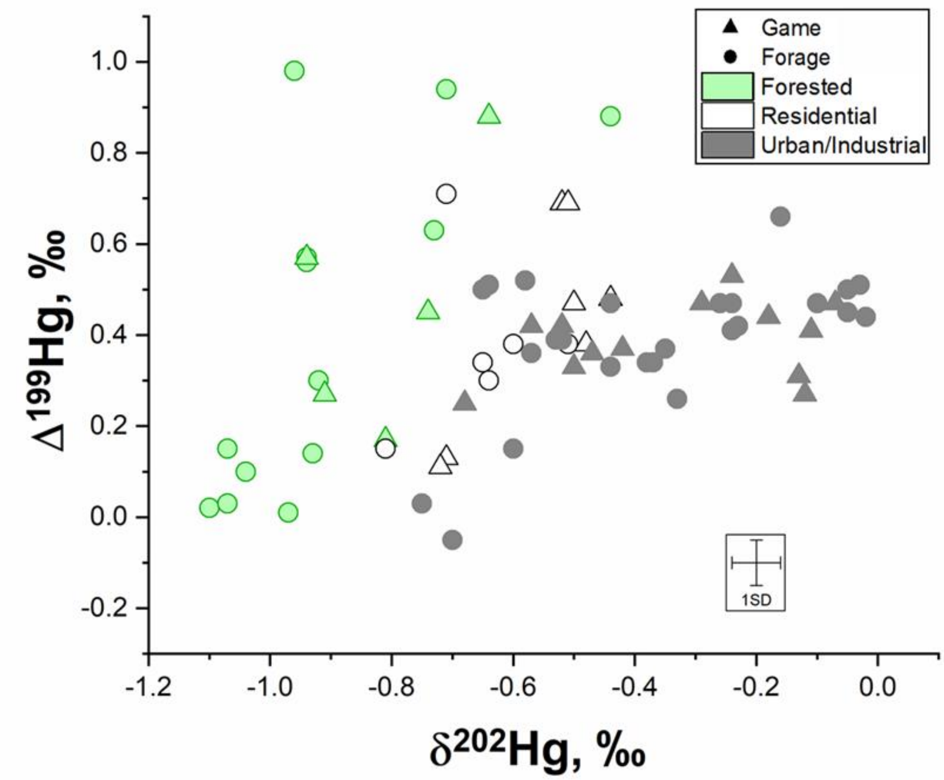

Fig. S1 Isotope biplot (δ^{202}Hg and Δ^{199}Hg) for NESQA fish collected from 23 streams across the Northeastern U.S.

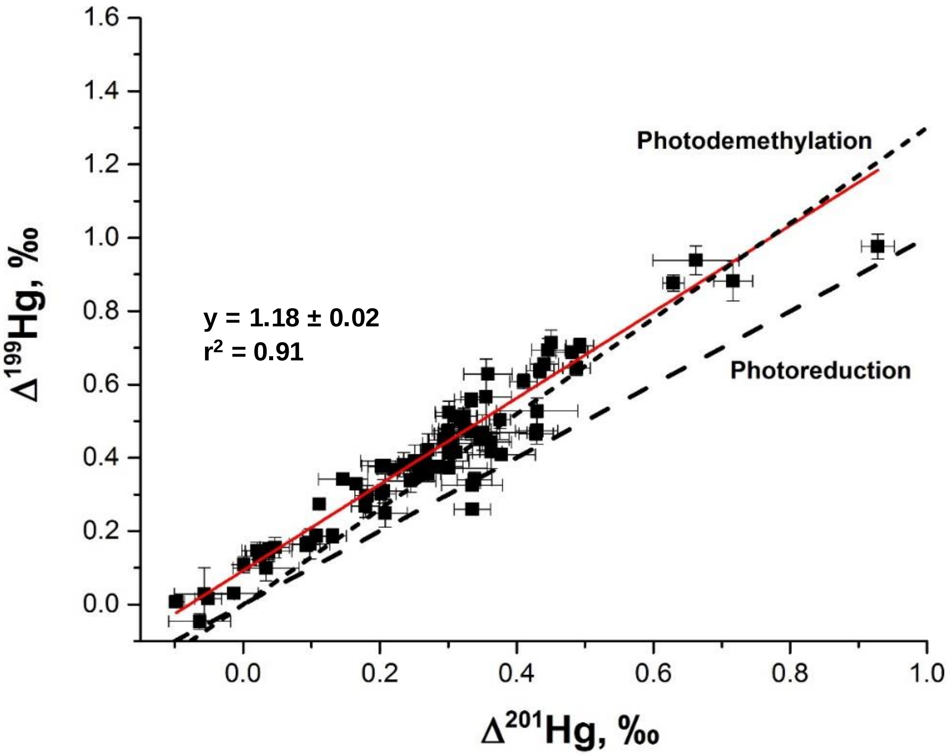

Fig. S2 Photochemical slope Δ^{199}Hg/ Δ^{201}Hg of NESQA fish. Slopes of photodemethylation (1.3) and photoreduction (1.0) are taken from Bergquist and Blum 2007[14] and use the low DOC conditions.

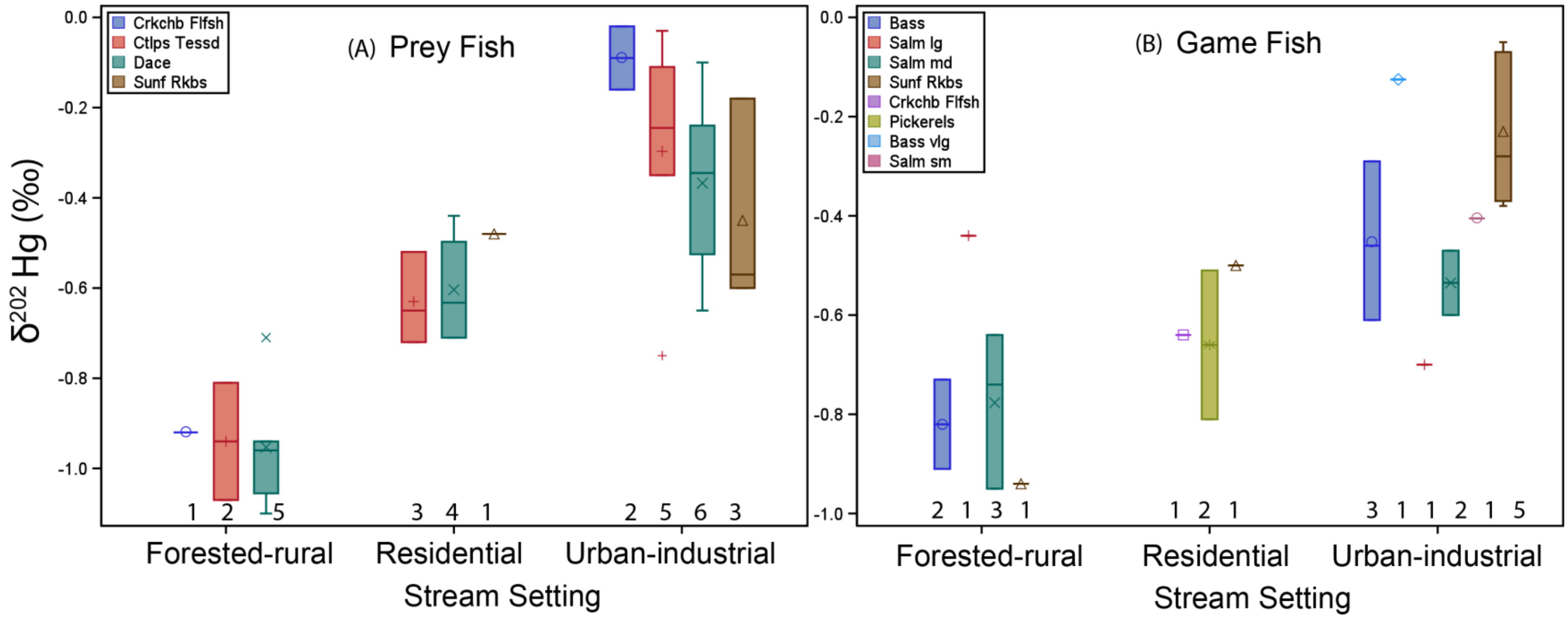

Fig S3 Mercury isotope composition (δ^{202}Hg) in (A) whole prey fish and (B) skinless fillets of game fish from three types of land-use settings across the northeastern U.S. Boxplots are comprised of mean values for each site; the numbers of sites are shown across the x-axis. Boxes show the 25^{th}-75^{th} quartiles, with medians and means denoted by the center line and symbol, respectively, and outliers as symbols above or below the boxes. Whiskers represent the 25th percentile less 1.5 times the interquartile range (IQR) and the 75th percentile plus 1.5 times the IQR, respectively. Species and species groups associated with abbreviations are as follows: in (A) Crkchb Flfsh – Creek Chub and Fallfish, Ctlps Tessd – Cutlips minnow and Tessellated Darter, Dace – Blacknose Dace and Longnose Dace, Sunf Rkbs – Sunfish species and Rock Bass; in (B) Bass – Smallmouth Bass and Largemouth Bass < 250 mm total length (TL), Salm md – salmonids 150-249 mm TL, Salm lg – salmonids ≥ 250 mm TL, Sunf Rkbs – sunfish species and Rock Bass, Crkchb Flfsh – Creek Chub, Pickerels – Pickerel species, Bass vlg – Largemouth Bass ≥ 250 mm TL, Salm sm – salmonids < 150 mm TL. Additional information regarding the scientific names can be found in the associated data release.[2]

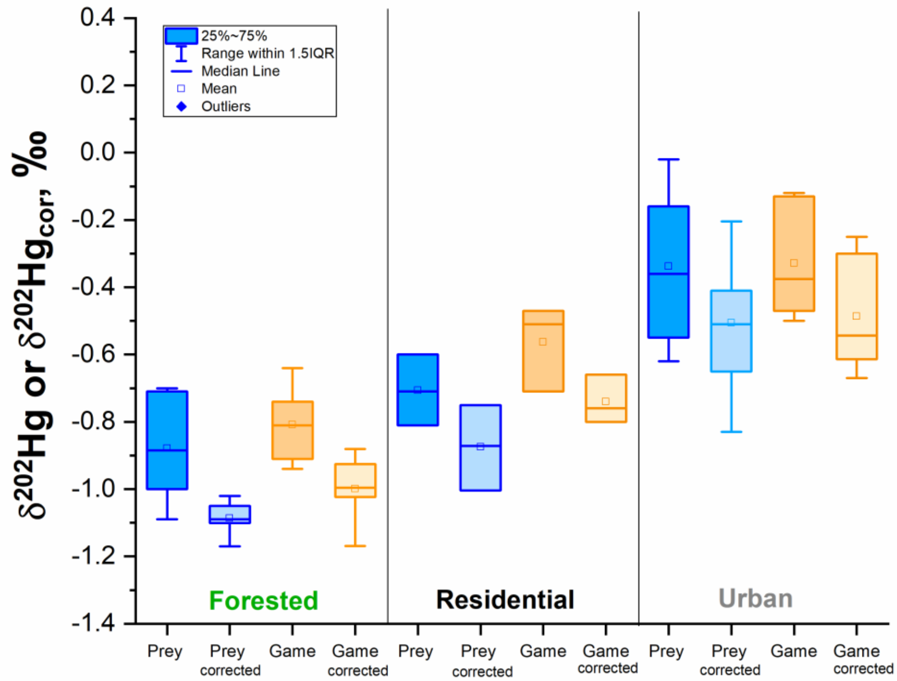

Fig. S4 Comparison of site mean δ^{202}Hg and $\delta^{202}Hg_{cor}$ for NESQA Fish. Sites are grouped according to land use characteristics. Photochemical corrected ($\delta^{202}Hg_{COR}$) values are denoted by "corrected" labels and with lighter colors. Boxplots represent the 25-75th quartile for data with medians and means denoted by the center line and symbol, respectively. Whiskers represent the 25th percentile less 1.5 times the interquartile range (IQR) and the 75th percentile plus 1.5 times the IQR, respectively. Corrected δ^{202}Hg values are more depleted than the true measurement across all groups. Numbers of sites are 7, 4, and 12 for forested, residential, and urban land-use groups, respectively.

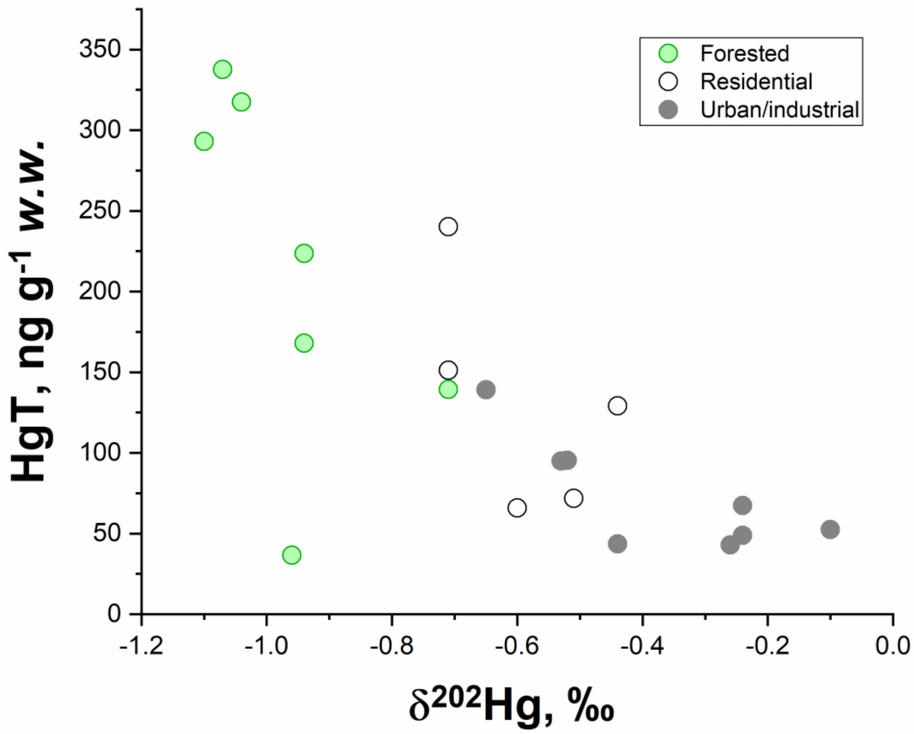

Fig. S5 Total mercury concentrations (HgT) vs δ^{202}Hg in dace (*Rhinichthys* sp.) collected from 15 sites across the three land-use groups (5 forested, 4 residential, and 6 urban). Dace are the most widely distributed species groups across the NESQA. Higher concentrations are observed in fish with depleted δ^{202}Hg signatures, representing forested regions.

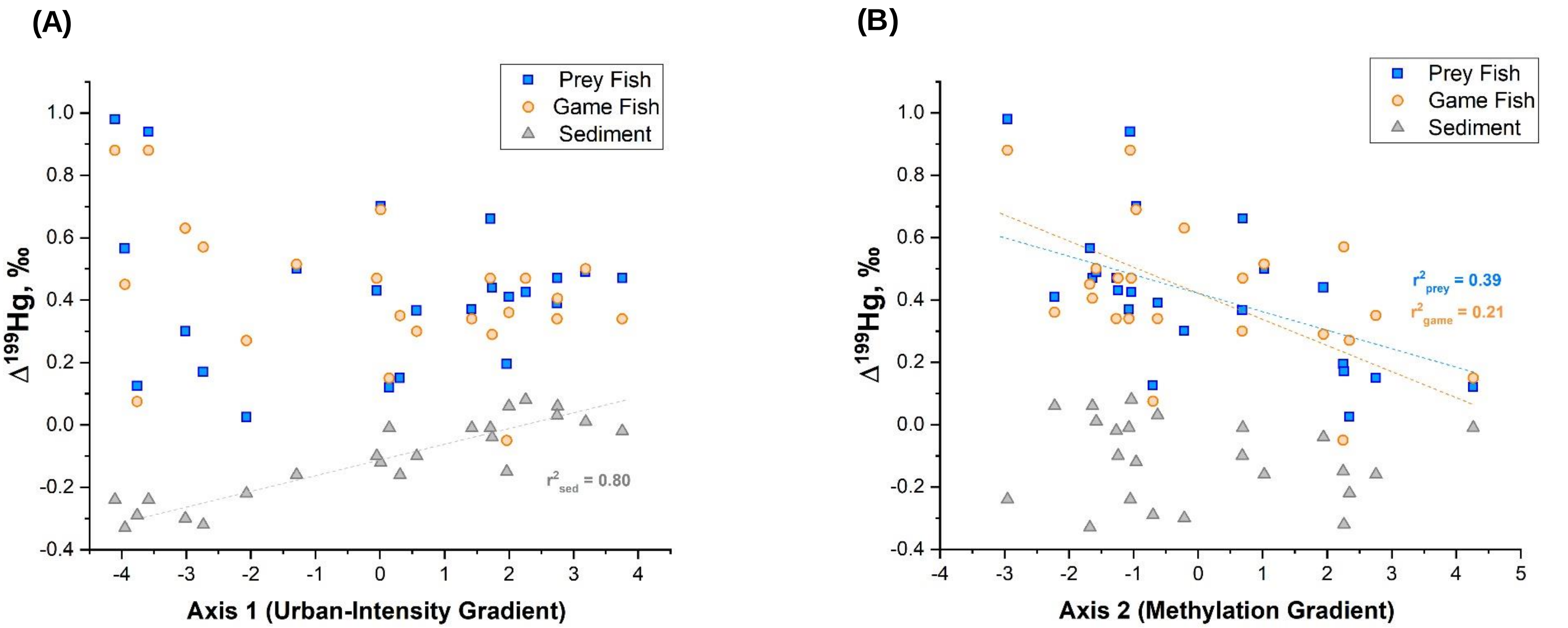

Fig S6. Correlations between site means of Δ^{199}Hg and PCA a) Axis 1 urban intensity gradient and b) Axis 2 methylation gradient. The dashed line represents the linear regression of the data; the Spearman rho is used as the reporting statistic. Fish Δ^{199}Hg shows a negative relation to Axis 2 and no relationship to Axis 1, the urban intensity gradient.

Table S1: Geomorphic and land-use characteristics [15-20] of sampling sites for 23 NESQA streams. More detailed site information, including lower watershed variables, can be found in the associated data release.[21]

Site abbreviation	Site Category	USGS Station Identifier	Latitude	Longitude	Watershed Drainage Area	Elevation	Modeled atmospheric Hg dry dep	Modeled atmospheric Hg wet dep	Calculated total Hg dep at site	Total wetland %	Industrial or military land usage	Total urban land cover	Road Density	Number of industrial facilities within 100 km
					km^2	m	ug m^{-2}	ug m^{-2}	ug m^{-2}	%	%	%	km km^{-2}	
							Ye et al. 2018	Ye et al. 2018	Ye et al. 2018	FWS NWI	NWALT 2012	MRLC NLCD 2011	U.S. Census TIGER 2016	USEPA FRS
CT_Hubbard	forested-rural	0118730	42.0375	-72.9393	53.5	191	11.79	5.15	16.94	6.0	0.0	2.8	2.7	0
CT_MillFair	residential	01208925	41.1655	-73.2700	74.0	2	16.11	6.60	22.70	4.4	0.0	30.4	13.0	1
CT_Rippowam	residential	01209901	41.0661	-73.5493	88.6	5	12.37	6.38	18.75	5.0	0.0	30.9	14.0	2
MA_Charles	urban-industrial	01103280	42.1400	-71.3897	169.0	41	16.85	6.29	23.14	10.9	2.1	44.3	6.3	27
MA_Ipswich	urban-industrial	01101500	42.5700	-71.0298	113.7	16	21.73	7.68	29.40	20.0	0.8	54.5	5.0	16
MA_Neponset	urban-industrial	01105000	42.1776	-71.2009	90.3	18	21.96	7.35	29.30	11.0	0.8	47.5	10.4	20
MA_OldSwamp	urban-industrial	01105600	42.1927	-70.9432	12.0	20	21.41	7.45	28.86	16.4	11.5	66.8	15.2	33
MA_Stillwater	forested-rural	01095220	42.4109	-71.7912	78.7	122	13.57	5.16	18.74	7.1	0.0	7.7	6.4	0
MA_WBSwift	forested-rural	01174565	42.4551	-72.3818	32.9	164	10.99	5.44	16.43	3.2	0.0	4.8	5.7	0
NH_Beaver	urban-industrial	010965852	42.7831	-71.3530	123.1	48	13.57	5.91	19.47	7.6	0.5	39.0	6.2	14
NY_Allen	urban-industrial	04232050	43.1303	-77.5186	79.7	103	25.96	7.30	33.26	4.1	1.9	66.4	15.8	24
NY_Bronx	urban-industrial	405242073521001	40.8785	-73.8693	138.1	17	63.83	8.44	72.27	1.0	1.9	73.1	23.9	19
NY_Casper	urban-industrial	413758073552401	41.6330	-73.9236	27.5	14	11.23	5.64	16.86	3.0	1.9	67.2	13.1	15
NY_Cross	forested-rural	01374890	41.2602	-73.6019	44.2	103	13.59	5.79	19.37	8.8	0.0	13.9	3.9	0
NY_Hallocks	residential	01374960	41.2845	-73.7740	25.0	115	13.52	6.30	19.82	8.0	0.1	55.3	10.0	0
NY_LBeaver	forested-rural	01362497	42.0194	-74.2664	43.2	206	10.19	5.67	15.86	1.6	0.0	2.2	2.4	0
NY_LeyCreek	urban-industrial	430524076084201	43.0901	-76.1451	61.7	113	10.35	10.43	20.79	5.0	15.1	72.1	12.7	84
NY_Mamaroneck	urban-industrial	405708073440301	40.9524	-73.7343	58.9	8	10.18	6.65	16.82	1.2	1.0	79.8	12.4	19
NY_Muscoot	residential	01374930	41.3381	-73.7687	34.3	156	13.43	5.96	19.39	8.0	0.0	37.6	8.2	0
NY_SawMill	urban-industrial	01376500	40.9382	-73.8897	66.1	29	37.79	7.66	45.45	1.1	5.3	75.0	19.8	54
NY_Scriba	forested-rural	04245840	43.2597	-76.0028	103.0	124	9.49	9.07	18.56	18.9	0.0	1.0	2.0	0
NY_Thomas	urban-industrial	430629077274701	43.1082	-77.4633	76.5	121	17.68	6.78	24.45	8.2	0.8	32.0	9.7	13
VT_Saxtons	forested-rural	01154000	43.1376	-72.4877	186.7	125	9.58	6.33	15.92	1.4	0.0	3.3	3.0	2

Table S2: Mercury concentrations, isotope composition, and collection information for NESQA fish tissue. $\delta^{202}Hg_{COR}$ values in parentheses represent the corrected value as performed using a 4.8 slope for sites with higher DOC contents as discussed in the SI methods.

Site abbreviation	Common name	Body part	HgT w.w.	δ^{202}Hg	1SD	Δ^{199}Hg	1SD	Δ^{200}Hg	1SD	Δ^{201}Hg	1SD	δ^{202}Hg cor	number of individuals in sample	mean specimen weight	median specimen total length
			ng g^{-1}	‰		‰		‰		‰		‰		g	mm
CT_Hubbard	Blacknose Dace	Whole	168.0	-0.94	0.05	0.57	0.1	0	0.03	0.36	0.04	-1.17	10	2	60.5
CT_Hubbard	Brook Trout	Skinless fillet	137.9	-0.74	0.03	0.45	0.03	0.05	0.02	0.29	0.02	-0.93	1	74	188
CT_Hubbard	Longnose Dace	Whole	223.6	-0.94	0.01	0.56	0.02	0	0.01	0.33	0.02	-1.17	10	4	67.5
CT_MillFair	Chain Pickerel	Skinless fillet	101.5	-0.51	0.02	0.69	0.01	0.03	0.01	0.48	0.02	-0.79	1	38.5	195
CT_MillFair	Longnose Dace	Whole	151.2	-0.71	0.03	0.71	0.04	0.04	0.01	0.45	0.01	-1	7	6	76
CT_MillFair	Tessellated Darter	Whole	87.3	-0.52	0.05	0.69	0.03	0.04	0.04	0.45	0.02	-0.8	4	2	59
CT_Rippowam	Blacknose Dace	Whole	129.1	-0.44	0.02	0.48	0.02	0.06	0.02	0.32	0.04	-0.64	10	4	67
CT_Rippowam	Redbreast Sunfish	Whole	81.9	-0.48	0.02	0.38	0.02	0.04	0.01	0.29	0.04	-0.64	5	20	102
CT_Rippowam	Redbreast Sunfish	Skinless fillet	365.4	-0.5	0.01	0.47	0.02	0.02	0.01	0.3	0.02	-0.69	1	88	158
MA_Charles	Fallfish	Whole	120.7	-0.02	0.03	0.44	0.01	0.04	0.02	0.3	0.04	-0.20 (-0.12)	13	14	114
MA_Charles	Largemouth Bass	Skinless fillet	448.8	-0.12	0.03	0.27	0.03	0.02	0.01	0.18	0.02	-0.23 (-0.18)	1	397	293
MA_Charles	Largemouth Bass	Skinless fillet	774.7	-0.13	0.05	0.31	0.03	0.02	0.02	0.21	0.03	-0.26 (-0.20)	1	748	395
MA_Ipswich	Largemouth Bass	Skinless fillet	136.6	-0.5	0.03	0.33	0.01	0.04	0.01	0.17	0.01	-0.64 (-0.57)	1	43.3	147
MA_Ipswich	Largemouth Bass	Skinless fillet	171.2	-0.42	0.04	0.37	0.01	0.02	0.02	0.22	0.02	-0.57 (-0.49)	1	42.1	146
MA_Ipswich	Redbreast Sunfish	Whole	78.0	-0.6	0.02	0.15	0.02	-0.02	0.02	0.04	0.03	-0.66 (-0.63)	2	19	99.5
MA_Neponset	Fallfish	Whole	76.7	-0.16	0.02	0.66	0.02	0.03	0.03	0.44	0.02	-0.43	10	14	108.5
MA_Neponset	Largemouth Bass	Skinless fillet	195.4	-0.29	0.02	0.47	0.06	0.01	0.01	0.3	0.02	-0.49	1	40.2	146
MA_OldSwamp	Bluegill	Whole	19.5	-0.57	0.02	0.36	0.02	-0.03	0.04	0.27	0.02	-0.72	2	1	37.5
MA_OldSwamp	Brook Trout	Skinless fillet	91.2	-0.7	0.03	-0.05	0.02	0.03	0.01	-0.06	0.05	-0.68	1	269	283
MA_OldSwamp	Tessellated Darter	Whole	100.1	-0.75	0.04	0.03	0.07	0.01	0.04	-0.06	0.04	-0.76	9	1	46
MA_Stillwater	Fallfish	Whole	96.3	-0.92	0.01	0.3	0.01	-0.02	0.02	0.2	0.02	-1.05	10	5	82.5
MA_Stillwater	Largemouth Bass	Skinless fillet	214.8	-0.73	0.02	0.63	0.01	0.04	0.02	0.36	0.04	-0.99	1	27.7	128
MA_WBSwift	Blacknose Dace	Whole	317.4	-1.04	0.04	0.1	0.03	0.04	0.02	0.03	0.05	-1.09	10	3	68
MA_WBSwift	Blacknose Dace	Whole	337.5	-1.07	0.03	0.15	0.02	0.02	0.02	0.02	0.02	-1.13	10	3	63.5
MA_WBSwift	Brook Trout	Skinless fillet	155.8	-0.93	0.02	0.14	0.02	0.06	0.01	0.04	0.01	-0.99	1	49.3	172
MA_WBSwift	Brook Trout	Skinless fillet	201.6	-0.97	0.02	0.01	0.01	0.03	0.01	-0.1	0.01	-0.97	1	45.8	166
NH_Beaver	Blacknose Dace	Whole	139.1	-0.65	0.04	0.5	0.02	0.03	0.01	0.32	0.01	-0.86	10	2	57.5
NH_Beaver	Smallmouth Bass	Skinless fillet	219.6	-0.64	0.03	0.51	0.02	0.01	0.01	0.32	0.02	-0.85	1	89	194
NH_Beaver	Smallmouth Bass	Skinless fillet	321.9	-0.58	0.03	0.52	0.03	0.03	0.01	0.3	0.02	-0.8	1	43	149
NY_Allen	Brown Trout	Skinless fillet	28.9	-0.68	0.03	0.25	0.04	-0.01	0.03	0.21	0.03	-0.78 (-0.73)	1	70	194
NY_Allen	Longnose Dace	Whole	43.6	-0.44	0.02	0.47	0.05	0.03	0.03	0.35	0.07	-0.63 (-0.53)	10	5	85
NY_Allen	Rainbow Trout	Skinless fillet	19.7	-0.24	0.03	0.53	0.04	0.12	0.02	0.43	0.06	-0.45 (-0.35)	1	31.7	139
NY_Allen	Rainbow Trout	Skinless fillet	27.1	-0.57	0.07	0.42	0.05	0.05	0.03	0.36	0.02	-0.74 (-0.66)	1	31.7	140
NY_Allen	Rainbow Trout	Skinless fillet	27.8	-0.52	0.09	0.42	0.04	0.03	0.03	0.27	0.01	-0.69 (-0.61)	1	54.1	172
NY_Bronx	Blacknose Dace	Whole	52.4	-0.1	0.04	0.47	0.04	0.05	0.02	0.43	0.03	-0.3	10	3	67
NY_Bronx	Redbreast Sunfish	Skinless fillet	55.9	-0.05	0.03	0.5	0.02	0.06	0.01	0.38	0.02	-0.26	1	73	144
NY_Bronx	Tessellated Darter	Whole	39.2	-0.03	0.05	0.51	0.01	0.02	0.03	0.31	0.03	-0.24	10	3	68
NY_Casper	Redbreast Sunfish	Skinless fillet	126.2	-0.37	0.03	0.34	0.02	0.05	0.02	0.34	0.03	-0.51	1	92	157
NY_Casper	Tessellated Darter	Whole	51.3	-0.35	0.04	0.37	0.01	0.1	0.01	0.3	0.06	-0.51	16	3	64.5
NY_Cross	Largemouth Bass	Skinless fillet	249.4	-0.91	0.01	0.27	0.01	0.05	0.01	0.11	0.01	-1.02	1	23.8	122
NY_Cross	Longnose Dace	Whole	292.9	-1.1	0.02	0.02	0.01	-0.02	0.01	-0.05	0.02	-1.11	9	6	72
NY_Cross	Tessellated Darter	Whole	240.6	-1.07	0.01	0.03	0.01	0.02	0.02	-0.01	0.04	-1.08	10	3	66
NY_Hallocks	Blacknose Dace	Whole	65.8	-0.6	0.03	0.38	0.03	0.02	0.04	0.24	0.04	-0.76 (-0.68)	10	2	58.5
NY_Hallocks	Creek Chub	Skinless fillet	90.6	-0.64	0.03	0.3	0.03	0.04	0.01	0.18	0.02	-0.76 (-0.70)	1	74	187
NY_Hallocks	Longnose Dace	Whole	71.7	-0.51	0.02	0.38	0.01	0.05	0.02	0.21	0.03	-0.67 (-0.59)	10	4	74.5
NY_Hallocks	Tessellated Darter	Whole	43.0	-0.65	0.02	0.34	0.01	0	0.03	0.15	0.04	-0.79 (-0.72)	10	3	63
NY_LBeaver	Blacknose Dace	Whole	139.4	-0.71	0.03	0.94	0.04	0.02	0.05	0.66	0.06	-1.1	6	2	64
NY_LBeaver	Brown Trout	Skinless fillet	151.5	-0.64	0.02	0.88	0.02	0.04	0.01	0.63	0.02	-1	1	113	220
NY_LeyCreek	Green Sunfish	Skinless fillet	44.6	-0.38	0.04	0.34	0.03	0.01	0.03	0.24	0.04	-0.52	1	48.7	126
NY_LeyCreek	Longnose Dace	Whole	95.0	-0.53	0.02	0.39	0.01	0.04	0.02	0.27	0.04	-0.69	10	5	82
NY_LeyCreek	Longnose Dace	Whole	95.4	-0.52	0.02	0.39	0.04	0.03	0.04	0.25	0.08	-0.68	10	5	80
NY_LeyCreek	Tessellated Darter	Whole	63.2	-0.44	0.02	0.33	0.01	0.06	0.01	0.33	0.04	-0.57	14	3	68.5
NY_LeyCreek	Tessellated Darter	Whole	71.3	-0.05	0.06	0.45	0.01	0.04	0.02	0.35	0.04	-0.24	13	3	71
NY_Mamaroneck	Redbreast Sunfish	Whole	121.6	-0.18	0.04	0.44	0.02	0.02	0.02	0.36	0.03	-0.36	4	37	129.5
NY_Mamaroneck	Redbreast Sunfish	Skinless fillet	107.3	-0.07	0.01	0.47	0.02	0.03	0.02	0.35	0.02	-0.26	1	110	168
NY_Mamaroneck	Tessellated Darter	Whole	74.4	-0.11	0.02	0.41	0.01	0.02	0.01	0.38	0.05	-0.27	5	5	79
NY_Muscoot	Blacknose Dace	Whole	240.1	-0.71	0.03	0.13	0.03	0.03	0.02	0.02	0.02	-0.76 (-0.74)	9	4	75
NY_Muscoot	Redfin Pickerel	Skinless fillet	153.1	-0.81	0.01	0.15	0.03	0.05	0.02	0.05	0.02	-0.87 (-0.84)	1	47.4	185
NY_Muscoot	Tessellated Darter	Whole	132.9	-0.72	0.05	0.11	0.02	0.04	0.04	0	0.01	-0.76 (-0.73)	10	3	65.5
NY_Sawmill	Blacknose Dace	Whole	67.3	-0.24	0.02	0.47	0.02	0.05	0.02	0.33	0.04	-0.43	7	4	64
NY_Sawmill	Longnose Dace	Whole	43.0	-0.26	0.02	0.47	0.02	0.01	0.03	0.43	0.03	-0.46	10	5	76
NY_Sawmill	Redbreast Sunfish	Skinless fillet	61.3	-0.23	0.02	0.42	0.02	0.02	0.01	0.31	0.02	-0.41	1	97	155
NY_Sawmill	Redbreast Sunfish	Skinless fillet	132.7	-0.33	0.03	0.26	0.01	0.01	0.01	0.34	0.03	-0.44	1	119	171
NY_Scriba	Cutlips Minnow	Whole	75.7	-0.81	0.06	0.17	0.04	0.04	0.03	0.1	0.03	-0.88 (-0.85)	10	12	102
NY_Scriba	Rock Bass	Skinless fillet	197.0	-0.94	0.05	0.57	0.1	0	0.03	0.36	0.04	-1.17 (-1.06)	1	109	167
NY_Thomas	Brown Trout	Skinless fillet	58.6	-0.47	0.05	0.36	0.01	0	0.02	0.26	0.03	-0.61	1	128	227
NY_Thomas	Longnose Dace	Whole	48.8	-0.24	0.02	0.41	0.01	0.07	0.01	0.3	0.03	-0.41	12	5	78
VT_Saxtons	Blacknose Dace	Whole	36.6	-0.96	0.06	0.98	0.03	-0.02	0.03	0.93	0.02	-1.36	10	2	53
VT_Saxtons	Rainbow Trout	Skinless fillet	35.2	-0.44	0.02	0.88	0.05	0.04	0.01	0.72	0.03	-0.8	1	224	293

Table S3: Median water chemistry parameters from weekly NESQA sampling sites between July 18th and August 5th, 2016 (n = 3). All raw data are publicly accessible by sampling site on the USGS National Water Information System (NWIS; https://waterdata.usgs.gov/nwis). Maximum parameters are listed in parentheses for variables directly tested in correlation analyses.

Site abbreviation	unfiltered MeHg	unfiltered HgT	dissolved organic carbon	ultraviolet absorbance at 254nm	specific ultraviolet absorbance at 254nm	percent unfiltered MeHg	chloride	sulfate	pH	Total nitrogen	Total phosphorous
	ng L^{-1}	ng L^{-1}	mg L^{-1}	abs cm^{-1}	L mg C^{-1} m^{-1}	%	mg L^{-1}	mg L^{-1}		mg L^{-1}	mg L^{-1}
CT_Hubbard	0.06 (0.07)	1.00 (1.21)	3.12 (3.25)	0.049 (0.054)	1.62 (1.73)	6 (7)	7.63 (8.11)	3.92 (4.27)	7.1	0.31	0.01
CT_MillFair	0.05 (0.06)	0.48 (0.52)	3.08 (4.46)	0.061 (0.09)	1.98 (2.96)	13 (14)	50.30 (51.57)	11.50 (11.53)	6.81	0.69	0.03
CT_Rippowam	0.05 (0.10)	0.67 (1.99)	3.36 (7.76)	0.075 (0.107)	2 (2.23)	7 (8)	154.10 (183.32)	16.62 (18.02)	7.3	0.93	0.06
MA_Charles	0.18 (0.22)	1.87 (2.48)	6.24 (6.36)	0.156 (0.193)	2.61 (3.09)	9 (11)	228.45 (250.56)	8.70 (10.02)	7.59	0.96	0.04
MA_Ipswich	0.07 (0.08)	0.56 (0.57)	8.09 (8.71)	0.191 (0.201)	2.31 (2.47)	12 (14)	204.72 (209.27)	6.26 (6.71)	7.06	0.55	0.03
MA_Neponset	0.12 (0.14)	2.28 (2.75)	4.36 (4.52)	0.107 (0.108)	2.43 (2.59)	5 (8)	164.27 (167.90)	6.48 (6.74)	7.22	0.55	0.03
MA_OldSwamp	0.12 (0.14)	1.55 (1.78)	4.11 (4.51)	0.132 (0.143)	3.17 (3.6)	7 (9)	243.14 (259.84)	11.80 (12.65)	6.51	0.78	0.05
MA_Stillwater	0.21 (0.23)	0.67 (0.76)	2.95 (4.86)	0.052 (0.057)	1.76 (2.82)	31 (37)	68.74 (71.46)	10.88 (10.91)	6.94	0.29	0.02
MA_WBSwift	0.19 (0.36)	1.19 (3.19)	2.61 (4.77)	0.05 (0.117)	2.28 (2.45)	16 (18)	23.41 (26.23)	5.27 (5.62)	6.61	0.30	0.01
NH_Beaver	0.14 (0.14)	0.86 (0.93)	4.32 (4.65)	0.104 (0.119)	2.38 (2.87)	16 (16)	121.12 (148.90)	9.44 (11.08)	6.95	0.49	0.02
NY_Allen	0.06 (0.06)	2.40 (2.40)	7.2 (15.6)	0.102 (0.139)	1.43 (1.93)	3 (3)	116.91 (166.84)	69.45 (83.14)	7.87	1.07	0.09
NY_Bronx	0.06 (0.06)	1.26 (2.46)	4.63 (4.63)	0.07 (0.094)	2.03 (2.08)	5 (6)	215.99 (269.96)	20.34 (22.15)	7.4	1.19	0.12
NY_Casper	0.09 (0.11)	1.53 (3.42)	4.62 (4.63)	0.098 (0.115)	2.12 (2.48)	6 (7)	139.39 (167.23)	19.96 (30.52)	8.05	0.90	0.11
NY_Cross	0.34 (0.42)	1.57 (2.06)	5.09 (6.37)	0.149 (0.17)	2.92 (2.93)	21 (27)	41.41 (51.59)	4.87 (4.95)	7.52	0.62	0.08
NY_Hallocks	0.06 (0.12)	0.70 (0.92)	6.61 (8.11)	0.124 (0.273)	2.39 (3.37)	9 (13)	137.69 (217.71)	7.08 (10.84)	7.83	0.80	0.09
NY_LBeaver	0.06 (0.28)	0.80 (1.34)	2.86 (3.33)	0.072 (0.085)	2.55 (3.21)	8 (21)	10.85 (10.95)	3.67 (3.82)	7.53	0.19	0.02
NY_LeyCreek	0.1 (0.10)	2.04 (3.91)	5.7 (7.42)	0.119 (0.127)	1.71 (2.19)	5 (7)	218.25 (330.46)	211.51 (372.24)	7.56	0.79	0.06
NY_Mamaroneck	0.05 (0.05)	1.66 (1.94)	5.14 (6.22)	0.109 (0.111)	2.16 (2.20)	3 (3)	184.55 (226.62)	12.64 (16.68)	7.1	1.15	0.10
NY_Muscoot	0.63 (0.86)	1.45 (1.51)	6.8 (8.49)	0.2185 (0.276)	2.91 (3.25)	43 (57)	160.97 (181.53)	6.20 (8.735)	6.97	0.78	0.15
NY_SawMill	0.11 (0.16)	10.2 (10.50)	4.55 (5.56)	0.108 (0.126)	2.27 (2.37)	2 (2)	147.397 (204.33)	14.66 (16.90)	7.9	1.04	0.14
NY_Scriba	0.16 (0.21)	0.93 (1.39)	6.11 (8.10)	0.149 (0.218)	2.44 (2.69)	16 (17)	12.57 (14.91)	2.92 (3.15)	7.4	0.38	0.02
NY_Thomas	<0.04 (0.20)	1.40 (7.61)	9.21 (9.88)	0.065 (0.180)	1.82 (1.86)	1 (2)	108.75 (130.31)	238.12 (326.14)	7.98	0.62	0.08
VT_Saxtons	0.09 (0.10)	0.64 (2.06)	2.21 (4.49)	0.045 (0.109)	2.04 (2.43)	4 (20)	13.48 (16.29)	4.33 (4.93)	8.52	0.18	0.02

Table S4: Mercury concentrations and isotope compositions for NESQA sediments

Site	HgT	MeHg	% MeHg	δ^{202}Hg	1SD	Δ^{199}Hg	1SD	Δ^{200}Hg	1SD	LOI
	ng g^{-1}	ng g^{-1}	%	‰		‰		‰		%
NY_Allen	60.0	0.9	0.02	-0.66	0.05	0.06	0.04	0.00	0.04	7.58
NH_Beaver	20.0	0.97	0.05	-1.06	0.04	-0.16	0.04	-0.01	0.04	7.4
NY_Bronx	60.0	0.61	0.01	-0.72	0.05	0.01	0.03	-0.04	0.04	2.41
NY_Casper	42.0	0.42	0.01	-0.78	0.04	-0.01	0.05	0.00	0.04	4.04
MA_Charles	120.0	1.23	0.01	-0.26	0.02	-0.04	0.01	-0.02	0.02	4.43
NY_Cross	13.0	1.06	0.08	-1.03	0.08	-0.22	0.06	0.02	0.05	3.72
NY_Hallocks	16.0	0.18	0.01	-1.13	0.1	-0.1	0.06	0.01	0.03	4.65
CT_Hubbard	8.5	1.11	0.13	-1.23	0.08	-0.33	0.03	-0.06	0.04	6.81
MA_Ipswich	100.0	2.57	0.03	-0.79	0.06	-0.16	0.04	0.03	0.02	33.2
NY_LeyCreek	130.0	1.33	0.01	-0.42	0.06	0.03	0.08	0.03	0.02	6.0
NY_LBeaver	15.0	0.17	0.01	-0.87	0.08	-0.24	0.07	-0.02	0.06	2.0
NY_Mamaroneck	46.0	0.31	0.01	-0.34	0.03	0.08	0.03	0.02	0.03	2.23
CT_MillFair	38.0	0.25	0.01	-1.03	0.04	-0.12	0.03	-0.01	0.01	2.89
NY_Muscoot	22.0	0.21	0.01	-0.59	0.08	-0.01	0.05	0.01	0.03	4.53
MA_Neponset	1500.0	10.4	0.01	-0.59	0.02	-0.01	0.03	-0.03	0.01	31.5
MA_OldSwamp	160.0	2.16	0.01	-0.92	0.06	-0.15	0.08	-0.03	0.01	5.06
CT_Rippowam	140.0	4.34	0.03	-0.69	0.06	-0.1	0.08	0.04	0.03	29.8
NY_SawMill	220.0	1.49	0.01	-0.51	0.06	-0.02	0.09	-0.01	0.03	7.97
VT_Saxtons	21.0	2.58	0.12	-1.28	0.06	-0.24	0.02	0.05	0.04	4.03
NY_Scriba	9.1	0.17	0.02	-1.27	0.1	-0.32	0.04	0.02	0.02	1.63
MA_Stillwater	54.0	3.44	0.06	-1.17	0.04	-0.3	0.03	-0.01	0.03	20.2
NY_Thomas	96.0	1.43	0.01	-0.63	0.05	0.06	0.04	0.04	0.02	14.6
MA_WBSwift	84.0	17.8	0.21	-1.24	0.06	-0.29	0.03	-0.01	0.04	9.64

Table S5: Spearman rank correlation results of Hg isotopes in prey fish, game fish, and bed sediments (Bsed) with all environmental variables and PCA results. ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns $p > 0.05$. Percent natural refers to the sum of forest, shrub, and wetland land cover. Bolded variables are included in PCA analysis.

Variable name (units)	Median (min-max)	Correlation with $\delta^{202}Hg_{cor}$			Correlation with $\Delta^{199}Hg$		
		Prey fish	Game fish	Bed sed	Prey fish	Game fish	Bed sed
Bed sediment variables							
$\delta^{202}Hg$ (‰)	-0.79 (-1.28 - -0.26)	0.86****	0.76****	---	ns	ns	0.83****
$\Delta^{199}Hg$ (‰)	-0.10 (-0.33 – 0.08)	0.87****	0.80****	0.83****	ns	ns	---
Total Hg (ng/g dw)	54 (8.5 – 1500)	0.61**	0.68***	0.61**	ns	ns	0.49*
Total Hg normalized to loss on ignition (ng/g LOI)	7.5 (1.25 – 47.6)	0.58**	0.65***	0.57**	ns	ns	0.53**
Methylmercury (ng/g dw)	1.11 (0.17 – 17.8)	ns	ns	ns	ns	ns	ns
Methylmercury normalized to loss on ignition (ng/g LOI)	0.15 (0.04 – 1.85)	ns	ns	ns	ns	ns	ns
Methylmercury: Total Hg (%)	1.4 (0.7 – 21.2)	-0.68***	-0.66***	-0.72***	ns	ns	-0.69***
Atmospheric deposition variables							
Mean Atmos Hg Total Deposition, (ug/ m^2)	20.52 (15.10-34.59)	0.70***	0.68***	0.58**	ns	ns	0.62**
Mean Atmos Hg Dry Depostion, (ug/ m^2)	13.86 (9.27-27.63)	0.61**	0.63**	0.52*	ns	ns	0.53**
Mean Atmos Wet Deposition (ug/ m^2)	6.32 (5.05-10.29)	0.53**	0.45*	0.41*	ns	ns	0.44**
Watershed and site variables							
Urban (%)	39 (0.95 – 80)	0.78****	0.83****	0.68***	ns	ns	0.78****
Natural* (%)	50 (16 – 96)	-0.81****	-0.78****	-0.67***	ns	ns	-0.88****
Industrial/military (%)	0.52 (0 – 15.1)	0.74****	0.84****	0.67***	ns	ns	0.70***
Forest and shrub (%)	36 (10 - 91)	-0.77****	-0.74****	-0.64***	ns	ns	-0.82****
Road density (km road/100 km^2)	9.7 (2.0 - 23.9)	0.64***	0.67***	0.54**	ns	ns	0.72***
Site elevation (m)	103 (2-206)	-0.59**	-0.64****	-0.43*	ns	ns	-0.49*
Drainage area (km2)	74.0 (12.0 – 187)	ns	ns	ns	0.42*	0.54**	ns
Channel width (m), md	11.3 (6.3 – 19.2)	ns	ns	ns	0.57**	0.56**	ns
Water quality variables							
Chloride (mg/L), md	138 (7.6 – 243)	0.76****	0.78****	0.71***	ns	-0.43*	0.63**
pH, md	7.4 (6.5 - 8.5)	ns	ns	ns	ns	ns	ns
Sulfate (mg/L) , md	9.4 (2.9 – 238)	0.70***	0.66***	0.58**	ns	ns	0.78****
Total nitrogen (mg/L), md	0.69 (0.18 - 1.19)	0.79****	0.73****	0.71***	ns	ns	0.79****
Dissolved Organic Carbon (mg/L) , md	4.62 (2.21 – 9.21)	0.60**	ns	0.51*	-0.52*	ns	0.57**
UVA_{254} (us/cm) ,md	0.104 (0.045 – 0.219)	ns	ns	0.42*	-0.54**	-0.52*	ns
SUVA (L/mgC/m), md	2.27 (1.43 - 3.17)	ns	ns	ns	ns	ns	ns
Unfiltered Methylmercury (ng/L) , md	0.09 (0.02 – 0.63)	ns	ns	ns	-0.49*	ns	ns
Unfiltered Total Hg (ng/L), md	1.26 (0.48 – 10.2)	0.56**	0.49*	0.67***	ns	-0.50*	0.58**
Unfiltered Methylmercury: Total Hg (%), md	7 (1 – 43)	-0.52*	-0.65***	-0.45*	-0.50*	ns	-0.59**
PCA Axes							
Axis 1: Urban Intensity Gradient	N/A	0.89****	0.83****	0.79****	ns	ns	0.87****
Axis 2: Methylation Gradient	N/A	ns	ns	ns	-0.63***	-0.44*	ns
Axis 3: Total Hg Gradient	N/A	ns	ns	ns	ns	ns	ns