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Mercury Cycling in Stream Ecosystems. 3. Trophic Dynamics and Methylmercury Bioaccumulation

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Trophic dynamics (community composition and feeding relationships) have been identified as important drivers of methylmercury (MeHg) bioaccumulation in lakes, reservoirs, and marine ecosystems. The relative importance of trophic dynamics and geochemical controls on MeHg bioaccumulation in streams, however, remains poorly characterized. MeHg bioaccumulation was evaluated in eight stream ecosystems across the United States (Oregon, Wisconsin, and Florida) spanning large ranges in climate, landscape characteristics, atmospheric Hg deposition, and stream chemistry. Across all geographic regions and all streams, concentrations of total Hg (THg) in top predator fish and forage fish, and MeHg in invertebrates, were strongly positively correlated to concentrations of filtered THg (FTHg), filtered MeHg (FMeHg), and dissolved organic carbon (DOC); to DOC complexity (as measured by specific ultraviolet absorbance); and to percent wetland in the stream basins. Correlations were strongest for nonurban streams. Although regressions of log[Hg] versus δ^{15}N indicate that Hg in biota increased significantly with increasing trophic position within seven of eight individual streams, Hg concentrations in top predator fish (including cutthroat, rainbow, and brown trout; green sunfish; and largemouth bass) were not strongly influenced by differences in relative trophic position. Slopes of log[Hg] versus δ^{15}N, an indicator of the efficiency of trophic enrichment, ranged from 0.14 to 0.27 for all streams. These data suggest that, across the large ranges in FTHg (0.14–14.2 ng L^{-1}), FMeHg (0.023–1.03 ng L^{-1}), and DOC (0.50–61.0 mg L^{-1}) found in this study, Hg contamination in top predator fish in streams likely is dominated by the amount of MeHg available for uptake at the base of the food web rather than by differences in the trophic position of top predator fish.

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Introduction

Because exposure to and uptake of mercury by fauna in aquatic ecosystems is primarily dietary (*1, 2*), Hg bioaccumulation must be evaluated in terms of (1) the quantity and chemical forms of Hg available to the base of aquatic food webs (*3, 4*) and (2) its transfer to successively higher trophic levels (*5*). The physicochemical factors that mediate Hg source, bioavailability, and bioaccumulation have been investigated primarily in lakes and reservoirs, where elevated Hg levels in biota have been associated with wetlands, fluctuating water levels, low pH, and high levels of dissolved organic carbon (DOC). Studies of trophic transfer of Hg in lakes with similar environmental settings and Hg loading rates suggest that both community complexity and the trophic pathway (pelagic versus benthic) drive the extent of methylmercury (MeHg) accumulation in top consumers, such as piscivorous fish (*6–8*). The apparent association of the degree of MeHg bioaccumulation in aquatic biota with community structure and function may be driven by food chain length, efficiency of the trophic transfer of Hg, or differences in the supply and availability of Hg. Studies conducted by Vander Zanden and Rasmussen (*8*) and Stewart et al. (*9*) have demonstrated that, for several lakes and reservoirs, the amount of Hg available to the food web base was the best predictor of Hg in top predator fish for both pelagic and benthic food webs. Recent stream studies have suggested that processes associated with mercury bioaccumulation in stream ecosystems may be similar to those in lakes (*10, 11*), but these studies have been limited in scope with respect to spatial and temporal coverage, environmental setting, and ecology. More extensive temporal and spatial studies are necessary to better understand the relative roles of community complexity, trophic pathway, and water and sediment geochemistry to MeHg bioaccumulation in stream ecosystems.

Streams are often more responsive than lakes to seasonality and local physical disturbance. Large fluctuations in flow, water chemistry, and bed sediment redox conditions in stream ecosystems make it difficult to associate discrete bed sediment and water samples with biota that integrate Hg over varying ranges of time and space (*12, 13*). Additional uncertainties in interpreting Hg concentrations in higher trophic level fish in streams may be generated by intraspecific differences in life stage and physical condition, high levels of omnivory and opportunistic feeding, and migration (*14*).

We evaluated Hg concentrations in aquatic biota in relation to water chemistry over a period of 3 years in streams spanning a range of environmental conditions—including atmospheric Hg deposition, contributing area of wetlands, amount of urbanization, and productivity—across the United States. This allowed us to assess seasonal and interannual variability in chemical constituents of interest [such as total Hg (THg), MeHg, DOC, nutrients, major ions] and to estimate the integrated environmental Hg exposure to aquatic biota. We also analyzed Hg concentrations and stable C and N isotopes [as indicators of carbon source (δ^{13}C) and trophic position (δ^{15}N)] in the tissue of aquatic biota to establish a temporally and spatially relevant assessment of food web complexity and trophic transfer of MeHg. The general objective of this study was to assess the relative roles of geochemistry and ecology in determining MeHg bioaccumulation in stream ecosystems. More specifically, we examined Hg in aquatic organisms spanning a range of trophic positions to determine the relative importance of fluvial MeHg concentrations and food-web structure among sites.

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TABLE 1. Study Streams, Basin Characteristics (*16*), and Selected Chemistry [mean(coefficient of variation)] for All Stream Water and Biological Samples Collected during 2002−2004[a]

Stream Name	Basin Area (km²)	Wetland %	Urban %	N_{sw}	FMeHg (ng L^{-1})[b]	FTHg (ng L^{-1})	DOC (mg L^{-1})	SUVA (L mgC-m^{-1})	PTHg (ng L^{-1})	PMeHg (ng L^{-1})	N_{inv}	Invertebrate MeHg (ng g^{-1}, dry wt.)	N_{ff}	Forage Fish THg (ng g^{-1}, dry wt.)	N_{pf}	Predator Fish THg (ng g^{-1}, dry wt.)	Predator Fish THg (mg kg^{-1}, wet wt.)
Lookout Creek, OR	62.4	0.00	0.00	40	0.023	0.648 (79.4)	0.937 (44.7)	2.50 (26.2)	0.309 (332)	0.0103 (17.7)	12	5.52 (63.9)	15	47.4 (68.4)	13	105 (30.9)	0.0185 (34.6)
Beaverton Creek, OR	95.6	0.30	85.8	39	0.042	1.40 (54.9)	4.43 (17.7)	2.95 (11.4)	1.59 (114)	0.0232 (81.2)	15	59.8 (59.6)	17	227 (42.8)	7	458 (48.7)	0.0881 (67.2)
Pike River, WI	660	17.9	4.80	39	0.104	1.81 (80.9)	7.74 (54.4)	3.70 (11.8)	0.947 (127)	0.0352 (114)	11	56.5 (40.8)	12	376 (34.3)	15	450 (37.0)	0.0970 (38.3)
Evergreen River, WI	167	13.4	4.17	38	0.053	1.09 (118)	4.74 (76.2)	3.05 (18.1)	1.26 (80.0)	0.0825 (59.9)	15	117 (114)	12	251 (39.5)	11	434 (25.0)	0.0973 (24.6)
Oak Creek, WI	64.8	3.50	58.9	33	0.057	1.35 (68.6)	6.65 (29.8)	3.05 (18.6)	3.84 (139)	0.0912 (122)	12	29.9 (43.9)	9	144 (24.3)	12	325 (30.7)	0.0628 (29.2)
St. Marys River, FL	1810	35.6	5.02	38	0.390	5.74 (47.0)	37.5 (37.7)	4.71 (6.14)	0.871 (76.7)	0.0381 (63.2)	27	157 (67.3)	16	723 (29.9)	10	5970 (26.8)	1.18 (26.7)
Santa Fe River, FL	2640	17.9	6.16	30	0.228	2.53 (117)	11.3 (103)	4.12 (21.2)	0.540 (82.6)	0.0358 (50.8)	32	183 (97.7)	24	525 (50.3)	15	2210 (51.2)	0.429 (48.4)
Little Wekiva River, FL	115	13.2	77.3	40	0.079	0.650 (82.6)	5.06 (55.4)	2.87 (15.2)	0.826 (60.3)	0.0390 (70.4)	39	71.7 (101)	23	312 (37.2)	8	1690 (24.7)	0.336 (25.9)

[a] *N* represents numbers of individual samples for surface water (N_{sw}) and predator fish (N_{pf}) and numbers of spatial/temporal composites for invertebrates (N_{inv}) and forage fish (N_{ff}). Detailed stream water chemistry and species−specific biological data (including % moisture for wet weight THg determinations) are presented in refs *22 and 27* and Supporting Information Tables S1 and S2. [b] For streams with values of FMeHg < method detection limit (MDL), means were calculated as maximum likelihood estimates (MLE; Supporting Information Table S3) (*15*), with the exception of Lookout Creek, OR, which had too few observations for the calculation of MLE. For Lookout Creek only, 0.5 MDL was substituted for values < MDL.

Experimental Design

Study areas were located in Oregon, Wisconsin, and Florida. Eight streams (one urban and one or two nonurban in each study area) were selected to represent wide ranges in basin size, landscape type, streamwater chemistry (Table 1; Supporting Information Table S1), and atmospheric Hg loading (*15, 16*). Nonurban streams were selected to represent a range in wetland coverage (Table 1) within each geographic study area. Urban sites (Portland, OR; Milwaukee, WI; Orlando, FL) were characterized as areas of rapid growth (*17*).

Naturally occurring stable isotopes (δ^{13}C, δ^{15}N) were used to identify carbon sources and describe site-specific feeding relationships for aquatic biota. However, while stable C and N isotope ratios have been used successfully in defining linkages between trophic dynamics and contaminant bioaccumulation in lakes and marine systems (*5, 18*), their application in stream systems—where source carbon and nitrogen isotopic ratios change over small spatial and temporal scales (*19, 20*)—has been limited. In continuous linear systems, such as streams, the use of stable C and N isotopes as diet indicators must be grounded by information on differences in source (food-web base) isotopic ratios both within and among systems. In the current study, bioaccumulation of Hg with successively higher trophic levels was evaluated using stream-specific regressions of log[Hg] versus δ^{15}N for aquatic food webs after establishing differences in base-level production within and among streams.

Aquatic Biota. Biological protocols for sample collection and processing are summarized here; they are described in detail by Scudder et al. (*21*). In each stream, sampling focused on two species of aquatic macroinvertebrates (one herbivore and one detritivore or omnivore), two species of midtrophic-level forage fish (primarily omnivores), and one species of piscivorous top predator fish. Invertebrate herbivores (grazers) included snails and baetid mayfly larvae; herbivore/detritivores and omnivores (grazer-gatherers) included amphipods, midge larvae, ephemerellid mayfly larvae, caddisfly larvae, grass shrimp, and crayfish. It was not possible to collect (1) sufficient numbers of the same invertebrate taxa across all seasons within specific streams or (2) the same taxa among all streams. Forage fish included mosquitofish, shiners, killifish, chubs, sculpin, dace, and juvenile sunfish. Top predators included largemouth bass, trout (cutthroat, rainbow, and brown), and green sunfish. These specific taxa were targeted in an attempt to collect species that were trophically linked and functionally equivalent within each trophic category (invertebrates, forage fish, and predator fish) across all streams, and selections were based on existing local or regional literature describing community structure, diet, and life history (*22*).

The timing, frequency, and intensity of biological sampling for each trophic category were selected on the basis of estimates of lifespan and tissue turnover for each functional feeding group, that is, weeks to months for invertebrates, months to years for forage fish, and multiple years for top predator fish (*12, 23*). Lower trophic levels (invertebrates and forage fish) were collected in both the spring and fall of 2003 to capture seasonality in forage or prey items that top predators were likely consuming during the active summer season. Invertebrates [three composites of approximately 30−120 individuals each (per species), except 4−12 for larger crayfish] and forage fish (12−24 individuals per species) were collected during streamwater sampling visits. Most predator fish were collected once in the second or third year of the study (summer or fall of 2003 or 2004) to allow the association of fish Hg concentrations with preceding multiyear streamwater data. Size−age relationships developed by local resource-management agencies were used to guide the collection of predator fish in a 3- to 4-year age range (6−12 individuals per species). All biota were processed the day of collection (with no depuration period) and frozen until analysis. Forage fish were processed whole (minus head and gut tract), and top predator fish were filleted for analysis of skinless axial muscle. Sagittal otoliths were removed and submitted to the U.S. Geological Survey (USGS) Cooperative Wildlife Research Unit Laboratory at Clemson University in Clemson, South Carolina, for age determination.

Invertebrate composites were freeze-dried, pulverized, and analyzed for MeHg by the USGS Wisconsin Mercury Research Laboratory, Middleton, Wisconsin (*15*). Hg in fish tissue is predominantly MeHg [>95% (*24*)], so tissue samples from individual fish were freeze-dried, pulverized, and

analyzed for THg by combustion and atomic absorption using a direct Hg analyzer (Milestone DMA-80) at the Texas A&M University Trace Element Research Laboratory, College Station, Texas (*25*).

Subsamples of all freeze-dried and ground tissue were sent to the USGS National Research Program Isotopic Tracers Laboratory (Menlo Park, CA) and analyzed for $\delta^{13}C$ and $\delta^{15}N$ ratios using a Carlo Erba 1500 elemental analyzer (to convert organic C and N into CO_2 and N_2 gas) interfaced with an Micromass Optima continuous-flow isotope ratio mass spectrometer (*26*). All samples were standardized against Pee Dee Belemnite (C) or N_2 in air. Instrument precision was 0.1‰ for C and 0.2‰ for N on the basis of replicate analyses of standard reference materials.

Stream Water. Stream water was sampled at each site approximately 18 times per year during 2002−2004 using trace-metal clean techniques. Samples were analyzed for filtered THg and MeHg (FTHg and FMeHg), particulate THg and MeHg (PTHg and PMeHg), major ions, nutrients, suspended sediment, DOC concentration, specific UV absorbance at 254 nm {SUVA, [L/(mg C·m)]}, and other constituents. Detailed analytical methods and quality control data are provided in ref *27*.

Data Analysis. Invertebrate Hg concentrations are reported as MeHg (ng g^{-1}, dry weight); forage fish and top predator fish Hg concentrations are reported as THg (ng g^{-1}, dry weight) (*22*). Because forage fish were oversampled relative to invertebrates and predator fish, they were mathematically pooled into three to four composites of four to six randomly selected individuals per species per collection date. Predator fish integrate lower trophic level prey over time, so most comparisons of Hg and stable isotopes in biota were made across broad trophic categories (invertebrates, forage fish, predator fish). Summary statistics for Hg and for C and N isotope data are provided in Supporting Information Table S2. Statistical analyses were conducted in SAS 9.1.3 and Systat 12. Hg concentrations in invertebrates and forage fish were compared (by stream, taxa, and collection date) using Kruskal−Wallis and Mann−Whitney tests on ranks. Correlations among concentrations of Hg in biota and ecological and environmental variables were conducted using both nonparametric and parametric tests, including Spearman's Rho (ρ) and linear regression. Regressions of log[Hg] versus $\delta^{15}N$ for biota (trophic enrichment) were tested for homogeneity of slopes among sites using PROC GLM in SAS. Correlations between mean Hg values for each trophic category and mean values for other environmental parameters were tested using Kendall's Tau (τ) (*28*). Spearman and Kendall rank correlation analyses were performed on raw data; simple and multiple linear regressions used base-10 logarithm-transformed data for both Hg in biota and water chemistry parameters. All statistical tests were performed at a significance level of $p < 0.05$ unless otherwise stated.

Hg concentrations in biota from lower trophic levels were compared to streamwater Hg concentrations (FTHg, FMeHg) averaged over restricted time intervals: 60 days immediately preceding sampling for invertebrates and 1 year for forage fish. Top predator fish were collected only once and were compared to streamwater data for the period of study (2002−2004). These intervals were selected on the basis of the average ages of forage fish (1.1 years) and predator fish (3.2 years) collected, and on literature-based estimates of tissue turnover times (*23*).

Because FMeHg concentrations sometimes fell below the method detection limit (MDL; 0.04 ng L^{-1}), FMeHg means were calculated as maximum likelihood estimates (MLE) for all streams except Lookout Creek, Oregon (Supporting Information Table S3). It was not possible to calculate MLEs for FMeHg at Lookout Creek because of the large number of nondetects at this site, and 0.5 MDL was substituted for all values below the MDL.

For log[Hg] versus $\delta^{15}N$ in food webs, regressions were conducted on two data sets: (1) all data for all streams and (2) data restricted to samples that fell within food webs that were operationally defined by dietary dependence among top predator fish, forage fish, and invertebrates within each stream using measurements of $\delta^{13}C$ and $\delta^{15}N$. Because $\delta^{13}C$ is a relatively conservative natural tracer [typically expressing <1‰ enrichment for each trophic level (TL) increase] (*29*), it serves as a good indicator of dietary carbon. The upper and lower bounds of potential contributing forage or prey were calculated using

$$\delta^{13}C_{consumer} = [\delta^{13}C_{forage} + (1‰ \times TL)] \quad (1)$$

In addition, $\delta^{15}N$ is frequently used to calculate a consumer's trophic position above the food web base and discrete TLs (1−4, primary producers through tertiary consumers) because it expresses relatively consistent fractionation with increasing TL (typically ≈ 3.4 ‰ enrichment per TL) (*30, 31*):

$$TL = \Delta\delta^{15}N \div 3.4 \quad (2)$$

where

$$\Delta\delta^{15}N_{consumer} = \delta^{15}N_{consumer} - \delta^{15}N_{base} \quad (3)$$

In this study, invertebrates with the lowest combined Hg and $\delta^{15}N$ (glossosomatid and hydropsychid caddisfly, baetid mayfly, and chironomid larvae and amphipods; Supporting Information Figure S2) were used to establish $\delta^{15}N_{base}$ for each stream. Autotrophic production is generally considered to be the base of aquatic food webs; however, invertebrates are frequently used as a proxy because of the difficulty in obtaining pure algal samples and the high degree of variability in algal MeHg concentrations and stable isotopic signatures over small temporal and spatial scales (*32*).

Predators may integrate prey across large ranges of carbon and nitrogen isotope values, and some prey items may not have been sampled. For these reasons, the ranges of $\delta^{13}C$ for forage or prey linked to top predator fish in each stream were conservatively estimated using the most ^{13}C-depleted individual top predator fish (lower bound), the most ^{13}C-enriched individual (upper bound), and the most ^{15}N-enriched individual to establish $\Delta\delta^{15}N$ (Supporting Information Figure S1).

Results and Discussion

Hg in Stream Biota. For most streams, variability of Hg across trophic categories was generally higher for invertebrates (all taxa, all dates; CV = 40.8−114) than for forage fish (CV = 24.3−68.4) or predator fish (CV = 24.7−51.2; Table 1). This higher variability is due in part to seasonality: for example, in Florida streams, temporal differences in both Hg and $\delta_{15}N$ were significant for several taxa (grass shrimp, amphipods, net-spinning cadisfly larvae, and midges). Even when analyzed by feeding strategy (e.g., herbivore, omnivore, invertivore) and collection date, variability was still greater for invertebrates (CV = 27.0−115) than for forage or predator fish in most streams. Across all sites, Hg concentrations ranged from 2.08 to 644 ng g^{-1} MeHg for invertebrates, 15.6 to 1090 ng g^{-1} THg for midtrophic level forage fish, and 61.3 to 7810 ng g^{-1} THg for top predator fish (*22*). Although there was no consistent geographic pattern in Hg concentration for specific trophic categories among streams, concentrations for all three categories were significantly different across broad geographic regions: Florida > Wisconsin > Oregon (Table 1; Figure 1; Supporting Information Figure S2).

Role of Water Chemistry in Hg Bioaccumulation. Hg concentrations in the tissue of all three trophic categories

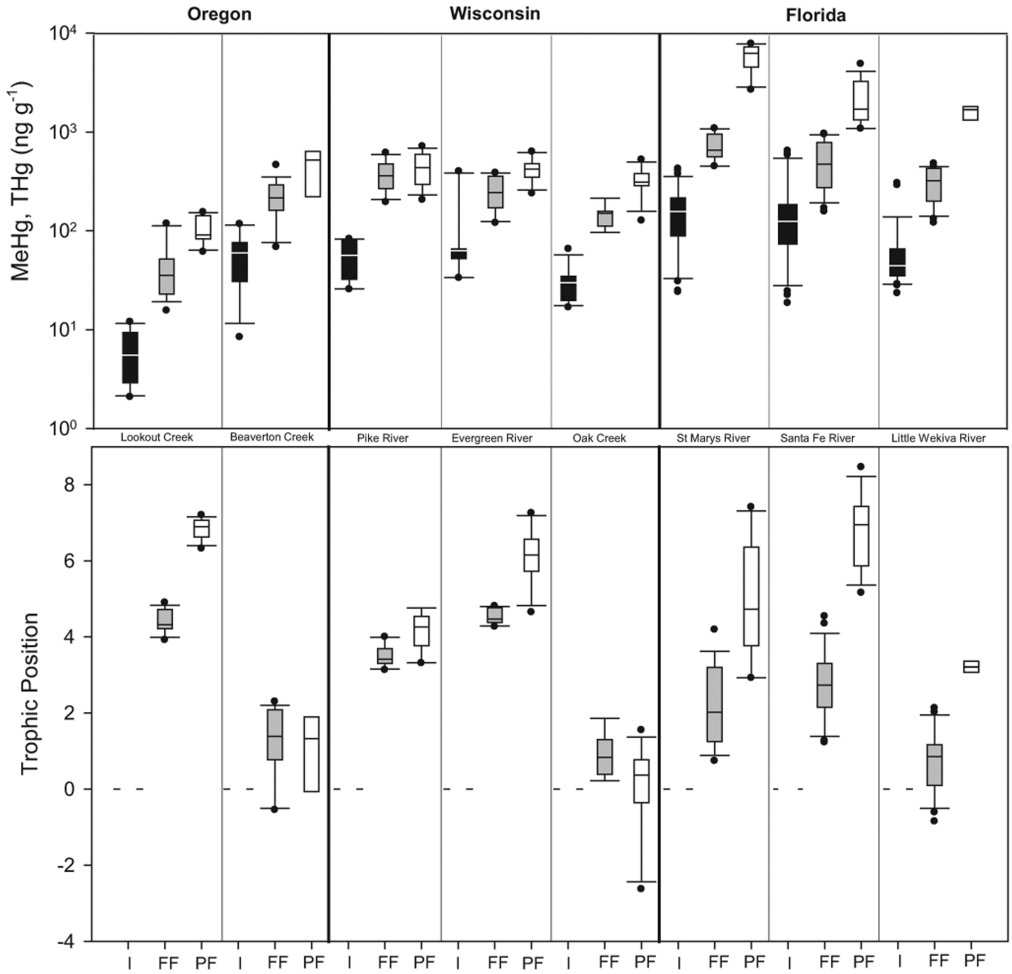

FIGURE 1. Biotic mercury (Hg) concentration and trophic position by geographic region, stream, and trophic category (black shading, I = invertebrate; gray shading, FF = forage fish; white shading, PF = predator fish). Invertebrate Hg concentrations are MeHg (ng g^{-1}, dry wt.); forage fish and predator fish concentrations are THg (ng g^{-1}, dry wt.). Trophic position of forage fish and predator fish is defined as $\Delta\delta^{15}N_{predatorfish\text{-}base}$, where base consumers included the lowest trophic level invertebrates in each stream (glossosomatid and hydropsychid caddisfly larvae, baetid mayfly larvae, chironomid larvae, and amphipods).

TABLE 2. Correlation of Tissue Hg Concentrations in Biota [MeHg (ng g^{-1}, dry wt.) for invertebrates and THg (ng g^{-1}, dry wt.) for forage and predator fish] to Selected Stream Water Constituents Across All Streams Based on Spearman's Rho [ρ (p value)][a]

trophic category	*N*	trophic position ($\Delta\delta^{15}N_{x\text{-}inv}$)	FMeHg (ng L^{-1})	FTHg (ng L^{-1})	PMeHg (ng g^{-1})	PTHg (ng g^{-1})	DOC (mg L^{-1})	SUVA (L mgC-m^{-1})	SS (mg L^{-1})
predator fish	90	0.05 (0.60)	**0.80 (<0.0001)**	**0.67 (<0.0001)**	**0.30 (0.003)**	−0.10 (0.34)	**0.74 (<0.0001)**	**0.79 (<0.0001)**	**0.43 (<0.0001)**
forage fish	128	−0.16 (0.080)	**0.79 (<0.0001)**	**0.70 (<0.0001)**	**0.38 (<0.0001)**	0.19 (0.83)	**0.72 (<0.0001)**	**0.80 (<0.0001)**	**0.53 (<0.0001)**
invertebrates	171	n/a	**0.59 (<0.0001)**	**0.55 (<0.0001)**	**0.33 (<0.0001)**	0.11 (0.14)	**0.53 (<0.0001)**	**0.63 (<0.0001)**	**0.43 (<0.0001)**

[a] Significant relations are indicated in bold.

were strongly and positively correlated to FTHg, FMeHg, and DOC (concentration and SUVA) and were less so to PMeHg and suspended sediment (SS; Table 2; Supporting Information Figure S3). Correlations were weakest for invertebrates and strongest for fish. As described previously, invertebrates probably reflect the biogeochemical variability associated with substrate type, flow, and redox conditions. Larger variability in Hg concentrations and weaker correlations with water chemistry in urban versus nonurban streams could also be associated with episodic inputs of wastewater and fertilizer in runoff (*19, 30*). Because fish are more mobile, larger, and longer-lived, they integrate this variability over greater temporal and spatial scales.

Many of the environmental variables tested exhibited multicollinearity, and stepwise linear regression analyses of log-transformed data suggested that FMeHg was the best single predictor of Hg in top predator fish when comparing among all streams ($r^2 = 0.76$):

$$\log[Hg_{predator\ fish}] = 3.6 + 1.1 \log[FMeHg] \quad (4)$$

This relationship was similar for Hg in forage fish among all streams ($r^2 = 0.50$):

$$\log[Hg_{forage\ fish}] = 3.2 + 0.8 \log[FMeHg] \quad (5)$$

Although FMeHg and DOC concentrations were strongly positively correlated across most sites (*15*), and DOC was

strongly correlated to predator fish Hg among all streams ($r^2 = 0.71$), DOC did not substantially improve either regression model. When regressions excluded extreme end-member streams—Lookout Creek (mean DOC = 0.94 mg L^{-1}) and St. Mary's River (mean DOC = 37 mg L^{-1})—Hg in predator fish remained strongly correlated to FMeHg ($r^2 = 0.51$) but not to DOC ($r^2 = 0.19$). Given the strong positive correlation of FMeHg with DOC across most streams (*15*), this lack of a consistent response of Hg in fish to these variables highlights the complexity of Hg bioaccumulation processes. Several recent studies have demonstrated the importance of not only DOC quantity (*33*) and quality [complexity (*34*); specific organic compounds (*35*)] to Hg bioavailability, but also most likely Hg speciation and partitioning in the water column (*15*).

Role of Food Web Processes in Hg Bioaccumulation. The basic relations between trophic level, $\delta^{15}N$, and Hg accumulation for stream biota were consistent with previous studies conducted in lakes and other aquatic ecosystems. Hg in biota increased with increasing trophic level for biota in all streams (Figure 2), with the exception of Oak Creek. Trophic position ($\Delta\delta^{15}N_{predator\text{-}base}$) for top predator fish was similar among nonurban sites (ranging from 4.2−6.8) and was lower for all urban streams (0.02−3.2). For all streams, slopes of Hg in biota versus trophic position (i.e., trophic transfer efficiency for Hg) ranged from 0.14 to 0.27 (Table 3); however, these slopes were not significantly different for six of the eight streams ($p < 0.122$), the exceptions being Evergreen River and Oak Creek. Regression slopes for most streams in this study were similar to slopes described for many other freshwater and marine food webs worldwide (*5, 36*).

Because of habitat heterogeneity in streams and localized feeding habits of some aquatic biota, $\delta^{15}N$ in consumers may be influenced by spatial differences in redox conditions as well as trophic level. Although the raw data (Table 3a; Figure 2) illustrate the ranges of all $\delta^{15}N$ values and tissue Hg concentrations sampled during the study, an operationally defined food web (i.e., trophically linked; Table 3b) should provide a more accurate representation of the actual trophic transfer of Hg. This approach removed high-leverage outliers and improved correlation coefficients for several sites (compare Table 3b to 3a). However, removing probable nondietary prey did not improve all regressions. Operationally defined regressions decreased the sample size and p value for Pike River (only 1 of 15 predator fish collected from this stream was apparently associated by diet to the prey organisms collected), but did not change the regression slope for Evergreen River, which was still significantly lower compared to all other streams. Reasons for the lower trophic transfer efficiency in Evergreen River are not apparent. The much lower slope for this stream could reflect unsampled prey or predators, the presence of migrating predators, lags in assimilation of Hg derived from rarely ingested or seasonally available prey, or a difference in trophic transfer efficiency associated with local geochemical and biological influences (*37, 38*). Bioaccumulation models for stream systems could be further improved by refining sample collections and employing more complex mixing models to estimate trophic relationships (*39, 40*).

Across the broad ranges of environmental conditions included in this study, Hg contamination in top predator fish was not strongly correlated to apparent trophic position among streams (Table 2; Spearman's $\rho = 0.05$, $p = 0.60$). This suggests that, among these systems, the supply of MeHg to the base of the food web is the strongest determinant of Hg in top-level predators (*7*), rather than differences in trophic transfer efficiency or trophic position. This is plausible considering that FMeHg concentrations vary over several orders of magnitude among streams, and given that biomagnification of MeHg from water to algae (10^5−10^6) is several orders of magnitude larger than from algae to successively higher trophic levels (<10 to 10^1 per trophic level) (*41*). When comparing streams among geographic regions, Hg in both forage and predator fish was positively correlated to trophic position only in Wisconsin (Spearman's $\rho = 0.48$, $p = 0.0024$), where FTHg and FMeHg vary by an order of magnitude or less among streams. These results suggest that differences in community composition, feeding relationships, and trophic transfer efficiencies are relatively more important to MeHg bioaccumulation in streams when evaluating long-term temporal trends within a specific stream or watershed, or when comparing across similar environmental settings with similar Hg loadings (*8*).

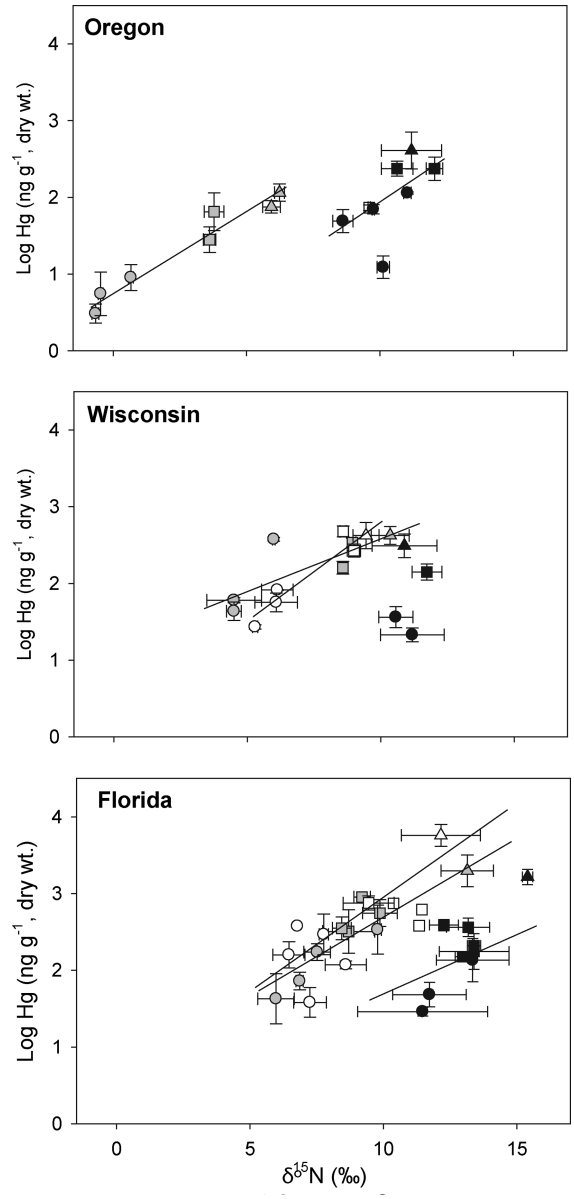

FIGURE 2. Trophic enrichment of Hg in Oregon, Wisconsin, and Florida streams. Data points are species means for MeHg in multiseason collections of all invertebrates (○) and for THg in forage fish (□) and top predator fish (Δ); regression lines are based on data from individual samples. Symbol shading represents nonurban streams: white (Pike River, St. Marys River); gray (Lookout Creek, Evergreen River, Santa Fe River); and urban streams, black (Beaverton Creek, Oak Creek, and Little Wekiva River).

Local, Regional, and Geographic Influences. Hg concentrations in biota were evaluated relative to integrated

TABLE 3. Trophic Enrichment of Mercury in Streams As Illustrated by the Slope of log[Hg] versus δ^{15}N for Biota[a]

			a. general food web				b. operationally defined food web			
	N	$\Delta\delta^{15}$N (‰)	slope ± 95% CI	SE	r^2	*p*	slope ± 95% CI	SE	r^2	*p*
Lookout Creek, OR	40	6.8	0.21 (0.19−0.24)	0.012	0.90	<0.0001	0.20 (0.17−0.24)	0.017	0.90	<0.0001
Beaverton Creek, OR	39	1.1	0.24 (0.15−0.32)	0.042	0.46	<0.0001	0.27 (0.20−0.33)	0.029	0.83	<0.0001
Pike River, WI	39	4.2	0.26 (0.21−0.28)	0.017	0.87	<0.0001	0.22 (0.06−0.38)	0.076	0.39	0.0124
Evergreen River, WI	38	6.2	0.14 (0.11−0.17)	0.015	0.70	<0.0001	0.15 (0.13−0.18)	0.013	0.84	<0.0001
Oak Creek, WI	33	0.02	n/a	n/a	0.01	0.553	n/a	n/a	0.08	0.265
St. Marys River, FL	57	4.9	0.23 (0.20−0.27)	0.026	0.63	<0.0001	0.24 (0.19−0.30)	0.026	0.63	<0.0001
Santa Fe River, FL	74	6.8	0.21 (0.18−0.23)	0.012	0.79	<0.0001	0.20 (0.17−0.22)	0.013	0.82	<0.0001
Little Wekiva River, FL	70	3.2	0.16 (0.09−0.23)	0.036	0.22	<0.0001	0.24 (0.15−0.33)	0.045	0.47	<0.0001

[a] Regressions include composites of invertebrates and forage fish, and individual predator fish. *N* represents the total number of observations in each regression, and SE is the standard error of the slope estimate. Community complexity is characterized by $\Delta\delta^{15}N = (\delta^{15}N_{predator} - \delta^{15}N_{invertebrates})$, which is an estimate of the difference in trophic position between top (predator fish) and base (herbivorous/detritivorous invertebrates) consumers. Regressions include (a) all data and (b) data restricted to biota operationally defined as nutritionally dependent [$\delta^{13}C_{predator} \pm (\Delta\delta^{15}N/3.4)$; Supporting Information Figure S1].

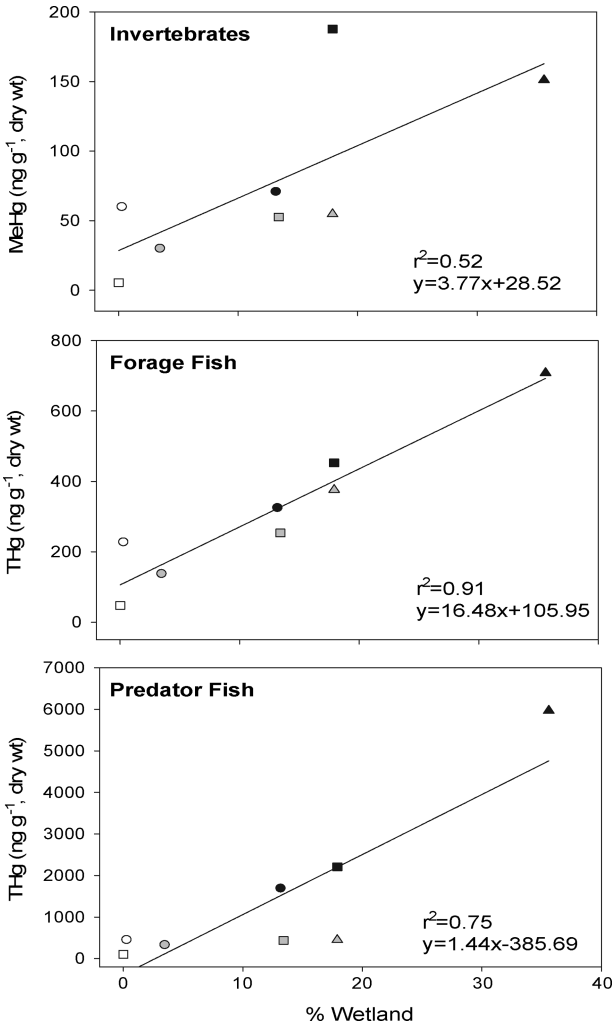

FIGURE 3. Mean Hg in biota versus wetland abundance. Symbol shading represents geographic area: white, Oregon; gray, Wisconsin; and black, Florida. Symbol shape represents land use: nonurban, Δ (Pike River, St Marys River) and □ (Lookout Creek, Evergreen River, Santa Fe River); and urban, ○ (Beaverton Creek, Oak Creek, and Little Wekiva River).

streambed sediment MeHg production potential (*42*), atmospheric depositional Hg loading (*15, 43*) (not shown), and wetland abundance as a percent of the watershed area in the stream basin (*16*). Mercury in biota was not significantly correlated to either in-stream MeHg production potential or depositional loading ($\tau < 0.5$, $p > 0.1$); however, Hg concentrations in invertebrates, forage fish, and predators were significantly and positively correlated to percent wetland (Figure 3). These results are not unexpected, given that (1) wetlands have been identified as important sources of MeHg and DOC (*44*), (2) FMeHg and DOC are highly correlated to percent wetland in the current study (*15*), and (3) Hg concentrations in biota are highly correlated to all of these variables (Table 2). The relationships between Hg in biota and wetland area may provide an expedient way to establish a preliminary characterization of potential MeHg contamination for a given stream.

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Supporting Information Available

Additional supporting figures and data tables are presented. This information is available free of charge via the Internet at http://pubs.acs.org.

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