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MODELING MERCURY BIOMAGNIFICATION (SOUTH RIVER, VIRGINIA, USA) TO INFORM RIVER MANAGEMENT DECISION MAKING

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Abstract—Mercury trophic transfer in the South River (VA, USA) was modeled to guide river remediation decision making. Sixteen different biota types were collected at six sites within 23 river miles. Mercury biomagnification was modeled using a general biomagnification model based on $\delta^{15}N$ and distance from the historic mercury release. Methylmercury trophic transfer was clearer than that for total Hg and, therefore, was used to build the predictive model ($r^2_{\text{prediction}} = 0.76$). The methylmercury biomagnification factors were similar among sites, but model intercept did increase with distance down river. Minimum Akaike's Information Criterion Estimation (MAICE) justified the incorporation of distance in the model. A model with a very similar biomagnification factor to the South River (95% confidence intervals [CI] = 0.38–0.52) was produced for a second contaminated Virginia river, the North Fork Holston River (95% CI = 0.41–0.55). Percent of total Hg that was methylmercury increased monotonically with trophic position. Trophic models based on $\delta^{15}N$ were adequate for predicting changes in mercury concentrations in edible fish under different remediation scenarios. Environ. Toxicol. Chem. 2010;29:1013–1020. © 2010 SETAC

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INTRODUCTION

Effective risk assessment and remediation require a clear understanding of contaminant movement through ecosystems to human or ecological receptors. Trophic processes are pivotal in such movement of Hg through aquatic systems. Generally, research on the trophic movement of Hg has focused more on lentic [1–3] than lotic systems, which tend to be structurally more heterogeneous. Mercury biomagnification slopes of Hg concentration (based on $\log_{10}$ of concentration) versus $\delta^{15}N$ (‰) in lentic systems have been measured all over the world: Africa (THg (wet wt): 0.13 [4], THg (wet wt): 0.20 [5]), Middle East (MHg (wet wt): 0.14 [6]), Canada (THg (dry wt: 0.2 [7]). Recently, Hg biomagnification in lotic systems has received more focus with studies done in the United States (THg (dry wt): 0.14 to 0.27 [8]) and Vietnam (THg (dry wt): 0.101 [9]). Stewart et al. [10] measured Hg biomagnification (MHg (dry wt): 0.20) within a reservoir. Relative to lentic systems, lotic systems, such as small rivers, have more exchange with the floodplain, and benthic-pelagic exchanges can be more pronounced. Different biota types are distinct in how they feed and are exposed to Hg, and therefore can vary widely in their realized Hg concentrations. This increased heterogeneity could make modeling Hg biomagnification in lotic systems potentially more difficult than in large lentic systems.

Trophic movement of Hg also depends on chemical speciation. Inorganic Hg is methylated in sediments by sulfur-reducing bacteria [11] that live in the anoxic zone [12]. These bacteria reduce sulfate for energy [13] and produce hydrogen sulfide as a byproduct. Most of the hydrogen sulfide remains in the sediment after binding with metals, but some diffuses to the oxic zone. In the oxic zone, sulfide re-oxidizes into sulfate by chemical reactions and chemotrophic bacteria [12]. If sulfate is limited and sulfur-reducing bacteria have a carbon source, Hg methylation can occur by using methylcobalamin as a methyl donor [11]. Methylmercury is more efficient at biomagnification than inorganic Hg (II) [14]. Invertebrates link periphyton and detritus to edible fish [15]. The longer the food chain, the higher the Hg concentrations are in top predators [16].

It was uncertain whether total Hg would provide a useful model for lotic systems. Total Hg analysis is less expensive than that for methylmercury, but methylmercury is more readily transferred among organisms than inorganic mercury [17]. Therefore, it was hypothesized that methylmercury might display a clearer trend in complex, heterogeneous systems.

The South River (northeast Virginia) was studied to determine whether trophic transfer models based on stable nitrogen isotope ratios ($\delta^{15}N$) could be generated that could be used to inform the remediation decision process. Stable nitrogen isotopes quantify trophic position of individuals in a community trophic web characterized by significant omnivory [16]. Predicative models enhance decision making, which allows for better ecosystem management, more informed health advisories, and efficient use of human and financial resources. The present study addressed five hypotheses.

Stable nitrogen isotopes will be used to model the biomagnification of Hg in the South River. An a priori criterion for acceptability for river management purposes will be a model

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with a prediction coefficient ($r^2_{\text{prediction}}$) in the range of 0.80. The range of 0.80 was based on what was judged to be realistically attainable yet useful to decision makers.

One Hg model will be sufficient for this contaminated reach of the South River: separate models for each location will be unnecessary. Distance downriver from the historical Hg point source could influence the model parameters because of differences in Hg concentrations and speciation. A p value for the null hypothesis that the coefficient for the effect of distance from the source was equal to 0 will be used to assess this hypothesis.

The proportion of Hg present as methylmercury will increase in a quantifiable manner with trophic position. Several sources suggested that the proportion of the total Hg that was methylmercury increases with trophic position because methylmercury biomagnifies most readily [18,19]. However, the literature is inconsistent on this point and most work has focused on lentic, not lotic, systems. A p value for the null hypothesis that the coefficient for the trophic level effect on proportion of Hg present as methylmercury was equal to 0 was used to assess this hypothesis.

Mercury biomagnification models will provide useful predictions of accumulation in the trophic web. Formal cross-validation [20] will be performed to determine how well the nitrogen isotope-based model predicts mercury concentrations.

The South River model will adequately predict bioaccumulation in piscivorous sportsfish of another contaminated mid-Atlantic river. If the models adequately predicted mercury biomagnification in one Virginia river, a reasonable extension would be to explore whether the same models would adequately predict mercury biomagnification in another. To assess this hypothesis, a biomagnification model will be built for another contaminated Virginia river (North Fork Holston River) and the model biomagnification factor estimate compared with that of the South River. The 95% confidence intervals for the estimated biomagnification factors will be used to suggest similarity of trophic transfer in the two rivers.

The South River is in the Potomac drainage within the Chesapeake Bay and Mid-Atlantic region. The river flows over Cambrian carbonate rocks. The North Fork Holston River is in the Tennessee Region which is part of the Ohio region. It flows over Paleozoic sedimentary rock.

MATERIALS AND METHODS

All plasticware into which smaller species were placed was prepared in the laboratory by soaking them in a 10% (v/v) nitric acid bath for at least 24 h. They were then rinsed seven times with Nanopure® deionized water [21].

In May 2007, triplicate samples of sixteen biota types were collected at each of five South River riffle sites and a pool site (river mile 9.1) within 23 river miles downriver of the historic release. These six sites were selected to measure the changing Hg concentrations and to complement other river studies. Three individuals of similar size within a site were used to produce triplicates for all fish and crayfish species; however, it was necessary to pool smaller organisms to obtain adequate tissue for triplicates. Chub (*Nocomis leptocephalus*) from North Park was the only fish species pooled. The range for total length of higher order fish among sites remained within twofold: largemouth bass (285–429 mm; $n = 15$), smallmouth bass (195–225 mm; $n = 3$), white suckers (275–490 mm; $n = 18$), red breasted sunfish (123–185 mm; $n = 9$), bluegill sunfish (146–204 mm; $n = 9$), and fallfish (198–270 mm; $n = 12$).

Organism types were selected to obtain an even distribution of isotopic ratios from low $\delta\ ^{15}N$ (e.g., primary producer) to a high $\delta\ ^{15}N$ (e.g., secondary consumer). Taxonomists from URS and U.S. Fish and Wildlife helped to identify the organisms. Three primary producers were collected: periphyton and two macrophytes. Six organism types that reflect primary consumers included: two gastropods (e.g., *Leptoxis carinata*), a caddisfly (Hydropsychidae), a mayfly (e.g., Stenonema), and a bivalve (e.g., *Corbicula flumenia*). A pilot study conducted a year earlier found a possible model gap where organisms have a mixed primary/secondary consumer diet. A predator insect (e.g., Zygoptera), a crayfish (Cambaridae) and three lower trophic fish (e.g., *Rhinicthys cataractae*) were selected. White suckers and bass were selected as higher trophic level fish. The North Fork Holston River was to be a simplified model of: periphyton from natural substrate, a primary consumer, a predator insect, a forage fish, and a predator fish. Opportunistic samples collected were also analyzed. A complete list of organisms analyzed can be seen in Table 1.

Procedurally defined periphyton was collected from both artificial and natural substrates. Artificial substrates were used to collect procedurally defined periphyton in an attempt to limit the amount of abiotic materials collected. Periphyton from natural substrates was collected in case the artificial substrates were vandalized or ineffective. Substrates were randomly placed at field locations selected a priori with Visual Sampling Plan version 4.7 (Battelle Memorial Institute, 2007). Additional substrates were placed in case the substrates were lost, vandalized, or washed away.

Aquatic invertebrates were collected by hand or by flipping over rocks and picking them off with tweezers. Predator insects were normally found along river edges and were collected using a net. Clams were collected within a specified shell length range (18–25 mm) to reduce the influence of age. Organisms were placed on ice in either acid-cleaned glass vials or plastic bags before being frozen at the laboratory. Only soft tissues were analyzed for the mollusks. All aquatic invertebrates were pooled in acid-washed containers in the laboratory, freeze dried (LABCONCO Freezone® 4.5 Liter Freeze Dry System) and then pulverized. Carbonates that can bias $\delta^{13}C$ values of the procedurally defined periphyton were removed from a ten-milligram aliquot by acidification with 2 M redistilled HCl [22]. Macrophytes were collected for background information only and were not used for modeling because they were not a major component of the general scraper/gatherer/collector-based trophic web being modeled.

Fish were collected by electroshocking and stored in plastic bags on ice until frozen in the laboratory. Prey items were removed from bass stomachs before homogenization. Small and larger fish were homogenized with a glass rod or food blender, respectively. The blender was washed thoroughly with tap water and then rinsed with Nanopure® deionized water between samples. The thick facial bones of the larger largemouth bass (*Micropterus salmoides*) required that a meat grinder was used before homogenization. An aliquot of each homogenized fish sample was frozen and freeze dried in a smaller acid-washed

Table 1. Organisms analyzed from the South and Holston Rivers (VA, USA)

River	Latin Name	Common Name	Feeding habits	Symbol
South River				
	N/A	Periphyton	Primary producer	A
	Baetidae	Mayfly	Collector/gatherer	B
	Cambaridae	Crayfish	Omnivore	C
	Catostomus commersoni	White sucker	Omnivore fish	D
	Corbicula fluminea	Clam	Filterer	E
	Ephemerellidae	Mayfly	Collector/gatherer	F
	Gomphidae	Dragonfly	Predator Insect	G
	Helisoma sp.	Snail	Scraper	H
	Hydrophsychidae	Caddisfly	Collector/filterer	I
	Lepomis auritus	Redbreast sunfish	Invertivore fish	J
	Lepomis macrochirus	Bluegill sunfish	Invertivore fish	K
	Leptoxis carinata	Snail	Scraper	L
	Micropterus dolomieu	Smallmouth bass	Piscivore fish	M
	Micropterus salmoides	Largemouth bass	Piscivore fish	N
	Nocomis leptocephalus	Bluehead chub	Omnivore fish	O
	Physidae	Snail	Scraper	P
	Pimephales notatus	Bluntnose minnow	Omnivore fish	Q
	Psephenidae	Water penny	Scraper	R
	Rhinichthys cataractae	Longnose dace	Invertivore fish	S
	Semotilus corporalis	Fallfish	Generalist fish	T
	Simuliidae	Blackfly	Collector/filterer	U
	Stenonema	Mayfly	Scraper	V
	Zygoptera	Damselfly	Predator insect	W
North Fork Holston River				
	N/A	Periphyton	Primary producer	
	Ambloplites rupestris	Rock bass	Piscivore fish	
	Campostoma anomalum	Stoneroller minnow	Herbivore fish	
	Corydalidae	Dobsonfly	Predator insect	
	Elimia (Goniobasis) clavaeformis	Snail	Scraper	
	Ephemeroptera	Mayfly	Collector/gatherer/scraper	
	Gomphidae	Dragonfly	Predator insect	
	Hypentelium nigricans	Northern hog sucker	Invertivore fish	
	Lepomis auritus	Redbreast sunfish	Invertivore fish	
	Micropterus dolomieu	Smallmouth bass	Piscivore fish	
	Nocomis micropogon	Largemouth bass	Piscivore fish	
	Notropis telescopus	Telescope shiner	Invertivore fish	
	Plectopera	Stonefly	Predator insect	
	Zygoptera	Damselfly	Predator insect	

container. Freeze-dried samples were pulverized and a small amount of each taken for isotope analysis.

The samples were sent to CEBAM (Seattle, WA, USA) for total Hg and methylmercury analysis. Because of the high cost of methylmercury analyses, only one sample from every set of triplicate riffle samples was randomly selected for methylmercury analysis. Nitrogen isotope ratios were generated at the University of California (UC)-Davis Stable Isotope Facility (Davis, CA, USA).

Biota from the North Fork Holston River, another mercury-contaminated river in Virginia, were taken in July 2008 and processed similarly. The sampling included triplicate samples of periphyton from natural substrate, a primary consumer, a predatory insect, a forage fish, smallmouth bass (*Micropterus dolomieu*), and additional samples as opportunity allowed. One site was sampled (46 miles downriver from the historical source) to produce the trophic transfer model. Triplicate smallmouth bass samples were collected from two additional sites 12.5 miles and 21 miles downstream of the historic source.

The predictive models were generated with the SAS® vers 9.1 PROC GLM general linear model procedure (SAS Institute). Prediction coefficients ($r^2_{\text{prediction}}$) for the models were estimated using the prediction sum of squares (PRESS) and model total sum of squares [20]. A general exponential model for biomagnification was used [23]:

$$e^{a+b\delta^{15}\text{N}} \quad (1)$$

where b is the biomagnification factor and e^a is the theoretical baseline concentration of a contaminant at the x intercept. This model can predict the contaminant concentration of an organism using the nitrogen stable isotope fractions and a baseline estimation.

Analytical quality control/quality assurance and methods

Analytical quality control (QC) measures were performed at the analytical laboratories. CEBAM measured total Hg and methylmercury concentrations with cold vapor atomic fluorescence spectrometry. Total Hg was reduced with tin chloride and collected on a gold trap, while methylmercury went through aqueous phase ethylation, collection on a Tenax® trap, and separation by a gas chromatograph. Laboratory duplicate splits were performed for total Hg (mean difference between splits = 5.0%, standard deviation [SD] = 4.0%; $n = 33$) and methylmercury (mean = 7.0%, SD = 4.4%; $n = 23$) analyses, and matrix spike for total Hg (mean recovery = 100.4%;

SD = 3.3%; $n = 38$) and methylmercury (mean recovery = 102.5%; SD = 7.4%; $n = 17$). They also analyzed standard reference materials (SRM 1566b, IAEA-350, DORM-2) for total Hg (mean recovery = 97.4%; SD = 2.8%; $n = 6$) and methylmercury (mean recovery = 96.6%; SD = 5.0%; $n = 6$). UC-Davis stable isotope facility analyzed isotope ratios by combusting the materials at 1020°C and removing the oxides with a reduction reactor before entering a magnesium perchlorate water trap. Nitrogen separation occurred on a Carbosieve™ GC column and then quantified with an isotope ratio mass spectrometer. They performed QC checks (mean = 1.32‰; SD = 0.17‰; $n = 79$) against an air standard (1.33‰). All analytical QC/quality assurance results were acceptable for the purpose of the present study.

RESULTS

The natural logarithm of total Hg concentration (natural logarithm mg/kg dry wt) versus δ ^{15}N (Fig. 1) produced inadequate predications for use in river management ($r^2_{\text{prediction}} = 0.31$) based upon the a priori criterion (r^2 prediction $\doteq 0.80$). The regression model based on methylmercury (Fig. 2) was materially better ($r^2_{\text{prediction}} = 0.78$; $r^2_{\text{prediction}} = 0.76$): adequate predictions were produced with methylmercury concentrations (Fig. 3). Mercury concentrations from black fly larvae (Simuliidae) and pulmonate snails lay outside of the general trend as discussed below, and as a consequence, were omitted during this general model building.

Parameter estimates were produced from the methylmercury predictive model: δ ^{15}N-based biomagnification factor (0.45, SE = 0.03; $n = 66$) and distance from source coefficient (0.054, SE = 0.010; $n = 66$). Parameter estimates were significantly different from 0 (biomagnification factor: $p < 0.001$, two-tailed t test, $t = 13.13$, *degrees of freedom* (df) = 65, and influence of distance: $p < 0.001$, two-tailed t test, $t = 4.90$; $df = 65$).

Backtransformation of the logarithmic-linearized model to its arithmetic form required correction of a bias that is inherent when converting an exponential model to an arithmetic model [24],

$$\text{Backtransformation Correction Factor} = e^{\text{MSE}/2} \quad (2)$$

where MSE = model mean square error. The baseline, e^a, was $e^{-5.216}$, and the backtransformation bias correction was $e^{0.53/2}$, or $e^{0.265}$. The parameter estimates and this bias correction were incorporated into the general biomagnification model (Eqn. 3) to predict mercury concentrations.

$$\text{Methylmercury (mg/kg dry wt)} = e^{-5.216+0.448(\delta^{15}N)+0.054(RM)}e^{0.265} \quad (3)$$

where δ ^{15}N has units of per mil and RM (river miles below the historic release) has units of miles. The estimated biomagnification factors for all sites were similar but the y-intercept increased with distance from the source. No interaction term was included in the model because the initial model exploration detected no significant ($\alpha = 0.05$; $p = 0.69$) interaction between δ ^{15}N and distance from source.

Minimum Akaike's Information Criterion Estimation (MAICE) was applied to determine if the simple nitrogen isotope-based model or a nitrogen isotope-based model with river mile included was the best model. The model with the maximum amount of information per estimated parameter was favored in this selection process [25]. The model including δ ^{15}N and river mile had a slightly smaller Akaike's Information Criterion (AIC = 237) than that with δ ^{15}N alone (AIC = 257); therefore, both variables were left in the final model.

The proportion of the total Hg that is methylmercury increased with trophic position (Fig. 4). The sigmoid relationship was linearized with the inverse cumulative normal function to generate a predictive model that had an $r^2_{\text{regression}}$ of 0.71. Inorganic mercury concentration appeared to decrease slightly with trophic position (95% CI of slope estimate = 0.00 to −0.28; $p = 0.04$ for null hypothesis that slope = 0).

The North Fork Holston River total Hg model was also judged unacceptable for river management purposes based on the a priori criterion of an approximate $r^2_{\text{prediction}}$ of 0.80. However, the $r^2_{\text{regression}}$ for the North Fork Holston River methylmercury model was 0.83 and the $r^2_{\text{prediction}}$ was 0.80 that met the criterion for useful predictions. The North Fork Holston River data produced the following biomagnification model that included a bias correction of $e^{0.15}$,

$$\text{Methylmercury(mg/kg dry wt)} = e^{-5.034+0.481(\delta^{15}N)}e^{0.15} \quad (4)$$

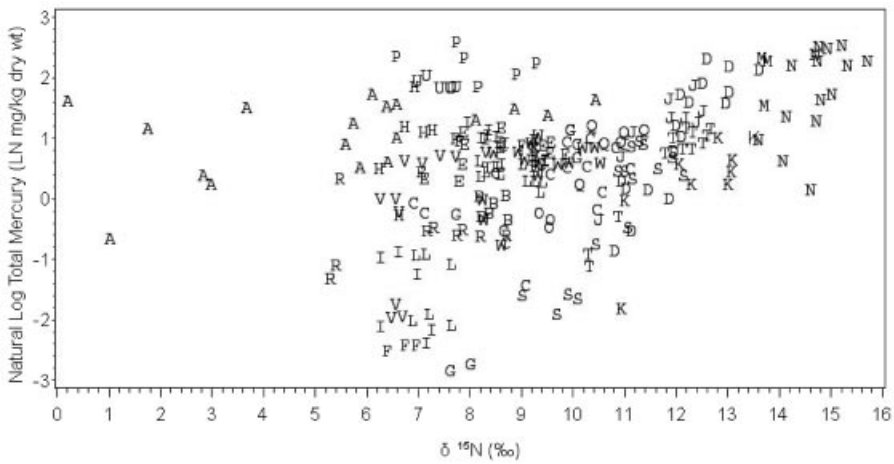

Fig. 1. The natural log of total mercury concentrations (ln mg/kg dry wt) versus δ ^{15}N (‰). The data were collected from five riffle sites and a pool site (VA, USA).

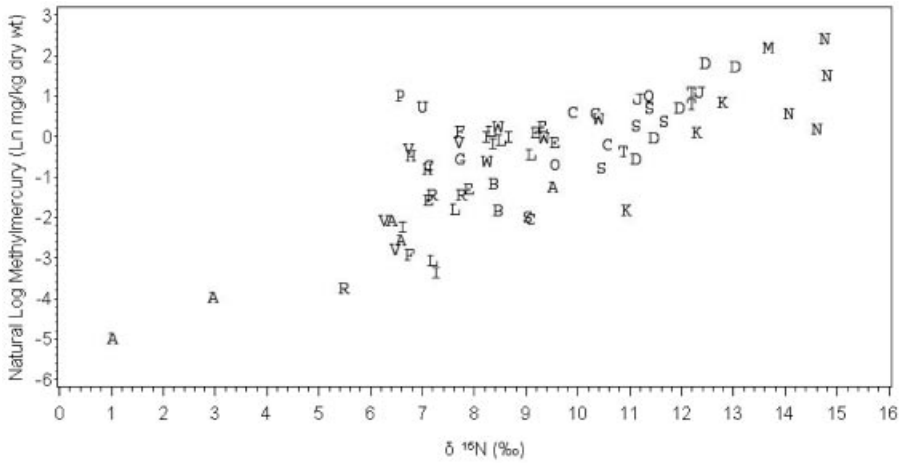

Fig. 2. Increase in methylmercury concentration (ln mg/kg dry wt) with increase in trophic position [δ ^{15}N (‰)] noted at the five riffle sites (VA, USA). Methylmercury was not measured at the pool site.

The slope (biomagnification factor) of the North Fork Holston River model (95% CI of estimate = 0.41 to 0.55) was very similar to the South River model slope (95% CI of estimate = 0.38–0.52) (Fig. 5).

DISCUSSION

Methylmercury concentrations were less variable than total Hg in trophic transfer graphs (Figs. 1, 2). Pulmonate snails and black fly larvae fit much better into the methylmercury model than total mercury but still had high mercury concentrations relative to the general trend. Total Hg-based models using river mile and nitrogen stable isotopes were inadequate for producing useful predictions, but the models were adequate if based on methylmercury concentrations.

The AIC values indicated that the model with both δ ^{15}N and river mile was more informative than that with δ ^{15}N alone; however, there was not a large difference in magnitude between the AIC values. Inclusion of distance downriver improved the South River model but a satisfactory model could have been built with δ ^{15}N alone. River mile was not relevant in the North Fork Holston River model because the model was built with samples taken primarily from one location. A distance downriver effect might not be seen if there was no material change in the methylmercury baseline. Holston River mercury baselines would decrease further down river, and a decrease in Hg concentrations in organisms would likely take place.

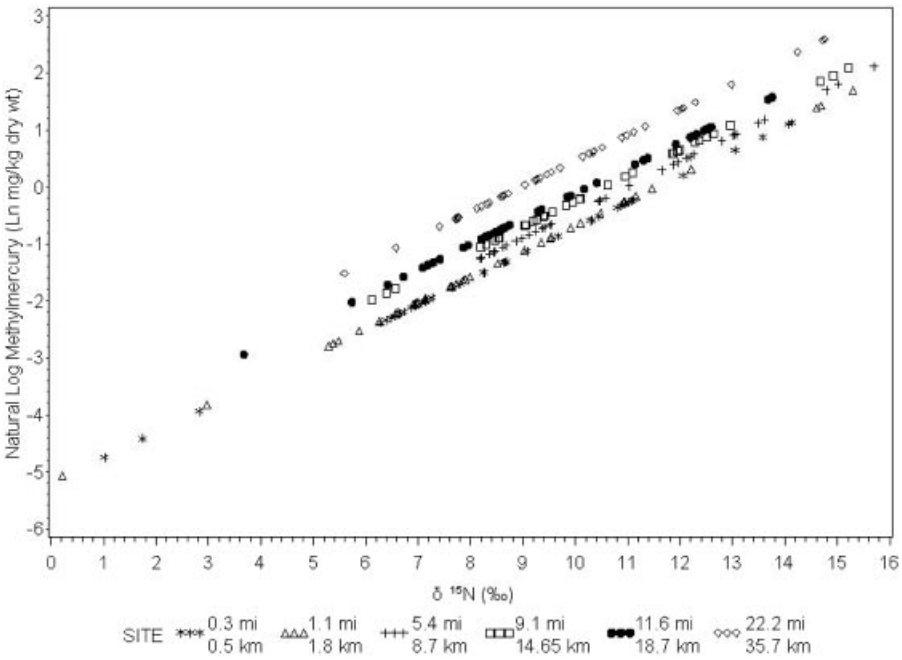

Fig. 3. Predictions of natural log of methylmercury concentration (ln mg/kg dry wt) from the model including river mile and δ ^{15}N (‰). The intercept increases with distance downriver from the historic source. Pulmonates, Simuliidae (black fly larvae), and macrophytes were not used to build this model. Although methylmercury concentrations were not analyzed for the pool site, the model predicted the methylmercury concentrations shown here from the pool site δ ^{15}N and river mile observation values.

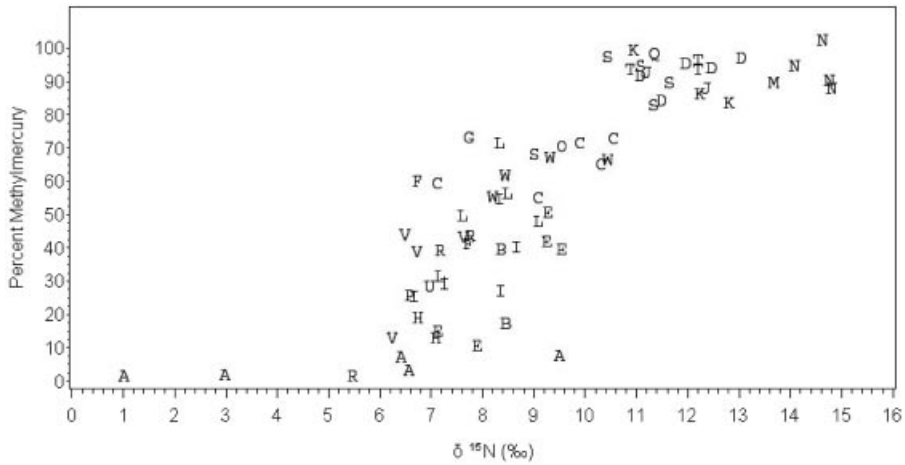

Fig. 4. The percent of total mercury that is methylmercury as a function of trophic position [δ ^{15}N (‰)]. The data were collected from five riffle sites: Methylmercury was not measured at the pool site (VA, USA).

Methylmercury baselines could increase with water depth due to higher anoxic conditions, as could a decrease in river width due to increasing significance of terrestrial inputs causing some variation.

Models based on stable nitrogen isotopes and methylmercury concentrations proved effective in modeling the South River mercury biomagnification to piscivorous sportsfish that might be consumed by humans, e.g., largemouth bass. A single coefficient accounting for trophic position sufficed for all sites. Influence of river mile likely reflected the increase of methylmercury at the base of the food web with increasing distance downriver from the source. This could have caused the distance-dependent increase of Hg concentration also seen in bass.

The estimated South River and North Fork Holston River biomagnification factors were similar, suggesting that a single biomagnification factor might be applied by river managers to estimate general remediation consequences for other Virginia rivers. The two study rivers differed slightly in species, microclimates, and physical properties but were typical of the region. With balance, the single biomagnification factor might be applied to similar rivers in the region.

The biomagnification slope for $\log_{10}$ of concentration versus δ^{15}N was very similar to those of other studies, which ranged from 0.13 to 0.27 [4–10]. Although previously mentioned studies did not use the same approach, i.e., using wet or dry weight, they all used $\log_{10}$. So to compare the present study with other studies, SAS was used to produce a $\log_{10}$-based model:

$$\text{Methylmercury}(\text{mg/kg dry wt}) = 10^{-2.265+0.195\left(\delta^{15}\text{N}\right)+0.0235(\text{RM})}10^{0.0498} \quad (5)$$

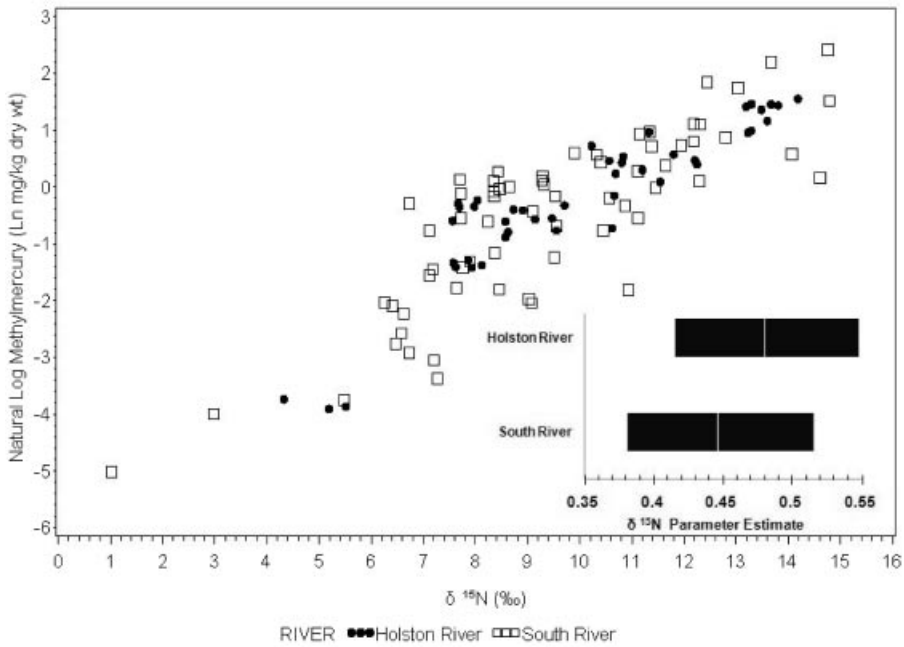

Fig. 5. Methylmercury biomagnification for both the North Fork the Holston and South Rivers (VA, USA). The 95% confidence intervals for the biomagnification factors of both rivers coincide (insert).

where the base 10 is used for the bias correction ($10^{0.0498}$) for $\log_{10}$ instead of e [24]. The biomagnification factor of 0.195 mg/kg dry weight fits within the range produced by other studies.

Some biota might be outside the general total Hg versus $\delta^{15}N$ trend because they contained large amounts of inorganic mercury that was not readily transferred to consumers. Mercury concentrations were higher in black fly larvae than initially expected from the trend of $\delta^{15}N$ versus Hg. In contrast to other biota at its general trophic position, these larvae consume dissolved organic matter directly from the water [26]. Mercury more readily accumulated in black fly larvae due to this unusual feeding mode, as documented in other studies [27].

Pulmonate snails (Physidae and *Helisoma* sp.) also appeared to deviate from the general scraper/gather/collector Hg biomagnification trend. We speculate that they graze more selectively on periphyton compared with other scrapers/gatherers [28] and this might influence bioaccumulation. *Helisoma* sp. mercury concentrations appeared lower than those of the Physidae, but were misleading because *Helisoma* sp. were only gathered at the two sites closest to the release. Physidae were collected at the four sites farthest from the source. Physidae, *Helisoma* sp., and black fly larvae were taken out of the aquatic model post hoc after considerable thought. Some invertebrates might consume less nutritious food which must then be consumed at a higher rate, exposing some organisms to higher Hg concentrations [29]. Also, because invertebrates did not clear their guts completely before being processed, it is possible that the high inorganic Hg concentrations might have been partially attributable to materials remaining in their guts.

Procedurally defined periphyton displayed considerable variation in inorganic Hg concentrations (1.93 mg/kg dry wt, SD: 1.21 mg/kg dry wt; $n = 5$) and inorganic Hg comprised most of the Hg in these materials. The percentage of Hg that is methylated in periphyton (1.3–7.5%) was similar to that noted by Žižek et al. [30] (1.6–8.8%). The percentage of total Hg present as methylmercury increased with trophic position until nearly all of the Hg in higher predators was methylmercury, which was similar to other studies [29–31].

Application of trophic transfer models facilitates prediction of remediation action consequences and can reduce uncertainty about benefits accrued for each dollar spent on remediation. Also, because the model biomagnification factors appeared to be similar for two Virginia rivers, their use in preliminary screenings could also reduce costs for other site investigations by providing a means of producing quick and inexpensive screening information. By collecting procedurally defined periphyton or a primary consumer, river managers can use the resulting $\delta^{15}N$ and methylmercury concentrations to estimate the baseline for the model. Using this estimated baseline with the established biomagnification factor, largemouth bass methylmercury concentrations can be predicted. It takes much less effort and expense to collect periphyton or snails than it does to collect fish that require heavy electroshocking units and much time.

Procedurally defined periphyton mercury concentrations can have considerable inherent variation because of this material being a mixture of different materials. Mercury concentrations of primary consumers such as snails vary less than periphyton, and are as simple and expedient to collect. Primary consumers still provide a suitable baseline to predict mercury concentrations of apex predators by using the biomagnification model. Chasar et al. [8] used filtered methylmercury to predict mercury in apex predators. Although predictions should not be used as proof of human health risks, they can help river managers focus on river stretches that are potentially hazardous to humans.

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REFERENCES

1. Atwell L, Hobson KA, Welch HE. 1998. Biomagnification and bioaccumulation of mercury in an Arctic marine food web: Insights from stable nitrogen isotope analysis. *Can J Fish Aquat Sci* 55: 1114–1121.
2. Cabana G, Tremblay A, Kaliff J, Rasmussen JB. 1994. Pelagic food chain structure in Ontario Lakes: A determinant of mercury levels in Lake Trout. *Can J Fish Aquat Sci* 51:381–389.
3. Bowles KC, Apte SC, Maher WA, Kawel M, Smith R. 2001. Bioaccumulation and biomagnification of mercury in Lake Murray, Papua New Guinea. *Can J Fish Aquat Sci* 58:888–897.
4. Campbell L, Verburg P, Dixon DG, Hecky RE. 2008. Mercury biomagnification in the food web of Lake Tanganyika (Tanzania, East Africa). *Sci Total Environ* 402: 184–191.
5. Kidd KA, Bootsma HA, Hesslein RH, Lockhart WL, Hecky RE. 2003. Mercury concentrations in the food web of Lake Malawi, East Africa. *J Gt Lakes Res* 29 (Suppl 2): 258–266.
6. Al-Reasi HA, Ababneh FA, Lean DR. 2007. Evaluating mercury biomagnification in fish from a tropical marine environment using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). *Environ Toxicol Chem* 26: 1572–1581.
7. Atwell L, Hobson KA, Welch HE. 1998. Biomagnification and bioaccumulation of mercury in an Arctic marine food web: Insights from stable nitrogen isotope analysis. *Can J Fish Aquat Sci* 55: 1114–1121.
8. Chasar LC, Scudder BC, Stewart AR, Bell AH, Aiken GR. 2009. Mercury cycling in stream ecosystems. 3. Trophic dynamics and methylmercury bioaccumulation. *Environ Sci Technol* 43:2733–2739.
9. Ikemoto T, Tu NPC, Okuda N, Iwata A, Omori K, Tanabe S, Tuyen BC, Takeuchi I. 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. *Arch Environ Contam Toxicol* 54:504–515.
10. Stewart AR, Saiki MK, Kuwabara JS, Alpers CN, Marvin-DiPasquale M, Krabbenhoft DP. 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Can J Fish Aquat Sci* 65:2351–2366.
11. Compeau GC, Bartha R. 1985. Sulfate reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. *Appl Environ Microb* 50:498–502.
12. Jørgensen BB. 1977. The sulfur cycle of coastal marine sediment (Limfjorden, Denmark). *Limnol Oceangr* 22:814–832.
13. Gilmour CC, Henry EA. 1991. Mercury methylation in aquatic systems affected by acid deposition. *Environ Pollut* 71:131–169.
14. Hill WR, Stewart AJ, Napolitano GE. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. *Can J Fish Aquat Sci* 53:812–819.
15. Cleckner LB, Gilmore CG, Hurley JP, Krabbenhoft DP. 1999. Mercury methylation in periphyton of the Florida Everglades. *Lemnol Oceanogr* 44:1815–1825.
16. Cabana G, Rasmussen JB. 1994. Modeling food chain structure and contaminant bioaccumulation using stable isotopes. *Nature* 372:255–257.
17. Hill WR, Stewart AJ, Napolitano GE. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. *Can J Fish Aquat Sci* 53:812–819.
18. Watras CJ, Bloom NS. 1992. Mercury and methylmercury in individual zooplankton: Implications for bioaccumulation. *Limnol Oceangr* 37: 1313–1318.

19. Anderson JL, Depledge MH. 1997. A survey of total mercury in edible fish and invertebrates from Azorean waters. *Mar Environ Res* 44:331–350.
20. Neter J, Wasserman W, Kutner MH. 1990. *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs*. 3rd ed. Richard D Irwin, Homewood, IL, USA pp 465–471.
21. Newman MC, Zhao Y. 2005. Measuring metals and metalloids in water, sediment and biological tissues. In Ostrander GK, Techniques in Aquatic Toxicology 2nd ed. Lewis /CRC, Boca Raton, FL, USA pp 591–615.
22. Søreide JE, Tamelander T, Hop H, Hobson KA, Johansen I. 2006. Sample preparation effects on stable c and n isotope values: A comparison of methods in Arctic marine food web studies. *Mar Ecol Prog Ser* 328: 17–28.
23. Broman D, Näf C, Rolff C, Zebühr Y, Fry B, Hobbie J. 1992. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the northern Baltic. *Environ Toxicol Chem* 11: 331–345.
24. Newman MC. 1993. Regression analysis of log-transformed data: Statistical bias and its correction. *Environ Toxicol Chem* 12: 1129–1133.
25. Newman MC. 1995. Quantitative Methods in Aquatic Ecotoxicology. CRC, Boca Raton, FL, USA
26. Pennak RW. 1953. *Fresh-water Invertebrates of the United States*. The Ronald Press Company, New York City, NY, USA
27. Harding KM, Gowland JA, Dillon PJ. 2006. Mercury concentration in black flies *Simulium* spp. (Diptera, Simuliidae) from soft-water streams in Ontario, Canada. *Environ Pollut* 143: 529–535.
28. Barnese LE, Lowe RL, Hunter RD. 1990. Comparative grazing efficiency of pulmonate and prosobranch snails. *J North Am Benthol Soc* 9: 35–44.
29. Tremblay A, Lucotte M, Rheault I. 1996. Methylmercury in a benthic food web of two hydroelectric reservoirs and a natural lake of Northern Quebec (Canada). *Water Air Soil Pollut* 91: 255–269.
30. Žižek S, Horvat M, Gibičar D, Fajon V, Toman MJ. 2007. Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. *Sci Total Environ* 377: 407–415.
31. Mason RP, Laporte JM, Andres S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Arch Environ Contam Toxicol* 38: 283–297.