Modeling Mercury Biomagnification in the South River

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Modeling Mercury Biomagnification in the South River

A Thesis
Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

by
Kyle R. Tom
2008
This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science

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Abstract
Mercury trophic transfer in a contaminated river was modeled using stable nitrogen isotopes. Predictive models were developed to guide river management in determining a sampling program to identify acceptable fish mercury concentrations. Methylmercury transfer through the food web was clearer than that for total mercury, so it was used to build the predictive model ($R^2_{\text{prediction}}=0.76$). Methylmercury concentrations increased with trophic position ($\delta^{15}N$) and distance down river (river mile). The model slopes of methylmercury concentration versus $\delta^{15}N$ were similar among the sites, but intercepts increased with distance down river. Methylmercury concentrations in fish increased with river mile due to methylmercury input at the base of the food web. A similar model was created for the Holston River. The biomagnification factors for both rivers were essentially the same. Quantitative models for percent methylmercury of total mercury showed an increase in the percent of organic mercury with trophic position. Inorganic mercury was diluted during trophic transfer.
Modeling Mercury Biomagnification in the South River
Introduction

Mercury Risks
Human activities have increased mercury concentrations throughout the biosphere. Mercury point sources, once numerous, have been eclipsed by pervasive coal burning in power plants, and residential and commercial boilers as the primary focus of concern (Orihel et al. 2007). Because humans use over 3 billion metric tons (oil equivalent) of coal (BP 2007), fossil fuel burning releases a large quantity of mercury. Anthropogenic sources now account for an estimated two-thirds of the 2190 tons of mercury emitted into the atmosphere every year (Pacyna et al. 2006).

Atmospheric mercury can travel far as an aerosol and is deposited in what were once considered remote, pristine environments, such as the Arctic (Atwell et al. 1998). Increasing mercury concentrations in Greenland ice core samples reflect increased mercury levels in worldwide precipitation over the past century (Weis et al. 1971). This is also reflected in bioaccumulation. By 1991, Sweden had approximately 10,000 lakes with mercury levels above the national consumption advisory limit of 1 mg Hg kg$^{-1}$ wet weight in fish (Lindqvist et al. 1991).

Mercury in the environment can be inhaled as a vapor, but the highest risk is posed by ingestion, with the primary ingestion concern involving fish consumption (EPA 1984). Once ingested, mercury enters the blood and circulates throughout the body (EPA 1984). It binds primarily to amino or sulphhydryl groups in proteins (Krantzberg 1989) and thus has diverse and widespread opportunities to bind to tissues (Major et al. 1991). Ionic mercury can be absorbed through specific receptor sites that facilitate
sodium transport. Mercury can interfere with this transporter, disrupting sodium gradients and membrane functionality (Sellinger et al. 1991). This is particularly important to nerve function. Mercury can damage or kill cells by binding with enzymes in mitochondria and microsomes (Goyer 1996). Mercuric chloride causes mitochondria to swell by disrupting cation transport. Matrix proteins inside the mitochondria denature, causing deformations. Eventually, cellular damage occurs to the organelles and cytosol, and the nuclear envelope ruptures leading to necrosis (Gritzka and Trump 1968).

Cell death occurs primarily from oxidative stress at mercury concentrations as low as 10 µg L⁻¹ in human neuroblastoma cells (Olivieri et al. 2000). Newman and Unger (2003) describe oxidative stress as “…damage to biomolecules from free oxyradicals…Free radicals such as the superoxide radical (O₂⁻) and hydroxyl radical (’OH) can damage proteins, lipids, DNA, and other biomolecules.” Aerobic organisms create free radicals when they use oxygen as an electron receptor, but additional oxidative stress can occur from toxicants producing hydroxyl radicals (Newman and Clements 2008). Mercury can reduce the cellular pool of glutathione, the most abundant intracellular antioxidant. If glutathione levels become insufficient, the body’s ability to remove free radicals produced by mercury is exceeded and biomolecular damage occurs. Necrosis can occur with significant amounts of oxidative damage (Olivieri et al. 2000).
Human body burdens average 13 mg of mercury, yet this element serves no known function to the body (Salonen et al. 1995). Salonen et al. (1995) reported that men who consumed over 30g of fish daily had a higher risk of death from coronary disease than men with lower fish consumption rates because of mercury contamination. The risk to the men in the study depended on the type of fish they consumed: Fatty fish had lower mercury concentrations (Salonen et al. 1995).

Mercury diffuses across membranes into the brain where it is retained for long periods (Hartung and Dinman 1972). Methylmercury in particular affects the central nervous system by destroying neurons, impairing vision and critical cerebral functions such as motor skills (EPA 1984). Methylmercury changes the levels of amino acid transmitters which can inhibit cerebellar and spinal neurons (Hirayama et al. 1985). It also affects neuron microtubules, stunting neuron development (Goyer 1996). Mercury can also cause damage to the liver and kidneys (Salonen et al. 1995). Mercury moves to the fetus through the placenta. High mercury exposures of women cause a concern that developmental harm to the fetus might occur (EPA 1984). These concerns make understanding mercury in the environment essential to protecting our well-being.

**Mercury in Water and Biota**

Inorganic mercury can change form after entering aquatic ecosystems. When salinity increases, mercury speciation shifts from mostly Hg (OH)$_2^0$ to mostly HgCl$_2^0$. During this shift in speciation, the octanol-water partition coefficient increases,
suggesting that mercury becomes more bioavailable to organisms. $D_{ow}$, the water partition coefficients, in the table below is the sum of all the mole fractions multiplied by their $K_{ow}$ (Mason et al. 1996),

$$D_{ow} = \sum f_i (K_{ow})_i$$ (1)

By increasing the pH of water that contains the chloride ion, ionic mercury and methylmercury form more chloro complexes. The overall octanol-water partition coefficients are higher as a result, but level off above a certain pH value depending on the water salinity.

![Graph](image)

Figure 1a and b and Table 1 of Mason et al. (1996). Figure 1a and b show the speciations of mercury and monomethylmercury, respectively, with increasing chloride concentrations ($pCl=-\log[Cl^-]$).

Methylmercuric chlorides might be more lipophilic than $\text{CH}_3\text{HgOH}$ because they interact more with lipid hydrophobic tails. The OH in $\text{CH}_3\text{HgOH}$ makes this complex more polar, and the complex interacts more with water than with lipids. Absorption
via the gut is enhanced by increased lipid solubility, thus mercury would be more readily absorbed as methylmercuric chlorides than as other forms (Faust 1992). Only 0.01% of elemental mercury is absorbed through the gastrointestinal tract, making it less toxic than organomercury species. Mercury speciation might also influence which body organs are most sensitive to the effects of mercury. Organic mercury causes more neurological damage than inorganic mercury salts, which cause more renal damage (Schnellmann 2008). Hydrophobicity is not the only characteristic that determines toxicity of mercury species - intracellular changes in speciation can also be important. For example, elemental mercury can be oxidized to divalent inorganic mercury in cells (Goyer 1996).

Inorganic mercury is methylated in sediments by sulfur-reducing bacteria (Compeau and Bartha 1985) that live below the oxic layer in the anoxic zone (Jørgensen 1977). These bacteria reduce sulfate for energy (Gilmour and Henry 1991) 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 (Jørgensen 1977). If sulfate is limited and sulfur-reducing bacteria have a carbon source, mercury methylation can occur with methylcobalamin acting as the methyl donor (Compeau and Bartha 1985).
Biomagnification
Predatory fish have very high mercury concentrations due to biomagnification (Cabana and Rasmussen 1994). Uptake by photosynthetic producers is the primary means by which mercury enters aquatic trophic webs. In shallow flowing systems, periphyton accumulates mercury and methylmercury to concentrations thousands of times greater than that in the surrounding water (Hill et al. 1996). Periphyton is defined by Newman and MacIntosh (1989) as “all aquatic organisms (microflora) growing on submerged substrates.” In practice, periphyton is procedurally defined during pollutant surveys as all material accumulating on submerged surfaces, which can include substantial abiotic material. Newman and MacIntosh (1989) recommended periphyton as a convenient material to monitor trace metals. Periphyton is easily linked to other shallow river community members because it is a major component of the food web base.

Invertebrates link procedurally-defined periphyton and other primary producers to edible fish (Cleckner et al. 1998). The longer the food chain, the higher the mercury concentrations will be in top predators, because more links account for more biomagnification (Cabana and Rasmussen 1994). Mercury bioaccumulates in each organism in the food web, so each additional trophic level causes the top predator to be exposed to higher mercury concentrations.

Methylmercury is more efficient at biomagnification then is ionic mercury (II) (Hill et al. 1996). Methylmercury is approximately fifty percent of the total mercury in marine and freshwater invertebrate primary consumers. Boudou and Ribeyre (1985)
reported that approximately twenty-three percent of inorganic mercury was transferred from prey to predator, but seventy-two percent of organic mercury was transferred. McCloskey et al. (1998) found that one third of methylmercury ingested by fish is bioavailable. Once assimilated, most mercury is found in the muscle tissue because muscle constitutes more than half of the fish and is most of the weight of the fish (McKim et al. 1976, Pentreath 1976). However, high methylmercury concentrations can be found in the brain. The intestinal wall is permeable to inorganic mercury, so inorganic mercury can accumulate in the posterior intestine (Boudou and Ribeyre 1985). It also accumulates in kidneys of fish (Goyer 1996).

Mercury in fish muscle is almost all (98%-100%) methylmercury. There is some analytical error in measurements of methylmercury and total mercury, and perhaps, future improvements in analytical methods will allow us to determine if all of the mercury in piscivorous fish is methylated (Bloom 1992). Some studies have focused on whole body samples instead of muscle or organ samples. Hill et al. (1996) observed methylmercury percentages as low as fifty percent in whole-body fish samples, when methylmercury should be eighty to ninety percent of the total mercury as in Watras and Bloom (1992). If the form of mercury differs among environments, there might be distinct spatial biomagnification dynamics in these environments (Bloom 1992).
**Variability in Biological Mercury Concentrations**

In a study by Lindvist et al. (1991), factors other than biomagnification influenced fish mercury concentrations. For example, mercury concentrations increased with size, and therefore presumably age, of roach (*Rutilus rutilus*), before leveling off at approximately 50 mg Hg kg\(^{-1}\) of fish. Season influenced mercury concentrations, though seasonal variation might have been due to confounding factors. Seasonal concentrations in roach peaked simultaneously with feeding activity, growth efficiencies, and growth rates. The effect of growth rate on mercury concentration was minor. Sex of the fish did not affect mercury concentrations, although spawning increased mercury concentrations because fish appeared to lose more body mass than mercury upon spawning. Fish eggs had accumulated little mercury, resulting in little mercury being removed from the mother after spawning. Such variables can cause mercury concentration to vary among individual fish within a population by 10-fold or more (Lindqvist et al. 1991).

Biomagnification cannot be fully defined by a simplistic approach such as multiplying a constant by a discrete trophic level occupied by the species of interest. Most organisms do not feed from a single trophic level. More often, consumers are omnivorous,\(^1\) feeding on many species from several trophic levels. Cabana and Rasmussen (1994) estimate that lake trout (*Salvelinus namaycush*), the top predator in their Canadian lake studies, showed three percent omnivory. The species that feed at the second highest levels showed a higher percentage of omnivory (19%).

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\(^1\) Conforming to current usage in the biomagnification literature, omnivory is used here to mean feeding on several food sources that occupy different trophic positions.
Stable nitrogen isotopes can be used to quantify trophic position in the presence of omnivory. Carbon (Mizutani et al. 1990) and sulfur isotope analyses also help differentiate the varying feeding habits of organisms (Cabana and Rasmussen 1994) and their assimilation efficiencies (Gagnon and Fisher 1997) but are generally less effective for quantifying trophic position.

Stable nitrogen isotope ratios change from prey to predator because most organisms release nitrogenous waste that is enriched in $^{14}\text{N}$ relative to $^{15}\text{N}$, and feces that are enriched in $^{15}\text{N}$ relative to $^{14}\text{N}$. Their diet, along with an overall net loss of more $^{14}\text{N}$ through their urine, causes the $^{15}\text{N}:^{14}\text{N}$ ratio to increase in each ascending trophic level (Steel and Daniel 1978). This permits the estimation of trophic positions with the $\delta^{15}\text{N}$ (see Equation (3)), which is essentially the $^{15}\text{N}:^{14}\text{N}$ ratio of a sample normalized to the $^{15}\text{N}:^{14}\text{N}$ ratio in the air. The $\delta^{15}\text{N}$ is used to quantify the trophic position of an organism in a food web. Thus stable isotope analysis has significant value when examining the biomagnification of mercury in the food web.

**Managing Mercury in the South River**

The South River is a point-source contaminated river in northwestern Virginia, USA. The Commonwealth of Virginia currently has a fish consumption advisory for a distance of approximately 130 river miles below the historic site of mercury release (Virginia Department of Health 2008). DuPont, the party responsible for the release,
is now required by legal settlement to extract information about mercury movement in this river with the goal of informing future remediation activities.

How mercury moves within rivers such as the South River influences human exposure. Once mercury gets into the water, its speciation and movement through the food web determine the concentrations in edible fish. The speciation of mercury affects uptake and assimilation through the food web to edible fish. Because mercury movement affects fish concentrations, we can facilitate stream management by understanding the trophic transfer of mercury in the aquatic food web. Ultimately, such understanding will help managers to determine by how much mercury concentrations at the base of the food web must be lowered to achieve acceptable fish mercury concentrations for human consumption.

For this study, mercury concentrations, coupled with stable nitrogen isotopes of a wide array of aquatic organisms, were gathered to build mercury trophic transfer models. These models were intended to inform river managers in their efforts to achieve edible fish concentrations in the South River. River managers can use base level mercury concentrations to determine mercury concentrations in fish if they are provided a clear, quantitative trophic biomagnification model. A model was developed based on samples from six locations in the most contaminated river reach.
**Project Background**
This research is the central component of a three-year study that began in 2005 to define mercury bioaccumulation and trophic transfer in South River biota. After the first year, the spatial distributions of mercury and methylmercury in procedurally-defined periphyton (natural surface coatings) were determined. Snail and periphyton samples were analyzed for mercury, and N and C isotopes during the first year to formulate a sound sampling design for the following year.

In the 2006 pilot survey, periphyton, invertebrate, and fish samples from a variety of South River studies were combined for each of three sites (Dooms, Augusta Forestry Center, and Grottoes Town Park (see Figure 2) to build preliminary models of mercury biomagnification using N isotopic ratios. This study was intended only as a feasibility study that would allow design of a definitive study the next year. The samples differed in numerous ways, e.g., processed by different analytical laboratories, different tissues analyzed from the various species, and samples taken at different times and locations. Samples for important species such as forage fish and
predatory insects were unavailable. This exploration of biomagnification generated crucial insight used to design a definitive study in 2007.

**Hypotheses**

Total mercury and methylmercury concentrations in the tissues of aquatic organisms were explored for potential modeling of trophic transfer. The following hypotheses were examined in the 2007 study:

1. **Stable nitrogen isotopes can be used to model the biomagnification of mercury in the South River.**

   Mercury biomagnification models can be built to model the distribution of the element for typical sites. Five riffle sites and a pool site were selected to address this question. A model was expected to fit to data with a prediction coefficient ($r^2_{\text{prediction}}$) in the range of 0.80, which was judged *a priori* to be adequate for the purposes of river management.

2. **One mercury model for this contaminated segment of the South River, instead of several, is sufficient.**

   A trend in the model parameters might exist relative to distance from the historic source. There are potential differences in mercury concentrations and speciation along the length of the river. A nitrogen isotope-based model was assessed to see if it would suffice for the entire river segment or if separate models were required for each site. The sites were chosen within the same river segment of concern and contained
similar taxa. Based on knowledge of the river reach, the sites were assumed to experience the same general micro-climate and geology. They deviated from each other relative to concentrations of inorganic mercury and methylmercury, species densities, and distance from the source.

Biomagnification models were produced by pairing each biotic sample trophic position with its total mercury or methylmercury concentration. Information criteria described below, i.e., Akaike’s Information Criterion (AIC), were used to assess the relative value of the model combining all sites (with and without including river mile in the model).

3. The proportion of mercury present as methylmercury increases with trophic position.

The literature (e.g., Watras and Bloom 1992) suggests that the percentage of the total mercury that is methylmercury increases with trophic position because methylmercury biomagnifies more readily than inorganic mercury. However, variation exists in the literature on this point and most studies focused on lentic, not lotic, systems. It is possible that a clear trend might not exist for the study location. This hypothesis was assessed specifically for the South River by using the total mercury, methylmercury, and \(^{\delta^{15}}\)N data from the five riffle sites. If the slope for a model predicting percentage of mercury present as methylmercury from \(^{\delta^{15}}\)N was large and significantly greater than 0 (\(\alpha = 0.05\)), the hypothesis would be judged to be supported for the South River.
4. The models for the biomagnification of mercury are viable to make useful predictions on the accumulation of the element up the food chain.

Cross-validation was performed to determine how well the nitrogen isotope-based model predicts mercury concentrations. Prediction residuals and sums of squares (PRESS) were calculated for the model. PRESS was used to estimate how well the model predicts mercury concentrations.

5. The South River biomagnification models can adequately estimate fish bioaccumulation in another Virginia river.

If the model(s) adequately predict(s) mercury biomagnification in one Virginia river, a reasonable extension of the modeling would be to explore whether the same model(s) can also be used to predict mercury biomagnification in another Virginia river. To assess this hypothesis, stable nitrogen isotopes and methylmercury concentrations were taken at another contaminated Virginia river (Holston River) to model methylmercury biomagnification in its biota. Predictions from the South River model were compared to observed concentrations in the Holston River by sampling periphyton, invertebrates, forage fish, and piscivorous fish in the Holston River during the summer of 2008. An increase or decrease in the biomagnification factor of the trophic web at the Holston River was compared to that of the South River using the 95% confidence intervals for the model biomagnification factor estimates. If successful, South River biomagnification models could be used by managers of other rivers for practical prediction of methylmercury in relevant fish species.
Predictions could also be made by using the mercury estimates for the easily-sampled periphyton or scraper species along with the knowledge of the species’ nitrogen isotope ratios. Associated predictions would facilitate an understanding of the consequences of potential remediation or regulatory decisions. The associated sampling would also take less tissue, time, and funds to measure nitrogen isotopic ratios than to measure methylmercury concentrations. The model could lead to sampling fish or other biota in a nondestructive manner. It could also be used for survey studies to most efficiently define river reaches requiring follow-up definitive studies. This would be especially useful for edible, endangered, or threatened species in Virginia waterways.

**Materials and Methods**

**Site Description**
The South River is located in northwest Virginia, United States. A DuPont plant located in Waynesboro used inorganic mercury as a catalyst and released it into this river from 1929 to 1950, contaminating over 100 miles of river. Instead of the bulk of the mercury being washed from the system as originally anticipated, mercury has remained at high levels for more than 50 years (South River Science Team 2008). Consequently, a fish consumption advisory exists for a river reach extending from Waynesboro to the confluence of the North and South rivers near Port Republic. The Virginia Department of Health has a two fish per month advisory for fish from Port Republic to the Warren Power Dam (2008).
The Holston River is a contaminated river located in the southwestern part of Virginia. It is different from the South River in that it has a different water chemistry, is wider, and is more uniform, i.e., fewer transitions from pool to riffle.

**Overview of Sampling**
Five riffle sites were sampled with emphasis on continuity with past and ongoing projects in the South River (see Figure 2). An additional pool site was chosen at the request of URS, a DuPont-funded environmental consulting firm. This additional site was chosen to explore whether mercury biomagnification was distinct in deep areas of the river that might have higher methylmercury concentrations in the periphyton than the shallow riffles. This pool site was not originally part of the intended study, but did provide some
valuable additional information. Fish were collected in the pool; however, some of the periphyton and invertebrates were collected along the edges of the pool.

A riffle site was defined as a fast-moving channel with a rough bottom (Hauer and Lamberti 2006). Riffles contained rocky substrates on which periphyton accumulated. Many of the organisms were collected by flipping rocks and picking them off with tweezers. Some organisms, such as Physidae snails and predatory insects, were found near banks on tree roots, macrophytes, or sediment. Crayfish and small fish were collected using a backpack electroshocking unit, but larger fish were collected with a boat-mounted electroshocking unit.

Triplicate samples of each organism type were collected at each site. If necessary, smaller organisms were pooled to obtain adequate tissue for triplicate analyses. The samples were sent to a private analytical laboratory, CEBAM (Seattle, WA), for total mercury analysis. CEBAM is DuPont’s contract laboratory for mercury and methylmercury analyses. Because of the high cost of methylmercury analyses, only one of every set of triplicate riffle samples was randomly selected for methylmercury analysis at CEBAM. Nitrogen isotope analyses were done at the UC-Davis Stable Isotope Facility (Davis, CA). Stable nitrogen isotopes were used to quantify the trophic position of each sampled organism. The efficiency of mercury movement through the trophic web was assessed by pairing nitrogen isotopes with total mercury or methylmercury concentrations, or percent methylmercury.
**Periphyton from Artificial Substrates**

Procedurally-defined periphyton contains a wide variety of materials such as microflora, settled clays, and detritus (Newman and McIntosh 1989). Artificial substrates were used in an attempt to limit the amount of various, non-living materials collected.

Clear vinyl carpet protector was used as an artificial substrate. It was a cheap, durable, uncontaminated surface that could be purchased locally. The vinyl carpet roll was torn every foot at a perforated line to obtain 68.6 cm by 30.5 cm rectangles. One side contained patches of rough surface used for traction. The smooth side was placed face-up in the river. Samples were eventually scraped from this smooth side.

Disrupting storms and natural processes, led to potential difficulty relocating substrates and meant that all of the substrates might not be found. Because riffle sites were partly chosen for ease of access, many were located in public parks and vandalism was a concern. Ten substrates were randomly placed in the field using locations selected *a priori* with a freeware program called Visual Sampling Plan version 4.7 (Battelle Memorial Institute 2007). Shapefiles were generated in ARCVIEW 3.1 atop of aerial photographs, river shapefiles, and river mile data points collected by URS. Sampling sites were located with ARCVIEW. The measuring tool was used to mark one hundred feet upstream and downstream of the sites. Shapefiles covering two hundred feet of river and following the contours of the river were built for each site. These shapefiles were exported into the Visual Sampling Plan program that randomly generated ten points within the shape. Substrates were placed in the field according to these randomly
generated points on 21-22 of May, 2007. A Garmin Etrax Legend GPS unit was used to establish the locations of the substrates.

Periphyton was scraped off the artificial substrates and into acid-washed Nalgene\textsuperscript{®} bottles on 9 July, 2007. All bottles were prepared in the laboratory by soaking them in a 10\% (v/v) nitric acid bath for at least 24 hours. They were then rinsed seven times with Nanopure\textsuperscript{®} deionized water (Newman and Zhao 2005). Nylon toothbrushes were used to scrape natural periphyton from rocks. Occasionally funnels were used to collect the periphyton in the bottles. GPS points were not accurate enough to relocate the substrates for retrieval, so the point files from the Visual Sampling Program were used to locate the substrates. Periphyton was scraped into separate bottles for substrates that were relocated. The samples were put on ice and then frozen once in the laboratory.

Most samples contained little material. Three substrates at each site were generally selected on the basis of having ample sample for analyses. If a site had more than three substrates with enough material, a 10-sided die was used to randomly select three of the available samples for analysis.

The stored frozen samples were thawed and placed into small, tared and acid-washed bottles. These new bottles containing samples were reweighed and refrozen. The samples were dried on a freeze-dryer (LABCONCO Freezone\textsuperscript{®} 4.5 Liter Freeze Dry System, Kansas City, MO). The first few samples were occasionally removed from the freeze dryer and weighed to determine how long it took to thoroughly dry them. Once
the weight stabilized, the samples were taken from the freeze-dryer. The samples were then weighed in the bottles for wet and dry weight calculations.

The dried samples were ground using a glass stirring rod, transferred into acid-washed microcentrifuge tubes, and ground again. Ten milligrams were taken of each dried sample and placed into another acid-washed microcentrifuge tube for acidification before isotope analyses. Procedurally-defined periphyton contains carbonates that can affect $\delta^{13}$C values and acidification removes these carbonates (Søreide et al. 2006).

The ten milligram aliquot was acidified by a 2M solution of redistilled HCl. The literature recommends using 1 to 2 M HCl and 2 M HCl was chosen because it was strong enough to drive off the carbonates, but also was weak enough to have minimal influence on $\delta^{15}$N. Some literature suggests that acidifying a sample might affect the nitrogen isotope ratio (Bunn 1995). Other literature states that acidifying does not affect stable isotope analysis (Waldron et al. 2001). The Søreide et al. (2006) recommendations were followed because these authors carefully considered both sides of the issue: the need to eliminate inorganic carbon from the sample while minimally changing $\delta^{15}$N values.

The periphyton was soaked in acid for two hours, spun in a microcentrifuge (Fisher Scientific Micro14, San Francisco, CA) at 2000 RPM for five minutes. After the acid supernatant was removed, the samples were rinsed with deionized water to remove residual acid solution. The samples were shaken, centrifuged again, and the supernatant
decanted. This rinsing was done twice. The samples were refrozen and redried on the freeze dryer. Each dried sample was homogenized, and 2 to 3 milligrams were transferred into 5 mm by 9 mm tin capsules. The capsules were closed, organized in a 96 well plate, and sent to UC-Davis Stable Isotope Facility for $\delta^{15}$N and $\delta^{13}$C analyses. The remaining dry sample was sent to CEBAM analytical laboratories for methylmercury and total mercury analysis.

Freeze dried samples were sent instead of wet samples. Samples such as periphyton were hard to thoroughly homogenize without freeze-drying. Some of the samples contained large amounts of water, and it was impossible to guarantee complete homogenization of the suspension. Samples, especially some of the smaller organisms, were more easily homogenized once dried. Patrick Pang and Liam Lang of CEBAM confirmed this decision (Pers Comm 18 July 2007), agreeing that the mercury samples would not be compromised by freeze-drying.

**Work Station**
The sample preparation area was decontaminated prior to use. A 68.6 cm by 30.5 cm sheet of thick plastic was washed with Citranox® Acid Cleaner and Detergent. It was then rinsed with Nanopure® deionized water. The surface was set on a clean table which was frequently cleaned with Citranox® detergent and Nanopure® deionized water, especially between processing of very different samples. Tweezers were similarly cleaned and rinsed.
Natural Periphyton
Natural periphyton was collected during the sampling in May in case ample material could not be collected from artificial substrates. Collection locations were randomly selected using a 10-sided die. The samples were scraped into acid-washed containers and placed on ice until they could be frozen in the laboratory. Natural periphyton samples were processed similarly to artificial periphyton samples, except 3-4 milligrams were sent for stable isotope analysis.

Macrophytes
Two macrophyte species were selected at each site. If possible, the same species type of macrophyte was selected at each site. Emergent macrophytes were selected at some sites, such as Augusta Forestry Center, instead of submerged macrophytes due to availability. Macrophytes were stored in clean zip-lock bags, placed on ice, and frozen in the laboratory. The samples were thawed and blotted in the laboratory with Kimwipes® EX-L (Kimberly-Clark®, Ontario, Canada) to remove excess water before estimating wet and dry weights. Each macrophyte was separated into triplicate samples, placed into acid-washed containers, frozen, and freeze dried. The samples were homogenized, and 2-3 milligrams were processed for isotope analysis.

Invertebrates
Snails

The snail *Leptoxis carinata* was abundant on many of the rocks in the South River and were collected by hand. The planorbid snail, *Helisoma* sp., was found along the river banks on logs, roots, and sediment. Snails in the Physidae family were also found near
the banks, but could also be found crawling on plants or sediment. Snails were stored in plastic bags on ice in the field and then frozen in the laboratory.

All snail types sampled were processed similarly. The soft tissue was removed from the shell using tweezers and transferred into acid-washed microcentrifuge tubes. Each species had several individuals pooled to produce triplicates. *Leptoxis* required 20-30 snails pooled for each sample. Pool size varied for Physidae and *H. trivolvis* samples depending on how many individuals were collected at each site. The number of pooled organisms ranged from 6-20. The soft tissue samples from the snails were frozen, freeze dried, and split into two aliquots: one for analysis of mercury and the other for analysis of stable isotopes.

**Aquatic Insects**

Mayflies were mostly sampled by flipping over rocks and picking them off the rocks with tweezers. Baetidae were often found on top of rocks in the periphyton. They were easily distinguishable by their shape and movement. *Stenonema* sp. were found in brisk riffles. They were identified by their flat bodies with horizontal stripes. Another less common mayfly looked similar, but had vertical stripes on their bodies. There were also differences in its gill movement. These might be *Stenacron* sp. mayflies (Chris Cole, Pers Comm 24 May 2007), so they were not mixed into the pooled *Stenonema* sp. samples. *Seratella* sp. and *Ephemerella* sp. are small mayflies from the Ephemerellidae family. They were sometimes mixed together in samples. They were found under rocks, but *Seratella* sp. was also commonly seen around caddisfly cases. A few other mayflies
were observed, but there were not enough to sample. Chris Cole helped identify several different species of mayflies.

Hydropsychidae were found either on or under rocks. They could be found inside cases or roaming on rocks. Psephenidae were generally found in shallow water in swift moving waters or on top of rocks. They were observed grazing on periphyton. Predator insects such as Gomphidae, dragonfly larvae, and Zygoptera, damselfly larvae, were found on or near the riverbanks.

Aquatic invertebrates were placed in glass vials or plastic bags, put on ice in the field, and then frozen at the laboratory. In the laboratory, they were thawed and dabbed on Kimwipes® EX-L to remove excess water so that accurate dry/wet quotients could be calculated. They were pooled into acid-washed microcentrifuge tubes, freeze dried, ground, and split into two aliquots for mercury and isotopes. The microcentrifuge tubes were weighed empty, with wet tissue, and later with dry tissue. A dry/wet quotient was calculated from these weights.

**Crayfish**

Crayfish were collected using a backpack electroshocking unit or catching them by hand in rocky areas of the riffle. Two main genera were sampled: *Cambarus* and *Orconectes*. Crayfish were put into plastic bags, placed on ice, and then frozen in the laboratory. Similar-sized crayfish were selected to reduce one source of variability. One whole crayfish was used for each sample. The samples were freeze dried and homogenized.
One aliquot was transferred to an acid-washed microcentrifuge tube for mercury analysis, and another aliquot was processed for stable isotope analysis.

**Corbicula**

*Corbicula fluminea* were gathered on the bottom of the riverbed at the top edge of the riffle using a net. We collected clams whose shell lengths were 18-25 mm, which was the size used in a previous South River study by URS. This size limit controls the specimens to 3 year old clams (Cohen et al. 1984). This range was determined from size-frequency distributions by Dresler and Cory (1980).

However, the ages gathered from the literature might not reflect the age of South River *Corbicula*. Growth is dependent on temperature, water movement, availability of food, and sediments (M. Newman Pers Comm 24 August 2007). The clams are probably 1-2 years old, but a narrow size range should limit the variability. *Corbicula* were taken out of their shell, dabbed with a Kimwipe, and placed into acid washed containers. Clams (*n* = 4-7) were pooled together for each sample. The samples were frozen, freeze dried, and ground. Two aliquots were taken out: one for mercury and the other for isotope analysis.

**Fish**

Personnel of URS and the Virginia Department of Environmental Quality used electroshocking to collect fish for this study. Larger fish, such as large bass and suckers, were collected using a boat electroshocking unit. Smaller fish were collected by a backpack unit. One type of bass, either *Micropterus salmoides* (largemouth bass) or
Micropterus dolomieu (smallmouth bass); Catostomus commersonii (white sucker), and three types of smaller fish were collected at each site. More of the smaller fish were collected in case pooling was required; however, Nocomis leptocephalus (chub) from North Park was the only species eventually pooled. For all other species, three individuals of similar size were used, one for each triplicate fish sample.

Edible-sized bass were used, with the possible exception of one M. dolomieu at Augusta Forestry Center which was small. Another M. dolomieu that had died after shocking was examined. It was smaller, but had developed gonads and contained fish contents in the stomach. Augusta Forestry Center was the only site at which we took M. dolomieu instead of M. salmoides. URS made multiple passes unsuccessfully to get three M. salmoides that were of edible and similar size. Scott Gregory of URS recalled that the Virginia Department of Environmental Quality had taken five adult M. salmoides out of the same site a few weeks earlier (Pers Comm 23 May 2007).

Fish were double sealed in Ziploc bags and stored on ice until frozen in the laboratory. Smaller fish were transferred into acid washed containers and freeze dried. They could then easily be ground. Larger fish were more difficult. The stomachs of bass were examined. Any large organisms, such as other fish or crayfish, were removed. Some species, Lepomis auritus (red-breasted sunfish), Lepomis macrochirus (blue-gill sunfish), Semotilus corporalis (fall fish), C. commersonii, and M. salmonides required homogenization with a food blender. The blender was rinsed with tap water and then washed thoroughly with Nanopure® deionized water thrice between samples.
Some fish, such as the bass, needed Nanopure® deionized water added in the blender to homogenize effectively. The added water was weighed so it could be subtracted later during estimation of dry/wet quotients. *Catostomus commersonii* were particularly easy to homogenize because they were oilier fish with fewer thick bones. Larger *M. salmoides* had thick facial bones which made homogenization difficult. Some of the bass were ground with a meat grinder prior to homogenization.

Once the fish were homogenized, an aliquot was placed into a smaller acid washed container, frozen, and freeze dried. The samples were homogenized again after freeze drying. One milligram of homogenized sample was taken out for isotopes, and one gram was taken out for mercury analysis.

**Methylmercury**
The sample measured for methylmercury was selected from triplicates using the Excel® random number generator. The sample selected was analyzed for both total mercury and methylmercury while the remaining two were analyzed only for total mercury.

**Stable Isotopes**
A small amount of each sample was taken for isotopes after the samples were homogenized. Animal tissue required 0.8 to 1.2 mg, plants required 2 to 3 mg, and soil required 10 to 75 mg for analyses. The same sample size required for plants were used for periphyton from the artificial substrates. Sample size used for natural periphyton (3-4
mg) was a little larger because there could be substantial amounts of sediment or abiotic material in these samples.

For isotope analyses, 5 x 6 mm tin capsules were tared on an analytical scale. Then an isotope preparation spatula was used to transfer material into the tin capsule. Once a weight of sample was within the acceptable range in the tin capsule, tweezers were used to move the capsule to a crimping plate where the capsule was pressed closed. The capsule was moved into a 96-well plate and the well number and sample information recorded in a notebook. The plates of pelletized samples were then sent to UC Davis for nitrogen and carbon stable isotope analysis.

**Holston River**

A similiar collection scheme was used to sample biota from the Holston River, another mercury-contaminated river in Virginia. The original plan was to collect triplicate samples of periphyton from natural substrate, a primary consumer, a predator insect, a forage fish, and a bass. These organisms and additional opportunistic samples were successfully collected. Because of limited funding, sampling was not as extensive as South River sampling. One site was sampled (46 miles from the source) to produce the trophic transfer model, and triplicate *M. dolomieu* samples were collected by U.S. Fish and Wildlife Service personnel from two additional sites upstream: 12.5 miles and 21 miles from the source.
General Biomagnification Model
The general equation for expression of the stable isotopes ratio expression is the following (Peterson and Fry 1987).

\[
\delta X = \left[ \left( \frac{R_{\text{Sample}}}{R_{\text{STANDARD}}} \right) - 1 \right] \times 10^3 \quad (2)
\]

Equation (2) gives a ratio of the fractionalized isotopes, heaviest over lightest, in a sample to be compared to the isotope ratio for a reference. The symbol \( \delta \) represents the amount of heavy isotopes per light isotope, \( X \) is the heavy stable isotope \((^{15}\text{N}, ^{13}\text{C}, \text{or} ^{34}\text{S})\), and \( R \) is the ratio \((^{15}\text{N}/^{14}\text{N}, ^{13}\text{C}/^{12}\text{C}, \text{or} ^{34}\text{S}/^{32}\text{S})\) for the sample or reference material.

The stable isotope reference for nitrogen is nitrogen gas in air; belenmite from the Pee Dee Formation is used as the carbon reference (Peterson and Fry 1987). For nitrogen, the final equation for \( \delta^{15}\text{N} \) is the following (Cabana and Rasmussen 1994).

\[
\delta^{15}\text{N} = 1,000 \left[ \left( \frac{^{15}\text{N}_{\text{Sample}}}{^{15}\text{N}_{\text{air}}} \right) / \left( \frac{^{14}\text{N}_{\text{Sample}}}{^{14}\text{N}_{\text{air}}} \right) - 1 \right] \quad (3)
\]

\( \text{N}_{\text{air}} \) represents the nitrogen isotope concentration found in the atmosphere of Earth.

Biomagnification models can be created using mercury concentrations and \( \delta^{15}\text{N} \). Broman et al. (1992) gave the general equation.

\[
e^{(a + b\delta^{15}\text{N})} \quad (4)
\]
where $b$ is the biomagnification factor and $e^a$ is the theoretical baseline concentration of a contaminant at the x-intercept. Fitting this equation to data can produce an estimate of the biomagnification factor at a site. It might also serve to predict the contaminant concentration in an organism if the only data available were the nitrogen stable isotope fractions and mercury or methylmercury concentrations at the base of the food web.

**Statistical Methods**

**Selection of Candidate Models - Akaike’s Information Criterion (AIC)**

There are several variables that could be used to build a biomagnification model; however, the stable nitrogen ratio-based model above is currently the most practical one. Equation (4) or simple elaborations of this model were explored here for the South River food web. Akaike’s Information Criterion was applied to determine if the simple nitrogen isotope model or the simple nitrogen isotope model with river mile added was the best model. In other words, the model containing the maximum amount of information per estimated parameter was selected. Having more variables generally decreases the model sum of squares but at the expense of increased standard errors for the estimated parameters. Minimum AIC estimation (MAICE) prevents inclusion of unnecessary variables into the model and unnecessary uncertainty into parameter estimates. In its simplest form, the Akaike’s Information Criterion for a model is the following (Newman 1995):
\[ AIC = n \left[ \ln \left( \sum_{i=1}^{n} w(Y_i - Y_{pi}) \right) \right] + 2p \quad (5) \]

where \( n \) is the number of data points; \( w_i \) is the \( i \)th observation weight; \( Y_i \) is the observed value of the \( i \)th term; \( Y_{pi} \) is the predicted value of the \( i \)th term; and \( p \) is the number of model parameters. Here, regressions were not weighted so \( w_i = 1 \) for all data.

**Cross-Validation**

Measures of goodness-of-fit do not necessarily reflect the ability of a model to produce useful predictions. Cross-validation is required to assess the predictive capability of models, like those from this study (Schwilk et al. 1998). The correlation coefficient (\( r^2 \)) quantifies how well the model fits the data by comparing data points to predicted points from a model that is made up of these same data (Cooil et al. 1987). Use of data to generate a model and then to assess that model’s ability to give close predictions for the same data inserts an undefined degree of circularity about judgment of a model’s ability to make accurate predictions for a new data point. A measure of fit, such as the model error mean square (MSE), tends to underestimate the true variability that will manifest in model predictions (Neter et al., 1990). Predictions from new data not used to build the model would be needed to assess the predictive capabilities of a regression model.

Cross-validation was developed to resolve this issue.

There are several ways to perform cross-validation. As described in Neter et al. (1990), a PRESS procedure can be used if there are only a small number of observations (less than 6-10 times the number of variables). For small data sets, squares of the residuals from the model predictions (when a data point is not used to generate the model) and observed
values for the validation data are summed to produce a PRESS estimate and divided by the original model’s total sum of squares (TSS). The quotient PRESS/TSS is subtracted from one to generate a prediction $r^2$. For example, a prediction $r^2 = 0.78$ infers that 78% of the variation in predictions for new data points will be captured by the model. However, a data splitting approach is preferable for large data sets. The data-splitting cross-validation approach in which a large data set is split in half and one subset of data points (training data) is used to build the model. Once the model is built, each point in the other half of the data, known as the validation set, is compared to the corresponding model prediction. Data-splitting has been used for a wide range of applications including assessing prediction accuracy of GPS disturbance corrections (Zhong et al. 2007) and orientation of lanthanide-substituted calcium binding proteins (Barbieri 2002).

In summary, triplicate samples of 16 biota types at each of the 6 sites were collected. The samples were processed and sent out for total mercury and stable isotopes. Single samples from the five riffle sites were analyzed for methylmercury. A biomagnification model was built using the mercury and isotope data, and the prediction capabilities of the model were estimated using PRESS. AIC was used to determine the model with the most information for prediction per parameter estimate.
**Results**

The mean total mercury concentration for each biota type was graphed against $\delta^{15}$N (Figure 5). Simuliidae, pulmonate snails and macrophytes were left out of the final regression model because they were not part of the general scraper/gatherer/collector-based food web relationship being modeled.

![Graph showing natural log total mercury concentration vs Del 15N with various biota groups represented](image)

**Figure 5:** The natural log of total mercury versus $\delta^{15}$N. The major biota groups are represented by asterisks (periphyton), filled circles (primary consumer invertebrates), predator insects (pluses), secondary consumer fish, (triangles) and bass (diamonds).
Periphyton had higher total mercury (but not methylmercury) concentrations than expected from the general trend of the other biota. Periphyton from artificial and natural substrates had high, but similar, total mercury concentrations. Natural periphyton was gathered during collection in case too many artificial substrates were vandalized or washed out. Natural periphyton was analyzed because periphyton from artificial substrates contained high total mercury concentration and stable nitrogen isotope ratios varied considerably. The range for periphyton grown on artificial substrates was higher than periphyton grown on natural substrates (Figure A1 in the appendix), but it was not enough to justify replacing the original periphyton grown on artificial substrates.

The model was built using the natural log of methylmercury concentrations. The transformation linearized the data so that simplified models could be created. However, converting natural log concentrations back to arithmetic concentrations produces a backtransformation bias. Multiplying the model predicted methylmercury concentrations by the correction factor eliminated the bias (Newman 1995),

$$\text{Backtransformation Correction Factor} = e^{\frac{\text{MSE}}{2}}$$  \hspace{1cm} (6)

where MSE stands for the model mean square error.
Figure 6: Increase in methylmercury concentration with increase in trophic position (δ\textsuperscript{15}N) is evident in this plot of data from all five riffle sites.

Table 2: Statistical information on the methylmercury model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Degrees of Freedom</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>t-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>-5.252</td>
<td>0.333</td>
<td>-15.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(\delta^{15})N</td>
<td>1</td>
<td>0.450</td>
<td>0.034</td>
<td>13.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>River Mile</td>
<td>1</td>
<td>0.054</td>
<td>0.011</td>
<td>4.85</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The parameter estimates used to predict methylmercury concentration from the \(\delta^{15}\)N and river mile (Table 2) were predicted in SAS and can be used in Equation (7). The standard
errors are relatively small for the parameter estimates of stable nitrogen isotopes 
(p<0.001, two-tailed t test, t=13.18, df=65) and distance from the source (p<0.001, two-
tailed t test, t=4.85, df=65) and were significantly greater than zero. The parameter 
estimates were incorporated into the general biomagnification model (Equation (7)) to 
predict mercury concentrations. The baseline, e^0, is e raised by the intercept (-5.25). The 
backtransformation bias correction was e^{0.5/2}, or e^{0.265}.

$$Methylmercury (mg/kg DW) = e^{-5.252 + 0.450_{\delta^{15}N} + 0.0541_{RM}} e^{0.5/2}$$  (7)

The slopes of all six sampling sites were similar. It was the y-intercept that gradually 
increased with distance from the source. The general linear model showed little 
interaction between $\delta^{15}N$ and river mile (p=0.65). The increase of mercury at the base of 
the food web could explain the resulting mercury concentration increase in bass down 
river noted here and in past studies.
Figure 7: Predictions of natural log methylmercury concentrations from the model that includes river mile and δ\textsuperscript{15}N. The intercept increases with distance downriver from the historic source. Pulmonates, simuliidae, and macrophytes were not used to build this model.

The influence of biomagnification (0.45) outweighs that of river mile (0.054) parameter as is evident from Figure 7. The \(r^2\) for the regression coefficient was 0.78, while the PRESS prediction coefficient was 0.76. Minimum Akaike’s information criterion estimation (MAICE) was used to determine if river mile was an informative variable in the model. The model built with δ\textsuperscript{15}N and river mile was compared to a model with only δ\textsuperscript{15}N. The model including δ\textsuperscript{15}N and river mile (AIC=238) had a smaller AIC than the
model with $\delta^{15}\text{N}$ alone (AIC=257); therefore, the model with both variables was considered the best of the two.

Strictly interpreting the AIC results, the AIC indicated that the model with both $\delta^{15}\text{N}$ and river mile was more informative than that with $\delta^{15}\text{N}$ alone; however, there was not a large difference in AIC values for the two models. River mile improves the South River model but a satisfactory model could have been built with $\delta^{15}\text{N}$ alone. River mile was not relevant in the Holston River model: The biomagnification factor was the sole parameter to compare in the two river models.

The percent of total mercury that is methylated increased with trophic position (Figure 8). Mercury in periphyton was predominantly inorganic but most of the mercury in fish was methylmercury. The other organisms varied depending on their trophic position on the sigmoid curve. An inverse cumulative normal function was used to linearize this sigmoid curve and to generate a predictive model (Figure 9) which had an $r^2$ of 0.71.

Inorganic mercury decreased with trophic position (95% confidence interval of slope estimate = 0.00 to -0.28, $p=0.0395$ for null hypothesis that slope = 0). Models for individual sites were not significant (Table A1) but, when the data from all sites were combined, the slope was significant (Figure A6 in the appendix).
Figure 8: The percent of total mercury that is methylmercury as a function of trophic position ($\delta^{15}$N).
Predicted Percent Methylmercury with 95% confidence Intervals Overlayed with Percent Methylmercury vs Del 15N

Figure 9: The inverse cumulative normal function of the proportion of total mercury predicted to be methylmercury based on trophic position (red line = prediction, blue line =95% confidence interval for individual predictions, black dots=data points).

Data from the Holston River showed similar trends. Mercury increased to high concentrations in South River, but started to drop after approximately 20 river miles. Holston River mercury concentrations are not as high, but the concentrations remained constant for more than 80 river miles. This effect is best observed in the periphyton data (Figure A7 and A8 in the appendix).
The data collected in 2008 from the Holston River were modeled using Equation (8),

\[ Methylmercury(\text{mg} / \text{kg DW}) = e^{-5.034 + 0.481(\delta^{15}N)} e^{0.15} \]  (8)

As noted for the South River, total mercury versus trophic position (Figure A9 in the appendix) was more variable than methylmercury versus trophic position (Figure 10). The total mercury model had a regression \( r^2 \) of 0.30, and a prediction \( r^2 \) of 0.24. The Holston River total mercury model was also judged nonviable for predictive purposes. Three biota types in particular were higher than the general trend: gomphidae, corydalidae and plecoptera. These three biota types were predator insects; a fourth predator insect, zygoptera, did not have higher than expected total mercury concentrations.
Figure 10: Methylmercury biomagnification for the Holston River.

Like the methylmercury-based model for the South River, the methylmercury model for the Holston River was viable for making predictions. The $r_{\text{residual}}^2$ was 0.83 and the $r_{\text{predicted}}^2$, calculated from the PRESS, was 0.80. *Micropterus salmoides* were not collected in the Holston River. Instead, *M. dolomieu* were collected at three Holston River sites. Because *M. dolomieu* feed lower in the trophic web than *M. salmoides*, the mercury biomagnification to bass of edible size was slightly lower than if *M. salmoides* had been present.
The slope of the Holston River model (95% confidence interval of estimate=0.413 to 0.549) was very similar to the South River model slope (95% confidence interval of estimate=0.382 to 0.518). The South River model was therefore tentatively judged to be viable for this second river system, and perhaps, still others. Even though the Holston River has a different contamination history, organisms, water chemistry, and river dynamics, mercury biomagnification was similar to that in the South River (Figure 11).

Figure 11: The biomagnification of methylmercury for both the Holston River and the South River. The 95% confidence intervals for the biomagnification factors of both rivers coincide (insert).
Discussion
The stable isotope model proved effective in modeling the bioaccumulation of methylmercury in the biota of the South River. Both stable nitrogen isotopes and river mile gave viable estimates of methylmercury trophic transfer in the South River model. Basically, one coefficient accounting for trophic position sufficed for all site data (Equation (7), Table 2). The influence of river mile reflected the increase of methylmercury at the base of the food chain with distance downriver. This caused an increase of mercury concentration in bass. That is, methylmercury from the lower portions of the food web were biomagnified to the bass. The model suggested minimal differences in the accumulation of methylmercury up the food web, i.e. the slope, among sites.

The South River data fit the aquatic system trophic structure expectations of Minigawa and Wada (1984). Primary producers in aquatic systems start at approximately δ^{15}N of 5 \%. Trophic levels increase every 3.4 \%: primary, secondary, and tertiary consumers are approximately 8.4 \%, 11.8 \%, and 15.2 \%, respectively. Aquatic primary consumer insects stay primarily between primary producer and consumer lines. Aquatic predator insects are midway between primary and secondary consumer. Forage fish start in the same area of the food chain as predator insects, but extend to midway between secondary and tertiary consumers. Piscivorous fish extend slightly past tertiary consumer. Brand and Cohen (1987) estimate that the average aquatic system is 3.7 trophic lengths; the South River was approximately 3.7 trophic lengths (Figure 12).
Figure 12: The natural log of total mercury without periphyton. The vertical lines represent the classical trophic levels derived by Minigawa and Wada (1984) from $\delta^{15}$N ratios. The dotted line marks 3.7 trophic chain lengths, the average length for an aquatic system (Briand and Cohen 1987).

The South River and Holston River predictive models were similar, suggesting that a single biomagnification factor might be applicable to other Virginia rivers. The rivers had differences such as different scraper and associated species, climates, and physical properties of the river. However, the rivers had similar food webs and community members, indicating a similarity in trophic structure. It would be hard to justify using these models outside riffled streams in the mid-Atlantic region of the United States without more research. It would be interesting to test the models in a similar low-order
river of New England or California to see what factors might affect the biomagnification parameter estimate. A European river with similar climate and trophic structure could also be informative. Further testing is needed to determine the applicability of the model to other river systems.

Periphyton from natural substrates had total mercury concentrations similar to periphyton from artificial substrates. The assimilation efficiency of mercury does not appear to be as high between these primary producers and primary consumers as it is in the rest of the food web. This is consistent with other publications on periphyton with mercury (Hill et al. 1996) and different metals (Newman and McIntosh 1982, 1989). One reason might be the low percentage of methylmercury in the periphyton; inorganic mercury is not as efficiently assimilated as methylmercury (Hill et al. 1996). An undefined portion of the mercury was bound to minerals or sediment in the periphyton that likely has lower bioavailability than that associated with the microflora.

Contrary to the original assumption, the range of the δ^{15}N was wider for the periphyton from artificial substrates than for periphyton taken from natural substrates (Figure A1 in the appendix). The use of artificial substrates was intended to reduce the amount of abiotic material in the periphyton samples, and thereby, reduce variation. But, artificial substrates made it more difficult to collect similar materials because collection was restricted to the few artificial substrates. Periphyton could grow for only a few weeks, whereas material taken from natural substrates accumulated over a longer time period. There was ultimately not enough evidence to support the original supposition that
periphyton grown on artificial substrates would be superior to the original samples of periphyton from natural substrates. This was a fortuitous finding because the use of periphyton from natural substrates simplifies and reduces costs for any future biomonitoring in mercury-contaminated Virginia rivers.

Total mercury was inadequate to develop a useful model for predicting biomagnification to edible fish. Preliminary data from 2006 produced good relationships between total mercury and δ¹⁵N, but these relationships did not hold up during the more rigorous sampling in 2007. Variation might have been reduced in the pilot study because of a selection of fewer biota types. This variation seems to be reduced even in the total mercury graphs in the Holston River (Figure A9). A few predator insects stand out in the figure, but only because there are not a lot of primary consumers to overshadow them like in the South River model (Figure 5).

Some biota types might have lower total mercury concentrations because they fed primarily on terrestrial sources such as leaves and other allochthonous detritus. That also could explain why some triplicate samples are similar within a site, but different among sites. Mercury concentrations changed at each site, but δ¹⁵N should have been the same among sites assuming biota fed similarly. The δ¹⁵N in a few biota varied among sites which could indicate different feeding habits or sources. Hydropsychidae, for example, had a mean δ¹⁵N of 6.76‰ (Standard Deviation: 0.43‰, n=6) in the City of Waynesboro (Constitution and North parks) but increased to 8.50‰ (Standard Deviation: 0.73‰, n=12) outside the city (Dooms, Pool, AFC, and GTP). Agricultural run-off was a large
source of nutrients to the river outside the city and might have increased the $\delta^{15}$N. More likely, an increase of nutrients from wastewater-treatment plants was introducing a new food source enriched in $^{15}$N isotopes to biota.

Methylmercury analysis was considerably more expensive than that for total mercury so single samples were analyzed for methylmercury instead of triplicate samples. Sixteen samples per site, minus one or two outlier taxa points, were judged suboptimal for building a methylmercury model for each site. The model produced by combining data from all sites was satisfactory for the predictive purposes of the study, that is, to quantitatively predict mercury concentrations in members of the food web, especially commonly consumed fish, in the impacted region of the South River.

Methylmercury concentrations were less variable than total mercury if plotted against trophic position ($\delta^{15}$N) (Figure 6). Periphyton had a considerable amount of inorganic mercury with considerable variation (average: 1.934 mg/kg DW, Standard Deviation: 1.213 mg/kg DW, n=5). So, the methylmercury concentrations reflected what was more readily bioavailable to primary consumers than the total mercury concentrations. Also, the percentage of total mercury in biota present as methylmercury increased with trophic position until nearly all of the total mercury in higher predators was methylmercury. For these reasons, methylmercury-based models were judged superior for predicting trophic movement of mercury to higher order predators than models that might be based on total mercury. Pulmonate snails and simuliidae fit much better into the methylmercury figure than total mercury, but were still high. Methylmercury is more prone to biomagnify than
inorganic mercury which tends to biodilute. This appears to generate more variation in the total mercury data than methylmercury data.

There was considerable variation in total mercury concentrations among organisms. The $r^2_{\text{regression}}$ value for the total mercury-based model with river mile and nitrogen stable isotopes (0.32) was judged insufficient for useful prediction. However, South River models using methylmercury versus $\delta^{15}N$ had an acceptable PRESS $r^2_{\text{prediction}}$ of 0.76 (based on an *a priori* criterion). Split validation would have been applicable if more data were available but PRESS was best for the methylmercury model because of the relatively small data set (66 data points). This was verified during a preliminary splitting of the data set. The $r^2_{\text{prediction}}$ coefficient for split validation is 0.86 (Figure A4 in the appendix). However, when the training points were used to build a second model, and the model points were used to validate the second model, the $r^2_{\text{prediction}}$ dropped from 0.86 to 0.69 (Figure A5 in the appendix). Split validation was not as accurate at predicting the true prediction coefficient as PRESS because if a model only has thirty-three model points, a few points can skew the model materially. This bias can give an inappropriately optimistic or pessimistic prediction.

Some biota might be outside the general total mercury vs $\delta^{15}N$ trend (Figure A2 in the appendix) because they contained large amounts of inorganic mercury that was not readily transferred to consumers. Mercury concentrations were higher in simuliiidae than initially expected from the trend of $\delta^{15}N$ versus mercury. Simuliidae consumed dissolved organic matter and concentrated mercury directly from the water, which was
different from the other organism types in its general trophic position (Pennak 1953). Mercury more readily bioaccumulated in simuliidae due to this unusual feeding mode as documented in other studies (Harding et al. 2006). However, South River simuliidae had low percentages of total mercury that was methylated while the data from Harding et al. (2005) suggested that simuliidae had proportions of methylmercury as high as predatory insects.

Pulmonate snails, such as Physidae and *Helisoma* sp., also appeared to deviate from the general scraper/gather/collector mercury biomagnification trend because they graze periphyton preferentially compared to other scrapers. They consume more plant material and less sediment, particulates, and various other materials that generalist consumers eat (M. Newman Pers Comm 19 Dec 2007). *Helisoma* sp. appeared lower than Physidae, but were misleading because they were only gathered at the two sites closest to the release: Pulmonates were collected at the four sites farthest from the source. These three species were taken out of the aquatic model post hoc, but only after considerable thought.

The outlier species data still convey important insight, especially regarding the adjacent terrestrial food web. Drift-feeding fish prey on stoneflies (Huhata et al. 1999) which then preyed on simuliidae (Tikkanen et al. 1997). Fish and other terrestrial organisms also consume Simuliidae directly (Allan 1981). Mallard ducks and tree swallows consume organisms from the same order as Simuliidae, that is, Diptera (Sugden and Driver 1980; Gerrard and St Louis 2001).
Macrophytes were not intended to be in the model because they were not considered an important part of this food web. They were collected to document the macrophyte concentrations relative to the observed periphyton concentrations. Some macrophyte biomass might get into the food web by collecting on natural surfaces, in which case they would be samples through the procedurally-defined periphyton.

Mercury contamination is becoming a global problem due to an increase of atmospheric deposition. Application of trophic transfer models, as done in this study, can reduce costs for other site investigations by providing a means of producing inexpensive, screening information and also for predicting consequences of various proposed remediation scenarios. By collecting procedurally-defined periphyton or a primary consumer, which were more easily defined and less variable, river managers can use the resulting δ\(^{15}\)N and methylmercury concentrations to estimate the baseline in the model. Using the newly estimated baseline with the established biomagnification parameter estimate, 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. This can help river managers, with their limited resources, look at larger stretches of river. The models can help narrow the attention to river stretches with the potential of bass with unhealthy mercury concentrations.
Figure A1: Periphyton grown on natural substrates (blue diamonds) versus periphyton grown on artificial substrates (purple squares). Both types of periphyton vary similarly in total mercury, but periphyton grown on artificial substrates had a wider nitrogen stable isotope range.
Figure A2: The mean total mercury concentration for each biota type versus its corresponding δ¹⁵N. Periphyton grown on artificial (*) and natural (*) substrates are both included in this figure.
Figure A3: The natural log of total mercury data in relation to $\delta^{15}$N. Site does create some variation, but still does not explain the majority of the variation.
Figure A4: Cross-validation using the modeling data points to create predicted methylmercury values (y-axis) and comparing them to the observed validation data.
Figure A5: Cross-validation using the same splits, but reversing their roles. This time the split data was used to create predicted methylmercury values (y-axis) and the original modeling data was used as the observed data.

\[ \text{Observed Ln Mercury Concentrations for Half A (Ln mg/kg DW)} \]

\[ \text{R}^2 = 0.69 \quad n=33 \]
\[ \text{Intercept: } -0.32 \pm 0.1 \ (p=.02) \]
\[ \text{Ln MHg: } 0.70 \pm 0.08 \ (p<.0001) \]
Natural Log Inorganic Mercury Concentration vs Del 15N

Figure A6: The natural log of inorganic mercury concentrations for organisms (without Helisoma sp., Physidae, macrophytes and simuliiidae) in relation to $\delta^{15}N$.
Figure A7: South River mercury concentrations in periphyton are low upriver of the source but rapidly increase below the source. More recent periphyton sampling showed a decrease in mercury around river mile 20.
Figure A8: Holston River mercury concentrations were high compared to reference samples upstream of the source. Mercury concentrations remained consistent over the 85 mile stretch below the source.
Figure A9: Holtson River total mercury concentrations increased with trophic position, but there were some biota that deviated from the general trend.
Figure A10: South River periphyton grown on natural substrates (diamonds) versus periphyton grown on artificial substrates (squares). Sites are colored in ROYGBIV order from closest to the source to furthest from the source.

Table A1: General linear model summary information on the natural log of inorganic mercury.

<table>
<thead>
<tr>
<th>Site</th>
<th>Intercept (SE, p)</th>
<th>Slope (SE, p)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitution Park</td>
<td>-0.31 (1.31, .8149)</td>
<td>-0.27 (0.14, .0894)</td>
<td>0.24</td>
</tr>
<tr>
<td>North Park</td>
<td>1.19 (0.89, .2091)</td>
<td>-0.28 (0.11, .0270)</td>
<td>0.40</td>
</tr>
<tr>
<td>Dooms</td>
<td>2.51 (1.40, .0921)</td>
<td>-0.30 (0.13, .0434)</td>
<td>0.35</td>
</tr>
<tr>
<td>Augusta Forestry Center</td>
<td>0.83 (0.71, .2673)</td>
<td>-0.13 (0.07, .0988)</td>
<td>0.21</td>
</tr>
<tr>
<td>Grottoes Town Park</td>
<td>1.69 (1.43, .2606)</td>
<td>-0.24 (0.14, .1056)</td>
<td>0.20</td>
</tr>
<tr>
<td>All Sites Data Combined</td>
<td>0.23 (0.64, .7184)</td>
<td>-0.14 (0.07, .0395)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table A2: Statistical information on the Holston River methylmercury-based model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Degrees of Freedom</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>t-value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>-5.034</td>
<td>0.348</td>
<td>-14.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>δ(^{15})N</td>
<td>1</td>
<td>0.481</td>
<td>0.034</td>
<td>14.30</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Analytical QC/QA and Methods**

Analytical QC/QA was performed at the analytical laboratories. CEBAM performed duplicates and matrix spikes for the mercury analysis (Table A3 and A7 in the appendix). They also analyzed standard reference materials (Table A4, A5, and A8 in the appendix). UC Davis stable isotope facility performed two checks against laboratory references after every twelve isotope samples (Table A6 and A9 in the appendix). All of the analytical QC/QA results were acceptable for the study. Field QC/QA was incorporated into the statistical models with replicate sampling.

CEBAM digested the samples in closed vials with alkaline for 3 hours at 75°C. Alkaline can be used for both mercury and methylmercury analysis and reduces the amount of mercury lost in the process. The digests were then diluted. An aliquot was taken for total mercury, oxidized with BrCl, and then reduced by SnCl₂. The mercury was collected with a gold trap and measured with cold vapor atomic fluorescence spectrometry (CVAFS). Methylmercury was measured by taking an aliquot of the diluted digestate and ethylating it in the aqueous phase. It is then purged and collected on a Tenax trap where it is run through GC separation and CVAFS detection. UC-Davis runs stable isotopes through a PDZ Europa ANCA-GSL elemental interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. After the isotopes are measured, the ratios are adjusted
according to the laboratory references. The references include NIST standard reference materials and are calibrated against different NIST standard reference materials.

Table A3: Summary of South River analytical QC/QA for total and methylated mercury (based on dry weight) from CEBAM.

<table>
<thead>
<tr>
<th></th>
<th>Percent Difference of Duplicate Samples</th>
<th>Percent Matrix Spike Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Average</td>
</tr>
<tr>
<td>Total Mercury</td>
<td>31</td>
<td>-1.2</td>
</tr>
<tr>
<td>Methylmercury</td>
<td>14</td>
<td>-2.6</td>
</tr>
</tbody>
</table>

Table A4: Standard reference material analyzed for total mercury during South River analysis at CEBAM. Dorm-2 and IAEA350 are both fish tissue and SRM 1566b is oyster tissue.

<table>
<thead>
<tr>
<th>Standard Reference Material</th>
<th>Total Mercury (Dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THg, ng/g</td>
</tr>
<tr>
<td>Dorm-2</td>
<td>4500.0</td>
</tr>
<tr>
<td>Dorm-2</td>
<td>4661.9</td>
</tr>
<tr>
<td>IAEA350</td>
<td>4396.2</td>
</tr>
<tr>
<td>IAEA350</td>
<td>4503.4</td>
</tr>
<tr>
<td>SRM 1566b</td>
<td>37.7</td>
</tr>
</tbody>
</table>
Table A5: Standard reference material analyzed for methylmercury during South River analysis at CEBAM. Dorm-2 and IAEA350 are both fish tissue and SRM 1566b is oyster tissue.

<table>
<thead>
<tr>
<th>Standard Reference Material</th>
<th>MeHg, ng/g</th>
<th>Dup</th>
<th>Mean</th>
<th>RPD</th>
<th>% Rec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorm-2</td>
<td>4322.1</td>
<td></td>
<td></td>
<td></td>
<td>96.7</td>
</tr>
<tr>
<td>Dorm-2</td>
<td>4234.9</td>
<td></td>
<td></td>
<td></td>
<td>94.7</td>
</tr>
<tr>
<td>IAEA350</td>
<td>3479.5</td>
<td>3339.2</td>
<td>3409.3</td>
<td>4.1</td>
<td>93.4</td>
</tr>
<tr>
<td>SRM 1566b</td>
<td>13.7</td>
<td>14.4</td>
<td>14.1</td>
<td>-4.9</td>
<td>106.5</td>
</tr>
</tbody>
</table>

Table A6: Summary of South River analytical QC/QA for Nitrogen Stable Isotopes from the UC-Davis Stable Isotope Facility.

<table>
<thead>
<tr>
<th>Stable Nitrogen Isotopes</th>
<th>( N )</th>
<th>Average*</th>
<th>Std Dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>66</td>
<td>1.33%</td>
<td>0.17%</td>
<td>0.82% - 1.63%</td>
</tr>
</tbody>
</table>

*Reference check 1.33%.

Table A7: Summary of South River analytical QC/QA for total and methylated mercury (based on dry weight) from CEBAM.

<table>
<thead>
<tr>
<th>Percent Difference of Duplicate Samples</th>
<th>Percent Matrix Spike Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Average</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
</tr>
<tr>
<td>Total Mercury</td>
<td>2</td>
</tr>
<tr>
<td>Methylmercury</td>
<td>9</td>
</tr>
</tbody>
</table>
Table A8: Standard reference material analyzed for total mercury and methylmercury during Holston River analysis at CEBAM. Dorm-2 and IAEA350 are both fish tissue.

<table>
<thead>
<tr>
<th>Standard Reference Material</th>
<th>Total or Methyl</th>
<th>ng/g (DW)</th>
<th>% Rec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA350 THG</td>
<td>4432.1</td>
<td>95.7</td>
<td></td>
</tr>
<tr>
<td>Dorm-2 MHG</td>
<td>4230.8</td>
<td>94.7</td>
<td></td>
</tr>
<tr>
<td>IAEA350 MHG</td>
<td>3380.4</td>
<td>93.4</td>
<td></td>
</tr>
</tbody>
</table>

Table A9: Summary of Holston River analytical QC/QA for Nitrogen Stable Isotopes from the UC-Davis Stable Isotope Facility.

<table>
<thead>
<tr>
<th>Stable Nitrogen Isotopes</th>
<th>N</th>
<th>Average*</th>
<th>Std Dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>1.31‰</td>
<td>0.17‰</td>
<td>1.11‰-1.65‰</td>
</tr>
</tbody>
</table>

*Reference check 1.33‰
Citations


Boudou A. and Ribeyre F. 1985. Experimental Study of Trophic Contamination of Salmo gairdneri by Two Mercury Compounds-HgCl₂ and CH₃HgCl- Analysis at the Organism and Organ Levels. Water Air Soil Poll 26:137-148.


United Nations Environment Programme Chemicals [Internet]. [Updated 2002].

Virginia Department of Health [Internet]. [Updated 2008]. Richmond (VA).
Shenandoah River Basin. [Cited 2008 Nov 7] Available from:
http://www.vdh.virginia.gov/Epidemiology/PublicHealthToxicology/Advisories/ShenandoahRiver.htm


**Vita**

Kyle Robert Tom