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EVALUATION OF TOTAL MERCURY AND METHYLMERCURY CONCENTRATIONS OF TERRESTRIAL INVERTEBRATES ALONG LOWER EAST FORK POPLAR CREEK IN OAK RIDGE, TENNESSEE

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To the Graduate Council:

I am submitting herewith a thesis written by Chelsea Lynden Standish entitled "EVALUATION OF TOTAL MERCURY AND METHYLMERCURY CONCENTRATIONS OF TERRESTRIAL INVERTEBRATES ALONG LOWER EAST FORK POPLAR CREEK IN OAK RIDGE, TENNESSEE." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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EVALUATION OF TOTAL MERCURY AND METHYLMERCURY CONCENTRATIONS OF TERRESTRIAL INVERTEBRATES ALONG LOWER EAST FORK POPLAR CREEK IN OAK RIDGE, TENNESSEE

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Learning is like mercury, one of the most powerful and excellent things in the world in skillful hands; in unskillful, the most mischievous.

-Alexander Pope

DEDICATION

This thesis is dedicated to my parents, Mike and Leslie Standish, as well as my two little sisters, Addie and Sophia. Without them, I would not be where I am today. Thank you for all the endless love and constant support over the years.

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ABSTRACT

Mercury (Hg) and methylmercury (MeHg) are environmental concerns due to their abilities to cause neurological, reproductive, and other physical damage to wildlife. Lower East Fork Poplar Creek (LEFPC), stemming from the Y-12 National Security Complex, located in Oak Ridge, TN, has elevated concentrations of inorganic mercury, a majority of which was released between 1950 and 1963. This inorganic mercury has been, and is currently, converted to methylmercury. An ecological assessment in 2011 revealed high concentrations of methylmercury in riparian spiders along LEFPC. These results suggested the transfer of mercury from aquatic to terrestrial systems may be higher than previously expected and suggested the need for a more complete study of the concentrations of total mercury and methylmercury in terrestrial invertebrates along LEFPC floodplain. A study was designed to address these needs.

The first objective was to quantify total mercury and methylmercury in terrestrial invertebrates along LEFPC floodplain. Four terrestrial invertebrate taxa were chosen for analysis: isopods (detritivore), leafhoppers (herbivore), wolf spiders (carnivore), and earthworms (detritivore). Earthworms (Family: Lumbricidae) had the highest concentration of total mercury at 7.04 ppm, while wolf spiders (Family: Lycosidae) and isopods (Family: Trachelipodidae) had the highest concentrations of methylmercury. The second objective was to investigate the relationship between total and methylmercury concentrations and trophic levels in terrestrial invertebrates. Methylmercury was found to increase with trophic level. Variation between methylmercury concentrations and trophic levels were seen in correlation with location along LEFPC. The third objective was to examine if the distance from the contaminated stream influenced mercury concentrations in the floodplain soils and invertebrates. Mercury

concentrations were higher at upstream locations, closest to the source of mercury, and decreased with distance downstream. The fourth objective was to examine the relationship between soil and biota mercury concentrations, bioaccumulation factors (BAF), and compare BAFs in LEFPC to those at other contaminated sites. Earthworms had the highest BAFs and overall, BAFs in this study were lower than reported at other sites. The information from this study will inform management decisions on conservation efforts to protect upper-level predators in areas where mercury and methylmercury are present.

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CHAPTER I

LITERATURE REVIEW

Mercury (Hg; atomic number 80, atomic mass 200.59) is a global environmental pollutant which has unique properties. In its elemental form, it is found as a silvery liquid at room temperature, giving it the nickname quicksilver. Between natural and anthropogenic activities, 2,900 to 8,000 metric tons of mercury are released into the environment annually. Human activity plays a chief role, contributing up to 80% of the mercury released into the environment (Pant et al. 2010a, Kaschak et al. 2014). Mercury is found naturally in the crust of the earth and is subsequently released into the environment through instances such as volcanic eruptions and the weathering of rocks. Anthropogenic sources of mercury into the environment are due to activities, such as coal and heavy metal mining (Han et al. 2012), and in products such as batteries, biocides used for paper production and pharmaceuticals, as well as paints, thermometers, fluorescent lamps, dental amalgams and uses as industrial catalysts (UNEP 2002).

Historical regulations, like the Clean Air Act of 1972 by the Environmental Protection Agency (EPA), have stated that mercury could be the greatest contaminant of concern to human health (Gray et al. 2015). The adverse effects of mercury and its compounds are diverse and include behavioral, neurochemical, hormonal, and negative cardiovascular effects to both wildlife and humans (Henderson et al. 2012). The EPA studies indicate that methylmercury (commonly denoted by the abbreviation MeHg), a harmful organic compound of mercury, may be carcinogenic to humans, whereas inorganic mercury is not known to cause cancer in humans

(ATSDR 2012). An additional concern is the fact that methylmercury is readily absorbed and transported through the placental barrier as well as the blood-brain barrier causing it to be a potential health concern. This transport across the placental barrier can have adverse effects for pregnant women, as it can cause long-term developmental problems in children born to women who have high concentrations of mercury in their bodies.

Elemental mercury is not as readily absorbed in the intestines but can be oxidized, in the tissue of the body, to the inorganic divalent form. Elemental mercury is primarily a concern when it is inhaled in vapor form. Up to 80% of the mercury vapors inhaled are absorbed by the lungs where mercury can enter the bloodstream and cross the blood-brain barrier. Mercury is well documented as a neurotoxin that has been reported to cause tremors, memory loss, neuromuscular issues, headaches and in extreme cases, mortality (UNEP 2002).

Methylmercury is one of the most toxic forms of mercury, especially relative to the health and development of humans, as it can accumulate in the tissue of living organisms. The most common way humans can be exposed to methylmercury is by consuming fish that have been contaminated with methylmercury, with as much as 95% absorbed into the gastrointestinal tract. From here, methylmercury can enter the blood stream easily where it can freely move to tissues and organs, including the brain (ATSDR 2012). Methylmercury is a great concern due to its ability to be quickly adhered to organ tissue because of the lipophilic character of the molecule, meaning it is extremely hydrophobic (Kaschak et al. 2014).

Mercury has been found in lakes and water sources worldwide (Gray et al. 2015). Regions in which insignificant natural or human releases of mercury have occurred, or currently occur, still have elevated concentrations of mercury. In the United States, over 1,840,000 km of streams are under human fish consumption warnings due to mercury contamination (Walters et

al. 2015). Atmospheric deposition as well as rivers and ocean currents contribute to the global movement of mercury to previously uncontaminated sites, indicating that no one area is excluded from the potential threat of mercury (UNEP 2002). The Colorado River, Grand Canyon, Mississippi River, and the Arctic stand as examples of contamination from the dispersion of mercury among waterways (Walters et al. 2015, Gray et al. 2015, Dos Santos et al. 2006).

Inorganic mercury is seldom found naturally in an ecosystem. When inorganic mercury is found, naturally-occurring concentrations of mercury in the soil can contain up to 0.06 ppm of mercury with about 1% being in the form of methylmercury (Kaschak et al. 2014). When found, however, inorganic mercury is typically bound with other compounds in either the monovalent (Hg(I)) or divalent form (Hg(II)), with a wide range of inorganic and organic compounds formed from the divalent molecule. Mercury released into the environment is often suspended or bound to organic compounds found in the surrounding soil or water (Sanborn and Brodberg 2006). Organic mercury, formed when mercury combines with carbon, in itself can be found in multiple forms: dimethylmercury ($(CH_3)_2Hg$), phenylmercury ($C_6H_5Hg^+$), ethylmercury ($C_2H_5Hg^+$), and methylmercury (CH_3Hg), with methylmercury, being the highest environmental and health concern (UNEP 2002). All forms listed above occur naturally at low concentrations (ATSDR 2012). Soil that contains mercury can be both a sink for the mercury and a continual source of it. All of these factors contribute to the solubility and how bioavailable the mercury is to the soil and ecosystem that lives in the environment of concern (UNEP 2002).

Methylmercury formation occurs in anoxic environments by anaerobic bacteria. These anaerobic bacteria are found in water systems (i.e., rivers, lakes, streams, oceans) as well as sources such as soil and sediment. Sulfate-reducing bacteria present in these habitats are the

primary producers of methylmercury. Iron-reducing bacteria and methanogens also contribute to methylmercury concentrations (Parks et al. 2013, Gilmour et al. 2013).

Historically, the majority of mercury research on food webs has been in aquatic ecosystems due to the high methylation rates in aquatic systems. Recent concern has risen about elevated concentrations of methylmercury in terrestrial consumers (Speir et al. 2014). One particular study focused on long-jawed orb weaver spiders (Araneae: Tetragnathidae), which feed on emerging aquatic insects like non-biting midges (Family: Chironomidae). The focus of the study was to assess methylmercury concentrations in the long-jawed orb weaver spider, as well as the impact mercury containing long-jawed orb weaver spiders can have on the surrounding terrestrial environment. Specifically, these researchers examined song birds that lived in the habitat along Caddo Lake, TX, another site that has been contaminated by mercury (Gann et al. 2015). This type of study suggests a connection between a polluted aquatic environment and a polluted terrestrial environment, as well as potential for the pollution of mercury to surrounding terrestrial ecosystems. Additional recent studies have shown that mercury and its many forms may have greater toxic effects to various organisms at much lower concentrations than previously thought, and the range of impact may extend into areas not known to be contaminated (i.e., the Arctic) (UNEP 2002).

Mercury in the Environment

One of the reasons that methylmercury is such a concern to the environment is its capability to bioaccumulate. Bioaccumulation occurs when methylmercury accumulates in organisms as it moves up the food chain. Microorganisms, found in sediment, naturally convert mercury from the environment into the more bioavailable forms, such as methylmercury (UNEP

2002). This process explains how spills of inorganic mercury from anthropogenic activities can lead to highly contaminated areas of methylmercury, especially in aquatic systems where the methylmercury can bioaccumulate in the animals living in the ecosystem, resulting in significantly higher concentrations of methylmercury in those organisms when compared to the surrounding environment.

Inorganic mercury in an aquatic environment is converted to toxic bioavailable methylmercury by sulfate or iron-reducing bacteria that reside in the aquatic ecosystem. Microorganisms, like periphyton, at the base of the aquatic food web concentrate methylmercury directly from the water, where methylmercury becomes available to higher level predators by their food consumption (Speir et al. 2014). Mercury found in aquatic systems poses a large and consistent threat to aquatic organisms living in the contaminated environment as well as the terrestrial organisms that feed on them (Walters et al. 2015). Higher level consumers may bioaccumulate more mercury in their tissues compared to organisms that are lower on the food web. In fish almost 100% of the mercury present is in the methylmercury form where it is covalently bonded to protein sulfhydryl groups (UNEP 2002).

Aquatic macroinvertebrates are essential to the trophic transfer of mercury especially to those vertebrates that consume them. How the conversion occurs, if it does, in terrestrial ecosystems is not well understood. Adults of some aquatic macroinvertebrates that emerge from the water are often eaten by terrestrial insectivores creating a key link of accumulation between aquatic environments to terrestrial ones (Henderson et al. 2012). Otters, minks, raptors, ospreys, and eagles are some of the upper-level predators that feed directly on the aquatic food web that are exposed to more mercury from contaminated aquatic ecosystems (UNEP 2002). Small organisms, such as black flies and small fish, with high concentrations of mercury are likely

links to the rising concentrations of mercury in terrestrial animals that feed in the riparian zone. It is in the riparian zone, the area along the side of a bank of water, where small aquatic organisms can be a primary source of food and energy for smaller birds.

Reproductive effects of mercury on birds that feed on aquatic life have been seen when mercury concentrations in some bird eggs reach concentrations as low as 0.05 to 2.0 ppm (UNEP 2002). Methylmercury can be most toxic to birds, with exposure potentially increasing the number of unfertilized eggs, decreasing the hatchability of eggs, or increasing embryonic death. Along with a decrease in the number of fertilized eggs, high concentrations of methylmercury can cause spinal cord degeneration, a reduction of food intake resulting in weight loss, and weakness in wings and legs; this weakness is due overall to an inability to coordinate muscle movement (Landrum et al. 1993). Similar negative health impacts can be seen in the upper-level predators that consume birds with high concentrations of mercury.

Accumulation of mercury in plants has been observed in various studies. In areas where inorganic mercury is present in the soil plants have been found to uptake mercury in the form of mercuric ions (ATSDR 2012). Atmospheric deposition and frequent flooding are sources of mercury to plants (Cocking et al. 1991). Unlike other heavy metals (e.g., zinc, lead, chromium, etc.) that cause diminished plant growth as well as other abnormalities, terrestrial plants are relatively unresponsive to the toxic impact of mercury and its compounds. In areas with high concentration of contamination, only a small fraction of the mercury present in the soil is integrated by the plant (Pant et al. 2010b). The highest mercury concentrations are documented in the roots of plants with decreasing mercury uptake seen in plant leaf tissue (Pant et al. 2010b). Soil characteristics as well as various geochemical factors greatly influence mercury concentrations in plants. Mercury has also been documented to accumulate in higher plants such

as perennials. However, mercury is still primarily seen in root tips in higher plants (Boening 2000). It is speculated, that ultimately higher level predators are more likely to come into contact with concerning concentrations of mercury when consuming invertebrates that live in the soil than those that feed on plant tissue in areas that have been contaminated by mercury (DOE 2014a).

Higher concentrations of mercury may be found in spiders, which are predatory, than in other terrestrial invertebrates that live in the soil or that feed on plant tissue. The Carolina wren (*Thryothorus ludoviciarus*) (Passeriformes: Troglodytidae) is an upper level consumer with a diet that includes various insects and spiders, a link in the food web that could increase its exposure to mercury. The composition of the wren's diet is likely to consist of 30% spiders, 60% insects (45% herbivores and 15% detritivores) and 10% plants (DOE 2014b, Cristol et al. 2008, Newman et al. 2011). The diet of the short tailed shrew (*Blarina brevicauda*) (Eulipotyphla: Soricidae), another terrestrial invertebrate predator, is primarily made up of detritivorous insects and earthworms, potentially causing a higher exposure to mercury (DOE 2014b). The composition of the shrew's diet is expected to be 20% vertebrates (mice only), 40% earthworms, and 40% detritivorous insects (DOE 2014b, Cristol et al. 2008, Newman et al. 2011).

Wolf spiders (Family: Lycosidae) are among the many families of spiders found in riparian zones (DOE 2014a). Other spiders that inhabit riparian zones have been used as key species in recent mercury contamination studies, such as those conducted by Speir et al. (2014) and Gann et al. (2015). Spiders, such as wolf spiders, found on land can cause movement of methylmercury to those that feed on them (i.e., birds). It is also understood that adult aquatic

insects can be a source of food for terrestrial feeders, specifically those in the riparian zone (Sanzone et al. 2003).

Earthworms, prey of the short-tailed shrew, are important organisms in the food chain by being sources of food for many smaller mammals and birds. Earthworms make up the most biomass in all of the invertebrates that inhabit the soil and are also an important source of food and protein to birds and small mammals, like the previously mentioned Carolina wren and short-tailed shrew (Zhang et al. 2009). They may also play an important role in the movement of contaminants, such as mercury, in the terrestrial environment (Han et al. 2012).

Recent studies have used isopods, a potential prey for both the Carolina wren and the short-tailed shrew, as a bioindicator of heavy metals like lead and cadmium (Dallinger et al. 1992). Udovic et al. (2009) used isopods to evaluate the effectiveness of ethylenediaminetetraacetic acid (EDTA) for remediation. Research has indicated that most of pollutants ingested by animals are stored in the midgut (Dallinger et al. 1992). Mercury stored in the midgut primarily comes from soil rather than litter or roots consumed (Ernst and Frey 2007). Studies like these indicate that isopods are viable sources to evaluate mercury impacts on terrestrial environments. All of the invertebrate taxa previously discussed in the paragraphs above are important food sources for upper-level terrestrial predators.

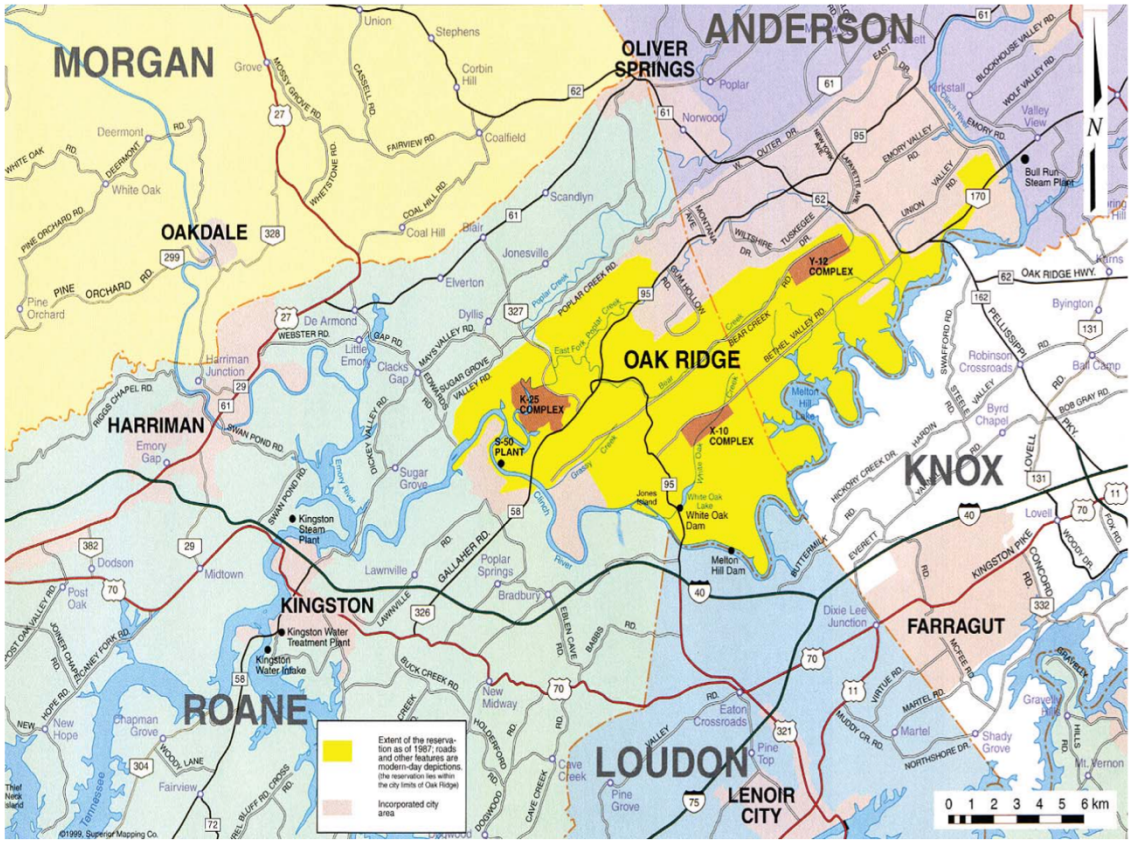
Bioaccumulation occurs when a substance of interest is taken up by an organism at a rate that is faster than the rate the organism can metabolize or excrete the substance. Bioaccumulation becomes a concern when the substance is harmful like a pesticide or certain toxic elements (i.e., mercury). To better understand how such toxic substances can impact an area that has been contaminated, bioaccumulation factors (BAFs) are used. BAFs can be defined by the process of dividing concentrations in biota by concentrations in the surrounding

environment (Han et al. 2012). BAFs have been used to evaluate organic compounds that are hydrophobic, like mercury, because it takes in account dietary factors as well as dermal and respiratory exposure (Costanza et al. 2012). BAFs can be an important tool in ecological evaluation because BAF values can provide results without the need for large sampling.

Mercury at Oak Ridge, TN

The Oak Ridge Reservation (ORR) rests on 1.3×10^{8} m^2 in Oak Ridge, TN, which is located in both Anderson and Roane Counties (Figure 1). From 1950 to 1963, the Y-12 National Security Complex (Y-12 Complex), located on the ORR, used roughly 11 million kg of mercury (Donovan et al. 2014). By 1989, the EPA added ORR to the National Priorities List (NPL) for clean-up due to the contamination of the area. Mercury is still present in old waste areas on the ORR, and these waste areas compose 5 – 10% of the vast area that ORR occupies. With plentiful rainfall as well as frequent flooding, mercury contamination still occurs in the surface and ground water as well as sediment, soil, and biota, with surface water being the greatest contributor of contamination to the soil and sediment of the surrounding area.

East Fork Poplar Creek (EFPC) originates within the Y-12 Complex on the ORR in Oak Ridge, TN with a total length of nearly 26 km. EFPC subsequently has been divided into Upper East Fork Poplar Creek (UEFPC), which constitutes the initial 2 km from the Y-12 Complex, and Lower East Fork Poplar Creek (LEFPC) which makes up the succeeding 24 km of water way. The creek runs through ORR as well as the city of Oak Ridge covering up to 7.69×10^{7} m^2 within its floodplain (DOE 2014a). An estimated 77,180 kg of mercury have been deposited in the sediments and soil of LEFPC (Pant et al. 2010a). However, more mercury may have been released into the surrounding environment than previously estimated (ATSDR 2012). The



Source: ChemRisk 1999a (with modifications)

Figure 1: Map of Oak Ridge, TN located in Roane and Anderson Counties.

majority of mercury use and activity at the Y-12 Complex ceased in 1963. Mercury that reaches LEFPC today is attributed to the storm drains from the Y-12 Complex (Donovan et al. 2014). Tennessee regulations state that mercury in the water has a limit of 51 ppm. In a majority of LEFPC, total mercury concentrations in the water are well above the Tennessee State limit and the mercury exposure limit of 12 ppm for aquatic life set by the EPA (Brooks and Southworth 2011).

Floodplain sources may continue to contaminate LEFPC (DOE 2014b). Storm drain pipes, as well as sediment along LEFPC, may be the sources of the continual mercury contamination (DOE 2014a). More than 80% of the mercury released from LEFPC comes from erosion of the stream bank as well as sediment from the streambed being resuspended. Knowing the forms of mercury present in the soils, water, and biota of EFPC can help obtain a better understanding of the threat that mercury poses on the environment of LEFPC (Donovan et al. 2014).

Remediation

Remediation actions have been taken in the past several years to remove highly contaminated floodplain soils surrounding LEFPC and replaced them with clean uncontaminated soil (Han et al. 2012). From July 8 to September 14, 1996 and from March 3 to October 24, 1997, soils in areas of the LEFPC floodplain with mercury concentrations exceeding 400 ppm were removed and replaced with uncontaminated soil (Han et al. 2012). In 1996 4,250 loose cubic meter (lcm) of contaminated soil were removed upstream. The following year, an additional 29,970 lcm of soil were removed upstream (ATSDR 2012). Although soils with mercury concentrations exceeding 400 ppm were remediated, there could still be areas with

concentrations ranging from 100 ppm to 400 ppm (Han et al. 2012, DOE 2014a). Recent studies of the area have revealed significant doubt as to whether even a decrease of 10 fold of total mercury would significantly impact methylmercury concentrations in the waters of the LEFPC. The mercury concentrations of fish from LEFPC have not significantly decreased, even though water quality has improved through remediation actions on LEFPC, as mercury concentrations in the water have declined (Brooks and Southworth 2011). Pant et al. (2010a) argue that the remediation actions along EFPC are expensive and may be ineffective.

With mercury as the primary contaminant in the LEFPC soils, ingestion of soil by animals has been identified as one of the biggest mercury exposure routes. When examining mercury uptake along the creek, previous studies of LEFPC have shown differences in upstream, midstream, and downstream sites. Mercury concentrations follow the general trends of higher concentrations upstream, close to the source, with decreasing values as distance increases away from the source. Mercury bioaccumulation in terrestrial invertebrates that live in the surrounding LEFPC habitat is an on-going source of uncertainty, especially in terms of threat to wildlife living in or using the floodplain.

Importance

A recent study found that the mercury released from the Y-12 Complex into the surrounding environment is a contributing source of mercury contamination found in the Mississippi River, indicating that the mercury problems at LEFPC are not limited to surrounding ecosystems but also other ecosystems at great distances away (Gray et al. 2015). Other countries that have managed mercury in the environment for an extended period of time have stated the need to better understand mercury and its impact on the environment in order to improve

assessments and establish effective management of mercury as a contaminant as well as other environmental hazards (UNEP 2002). Evaluating for both total mercury and methylmercury is also vital to better understanding the biomagnification of mercury. Newman et al. (2011) found that though methylmercury and total mercury did increase as trophic level increases, the variation among the food web, especially in respect to methylmercury, was much wider and diverse in the terrestrial invertebrates when compared to the aquatic ecosystem which accumulates methylmercury in a more direct route.

Mercury is both a historical and current contaminant at LEFPC and its surrounding floodplain. The question arises: where is the mercury contamination source in regards to the terrestrial invertebrates as well as the upper level predators that feed on both? A 2011 ecological assessment of LEFPC revealed new data indicating high concentrations of mercury in spiders in the LEFPC floodplain which included a high concentration of the bioavailable methylmercury in spiders (Mathews et al. 2011). This assessment also raised the concern that mercury still present in the LEFPC floodplain could accumulate in the flora and fauna of the ecosystem. This mercury accumulation may be harmful, especially to upper-level predators in the area.

Objectives

The overall goal of this research is to examine mercury bioaccumulation in resident invertebrate species in the LEFPC floodplain. The objectives are to 1) quantify total and methylmercury bioconcentrations in invertebrates living in soils with a range of mercury concentrations; 2) investigate the relationship between total and methylmercury accumulation, feeding guild, and trophic level in terrestrial invertebrates; 3) determine if distance from the contaminated stream (LEFPC) influences mercury concentrations in floodplain soils and/or

resident invertebrate species; and 4) examine the relationship between soil total mercury concentrations and biota total mercury concentrations (BAFs) and compare BAFs of terrestrial invertebrates along LEFPC to those at other contaminated sites.

The invertebrates living in the LEFPC floodplain could be an important mechanism for transfer between the aquatic and terrestrial food chains. Because insects and other invertebrates form key links in terrestrial food chains, understanding how mercury is transferred to these species is critical to evaluating threat to higher trophic levels.

CHAPTER II

BIOCONCENTRATIONS OF TOTAL AND METHYLMERCURY IN TERRESTRIAL INVERTEBRATES NEAR LOWER EAST FORK POPLAR CREEK

Introduction

Between 1950 to 1963, mercury was released at the Y-12 National Security Complex (Y-12 Complex), located on the Oak Ridge Reservation (ORR) in Oak Ridge, TN (DOE 2014a). During these years an estimated 108,000 – 212,000 kg of mercury were released into the environment surrounding the Y-12 Complex, specifically East Fork Poplar Creek (EFPC). EFPC has subsequently been divided to consist of Upper East Fork Poplar Creek (UEFPC) and Lower East Fork Poplar Creek (LEFPC). UEFPC is restricted to the first 2 km from the Y-12 Complex and is not accessible to the public. LEFPC runs approximately 24 km through the city of Oak Ridge which lies in both Anderson and Roane Counties in East Tennessee (Figure 1).

In the EFPC watershed, mercury is dispersed extensively in a wide range of concentrations in floodplain soils (Pant et al. 2010a). Despite an observed decrease in aqueous mercury concentrations in EFPC during the 1980s, in the years following, fish and aqueous methylmercury concentrations did not continue to decrease; in some instances methylmercury concentrations increased (Brooks and Southworth 2011). The mercury that has contaminated LEFPC is concentrated mostly in the top 3 m of the floodplain (Han et al. 2012). Mercury contamination at the Y-12 Complex is not limited to the soil but is currently also found in

groundwater, buildings, drains, and sumps causing the continual release of mercury into the surrounding environment (DOE 2014a).

The aquatic and terrestrial ecosystems of LEFPC may be currently threatened by the significant mercury spill that occurred over 50 years ago. Mercury is known and acknowledged as a toxin, especially when found in the environment, where it poses a greater threat to wild mammals, birds, and humans, due to its ability to biomagnify as it transfers up the food chain (Zhang et al. 2009). Tissue of fish in LEFPC have methylmercury concentrations that make up 85% - 95% of the total mercury concentrations (Brooks and Southworth 2011). Lasorsa and Allen-Gil (1995) examined the methylmercury fractions (i.e., the methylmercury to total mercury ratios) in a variety of species to determine if the fraction reveals higher exposure to organic or inorganic mercury in the environment, and how the fraction of methylmercury compares to the trophic positions of specimens in the food chain. They found that terrestrial mammals feeding on plant material had low methylmercury fractions. Another recent study found that detrital isopods had a higher concentration of methylmercury fractions compared to those of ground beetles. This study shows diversity in the ecosystems of floodplains, and emphasizes the diversity of mercury concentrations among taxa living in those ecosystems (Ortiz et al. 2015).

Stable Isotopes

Carbon and nitrogen stable isotope measurements have often been used to determine sources of food for organisms and for aiding in understanding trophic levels of sampled biota (Bisi et al. 2012). Evaluating trophic levels can be a key step to understanding bioaccumulation of contaminants, such as mercury. The ratio of carbon-12 (^{12}C) to carbon-13 (^{13}C) stable isotopes, $\delta^{13}C$, is calculated by dividing the ratio of ^{12}C to ^{13}C in the sample which is in turn

divided by the ratio of ^{12}C to ^{13}C in the standard used, all of which is subtracted by 1 multiplied by 1000.

$$\delta^{13}C = \left(\frac{\frac{^{13}C}{^{12}C}\text{ Biota}}{\frac{^{13}C}{^{12}C}\text{ Standard}} - 1 \right) \text{x } 1000$$

$\delta^{13}C$ is used to determine sources of organic matter and food for an organism. The ratio of nitrogen-14 (^{14}N) to nitrogen-15 (^{15}N), $\delta^{15}N$, is calculated in a similar method as $\delta^{13}C$ stated above. $\delta^{15}N$ is used to determine trophic position of an organism in an ecosystem. By using these two values, the relationship of the organism to the surrounding environment can be understood. When evaluating stable isotopes, a trend has been observed: as mercury concentration increases, so does trophic position (Newman et al. 2011, Walters et al. 2015).

Naturally-occurring stable isotopes are often used in studies to understand trophic levels, as well as food web interactions, in both aquatic and terrestrial ecosystems (Sanzone et al. 2003). Aquatic organisms are important food and energy sources for organisms, such as spiders and other terrestrial carnivorous predators. Nutrients move from aquatic ecosystems to the surrounding environment. Organisms in higher trophic levels have higher levels of ^{15}N (Bisi et al. 2012). This increase in ^{15}N reflects an overall increase in trophic levels, especially when viewing the relationship between prey and consumer (Speir et al. 2014). It has been documented that $\delta^{15}N$ increases 3-4% with increase in trophic position (Post 2002).

In recent studies, stable isotope ^{15}N has been used to confirm and identify trophic levels on terrestrial invertebrates (Speir et al. 2014). Researchers use nitrogen stable isotopes to distinguish between aquatic and terrestrial food webs alongside methylmercury. Paetzold et al. (2005) examined wolf spiders (Family: Lycosidae) as well as three other terrestrial invertebrates

from April to October to help understand the pathway of energy from aquatic to terrestrial food webs. $\delta^{15}N$ and $\delta^{13}C$ from their study revealed that wolf spiders and ground beetles (Family: Carabidae) collected 50 m from the stream were sustained completely on a terrestrial diet. Sanzone at al. (2003) found that 68% of the carbon from hunting spiders (Lycosidae and some Gnaphosidae) came from the stream (Sycamore Creek, located in Arizona). Methylmercury concentrations, when compared to $\delta^{15}N$ in the long-jawed orb weaver spider, supported evidence that orb weaver spiders are connected to the aquatic food chain, especially when compared to other terrestrial organisms (Speir et al. 2014). Bisi et al. (2012) found that detritivorous organisms had the lowest $\delta^{15}N$ in areas that were examined, also noting that invertebrates, in general, had lower levels of $\delta^{15}N$ when compared to vertebrates.

To understand the extent of mercury contamination in the LEFPC floodplain a study was designed to collect terrestrial invertebrate taxa representing different feeding guilds that included varying trophic levels (i.e., carnivore, detritivore, and herbivore). These animals form an important part of the diet of two key species in the LEFPC floodplain, the Carolina wren (*Thryothorus ludoviciarus*) (Passeriformes: Troglodytidae) and the short-tailed shrew (*Blarina brevicauda*) (Eulipotyphla: Soricidae). The invertebrate taxa were selected based on the need for an updated wildlife risk assessment that mercury poses on the LEFPC terrestrial environment. The objectives of this study are to quantify total and methylmercury concentrations in invertebrates living on and in LEFPC floodplain soils with a range of mercury concentrations, and to investigate the relationship between total and methylmercury accumulation, feeding guild, and trophic level in terrestrial invertebrates.

Materials and Methods

Sampling Sites and Plots

Sites were selected in the floodplain of LEFPC to provide a range of mercury concentrations and to assess possible differences in bioaccumulation trends with distance from the mercury source. Three sites were selected along the LEFPC floodplain representing upstream, midstream, and downstream sites (Figure 2). Initially four or five areas were chosen at each site; these areas were further separated into 25 to 49 plots that were 10 m x 10 m. Within each of the four or five areas, three plots had been randomly selected for the collection of soil samples for mercury analysis in a previous study (DOE 2014b). Upstream, midstream, and downstream sample areas with their respective sites and collecting plots are depicted in Figures 3, 4, and 5, respectively. Based upon the results of the soil sample analyses (DOE 2014b), three areas were selected from each site for the collection of terrestrial invertebrates for analysis of mercury. The same plots, within the three areas chosen, where soil samples were collected were used for invertebrate sampling. The reference site was along Brushy Fork which is located approximately 6 km (direct measurement) away from LEFPC, and has not been impacted by the mercury contamination (Figure 6). This site is located in Anderson County, Tennessee, next to a field used for livestock grazing. Three main characteristics were considered when choosing sampling plots: they must 1) have total mercury soil concentrations ranging from 0 ppm to 400 ppm, 2) have desired taxa present, and 3) be readily accessible. All collecting plot coordinates can be found in the Appendix (Table A1).

The upstream site, Area B closest to the Y-12 Complex (at the 4 km mark downstream from the origin) was near a small unnamed tributary (Figure 3). Though the plots in Area B

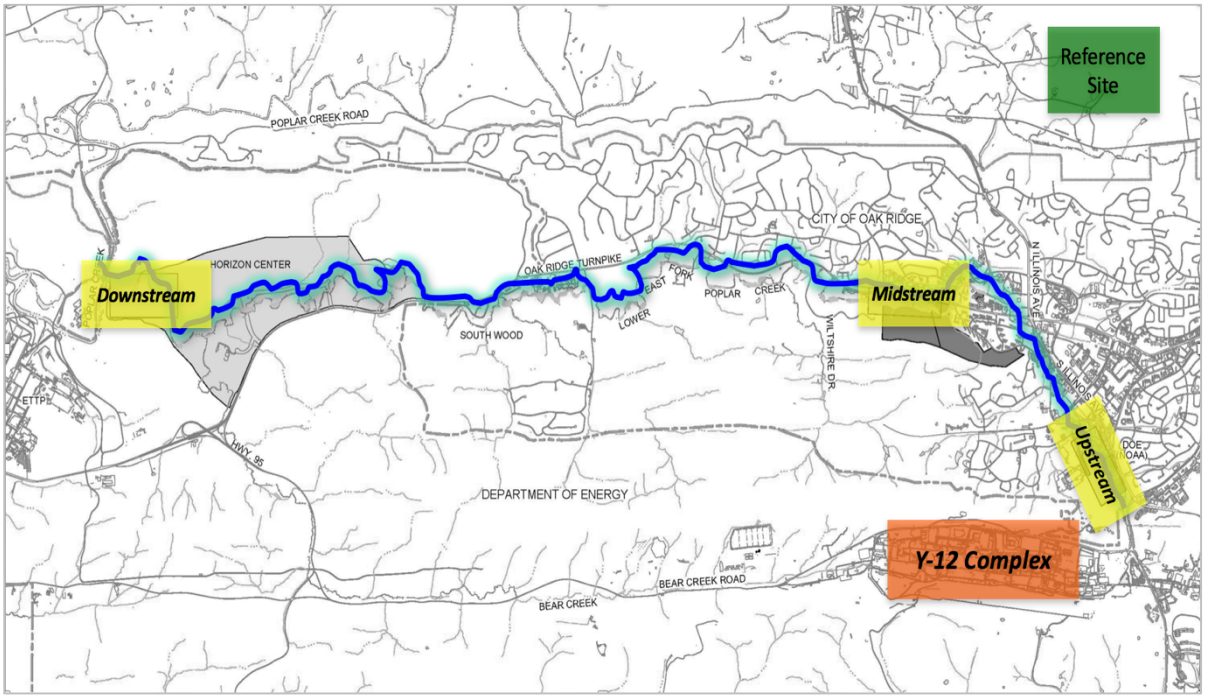


Figure 2: Map showing Lower East Fork Poplar Creek (LEFPC) and upstream, midstream, and downstream sites, as well as the origin of mercury, the Y-12 National Security Complex (Y-12 Complex) and reference site, Brushy Fork, located approximately 6 km from the stream.

Figure 3: Map of upstream collecting sites A, B, C, D and E. Biota were collected from sites B (plots 12, 18, and 20), C (plots 09, 12, and 41), and D (plots 06, 21, and 26). Additional soil samples were taken from A and E in 2014 as part of Phase 1 of the Lower East Fork Poplar Creek Mercury Biouptake Study. Map courtesy of Leidos.

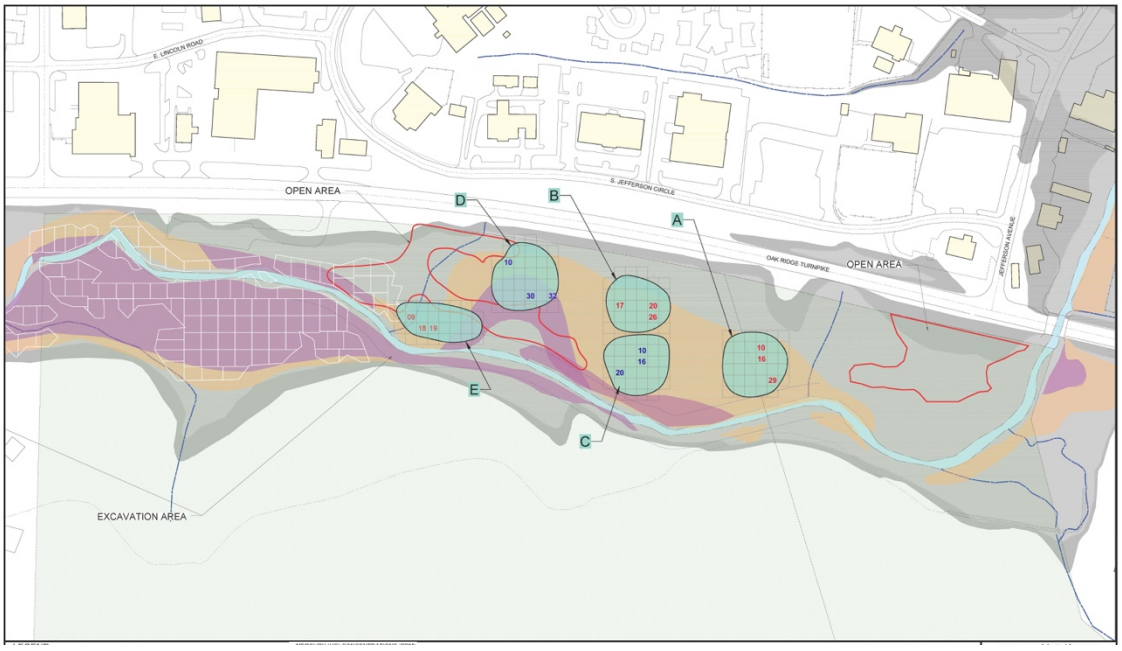


Figure 4: Map of midstream collecting sites A, B, C, D and E. Biota were collected from sites A (plots 16, 19, and 29), B (plots 17, 20, and 26), and E (plots 09, 18, and 19). Additional soil samples were taken from sites D and C in 2014 as part of Phase 1 of the Lower East Fork Poplar Creek Mercury Biouptake Study. Map courtesy of Leidos.

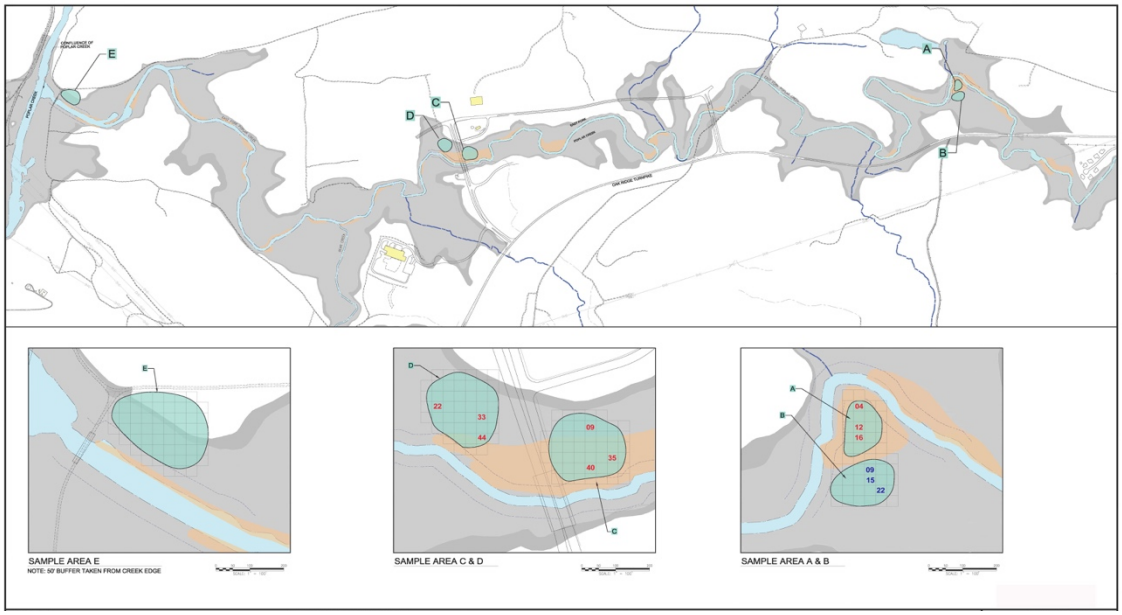


Figure 5: Map of downstream collecting sites A, B, C, D and E. Biota were collected from sites A (plots 04, 12, 16), C (plots 09, 35, 40), and D (plots 22, 33, 44). Additional soil samples were taken from site B in 2014 as part of Phase 1 of the Lower East Fork Poplar Creek Mercury Biouptake Study. Map courtesy of Leidos.

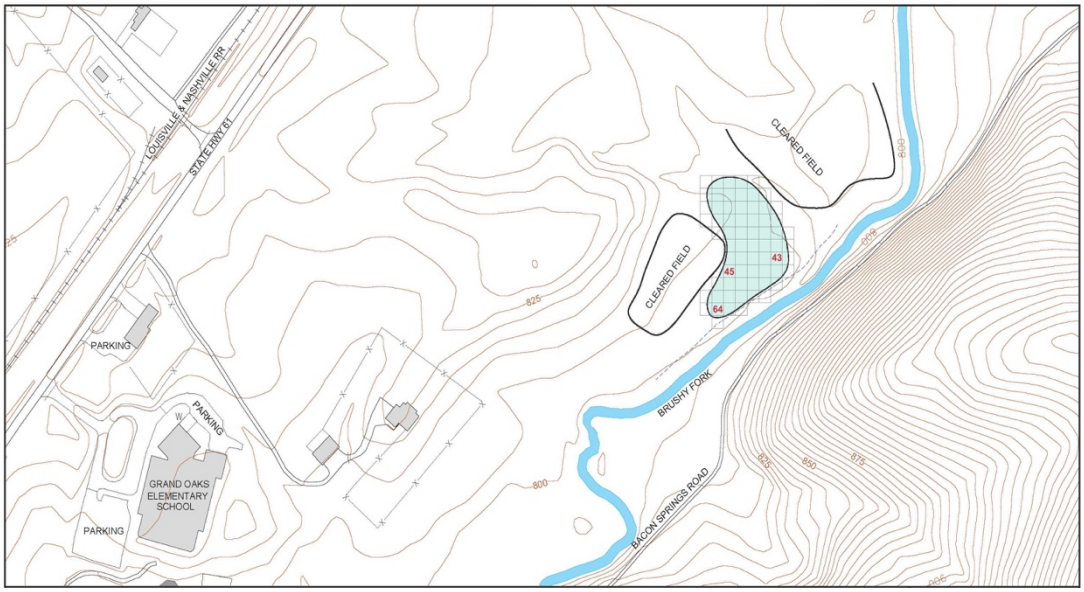


Figure 6: Map of reference collecting site (Brushy Fork) and collecting plots (43, 45, and 64) located in Anderson County, TN, 6 km (direct measurement) from the mercury source, Y-12 National Security Complex. Map courtesy of Leidos.

(B20, B18, and B12) were not the closest to LEFPC (34, 29, and 38 m, respectively, from the stream channel), evidence of frequent flooding was observed. The collection of taxa at these plots was difficult due to the lack of low-level vegetation, frequent flooding, which may have displaced invertebrate specimens away from the plots, and the presence of a dense thicket of privet (Oleaceae: Lamiales: *Ligustrum* sp.), which is not a host of desired herbivore taxa and hindered sweep netting. Of the nine total plots upstream, six plots (C09, C12, C41, D06, D21, and D26), at approximately the 4.5 km mark downstream from the Y-12 Complex, also had a slight tendency for flooding with sparse vegetation. Upstream plot D06 had the greatest tendency for flooding of all upstream sites.

Plots at the midstream site were at the 8 km mark away from original source of mercury, the Y-12 Complex (Figure 4). Overall, these nine midstream plots showed less tendency to flood. At all nine plots there was also more low-level vegetation, with less privet, and an abundance of tall grass, such as Panicgrass (Poaceae: Poales: *Panicum* sp.), which made collecting leafhoppers at significant biomass considerably easier than at the other collecting plots.

Downstream plots in Area A (A04, A12, and A16) were adjacent to the 19 km mark of LEFPC, and were located somewhat on a small island created by the main channel of EFPC and a small channel that carried water only during high flows (Figure 5). This "island" had a low tendency for flooding. A lot of low-level trees as well as bamboo (Family: Poaceae, Tribe: Bambuseae) were present. Collecting was challenging at these plots, but with the lack of frequent flooding, taxa were present in adequate numbers. The last six plots located farthest downstream in Areas C and D (C09, C35, C40, D22, D33, and D44) at km marker 23 had a greater tendency for flooding. Evidence of flooding with standing water at approximately 0.30

m could be seen based on water marks on trees in the area. The vegetation in this area was denser with more privet and some low-level vegetation. Sampling was challenging at these six plots.

Taxa and Sampling

The four targeted taxa of terrestrial invertebrates used in this study represent three feeding guilds: wolf spiders (carnivore, Family: Lycosidae), isopods (detritivore, Family: Trachelipodidae), earthworms (detritivore, Family: Lumbricidae), and leafhoppers (herbivore, Family: Cicadellidae). Wolf spiders primarily feed on small insects, such as leafhoppers, crickets, or beetles, that are found in their habitat. Wolf spiders tend to have one generation per year. Isopods feed on the detritus in their habitat, including leaf litter, fungi, and dead decaying plants and animals. Isopods also have a simple gut and lack a midgut section. Isopods tend to have one to three generations per year. Earthworms also feed on the detritus in their habitats as well as live and dead organic matter. Though they consume soil while they make burrows, their source of nutrition is primarily leaf litter. Earthworms tend to have only one generation per year. Leafhoppers have a beak-like feeding structure called a rostrum, which is used to suck nutritious content from plants. Some leafhoppers feed on the stem of plants, imbibing phloem sap, while other small leafhoppers feed on leaves, consuming cell content. Leafhoppers typically have one to three generations per year.

To adequately analyze and assess samples for mercury and methylmercury, sufficient sampling was necessary. To meet analytical reporting limits of 0.1 to 0.4 ng/g for total mercury and 0.2 to 3.0 ng/g methylmercury, a minimum biomass goal for each sample from each plot was 1.20 g. The minimum sample biomass necessary to allow analysis of mercury was 0.12 g.

However, because some targeted taxa, such as leafhoppers, were not as abundant and had a lower biomass, a weight minimum was established at 0.12 g. A minimum biomass of 0.12 g also provided sufficient biomass for analysis of both $\delta^{15}N$ and $\delta^{13}C$.

Sampling for targeted taxa began in April 2015 and ended in August 2015. Earthworms were primarily collected in April and May before the soil became too dry. Most of the isopods were collected in May, with wolf spiders collected May through June. Leafhoppers were primarily collected in July and August when populations were the most abundant, especially adults, in correlation with dense vegetation. These sampling dates were ideal because they included the reproductive period (late spring to early fall) of both the Carolina Wren and the short-tailed shrew.

Various methods were used to collect the four different targeted taxa increasing the chances of collecting desired taxa. Collecting methods were consistent among all 30 collecting plots. To prevent cross contamination of samples among plots, new clean nitrile gloves were used at each plot. Tools used for collecting were washed between plots in a 10% Liquinox solution followed by a thorough rinse with deionized water to also prevent cross contamination between plots. The specifics for collecting each of the taxa are described below.

Earthworms were collected with the aid of stainless steel trowels and searching under rocks and woody debris. The depth of digging for worms was limited to 0.00 – 0.15 m from the surface. Slender stainless steel forceps were also used to help collect earthworms, which were placed into a small, clean, plastic bag. The specimens were then placed in a cooler filled with ice for transport to the laboratory. Once in the laboratory, samples were then washed with clean deionized water to remove any surface soil and mud.

Isopods were collected using similar techniques as those described for earthworms,

primarily with the use of trowels and forceps. Hand collecting was also an effective method, which required examination of rotting wood, tall grass (specifically around the soil surface and leaf litter), and other areas where vegetation was thick. Isopods were often found under leaf litter. Isopods were placed into small, clean plastic bags and placed on ice for transport to the laboratory. Unlike earthworm specimens collected, rinsing of isopod specimens was not necessary because soil was not observed to have adhered to them.

Collection of wolf spiders was achieved by visual searches of the sampling plots and hand collecting. When a specimen was found, it was either grabbed with forceps, or an unused I-Chem bottle (i.e., a sample bottle certified by the manufacturer as free of chemical contaminants) or a glass bottle (125 mL) that had been acid washed was used to trap specimens and transfer them to a small, clean plastic bag that was placed on ice for transport to the laboratory. Spiders required no further rinsing because soil was not observed to have adhered to the collected specimens.

Leafhoppers were collected with a standard size sweep net (38.1 cm diameter). Sweeps were performed to optimize collection from the entire plot, beginning in the center and working outward. However, often due to thick woody vegetation and the presence of thorns, herbivore collecting was sometimes limited to certain areas of a plot. These factors, along with sometimes limited amounts of herbaceous vegetation, greatly restricted the number of leafhoppers collected (subsequently limiting the weight collected). Sweep-net samples were transferred from the net into large, clean plastic bags. To prevent cross contamination between plots with the sweep net, clean sweep nets were used at every plot. Sweep nets were cleaned in a 10% Liquinox solution, followed by a thorough rinsing with deionized water. This rinsing process was repeated until the nets washed clean.

For passive collection of additional specimens, plastic Nalgene containers (11 cm wide and 12.5 cm deep) were used as pitfall traps in the plots. The containers were placed flush with the ground at the south, north, and center points of each of the 27 plots along LEFPC. Pitfall traps were not placed at the three reference plots to prevent injury to livestock present at those plots. Holes were drilled at the bottom of each container to prevent accumulation of water, particularly in plots that were susceptible to frequent flooding. A red plastic plate (20 cm in diameter) was used to cover the top of the pitfall traps to prevent entry of excess water and discourage foraging animals in the area from eating trapped specimens (Figure 7). To maintain the position of these plates, u-shaped wire was placed on two sides into the soil. Pitfall traps were checked weekly for a period of 17 weeks.

All specimens collected in the field and placed in plastic bags were labeled with the area (upstream, midstream, downstream, or reference), site, and plot ID as well as date of collection (month/day/year). Similar taxa were kept in their respective bags to prevent cross contamination as well as prevent higher taxa from consuming desired specimens of lower feeding levels. The specimens were then placed on ice in a cooler for transport to the laboratory. Once in the laboratory, specimens were either washed with deionized water (i.e., worms) or placed in a freezer at -20℃ until further processing and identifications were conducted.

Figure 7: Pitfall trap used to collect ground-dwelling organisms.

Sorting

Samples were removed from the freezer for preprocessing and sorting. Earthworms were defrosted to prevent damage and tearing of specimens. Earthworm specimens were defrosted either by adjusting to room temperature over time (requiring an average of 1 to 2 hours) or by placing the specimen bags into a clean acid washed jar (250 ml) containing 200 ml of room temperature deionized water. As with field sampling, clean nitrile gloves were worn during all sorting, especially taking extra care to use new gloves between each plot and taxa. Clean forceps were also used for each sample to decrease the likelihood of cross contamination. Established cleaning methods for tools, described above for field collecting, were used in the laboratory as well. Once specimens were processed and identified they were separated into morphospecies and placed in separate clean 20 ml or 40 ml glass vials and returned to the freezer (at -20℃) and stored until being freeze dried.

One morphospecies of isopod was used for mercury assessment. The morphospecies used was distinguishable by the five pairs of white book lungs on the last ventral segments of the abdomen. Other defining characteristics include two segmented flagella on the antenna and alternating light and dark bands on the outer sides of the dorsal segments running the length of the body (Figure 8).

One morphospecies was used for earthworm specimens as well. This morphospecies is distinguishable by the four pairs of setae on each of the segments. The particular morphospecies used had 26 segments until the clitellum. To establish as much consistency in samples for plots as a whole and individually, only mature earthworms were used for all sample analysis, where mature worms are typically identifiable by the presence of a clitellum. The particular morphospecies of earthworm used was identifiable by other characteristics. such as the dark

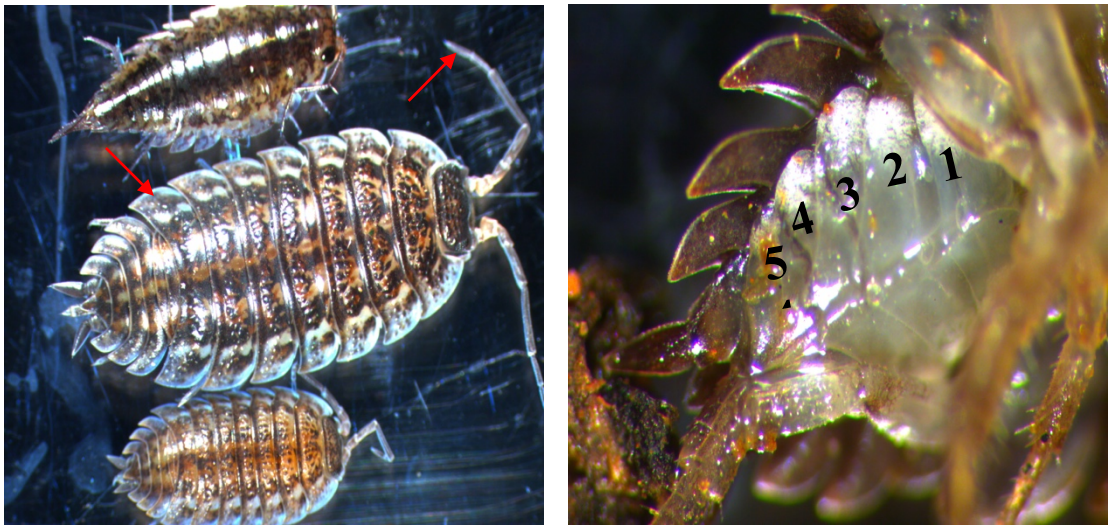


Figure 8: Isopod morphospecies identification characteristics (Family: Trachelipodidae). The photo on the left contains arrows pointing to the two antennal flagellum and the alternating white and dark lines running the length of the isopod body. The photo on the right showing the five white book lungs located on the last segments of the isopod abdomen.

dorsal segments and two tubercula pubertatis (Figure 9).

Wolf spiders are generally identifiable by their overall typical banded brown and black color, typically alternating in a semi-striped pattern. However, the most distinguishable characteristic of wolf spiders is the unique eye pattern (i.e., four smaller eyes aligned on the bottom, two larger eyes above those four and two eyes offset behind to the side of those six) (Figure 10). Thirteen morphospecies were used due to diversity along LEFPC floodplain. To achieve maximum weight goal for each plot, adult and immature spiders were used as well as both male and female wolf spiders. Each morphospecies used for each plot composite sample was documented and weighed individually for future reference. The number of male and female wolf spider specimens used for each plot composite sample was also documented and recorded for future reference.

Leafhoppers are distinguishable from other Hemiptera by their small slender bodies and specifically one or more row of spines on their hind tibia (Figure 11). Due to the great diversity along LEFPC floodplain, 12 morphospecies were used to achieve minimum weight goals at each plot. To achieve the minimum weight for some plots, both adult and immature leafhoppers were used. All 12 morphospecies were characterized, photographed, weighed by morphospecies, and documented for future reference.

Through the entirety of field collecting more than 6,000 specimens of varying class, order, and family amounting to more than 500 g of biomass, were collected. Ultimately 192 earthworms from all 30 collecting plots of one morphospecies were used. Collections yielded almost 90 g of earthworm weight with an average specimen weight of 0.5 g per earthworm. Isopods were collected from all 30 sampling plots. Because their average weight was 0.05 g,

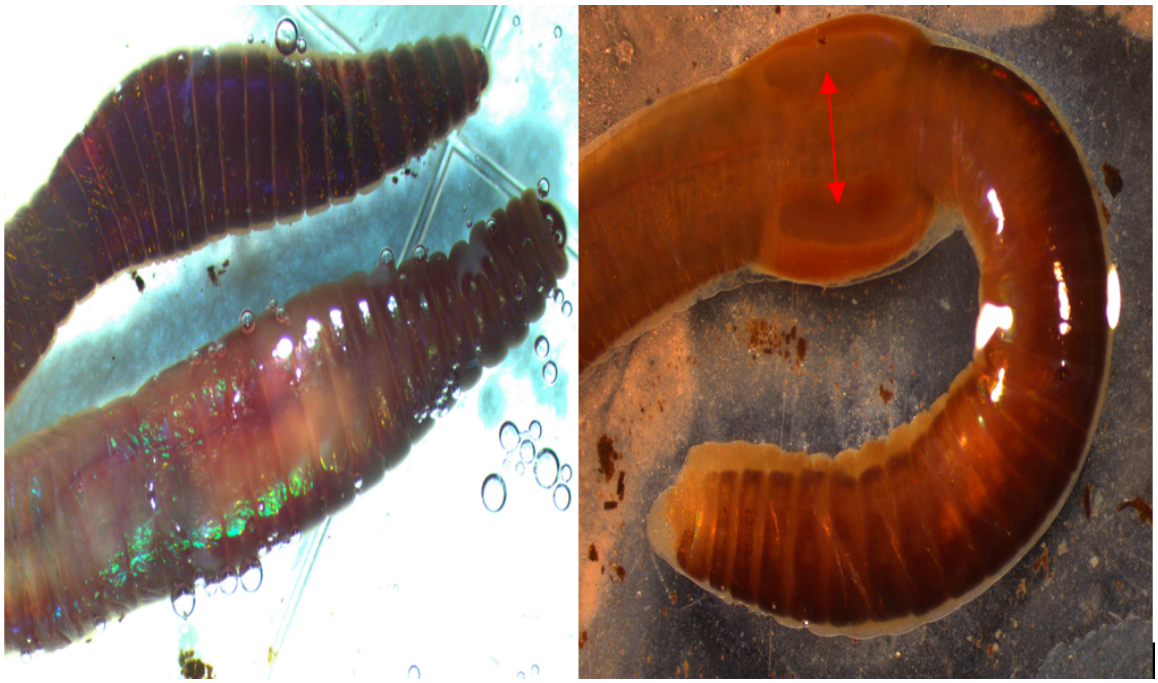


Figure 9: Earthworm morphospecies identification characteristics (Family: Lumbricidae). The photo on the left shows the 26 segments to the clitellum as well as the dark purple ventral side. The photo on the right shows the pair of tubercula pubertatis.

Figure 10: Wolf spider identification characteristics (Family: Lycosidae). The photograph on the left shows the dark and light brown alternating pattern. The photograph on the right shows their unique eye pattern.

Figure 11: Characteristics of leafhoppers (Family: Cicadellidae). The top left photograph shows the characteristic spines on the hind tibia. The top right photograph as well as the bottom two represent some of the morphospecies collected along LEFPC.

844 specimens needed to be collected to achieve weight goals for analysis. All specimens used were of the same morphospecies.

Wolf spiders were collected in significant numbers at each plot as well. A total of 837 wolf spider specimens were used for analysis, equaling 50 g with an average specimen weight of 0.1 g. Due to the great diversity of wolf spiders present throughout the LEFPC floodplain, 13 morphospecies, male and female, were used to achieve the analysis weight required.

After processing all sweep-net samples, the herbivorous, terrestrial invertebrate group collected with the highest numbers, consistently, among all plots was leafhoppers. More than 1,900 specimens of leafhoppers were used yielding 15.38 g with an average weight of 0.003 g. As with wolf spiders, diversity within leafhoppers was high along LEFPC. Twelve leafhopper morphospecies were used to achieve desired detection limits for total and methylmercury analysis. The total number of specimens used for all analyses was 3,807 with the total biomass equated to 203.24 g. The average percent moisture content for earthworms, wolf spiders, isopods, and leafhoppers was 81%, 70%, 69%, and 49%, respectively.

Sample Analysis

To prepare samples for analysis of total mercury, methylmercury, and stable isotopes $\delta^{13}C$ and $\delta^{15}N$, a composite of specimens of each taxon from each plot was weighed to achieve desired minimum and maximum weights (0.12 – 1.20 g). Once samples were weighed they were placed in either clean 20 ml or 40 ml vials depending on sample size, and the wet weight was recorded. Samples were refrozen before drying them in a Labconco FreezeZone freeze dryer. The weight of a subset of samples was periodically checked for progress in drying. After a constant weight was achieved, the samples were removed from the freeze dryer and weighed a

final time. This process took 117 hours on average. The change in weight was used to calculate the moisture content of each sample. All samples were then homogenized to prevent biased analysis. A tissue grinder was used directly in the glass vials to prevent tissue loss. The pestle was cleaned using 10% Liquinox, rinsed with deionized water, and dried thoroughly between each sample. Stainless steel scissors were used to help with homogenizing earthworm samples due to the leathery texture of their exoskeleton caused by the freeze drying process. Samples that were analyzed for total mercury and methylmercury were shipped directly to an analytical laboratory in the glass vials. Samples that were analyzed for stable isotopes were prepared in tin capsules. This process is described below.

To analyze for mercury, samples were shipped to Bothell, WA and evaluated by Brooks Applied Labs (http://brooksrand.com/services/mercury-hg/) using a modified EPA Method 1631 (EPA 1998b). In this method, mercury was analyzed using a MERX Analyzer with Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS). The use of a MERX Analyzer along with CVAFS allows methylmercury to be detected in sub-parts per trillion. Samples were prepared for analysis to oxidize all mercury present in the sample to Hg(II). The samples were then transferred to 40 ml vials. By adding hydroxylamine hydrochloride and stannous chloride samples were pre-reduced. Samples were purged using nitrogen gas. The addition of the two acids mentioned previously volatilized the elemental mercury to the gold amalgamation traps. The trap was then ballistically heated to discharge and transmit the mercury to the analytical trap. The analytical trap was then heated to release the mercury to the detector where the mercury was detected using CVAFS. Mercury concentrations were then determined using Mercury Guru software which was combined with the detector signal. Sample results were reported in a dry-

weight basis. Detection limit for total mercury was as low as 0.12 ppm (Brooks Rand Instruments 2016b).

To analyze for methylmercury, samples were shipped to and evaluated by Brooks Applied Labs (http://brooksrand.com/services/mercury-hg/) using a modified EPA Method 1630 (EPA 1998a). In this method methylmercury was analyzed using MERX Analyzer with Cold Vapor Atomic Fluorescence Absorption. Samples were prepared by distillation for water, and then placed into 40 ml vials where they were buffered to a pH of 4.9 and ethylated. The liquid samples were then transferred via gas pressure to a purge vessel. Here nitrogen gas was used to volatilize the ethylated mercury samples to a porous polymer resin, Tenax trap. This trap was then dried and ballistically heated. The ballistic heating allowed the thermal release of the methylmercury into an argon carrier gas stream and separated on a gas chromatography column followed by being reduced thermally to elemental mercury. The mercury was detected using CVAFS where Mercury Guru software was utilized along with the detector signal allowing for the calculation of mercury abundance. Sample results were reported in a dry-weight basis. Detection limit for methylmercury was as low as 0.07 ppm (Brooks Rand Instruments 2016a).

For the stable isotope analysis an optimal 0.08 – 0.12 g of each of the homogenized sample was weighed and placed into tin caps. These tin caps were then folded into cylindrical spheres and placed in an uncontaminated plastic well plate. This plate was shipped for analysis to the Stable Isotope Facility at the University of California, Davis (http://stableisotopefacility.ucdavis.edu/13cand15n.html) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS). In a reactor that is filled with chromium oxide and silver copper oxide, samples were combusted at 1000℃. Subsequently, the oxides were removed in a reduction reactor. A helium carrier then

flowed through the water trap. When analyzing for nitrogen stable isotope analysis a CO_2 trap was used. The use of a carbosieve gas chromatography column was used at 65°C at a rate of 65 ml per minute. Samples then entered the IRMS. Quality control was established between samples using laboratory standards that were selected to be similar to the samples being analyzed which had been calibrated against National Institute of Standards and Technology (NIST) standard reference materials. The initial isotope ratio for each sample was measured relative to the reference gases. The initial isotope ratios were then finalized by correcting them based on the known values of the laboratory standards used. The standard deviation for $\delta^{13}C$ is 0.2 per ml and 0.3 ml for $\delta^{15}N$. The delta (δ) results were reported relative to international standards where δ is equal to the sample divided by the standard minus one times 1000 (‰) (UC Davis 2016).

Data Analysis

Data were analyzed using SAS. A standard ANOVA as well as linear regression analysis was performed on appropriate data. Least Square Means (LSM) tables were derived from the standard ANOVA. LSM examined each taxa, i.e., isopods, earthworms, wolf spiders, and leafhoppers, against all factors (i.e., total mercury, methylmercury, $\delta^{13}C$, and $\delta^{15}N$).

Results and Discussion

Biota Concentrations

Total mercury concentrations resulted in averages of 0.10 ppm, 2.33 ppm, 3.92 ppm, 7.80 ppm for leafhoppers, wolf spiders, isopods, and earthworms, respectively. Methylmercury concentrations averaged 0.02 ppm, 0.18 ppm, 1.18 ppm, 1.29 ppm for leafhoppers, earthworms,

isopods, and wolf spiders, respectively. Methylmercury and total mercury concentrations for leafhoppers, wolf spiders, isopods, and earthworms are visually represented in Figures 12, 13, 14, and 15, respectively. The dark grey bars represent the total mercury concentrations minus the methylmercury concentrations. The light grey bars indicate the methylmercury concentrations. The dark grey bars and the light grey bars together represent the total mercury concentrations found in each of the total taxa, where total mercury concentrations consists of methylmercury and all other forms of mercury found in the biota that was collected and analyzed.

The average methylmercury and average total methylmercury concentrations of the composite samples of leafhoppers collected at each of the 27 collecting plots along LEFPC as well as the three reference plots located at Brushy Fork are shown in Figure 12. Note that total mercury for leafhoppers does not exceed 0.45 ppm at any plot. Leafhopper methylmercury concentrations are even lower. Leafhopper total mercury and methylmercury concentrations were the lowest among all taxa collected and analyzed along LEFPC in this study. Even though some methylmercury and total mercury concentrations for leafhoppers are relatively higher at some plots (i.e., US-B20) concentrations are still low in comparison to all other taxa collected.

The average methylmercury and average total mercury concentrations of the composite samples of wolf spiders for each of the 27 collecting plots along LEFPC as well as the three reference plots, are shown in Figure 13. Note that total mercury concentrations for wolf spiders are higher than leafhoppers. Total mercury concentrations for wolf spiders are greater than 5 ppm at some upstream plots and methylmercury levels reached almost 4 ppm.

The isopod total mercury concentrations reached even higher values than the wolf spiders as some midstream plot composite samples reached over 10 ppm (Figure 14). Isopod

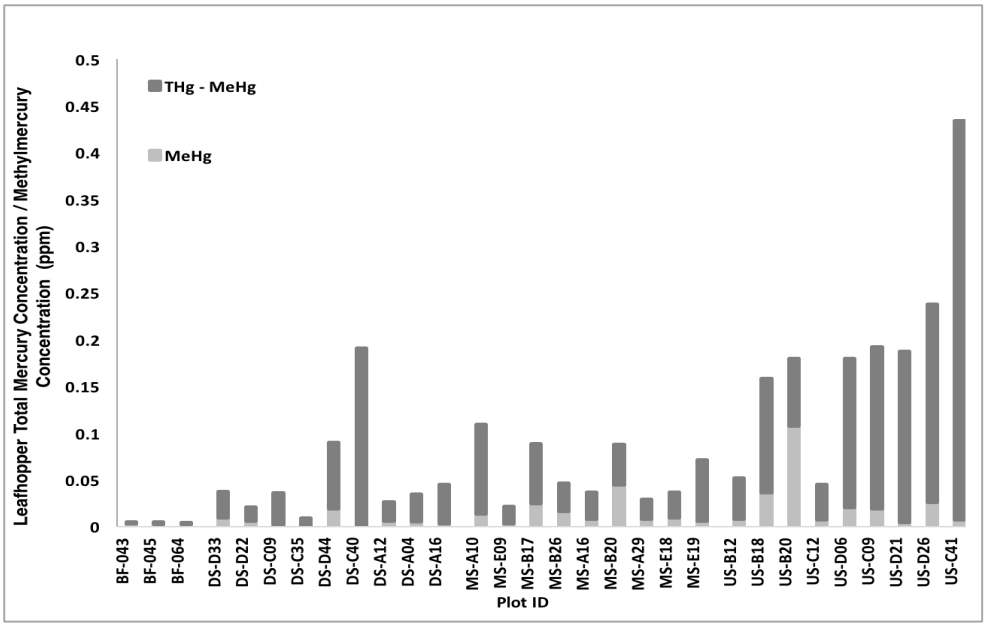


Figure 12: Average methylmercury and total mercury concentrations plotted for all leafhoppers collected and analyzed. Plot IDs represented by BF for the reference site, Brushy Fork, DS for downstream collecting area, MS for midstream collecting area, and US for upstream collecting area.

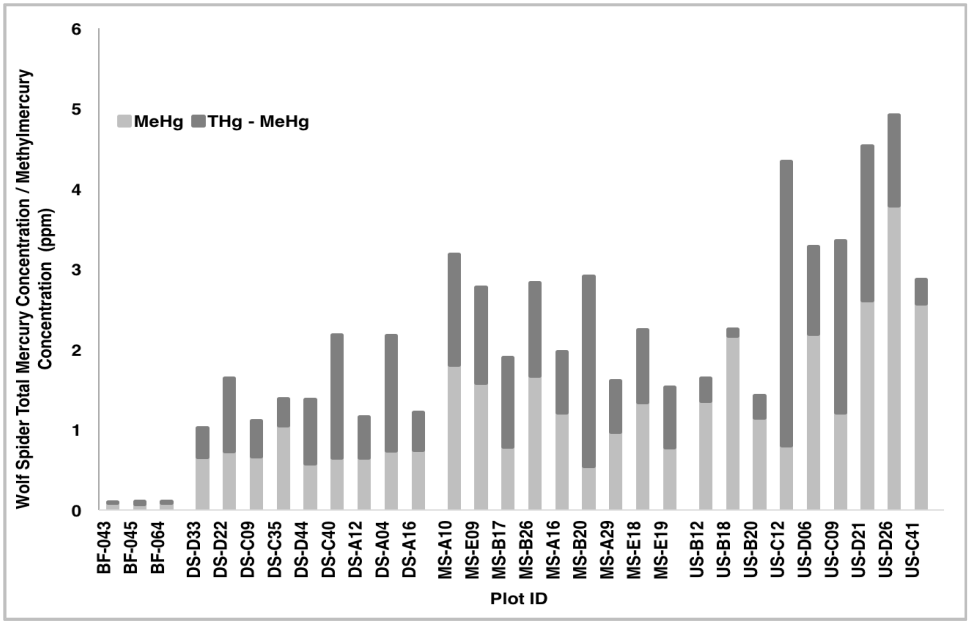


Figure 13: Average methylmercury and total mercury concentrations plotted for all wolf spiders collected and analyzed. Plot IDs represented by BF for the reference site, Brushy Fork, DS for downstream collecting area, MS for midstream collecting area, and US for upstream collecting area.

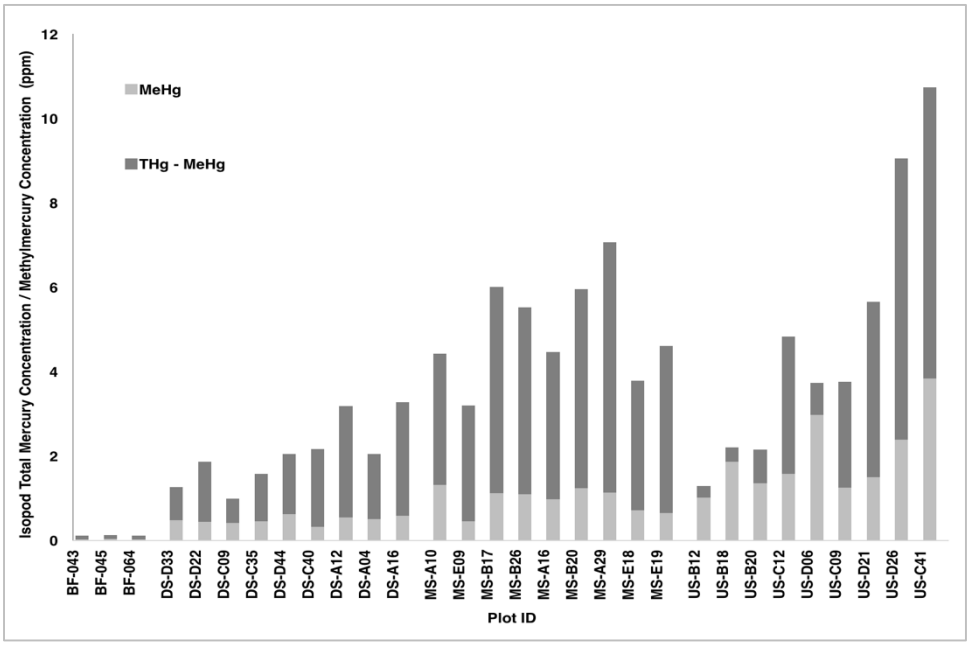


Figure 14: Average methylmercury and total mercury concentrations plotted for all isopods collected and analyzed. Plot IDs represented by BF for the reference site, Brushy Fork, DS for downstream collecting area, MS for midstream collecting area, and US for upstream collecting area.

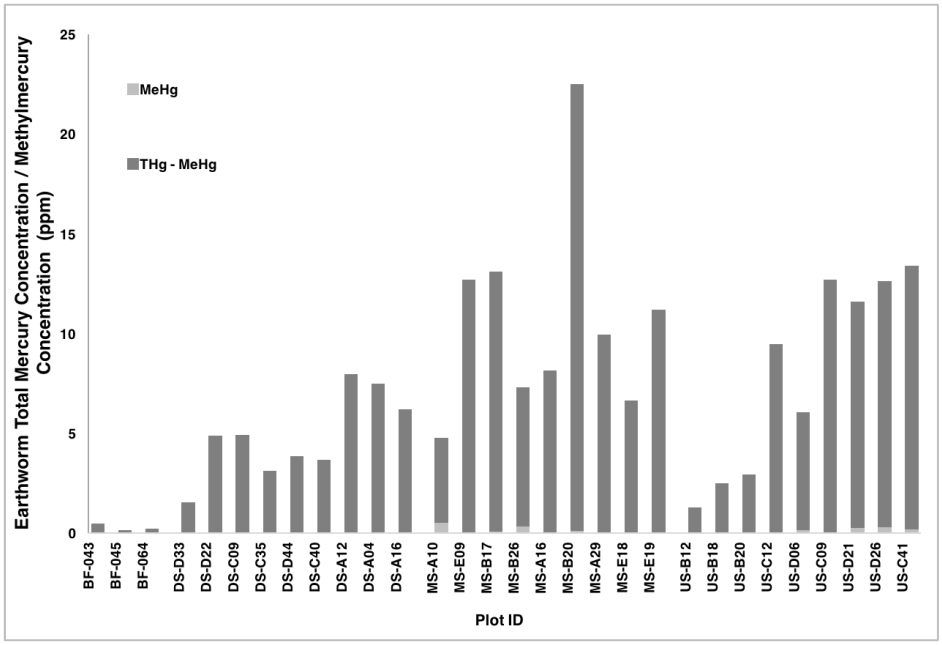


Figure 15: Average methylmercury and total mercury concentrations plotted for all earthworms collected and analyzed. Plot IDs represented by BF for the reference site, Brushy Fork, DS for downstream collecting area, MS for midstream collecting area, and US for upstream collecting area.

methylmercury values were similar to those of wolf spiders, reaching around 4 ppm. Total mercury concentrations found in isopods along LEFPC were higher than those found in leafhoppers.

Earthworm total mercury concentrations, as well as methylmercury concentrations, are show in Figure 15. Earthworm total mercury concentrations were the highest on average of all taxa. The highest total mercury concentration was greater than 20 ppm and was found at a midstream location. However, earthworm methylmercury values were much lower than those of the isopods and wolf spiders collected at all the sample collecting plots in this study along LEFPC. However, earthworm methylmercury concentrations were higher than leafhoppers in this study.

Methylmercury concentrations varied among taxa collected, but differently than the variation observed among taxa for total mercury concentrations. Total mercury concentrations were lowest in leafhopper samples with concentrations in earthworms, isopods and spiders almost 80X, 40X and 25X greater, respectively, than those reported for leafhoppers. Methylmercury concentrations were also lowest in leafhopper samples with earthworms only 9X greater and isopods and spiders almost 60X and 65X greater than the average methylmercury leafhopper concentrations. These differences of total mercury concentrations among taxa may be attributed to the feeding guilds of these organisms. Leafhoppers feed on plant sap causing them to be unlikely to obtain large quantities of mercury in their diet. Taxa of relatively higher trophic position, such as the isopods and wolf spiders collected, have feeding habits that may have provided them with greater opportunity to consume mercury in their diet.

The percentage of methylmercury that comprised the total concentration of mercury, methylmercury fractions, were highest in wolf spiders. The methylmercury fractions in the four

taxa resulted in averages of 2.89% for earthworms, 20.57% for leafhoppers, 34.28% for isopods, and 56.80% for wolf spiders (Table 1). The low methylmercury fraction value for earthworms indicates that earthworms may not uptake mercury as readily, compared to other taxa analyzed, despite having the highest concentration of total mercury.

Significant differences in methylmercury concentrations were detected among taxa. Methylmercury concentrations were significantly different among all taxa except between wolf spiders and isopods (p=0.0641) (Table 2). These results suggest that isopods may pose a threat to upper level consumers to a similar extent as wolf spiders, despite the difference in their feeding habits. Low concentrations of methylmercury in earthworms and leafhoppers may mean that these organisms pose less of a threat to higher level consumer organisms that feed on them. All individual plot biota composite sample results for total mercury and methylmercury concentrations can be found in the Appendix (Table A2).

Stable Isotopes

Stable isotope analysis revealed that isopods had a mean $\delta^{13}C$ level of -25.08‰, spiders at -26.27‰, earthworms at -26.36‰, and leafhoppers at -29.51‰. For each taxa the $\delta^{15}N$ was 7.23‰, 6.68‰, 3.73‰, and 2.71‰ for isopods, spiders, leafhoppers, and earthworms, respectively. The relationship between $\delta^{13}C$ and $\delta^{15}N$ for each taxa is shown in Figure 16, which shows that the herbivores (leafhoppers) are distinct from the detritivores (earthworms and isopods) and carnivores (wolf spiders). The distinguishing separation of leafhoppers from all other taxa collected reflects leafhoppers distinctly different feeding habits. While the

Table 1: Average total mercury, methylmercury, and methylmercury fractions[1] for each of the four taxa collected and analyzed.

Biota	*Total Mercury (ppm)*	*Methylmercury (ppm)*	*Methylmercury Fraction*
Earthworms	7.780	0.18	2.89
Isopods	3.92	1.18	34.28
Wolf spiders	2.33	1.29	56.80
Leafhoppers	0.10	0.02	20.57

[1] Methylmercury fractions were calculated by dividing methylmercury concentrations by total mercury concentrations for each taxon and multiplying by 100. Methylmercury fractions were averaged by plot. The total averages of these plot methylmercury fraction values are reported above by taxa.

Table 2: Statistical comparisons of methylmercury concentrations between taxa (Least square means, α = 0.05).

	Isopods	*Leafhoppers*	*Wolf Spiders*	*Earthworms*
Isopods		<0.0001	0.0641	<0.0001
Leafhoppers	<0.0001[1]		<0.0001	<0.0001
Wolf Spiders	0.0641	<0.0001		<0.0001
Earthworms	<0.0001	<0.0001	<0.0001	

[1] Taxa in individual rows are compared to taxa in individual columns to assess differences in methylmercury concentrations between taxa.

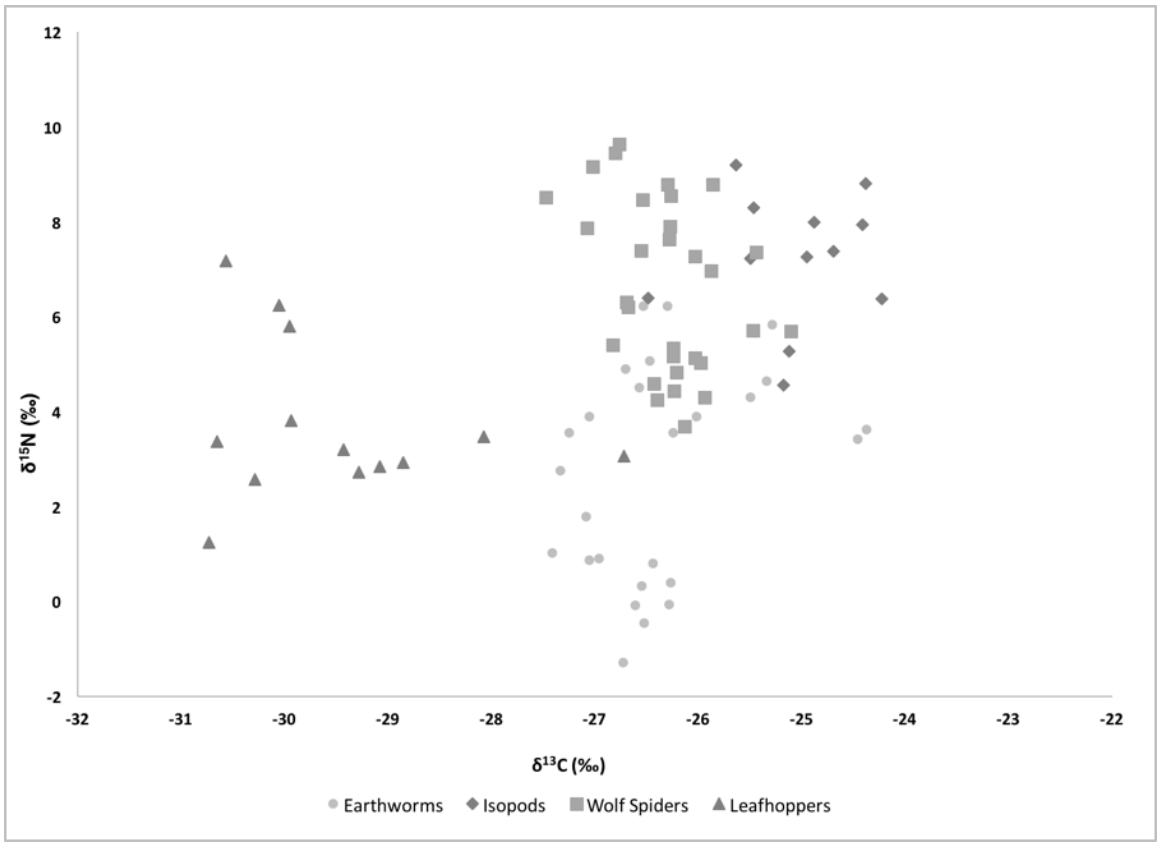


Figure 16: Stable isotopes $\delta^{15}N$ versus $\delta^{13}C$ indicating trophic levels of earthworms, leafhoppers, isopods, and spiders collected.

overlapping feeding habits of wolf spiders, isopods, and earthworms is reflected in the overlapping $\delta^{13}C$ and $\delta^{15}N$ values (Figure 16).

$\delta^{15}N$ was found to increase as methylmercury increased among all taxa collected and analyzed. The trend of $\delta^{15}N$ increasing as methylmercury in taxa increased was observed at upstream, midstream, and downstream areas, respectively (Figures 17, 18, and 19). The methylmercury concentrations for all upstream taxa, where earthworms and leafhoppers have the lowest methylmercury concentrations and are lower in trophic position, are shown in Figure 17. Again, this same trend can be seen for taxa midstream (Figure 18) and downstream (Figure 19).

The total mercury concentrations in biota plotted against $\delta^{15}N$ suggest that the biota with the highest total mercury do not have the highest $\delta^{15}N$ (Figures 20, 21, and 22). Earthworms have some of the highest concentrations of total mercury, but did not have the highest levels of $\delta^{15}N$ and therefore are not in the highest trophic position (Figure 20). Midstream and downstream collecting sites reflect this statement as well, that an increase in total mercury concentrations is not always associated with an increase in trophic position, especially in regards to earthworms (Figures 21 and 22). It should be noted that in Figures 17-22 limited data were collected for leafhoppers and isopods at some stream locations due to insufficient biomass present at all collecting plots for stable isotope analysis. All $\delta^{13}C$ and $\delta^{15}N$ results from each of the collecting plots for all of the taxa collected can be found in the Appendix (Table A2).

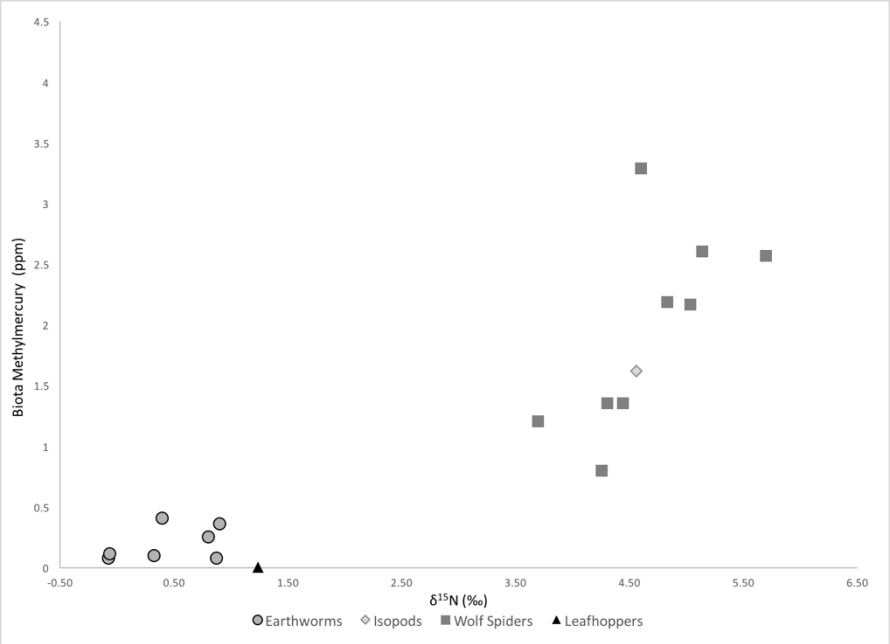


Figure 17: Methylmercury concentrations of all upstream taxon collected and analyzed plotted against the respective $\delta^{15}N$.

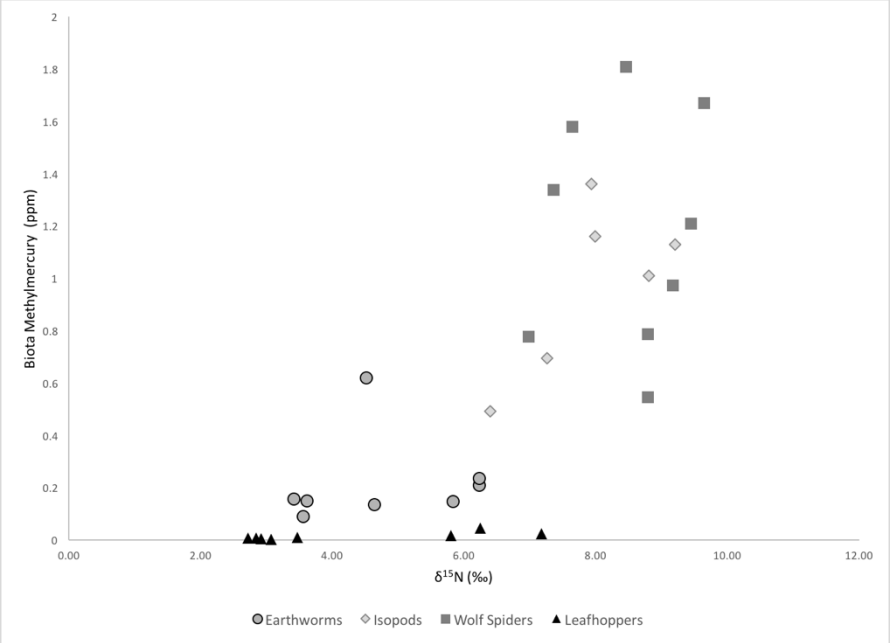


Figure 18: Methylmercury concentrations of all midstream taxon collected and analyzed plotted against the respective $\delta^{15}N$.

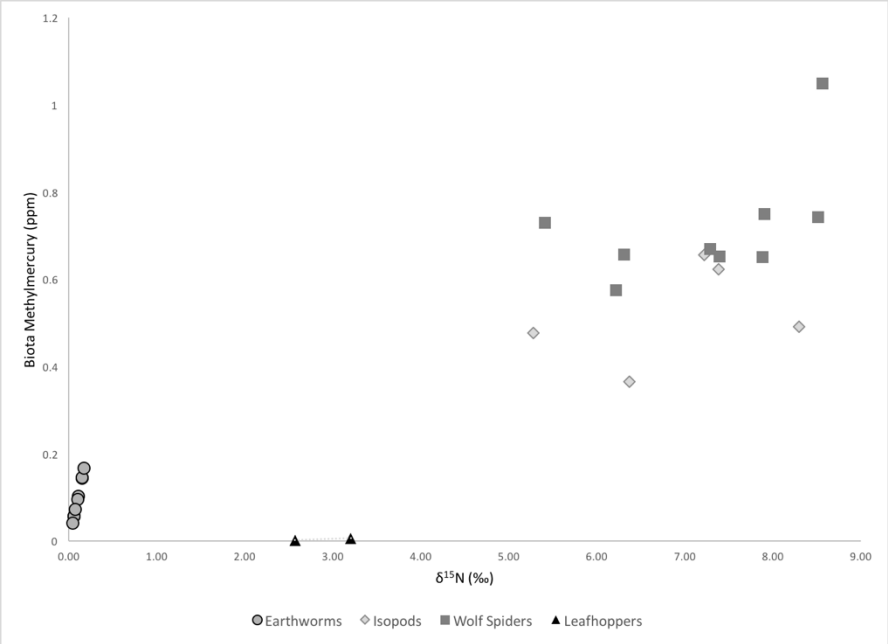


Figure 19: Methylmercury concentrations of all downstream taxon collected and analyzed plotted against the respective $\delta^{15}N$.

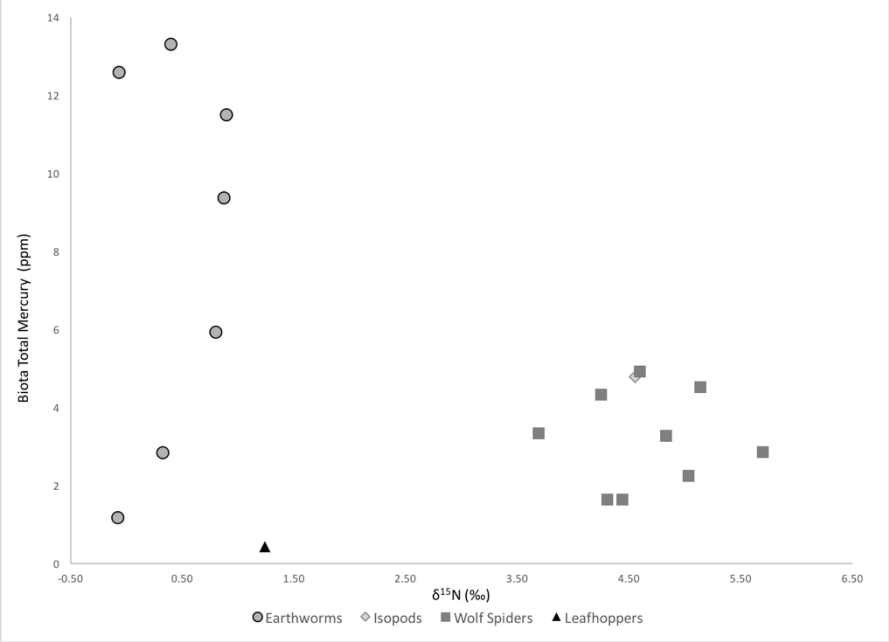


Figure 20: Total mercury concentrations of all upstream taxon collected and analyzed plotted against the respective $\delta^{15}N$.

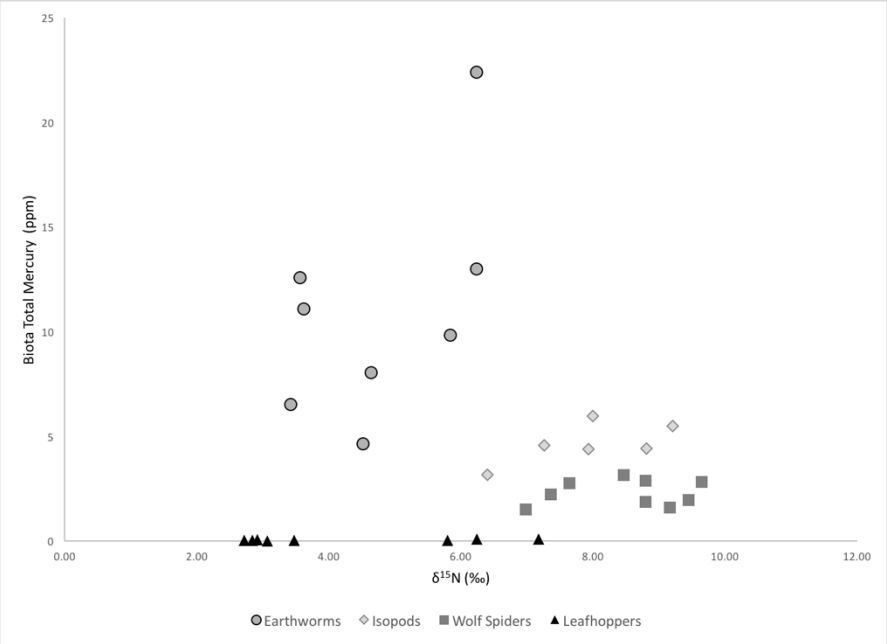


Figure 21: Total mercury concentrations of all midstream taxon collected and analyzed plotted against the respective $\delta^{15}N$.

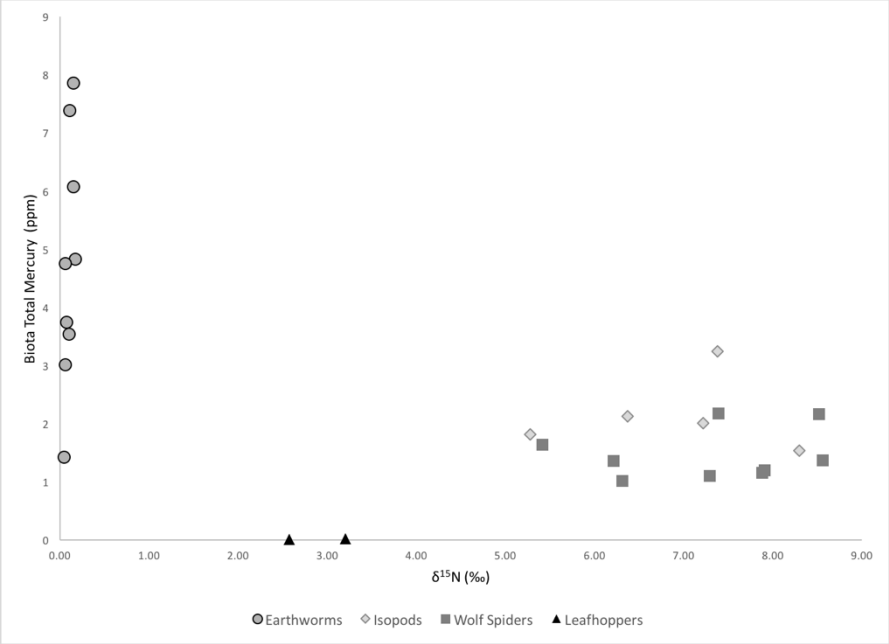


Figure 22: Total mercury concentrations of all downstream taxon collected and analyzed plotted against the respective $\delta^{15}N$.

Summary

The leafhopper taxa had the lowest concentrations of total mercury and methylmercury. The earthworms had the highest average concentration of total mercury, but one of the lowest average concentrations of methylmercury. Though average methylmercury concentrations in wolf spiders were the highest at 1.29 ppm, methylmercury concentrations in isopods were just slightly lower at 1.18 ppm. Both are significantly greater than the average methylmercury concentrations in leafhoppers and earthworms and are not significantly different from each other. The percent methylmercury was greatest in wolf spiders. In organisms, such as fish, methylmercury fractions can reach 100%. Methylmercury is covalently bonded to sulfhydryl groups on proteins within the organism that the methylmercury has accumulated in. Isopods and wolf spiders, with percentages reaching 70% on average, may absorb methylmercury in a similar fashion with such high percentages of methylmercury to total mercury.

Lumbricus spp., in the family Lumbricidae, were found to contain high concentrations of mercury when compared to other flora and fauna collected (Cocking et al. 1991). This study also found high concentrations of mercury in larvae of the beetle Family Scarabaeidae, especially when compared to those in the Family Elateridae. The findings by Cocking et al. (1991) reflect similar results as those seen in this current study. Higher concentrations of total mercury were seen in earthworms from the same family analyzed by Cocking et al. (1991) compared to all other biota collected in this current study.

Mercury contamination from flooding could be significant in the terrestrial food web of the South River floodplain in Waynesboro, VA (Cocking et al. 1991). It is hypothesized that high concentrations of mercury in invertebrates that inhabit the soil reveal bioaccumulation occurs through pathways via the detritus food web (Cocking et al. 1991). Researchers also found

that the kidney tissue of shrews contained high concentrations of mercury, as did spiders and preying mantids at South River, VA (Cocking et al. 1991). Studies like these allow for further implications that biomagnification occurs within other food webs outside that of detritus and outside the aquatic food web. These results also reflect similar results as seen in this current study of increasing mercury concentrations with increasing trophic position.

This study provides important information. First, multiple sources of mercury may contaminate the terrestrial environment and food web in, and surrounding, LEFPC. Sources of mercury to the terrestrial environment and food web in and surrounding LEFPC include: sediment along LEFPC, the surface waters of LEFPC, emerging aquatic insects from LEFPC, and frequent flooding. These sources, the LEFPC and the aquatic life present in it, alongside frequent flooding, contribute to the continual exposure of terrestrial invertebrates to mercury. These multiple sources of mercury are reflected by the fact that the isopods (detritivores) analyzed in this study had methylmercury concentrations that were not found to be significantly different than the carnivores analyzed in this study (wolf spiders). Second, methylmercury concentrations do increase as $\delta^{15}N$ increases. However, variable concentrations of mercury found in the analyzed trophic level samples indicate that total mercury bioaccumulation in the terrestrial environment does not always increase as $\delta^{15}N$ increases. This variation of total mercury and methylmercury trends for $\delta^{15}N$ is reflected in the earthworms collected and analyzed in this study, they had the highest total mercury concentrations but the lowest $\delta^{15}N$ values. Third, when examining a contaminated site using varying trophic levels, it cannot be presumed that certain taxa align within a particular trophic level and therefore uptake contaminants in a predictive way. The need for a wider range of collected taxa within a feeding group at a contaminated site is reflected by the earthworm (detritivores) samples collected, which

had the highest concentrations of total mercury, but had one of the lowest concentrations of methylmercury. Isopods, another detritivore taxa collected and analyzed in this study, had lower total mercury concentrations than the other detritivores collected and analyzed in this study (earthworms). However, isopods had methylmercury concentrations that were not significantly different than those of the carnivores collected in this study, wolf spiders. Thus, it is important to collect multiple taxa representing specific trophic levels of interest. A wider range of taxa collected allows for a better understanding of the movement of mercury through the food web in a contaminated environment.

CHAPTER III

DETERMINING INFLUENCE OF DISTANCE FROM THE CONTAMINATED STREAM ON MERCURY CONCENTRATIONS IN FLOODPLAIN SOILS AND RESIDENT TERRESTRIAL ARTHROPOD SPECIES

Introduction

East Fork Poplar Creek (EFPC) runs through the city of Oak Ridge, TN, which resides in both Anderson and Roane Counties (Figure 1). EFPC originates at the Y-12 National Security Complex (Y-12 Complex) which is located on the Oak Ridge Reservation (ORR) (Figure 23). EFPC has subsequently been divided into Upper East Fork Poplar Creek (UEFPC) which consists of the first 2 km within the ORR and Lower East Fork Poplar Creek (LEFPC) which runs for 24 km through Oak Ridge, TN. The Y-12 Complex was a source of mercury contamination to EFPC as well as the LEFPC floodplain. An estimated 108,000 – 212,000 kg of mercury was released into LEFPC between the years of 1950 to 1963. The LEFPC floodplain occupies 7,689 ha of land (DOE 2014b). Of the mercury released into LEFPC, almost 77,000 kg have been deposited into the soil and sediment along LEFPC in the floodplain soils (Pant et al. 2010b). A study in 1982 found mercury concentrations in fish in LEFPC four times higher than the limit set by the Food and Drug Administration (FDA) at that time.

In the 1982 study, aqueous mercury samples decreased as distance from the origin source increased (Brooks and Southworth 2011). However, in succeeding years, 1997-1999,

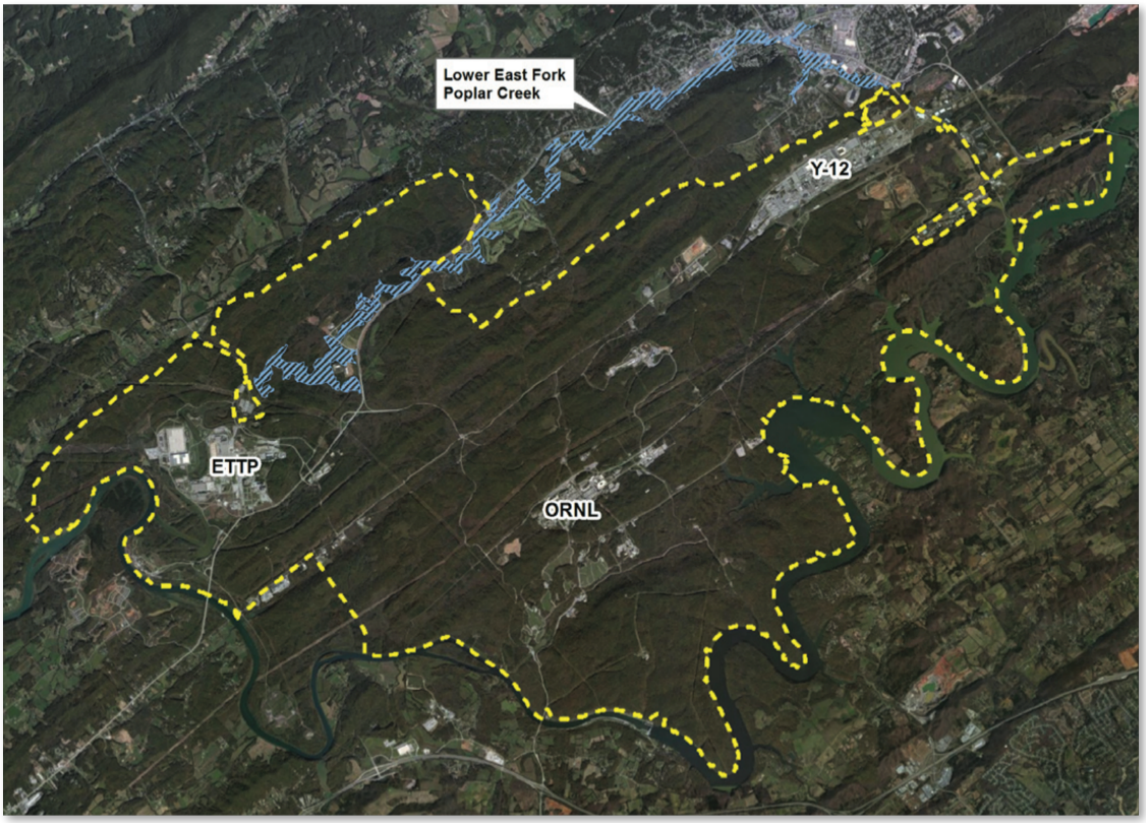


Figure 23: Map showing the Oak Ridge Reservation boundaries (denoted by the yellow dotted line) and Lower East Fork Poplar Creek stemming from the Y-12 Complex (Y-12) running through Oak Ridge, TN.

methylmercury concentrations increased as distance downstream increased (Southworth et al. 2000). A 1995 clean up action called for the excavation of mercury in the LEFPC floodplain soils (DOE 2014b). The Department of Energy (DOE) performed remediation actions resulting in the removal of soils in the LEFPC floodplain that had total mercury concentrations exceeding 400 ppm and replaced them with clean soils (Pant et al. 2010a). Remediation actions were taken at upstream and midstream sites along LEFPC (ATSDR 2012). The high concentrations of mercury in LEFPC waters and resident fish are above guidelines for human and ecological risk. The high concentrations of mercury present in the soil and sediment along LEFPC also potentially pose a threat to the terrestrial environment of LEFPC.

To understand mercury impact in contaminated areas, bioaccumulation factors (BAFs) are used. BAFs are defined as the ratio of mercury concentrations in biological tissue to that in the surrounding environment (Han et al. 2012). BAFs can be important in ecological evaluation by providing results without the use of large sample numbers for evaluation. The use of BAFs has been found to be applicable to evaluate organic compounds that are hydrophobic, like mercury, due to the fact that BAFs include dietary, dermal, and respiratory exposure where dietary routes that lead to exposure are of great importance in lipophilic compounds (Costanza et al. 2012).

The concentration of mercury has been an ongoing concern at LEFPC for the past several years. Actions have been taken to address this concern, however the issue of mercury still persists. The concern has also extended to include areas outside of LEFPC and the LEFPC waters to the surrounding floodplain soils, as well as to the terrestrial organisms living in the habitat. One objective of this study is to determine if the distance from the contaminated stream (LEFPC) influenced mercury concentrations in floodplain soils and/or resident invertebrate

species and to compare bioconcentrations of total and methylmercury in invertebrates from the LEFPC and other contaminated and reference sites. A second objective is to use soil total mercury concentrations from Phase 1 of a 2014 LEFPC Mercury Biouptake Study (DOE 2014b) and biota total mercury concentrations for the current study to evaluate BAFs for biota collected along LEFPC and compare the calculated BAFs to those of other contaminated sites.

Materials and Methods

Distance

This study used the same plots described in Chapter II, so the details and methodologies of those plots are not repeated here (see Chapter II for specific details). ArcGIS software was used to measure the distance of each of the 27 plots along LEFPC from the contaminated stream, LEFPC, as well as the distance of the three reference plots at Brushy Fork from their associated stream. Plots were located at upstream, midstream, and downstream sites. The distance of each plot from the contaminated stream, LEFPC, was measured three times using ArcGIS software. The average distance of the three measurements was calculated and used for comparisons. The plots and their location relative to LEFPC as depicted by the ArcGIS software are shown in Figures 24, 25, 26, and 27. ArcGIS software was also used to measure the distance of each of the 27 plots along LEFPC from the origin source of mercury, i.e., the Y-12 Complex. When measuring plot distances from the origin source, distances were measured to the closest km marker from the Y-12 Complex.

Figure 24: Upstream collecting plots (n=9) with IDs at km marker 4 and 4.5, along Lower East Fork Poplar Creek.

Figure 25: Midstream collecting plots (n=9) with IDs at km marker 8, along Lower East Fork Poplar Creek.

Figure 26: Downstream collecting plots (n=3) with IDs at km marker 19, along Lower East Fork Poplar Creek.

Figure 27: Downstream collecting plots (n=6) with ID at km marker 23, along Lower East Fork Poplar Creek.

Soil Total Mercury Concentrations

A soil survey of total mercury concentrations along LEFPC was performed by Oak Ridge National Laboratory (ORNL) as Phase 1 of the LEFPC Mercury Biouptake Study prior to the beginning of this study. Total mercury soil concentrations in this soil survey were determined using a direct mercury analyzer (DMA) (DOE 2014b). Results were reported on a dry-weight basis. Complete data for total mercury concentrations in the soil for each plot where taxa were collected can be found in Table A2 in the appendix. These soil mercury values were used in conjunction with biota mercury concentrations from this study to calculate BAFs and better understand the relationship between biota total mercury concentrations and soil total mercury concentrations. Because of the relationship used in calculating BAFs, it was important to collect biota in areas with varying soil mercury concentrations as well as biota that was readily available. Soil total mercury concentration ranges were set during Phase 1 of the 2014 LEFPC Mercury Biouptake Study from high to low with high values defined as 100-400 ppm, medium values defined as 25-100 ppm, and low concentrations defined as 0-25 ppm (DOE 2014b).

Data Analysis

Once distances were measured using the ArcGIS software, SAS was used to conduct a standard ANOVA with an alpha error level at 0.05. Significant differences in distance from both the contaminated stream and the source of contamination, the Y-12 Complex, among total mercury concentrations in the soil, total mercury concentrations in the biota (separated by taxa) and methylmercury concentrations in the biota (separated by taxa), were determined. BAFs and the fraction of methylmercury to total mercury (methylmercury fractions) were also evaluated for significant differences among distances from both LEFPC and the Y-12 Complex using SAS and

a standard ANOVA with an alpha error level at 0.05.

Bioaccumulation factors were calculated by dividing the total mercury concentrations in each taxon at each plot collected in 2015 by the total mercury concentrations in the surrounding soils for each plot as determined in the 2014 Phase 1 of LEFPC Mercury Biouptake Study (DOE 2014b). Average BAFs were calculated for each taxon. To understand the relationship between the total mercury concentrations found in the biota and the total mercury concentrations in the soil in regards to stream location, linear regression analysis was used for each of the taxa collected and analyzed where each taxon was arranged into upstream, midstream, and downstream collecting sites. To understand the relationship between BAFs for each of the taxa and their location along the stream (i.e., upstream, midstream, and downstream), SAS was used to conduct a standard ANOVA with an alpha error level of 0.05 as well as linear regression analysis.

Results and Discussion

All numerical plot distances from the contaminated stream (LEFPC) and the mercury origin (Y-12 Complex) can be found in the Appendix (Table A3). No statistical differences among numeric plot distances (meters and kilometers) and mercury concentrations of biota samples or soil samples, i.e., total mercury and methylmercury, were documented (Table A3). However, significant differences in total mercury concentrations of biota samples and soil samples when LEFPC collecting sites were viewed by stream location (i.e., downstream, midstream, and upstream) were observed. Total mercury concentrations in the soil along LEFPC were significantly different among upstream, midstream, and downstream sites. For example, total mercury concentrations were significantly different in downstream sites when compared to

upstream and midstream. However, no significant difference in total mercury concentrations in the soil between upstream and midstream sites ($p = 0.4817$) were found (Table 3). No significant difference between upstream and midstream sites was also found for total mercury, where total mercury concentrations for the biota sampled were significantly different in downstream concentrations when compared to upstream and midstream sites. However, no significant difference in total mercury concentrations in biota was observed between upstream and midstream sites ($p=0.9862$) (Table 4). Upstream and midstream collecting sites that are not significantly different in total mercury concentrations for both soil and biota may be attributed to their relative closeness to each other and relative proximity to the mercury source, the Y-12 Complex. Upstream sites are at 4 and 4.5 km from the Y-12 Complex and only 4 km from the midstream collecting sites and plots. Downstream sites are an additional 11 and 15 km from the midstream collecting sites and plots. This increase in downstream distance may contribute to the significant difference of total mercury values in both soil and biota.

Methylmercury concentrations in the biota samples collected from the floodplain along LEFPC were significantly different among upstream, midstream, and downstream sites (Table 5). Methylmercury fractions in the biota samples were significantly different ($p=0.0240$) between upstream and midstream sites. However, when examining BAFs, no significant difference was seen among collecting site location (upstream, midstream, and downstream) and taxa (Table 6). For example, upstream isopod BAFs are not significantly different than midstream or downstream isopod BAFs. No significant difference of this manner is documented for any of the four taxa collected in this study. Upstream and midstream collecting sites are not significantly different when examining methylmercury fractions in the biota samples. This

Table 3:Comparison of soil total mercury concentrations between stream location along Lower East Fork Poplar Creek (LEFPC) in Oak Ridge, TN, 2014 .

	Downstream	***Midstream***	***Upstream***
Downstream		<0.0001	<0.0001
Midstream	<0.0001[1]		0.4817
Upstream	<0.0001	0.4817	

[1]SAS was used to conduct standard ANOVA; value represents probability level between stream location for soil total mercury concentrations.

Table 4: Comparison of biota total mercury concentrations between stream location along Lower East Fork Poplar Creek (LEFPC) in Oak Ridge, TN, 2015.

	Downstream	***Midstream***	***Upstream***
Downstream		<0.0001	<0.0001
Midstream	<0.0001[1]		0.9862
Upstream	<0.0001	0.9862	

[1]SAS was used to conduct standard ANOVA; value represents probability level between stream location for biota total mercury concentrations.

Table 5: Comparison of biota methylmercury concentration between stream location along Lower East Fork Poplar Creek (LEFPC) in Oak Ridge, TN, 2015.

	Downstream	***Midstream***	***Upstream***
Downstream		<0.0001	<0.0001
Midstream	<0.0001[1]		0.0003
Upstream	<0.0001	0.0003	

[1] SAS was used to conduct standard ANOVA; value represents probability level between stream location for biota methylmercury concentrations.

Table 6: Comparisons of biota bioaccumulation factors between stream location along Lower East Fork Poplar Creek (LEFPC) in Oak Ridge, TN, 2015.

	US[1]- I[2]	***US-L***	***US-W***	***US-E***	***MS-I***	***MS-L***	***MS-W***	***MS-E***	***DS-I***	***DS-L***	***DS-W***	***DS-E***
US- I		<.0001[3]	0.9598	0.0058	0.9993[4]	<.0001	0.0919	0.0016	1.0000	<.0001	1.0000	0.2427
US-L	<.0001		<.0001	<.0001	<.0001	0.9999	0.0003	<.0001	<.0001	0.9983	<.0001	<.0001
US-W	0.9598	<.0001		<.0001	0.5145	<.0001	0.8416	<.0001	0.8233	<.0001	0.9997	0.0054
US-E	0.0058	<.0001	<.0001		0.0731	<.0001	<.0001	1.0000	0.0191	<.0001	0.0007	0.9633
MS-I	0.9993	<.0001	0.5145	0.0731		<.0001	0.0078	0.0257	1.0000	<.0001	0.9432	0.7836
MS-L	<.0001	0.9999	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.9005	<.0001	<.0001
MS-W	0.0919	0.0003	0.8416	<.0001	0.0078	<.0001		<.0001	0.0333	0.0092	0.3392	<.0001
MS-E	0.0016	<.0001	<.0001	1.0000	0.0257	<.0001	<.0001		0.0058	<.0001	0.0002	0.8326
DS-I	1.0000	<.0001	0.8233	0.0191	1.0000	<.0001	0.0333	0.0058		<.0001	0.9974	0.4653
DS-L	<.0001	0.9983	<.0001	<.0001	<.0001	0.9005	0.0092	<.0001	<.0001		<.0001	<.0001
DS-W	1.0000	<.0001	0.9997	0.0007	0.9432	<.0001	0.3392	0.0002	0.9974	<.0001		0.0577
DS-E	0.2427	<.0001	0.0054	0.9633	0.7836	<.0001	<.0001	0.8326	0.4653	<.0001	0.0577	

[1] Stream location denoted by US for upstream, MS for midstream, and DS for downstream

[2] Biota denoted by: I for isopods, L for leafhoppers, W for wolf spiders, and E for earthworms.

[3] SAS was used to conduct standard ANOVA; value represents probability level between stream location for individual taxa bioaccumulation factors.

[4] Shaded squares represent the probability level of each individual taxon to each other at each stream location (i.e., upstream isopod bioaccumulation factor compared to midstream isopod bioaccumulation factor).

insignificance may be attributed to the relative closeness to each other and proximity to the Y-12 Complex.

The average total mercury concentrations collected within the floodplain soils during the 2014 Phase 1of the LEFPC Mercury Biouptake Study fell between 0.042 ppm to 259 ppm from plots where taxa were collected and analyzed (DOE 2014b). Total mercury soil concentrations were highest at the upstream sites, closest to the source of contamination (the Y-12 Complex) and decreased with downstream distance. However, concentrations varied greatly within sampling areas resulting in a wide range of upstream mercury concentrations as well as a wide range of mercury concentrations throughout the LEFPC floodplain (DOE 2014b).

Total mercury concentrations in the soil in upstream plots ranged from 4.68 ppm to 295.38 ppm. In midstream plots, total mercury soil concentrations ranged from 38.33 ppm to 73.82 ppm. Total mercury concentrations in the soil ranged from 4.71 ppm to 59.15 ppm at the downstream plots. The reference plots at Brushy Fork had mercury concentrations that were low. Total mercury concentrations in the soil along LEFPC floodplain had great variation with both the lowest and highest values found upstream. The lowest total mercury concentration (4.68 ppm) in the soil was found upstream at plot B12. The highest concentration (259.39 ppm) of total mercury in the soil was also found upstream at plot C41. Overall, total mercury concentrations in the soil were 57.94 ppm (SD$\pm$55.53) (DOE 2014b). All total mercury concentrations in the soil of LEFPC floodplain, collected in 2014 as Phase 1 of the LEFPC Mercury Biouptake Study, are represented in Figure A5 in the Appendix.

By dividing the total mercury concentrations of the biota to the total soil concentrations, BAF averages for each taxon were derived. Average BAFs were 0.003 for leafhoppers, 0.076 for wolf spiders, 0.099 for isopods and 0.181 for earthworms. When examining the relationship

between total mercury concentrations in the biota and the total mercury concentrations in the soil using linear regression, it is interesting to note that R^2 values were 0.57 for leafhoppers, 0.72 for isopods, 0.31 for wolf spiders, and 0.32 for earthworms (Figure 28). A strong relationship between total mercury concentrations in the soil and total mercury concentrations in biota collected along LEFPC for isopods was observed. However, the relationship between total mercury concentrations in the biota compared to the total mercury concentrations in the soil segmented by stream location (i.e., upstream, midstream, and downstream) provided an R^2 value that indicates a stronger relationship at upstream sites (Table 7). For example, isopod total mercury concentration versus soil total mercury concentration R^2 value when not segmented by stream location is 0.72, indicating an overall strong relationship between isopod total mercury concentrations and soil total mercury concentrations. However, when isopod total mercury concentrations versus soil total mercury concentrations are segmented by stream location (upstream, midstream, and downstream) a strong relationship is primarily seen upstream where the R^2 is 0.9808 (Table 7). The R^2 value (0.0124) for isopod total mercury concentration versus soil total mercury concentration midstream does not indicate a strong relationship, and the downstream R^2 value for isopods does reflect a stronger relationship at 0.64546. Regression analysis for total mercury concentrations for each taxon segmented by stream location along LEFPC is visually represented in the Appendix (Figures A1-A4).

Summary

Results from this study indicated no significant difference of total mercury in the soil and total mercury in biota among upstream and midstream sites. However, concentrations of total

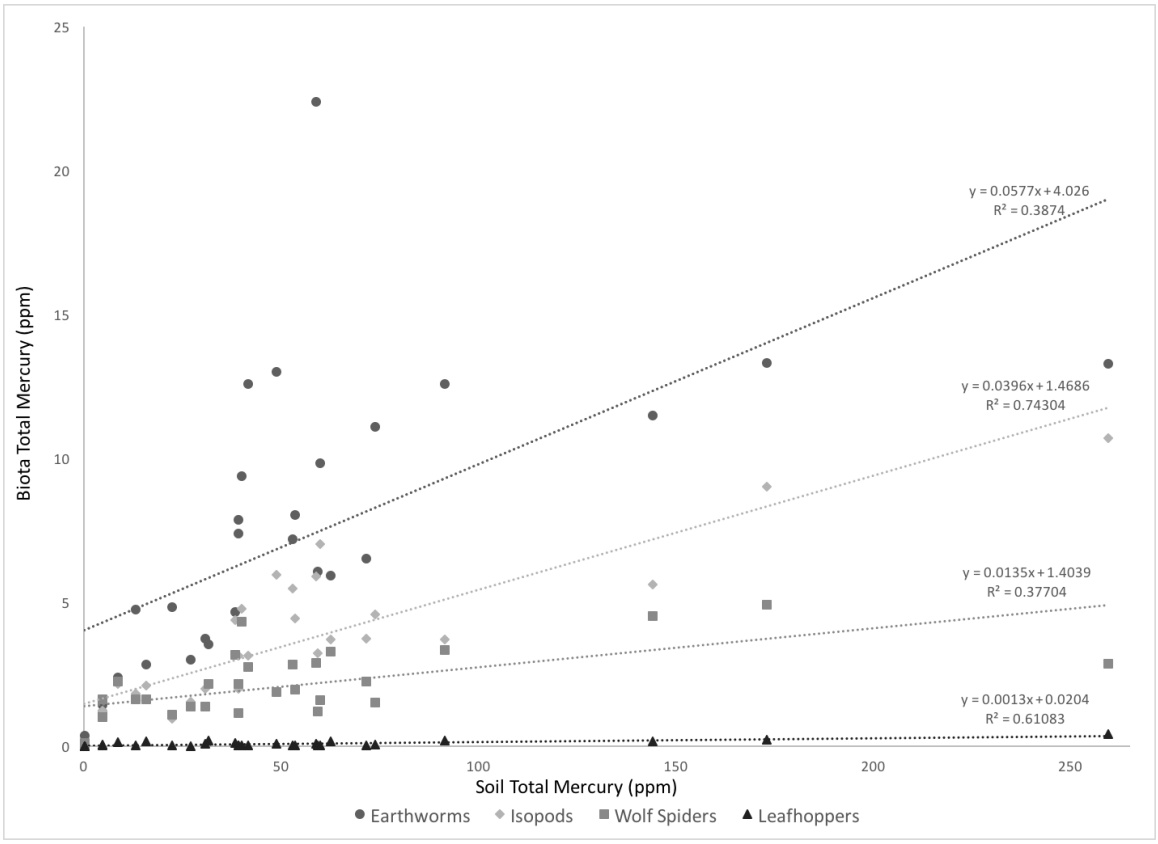


Figure 28: Biota total mercury concentrations for each taxon compared to soil total mercury concentrations. R^2 values reported for each taxon.

Table 7: R^2 values for total mercury concentrations for each taxon compared to total mercury concentration in the soils. Figures illustrating regression for each taxon is provided in the Appendix (Figures A1 – A4).

	Upstream	*Midstream*	*Downstream*
Earthworm	0.7067	0.0104	0.2254
Isopod	0.9808	0.0124	0.6454
Wolf Spider	0.2484	0.3648	0.0236
Leafhopper	0.7289	0.0131	0.0417

mercury in the soil and in the biota at downstream sites were significantly different from both upstream and midstream sites. Methylmercury concentrations in biota samples were significantly different among all stream locations (i.e., upstream, midstream, and downstream). No significant difference among BAFs and taxa at different stream locations (e.g., upstream isopods, midstream isopods, and downstream isopods) were observed. The relationship between concentrations of biota total mercury and soil total mercury also varied among stream location where in general a stronger relationship was seen upstream and downstream. Factors contributing to the variation in BAFs and stream location might include remediation actions that occurred at midstream sites, potentially less frequent flooding occurring midstream and downstream, and the close proximity of upstream collecting sites to the mercury origin, the Y-12 Complex.

Overall variation of mercury concentrations among sites along LEFPC could be due to the copious amount of rainfall in the area, with annual rainfall totaling 140 cm. This rainfall subsequently results in a secondary type of continuous mercury contamination, i.e., flooding. Some of the potential areas affected by frequent flooding include: surface waters, sediment along LEFPC, groundwater, and organisms living in the area (ATSDR 2012). Previous assessments have shown that due to the expanse of mercury in the area, future remediation actions may not provide enough benefit to outweigh the cost of remediation in areas where mercury concentrations are high in the soil (Pant et al. 2010a). The unforeseen increase in methylmercury availability to downstream food webs may be a key contributing factor to higher concentrations than expected in the LEFPC contaminated site. Future studies could examine the relationship between mercury concentrations and plot elevations along LEFPC to better understand the the impact that flooding may have on mercury concentrations in taxa and their location along stream.

The soil total mercury concentrations found during the 2014 Phase 1 of the LEFPC Mercury Biouptake Study revealed diverse mercury concentrations in the soil of the LEFPC floodplain (DOE 2014b). The current research reveals diverse mercury concentrations in the terrestrial invertebrates living along the LEFPC floodplain. Downstream soil and taxa tend to have lower concentrations of mercury than upstream and midstream sites, where remediation actions have taken place. However, when examining values (BAFs and methylmercury fractions), no significant differences among locations along LEFPC were observed. This finding indicates that the relationship between mercury concentrations in the soil and mercury concentrations in the biota living in the area is complex. Results also indicate that remediation actions may help lower environmental mercury concentrations but may not help reduce the overall concentrations of mercury in the organisms living in the terrestrial environment.

CHAPTER IV

CONCLUSIONS

The highest concentration (22.4 ppm at midstream) of total mercury in biota was found in the earthworms, while the lowest concentration (0.009 ppm downstream) of total mercury was found in leafhoppers. The highest concentration of total mercury for leafhoppers reached only 0.24 ppm. Even though earthworms had the highest concentrations of total mercury on average and overall individually, the highest individual concentration (3.38 pm) of bioavailable methylmercury was found in an isopod sample collected upstream. The lowest concentration of methylmercury, averaging 0.002 ppm, was found in leafhoppers. These results may indicate that isopods uptake more methylmercury than do earthworms. It may also indicate that isopods feed on organic matter that consists of higher concentrations of methylmercury. High concentrations of methylmercury in isopods and wolf spiders may pose a greater danger to upper-level predators that feed on them. Predators, such as the short-tailed shrew and the Carolina wren, may be less likely to uptake high concentrations of methylmercury from earthworms and leafhoppers.

Wolf spiders had the highest methylmercury fraction; one sample from an upstream site reached 96.4%. The lowest methylmercury fraction (0.07%) was found in an individual sample of earthworm from a midstream site. Earthworms had, on average, the lowest methylmercury fractions at 2.9%. The high methylmercury fractions in wolf spiders may indicate that this taxa uptakes methylmercury more than taxa at lower trophic levels. Higher methylmercury fractions in taxa may also indicate a greater threat to higher consumers that feed on them, where taxon

such as earthworms with lower methylmercury fractions that make up a larger percentage of some higher level consumers may not pose as great of a threat.

Mercury concentrations among taxa revealed interesting results, indicating that assumptions cannot be made about how taxa of certain trophic positions will uptake a contaminant, such as mercury. Methylmercury concentrations were observed to increase with trophic level, but total mercury concentrations where not observed to increase with trophic level. For example, earthworms had some of the highest total mercury concentrations but had some of the lowest $\delta^{15}N$ values. These results also indicate that, when evaluating contaminants in the environment, a wide range of sampling among ecosystems of interest is necessary to obtain a clear understanding of the impact that the contaminant of concern poses (e.g., multiple taxa from various trophic levels, multiple collecting locations, etc.), especially in contaminated sites that extend to large areas, such as Lower East Fork Poplar Creek (LEFPC). The need for collecting multiple taxa from various trophic levels is reflected by the detritivores in this current study. Earthworms had high concentrations of total mercury but low concentrations of methylmercury (average methylmercury fraction for earthworms equaling 2.89%), potentially indicating that detritivores do not pose as great of a threat to upper level consumers of which earthworms are a part of their diet. However, another detritivore taxa collected and analyzed in this study, isopods, revealed higher methylmercury concentrations (average methylmercury fractions for isopods equaling 34.25%) indicating the potential of detritivores to be an elevated source of methylmercury to higher organisms that consume them.

A 2011 assessment of mercury in LEFPC biota revealed high concentrations of methylmercury in spiders of the genus *Dolomedes* (Mathews et al. 2011). The elevated concentrations of methylmercury relative to total mercury caused concern about previous

assumptions about mercury transfer to the terrestrial environment and specifically the threat that mercury poses to the upper-level predators that live along LEFPC. The use of bioaccumulation factors (BAFs) derived from earlier data was also doubted during the 2011 ecological assessment. Because of these concerns, BAFs and methylmercury fractions were recalculated in 2014 as part of Phase 1 of the LEFPC Mercury Biouptake Study for terrestrial invertebrates living in the LEFPC floodplain (Table 8). These recalculated values reflected predicted values for threat that certain taxon may pose on LEFPC and the higher-level consumers that feed on them.

Predicted values, reported in Table 8, were calculated using values derived from recent studies of EFPC (Han et al. 2012, Pant et al. 2010b) and the South River, VA, another water source that had been contaminated with mercury and subsequently the surrounding environment (Newman et al. 2011, Cristol et al. 2008). Specifically, earthworm BAFs were reported from Han et al. (2012). Other BAFs were derived by using plant – soil BAFs from East Fork Poplar Creek (EFPC) reported by Pant et al. (2010b) and soil-biota values from South River, VA by Cristol et al. (2008) and Newman et al. (2011). Using these two sources (EFPC and South River) for BAF data, a hybrid BAF for each taxa was derived to create predicted BAF values for taxa along LEFPC. Predicted methylmercury fraction values were reported from a previous study on the South River, VA (Newman et al. 2011).

In this current study, total mercury concentrations in the four taxa (earthworms, isopods, leafhoppers and wolf spiders) along LEFPC were not directly associated to any of the soil total mercury concentrations from the 2014 Phase 1 of the LEFPC Mercury Biouptake Study. BAFs were lower than the predicted values from the 2014 Phase 1 of the LEFPC Mercury Biouptake Study, indicating that terrestrial invertebrates may not pose a threat to upper-level predators in

Table 8: Predicted Bioaccumulation Factors (BAFs) and percent methylmercury to total mercury for all taxa collected and analyzed. Predicted values were derived from previous studies on East Fork Poplar Creek and values from another contaminated site, South River, located in Virginia.

Biota	Predicted[a] Total Mercury BAFs[abcd]	Actual Total Mercury BAFs	Predicted[b] Methylmercury Fraction	Actual Methylmercury Fraction
Earthworms	1.000	0.180	4.0 %	3.2%
Spiders	1.350	0.58	45.0 %	58.1%
Detritivores	1.520	0.099	16.0 %	39.0%
Herbivores	0.017	0.003	30.0 %	28.0%

[a] Pant, P., M. Allen, and B. Tansel. 2010b. Mercury Uptake and Translocation in *Impatiens walleriana* Plants Grown in the Contaminated Soil from Oak Ridge. International Journal of Phytoremediation 13:168-176.

[b] Cristol, D. A., R. L. Brasso, A. M. Condon, R. E. Fovargue, S. L. Friedman, K. K. Hallinger, A. P. Monroe, and A. E. White. 2008. Movement of Aquatic Mercury Through Terrestrial Food Webs. Science 320:335.

[c] Han, F. X., Y. Su, Y. Xia, W. Tian, V. Philips, Z. Shi, D. L. Monts, M. Gu, and Y. Liang. 2012. Mercury Distribution and Speciation in Floodplain Soils and Uptake into Native Earthworms (*Diplocardia* spp.). Geoderma 170:261-268.

[d] Newman, M. C., X. Xu, A. Condon, and L. Liang. 2011. Floodplain Methylmercury Biomagnification Factor Higher than that of the Contiguous River (South River, Virginia USA). Environmental Pollution 159:2840-2844.

the LEFPC environment (DOE 2014b). However, a lower BAF value does not always indicate a lower threat as a higher BAF does not always indicate a higher threat. With BAFs varying among locations along LEFPC in regards to taxa and BAFs not being necessarily comparable among different contaminated sites (i.e., LEFPC compared to the South River in Virginia), BAFs might not be the best indicator of the threat that mercury poses to a contaminated area.

In contrast methylmercury fractions found in this study were close to the predicted values based on values from studies conducted at the South River in Virginia (DOE 2014b). Isopods and wolf spiders in the current study did have slightly higher than predicted methylmercury fractions, but were relatively close. This close prediction of methylmercury fractions may indicate that methylmercury fractions are similar among contaminated sites, in that taxa may uptake methylmercury at consistent rates among contaminated sites. This fact may be consistent even in sites with different amounts of mercury contamination and background soil mercury levels. The usefulness of methylmercury fractions found in this study is in contrast to BAFs which are directly impacted by the environmental mercury concentration (e.g., soil total mercury concentration) where extremely high soil concentrations will result in low BAFs but do not directly indicate a lower threat to the environment. In the future methylmercury fractions may be used as an indicator of the threat a taxon may pose to organisms that consume them in areas that have been contaminated. Methylmercury fractions may provide use in their potential ability to compare taxa directly to similar taxa in other contaminated areas.

BAFs were calculated in this study by dividing 2015 biota total mercury concentration to soil total mercury concentrations reported in a 2014 Phase 1 of the LEFPC Mercury Biouptake Study which also reported predicted BAFs for some terrestrial invertebrates along the LEFPC floodplain (DOE 2014b). BAFs were relatively low in all taxa. Leafhoppers had the lowest

BAF, with a sample from midstream at 0.0005. Earthworms had the highest BAF, with a sample from midstream at 0.3807. Generally, BAFs were lower than the predicted values reported in the 2014 Phase 1 of the LEFPC Mercury Biouptake study and indicate that the terrestrial invertebrate taxa collected possibly will not pose a substantial threat to upper-level predators in this area. However, low BAFs along LEFPC may be due to higher soil total mercury concentrations meaning that these low BAFs may indicate a potential for greater danger to higher consumers of the taxa collected. Future studies should include evaluation of methylmercury concentrations in the soil and comparing these data to the methylmercury concentrations found in the biota of that area since methylmercury is the more bioavailable form of mercury and of the greatest concern in areas that have been contaminated.

A previous study conducted on LEFPC to assess native earthworm populations agree with the findings in this study. Han et al. (2012) found concentrations of total mercury in earthworms similar to those in this study. These results suggest that the threat earthworms pose to upper-level predators is low due to the relative insignificant amount of methylmercury and the ratio of methylmercury to total mercury in this taxa. Findings from previous and current studies also show that earthworms could potentially be a good bioindicator of mercury availability at terrestrial sites. However, an analysis of total mercury may not be sufficient to reflect the actual threat a specific taxon poses to the environment. If total mercury concentrations had been the only variable evaluated for taxa in this study, earthworms would have been the greatest concern with one earthworm sample reaching 22 ppm for total mercury. But because both total mercury and methylmercury were evaluated for all taxa, earthworms were not the greatest concern due to their relatively low methylmercury concentrations. This finding reaffirms the importance of conducting analyses of both total mercury and methylmercury in order to obtain a better

understanding of mercury contamination in an area as well as the uptake of mercury by the biota it impact.

Researchers are able to predict that up to 100% of the mercury in upper-level fish species from aquatic systems that have been contaminated by mercury is in the form of methylmercury (Landrum et al. 1993). The lipophilicity of methylmercury can cause it to be absorbed more easily than inorganic mercury. The ease of methylmercury uptake, compared to inorganic mercury, allows some organisms to uptake methylmercury more readily than others. Evidence of this difference in absorption of methylmercury may be seen in the results of the current study; though earthworms had higher concentrations of total mercury, they did not have the highest concentrations of methylmercury. The low concentrations of methylmercury in earthworms indicate that they may not uptake lipophilic methylmercury or that they are consuming organic matter that is lower in methylmercury and higher in total mercury. In contrast, the higher concentrations of methylmercury in isopods indicate that isopods may absorb and uptake more methylmercury, despite the concentration of total mercury in isopods being significantly smaller than that of the earthworms collected and analyzed. These results might also indicate that the concentrations of mercury in the diet that each taxon consumes might have a role in the overall concentrations seen in each taxon.

Studies that have examined a wide range of geographical locations show that a large number of humans, as well as wildlife and mammals, are exposed to concerning amounts of methylmercury through the consumption of contaminated fish (UNEP 2002). Similarly, upper level predators could be exposed to methylmercury by consuming contaminated terrestrial organisms. However, this threat by consumption in the terrestrial environment is not well understood. Do contaminated terrestrial organisms pose a threat to animals that consume them?

Do they pose the same level of concern as to humans and other animals from contaminated fish? This current study does reveal the complexity of the movement of mercury among ecosystems and provides a reference of mercury accumulation in terrestrial taxa.

After examining long-jawed orb weaver spiders, Speir et al. (2014) found low concentrations of methylmercury in the terrestrial food chain and concluded that the terrestrial food web did not seem to be a source of methylmercury to the long-jawed orb weaver spiders. Based on the results of the study by Speir et al. (2014), the same conclusions may be observed from the results from the contaminated area, LEFPC, in that though isopods have higher concentrations of methylmercury than previously predicted, detritivores are not actively feeding from the terrestrial food web. Therefore, the terrestrial food web is not the direct source of methylmercury in this taxa.

Although the aquatic organisms (e.g., emerging aquatic insects) may be a source of mercury to some terrestrial invertebrates (e.g., spiders), terrestrial invertebrates that do not directly consume aquatic life still have noteworthy concentrations of mercury. This contamination could be due to 1) frequent flooding from the contaminated LEFPC or 2) the consumption and absorption of mercury from dead organic matter that contains methylmercury from the aquatic environment and/or the contaminated soils. Another potential source of mercury in the surrounding environment of the Y-12 Complex is through an air stack where an estimated additional 3,700 kg of mercury was released into the air (Brooks and Southworth 2011). Mercury contamination to terrestrial invertebrates in this study was focused on the soil, followed by water. However, it is important to consider secondary impacts, such as atmospheric deposition, as well as long-term impacts with anthropogenic emissions. Though the amount of mercury released into the atmosphere from the Y-12 Complex is small compared to the amount

released in the soil, it is not an amount that can be disregarded. Results from this study can be used for future contamination assessment at these sites as well as potentially for other similarly contaminated sites.

This study contributed to knowledge on mercury transfer and its movement in taxa and between trophic levels. Results from this study will ultimately inform decisions concerning site contamination management by the Department of Energy (DOE), Environmental Protection Agency (EPA), and Tennessee Department of Environment and Conservation (TDEC) in regards to the impact of mercury on the Oak Ridge Reservation (ORR) and the city of Oak Ridge. This study provides important information to answer future questions, such as how far does mercury contamination go in the LEFPC floodplain and what is the potential for mercury in the floodplain soil to be transferred to the terrestrial food web that inhabits the area. Preventative steps should be taken for future reduction of mercury contamination into the environment and other various ecosystems that are currently impacted by mercury. The current amount of mercury present in LEFPC, its surrounding floodplain, sediment, soil, and fish is, and will continue to be, of great environmental concern until concentrations are significantly reduced; however, how the reduction of mercury can be accomplished warrants further investigation. This study indicates that current concentrations of mercury may not pose immediate danger to terrestrial invertebrates living in the riparian zone, floodplain soils, or feeding on plants along LEFPC nor the upper-level predators that feed on these terrestrial invertebrates. Mercury is a persistent toxin in the environment, and should therefore not be ignored; monitoring should continue because this concern will not disappear on its own.

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APPENDIX

Table A1: Stream locations, IDs, and coordinates for collecting plots along Lower East Fork Poplar Creek, located in Oak Ridge, TN.

Plot Location	*Plot ID*	*Coordinates*
Upstream	B-12	36.0020996, - 84.2501872
Upstream	B-18	36.0020758, - 84.2500332
Upstream	B-20	36.0021771, - 84.2498498
Upstream	C-09	36.0027178, - 84.2537972
Upstream	C-12	36.0028698, - 84.2535221
Upstream	C-41	36.0026224, - 84.2531811
Upstream	D-06	36.0031147, - 84.2546069
Upstream	D-21	36.0028912, - 84.2544199
Upstream	D-26	36.0028166, - 84.2543575
Midstream	A-10	36.0045312, - 84.2830912
Midstream	A-16	36.0044567, - 84.2830289
Midstream	A-29	36.0043584, - 84.2828124
Midstream	B-17	36.0041628, - 84.2844910
Midstream	B-20	36.0043148, - 84.2842160
Midstream	B-26	36.0042403, - 84.2841536
Midstream	E-09	36.0031363, - 84.2861493
Midstream	E-18	36.0031125, - 84.2859953
Midstream	E-19	36.0031569, - 84.2859186
Downstream	A-04	35.9707170, - 84.3501919
Downstream	A-12	35.9705681, - 84.3500671

Table A1 Continued

Plot Location	*Plot ID*	*Coordinates*
Downstream	A-16	35.9704936, - 84.3500047
Downstream	C-09	35.9572113, - 84.3682251
Downstream	C-35	35.9570894, - 84.3678547
Downstream	C-40	35.9569135, - 84.3679754
Downstream	D-22	35.9566461, - 84.3695982
Downstream	D-33	35.9567746, - 84.3691695
Downstream	D-44	35.9566257, - 84.3690447
Reference	BF-43	36.0543216, - 84.2337422
Reference	BF-45	36.0542413, - 84.2341708
Reference	BF-64	36.0539700, - 84.2342913

Table A2: Total mercury (THg) for both biota and soil samples, methylmercury (MeHg) and calculations for percent of methylmercury that contributes to the total mercury values, and bioaccumulation factors (BAFs) of specimens collected along Lower East Fork Poplar Creek, in Oak Ridge, TN.

	Plot	*Soil THg*[a]	*Biota*	*THg*	*MeHg*	*MeHg/THg%*	*BAFs*
Upstream	B12	4.68	Earthworm	1.18	0.0811	6.9	0.2521
Upstream	B18	8.454	Earthworm	2.38	0.0761	3.2	0.2815
Upstream	B20	15.62	Earthworm	2.85	0.101	3.5	0.1825
Upstream	C09	91.302	Earthworm	12.6	0.119	0.9	0.1380
Upstream	C12	39.94	Earthworm	9.38	0.0804	0.9	0.2349
Upstream	C41	259.386	Earthworm	13.3	0.307	2.3	0.0513
Upstream	D06	62.452	Earthworm	5.94	0.256	4.3	0.0951
Upstream	D21	143.948	Earthworm	11.5	0.363	3.2	0.0799
Upstream	D26	172.836	Earthworm	13.32	0.41	3.1	0.0771
Midstream	A10	38.332	Earthworm	4.66	0.621	13.3	0.1216
Midstream	A16	53.53	Earthworm	8.05	0.137	1.7	0.1504
Midstream	A29	59.746	Earthworm	9.84	0.148	1.5	0.1647
Midstream	B17	48.706	Earthworm	13	0.21	1.6	0.2669
Midstream	B20	58.844	Earthworm	22.4	0.237	1.1	0.3807
Midstream	B26	52.802	Earthworm	7.2	0.447	6.2	0.1364
Midstream	E09	41.47	Earthworm	12.6	0.0921	0.7	0.3038
Midstream	E18	71.552	Earthworm	6.53	0.157	2.4	0.0913
Midstream	E19	73.82	Earthworm	11.1	0.15	1.4	0.1504

Table A2 Continued

	Plot	*Soil Hg*	*Biota*	*THg*	*MeHg*	*MeHg/THg%*	*BAFs*
Downstream	A04	39.156	Earthworm	7.39	0.103	1.4	0.1887
Downstream	A12	39.112	Earthworm	7.86	0.145	1.8	0.2010
Downstream	A16	59.152	Earthworm	6.08	0.146	2.4	0.1028
Downstream	C09	22.376	Earthworm	4.83	0.168	3.5	0.2159
Downstream	C35	26.96	Earthworm	3.02	0.0573	1.9	0.1120
Downstream	C40	31.566	Earthworm	3.55	0.0959	2.7	0.1125
Downstream	D22	13.032	Earthworm	4.76	0.058	1.2	0.3653
Downstream	D33	4.712	Earthworm	1.43	0.0422	3.0	0.3035
Downstream	D44	30.762	Earthworm	3.75	0.0733	2.0	0.1219
Upstream	B12	4.68	Isopods	1.25	1.06	84.8	0.2671
Upstream	B18	8.454	Isopods	2.16	1.9	88.0	0.2555
Upstream	B20	15.62	Isopods	2.11	1.39	65.9	0.1351
Upstream	C09	91.302	Isopods	3.72	1.29	34.7	0.0407
Upstream	C12	39.94	Isopods	4.79	1.62	33.8	0.1199
Upstream	C41	259.386	Isopods	10.7	3.88	36.3	0.0413
Upstream	D06	62.452	Isopods	3.7	3.02	81.6	0.0592
Upstream	D21	143.948	Isopods	5.61	1.54	27.5	0.0390
Upstream	D26	172.836	Isopods	9.01	2.43	27.0	0.0521
Midstream	A10	38.332	Isopods	4.39	1.36	31.0	0.1145
Midstream	A16	53.53	Isopods	4.43	1.01	22.8	0.0828

Table A2 Continued

	Plot	*Soil Hg*	*Biota*	*THg*	*MeHg*	*MeHg/THg%*	*BAFs*
Midstream	A29	59.746	Isopods	7.03	1.17	16.6	0.1177
Midstream	B17	48.706	Isopods	5.97	1.16	19.4	0.1226
Midstream	B20	58.844	Isopods	5.91	1.27	21.5	0.1004
Midstream	B26	52.802	Isopods	5.49	1.13	20.6	0.1040
Midstream	E09	41.47	Isopods	3.16	0.493	15.6	0.0762
Midstream	E18	71.552	Isopods	3.75	0.757	20.2	0.0524
Midstream	E19	73.82	Isopods	4.57	0.694	15.2	0.0619
Downstream	A04	39.156	Isopods	2.01	0.548	27.3	0.0513
Downstream	A12	39.112	Isopods	3.14	0.586	18.7	0.0803
Downstream	A16	59.152	Isopods	3.24	0.623	19.2	0.0548
Downstream	C09	22.376	Isopods	0.951	0.455	47.8	0.0425
Downstream	C35	26.96	Isopods	1.54	0.491	31.9	0.0571
Downstream	C40	31.566	Isopods	2.13	0.366	17.2	0.0675
Downstream	D22	13.032	Isopods	1.82	0.477	26.2	0.1397
Downstream	D33	4.712	Isopods	1.22	0.516	42.3	0.2589
Downstream	D44	30.762	Isopods	2.01	0.656	32.6	0.0653
Upstream	B12	4.68	Spiders	1.64	1.36	82.9	0.3504
Upstream	B18	8.454	Spiders	2.25	2.17	96.4	0.2661
Upstream	B20	15.62	Spiders	1.64	1.36	82.9	0.1050
Upstream	C09	91.302	Spiders	3.35	1.21	36.1	0.0367
Upstream	C12	39.94	Spiders	4.34	0.804	18.5	0.1087
Upstream	C41	259.386	Spiders	2.87	2.57	89.5	0.0111
Upstream	D06	62.452	Spiders	3.28	2.19	66.8	0.0525
Upstream	D21	143.948	Spiders	4.53	2.61	57.6	0.0315

Table A2 Continued

Plot	*Soil Hg*	*Biota*	*THg*	*MeHg*	*MeHg/THg%*	*BAFS*	*Plot*
Upstream	D26	172.836	Spiders	4.92	3.29	66.9	0.0285
Midstream	A10	38.332	Spiders	3.18	1.81	56.9	0.0830
Midstream	A16	53.53	Spiders	1.97	1.21	61.4	0.0368
Midstream	A29	59.746	Spiders	1.61	0.974	60.5	0.0269
Midstream	B17	48.706	Spiders	1.9	0.789	41.5	0.0390
Midstream	B20	58.844	Spiders	2.91	0.548	18.8	0.0495
Midstream	B26	52.802	Spiders	2.83	1.67	59.0	0.0536
Midstream	E09	41.47	Spiders	2.77	1.58	57.0	0.0668
Midstream	E18	71.552	Spiders	2.24	1.34	59.8	0.0313
Midstream	E19	73.82	Spiders	1.53	0.779	50.9	0.0207
Downstream	A04	39.156	Spiders	2.17	0.743	34.2	0.0554
Downstream	A12	39.112	Spiders	1.16	0.652	56.2	0.0297
Downstream	A16	59.152	Spiders	1.21	0.751	62.1	0.0205
Downstream	C09	22.376	Spiders	1.11	0.67	60.4	0.0496
Downstream	C35	26.96	Spiders	1.38	1.05	76.1	0.0512
Downstream	C40	31.566	Spiders	2.18	0.653	30.0	0.0691
Downstream	D22	13.032	Spiders	1.64	0.731	44.6	0.1258
Downstream	D33	4.712	Spiders	1.02	0.657	64.4	0.2165
Downstream	D44	30.762	Spiders	1.37	0.576	42.0	0.0445
Upstream	B12	4.68	Leafhopper	0.0521	0.0083	15.9	0.0111
Upstream	B18	8.454	Leafhopper	0.158	0.0367	23.2	0.0187
Upstream	B20	15.62	Leafhopper	0.18	0.108	60.0	0.0115
Upstream	C09	91.302	Leafhopper	0.192	0.0195	10.2	0.0021
Upstream	C12	39.94	Leafhopper	0.0449	0.0074	16.5	0.0011
Upstream	C41	259.386	Leafhopper	0.434	0.0074	1.7	0.0017

Table A2 Continued

Plot	*Soil Hg*	*Biota*	*THg*	*MeHg*	*MeHg/THg%*	*BAFS*	*Plot*
Upstream	D06	62.452	Leafhopper	0.18	0.0207	11.5	0.0029
Upstream	D21	143.948	Leafhopper	0.187	0.0099	5.3	0.0013
Upstream	D26	172.836	Leafhopper	0.238	0.0526	22.1	0.0014
Midstream	A10	38.332	Leafhopper	0.109	0.0136	12.5	0.0028
Midstream	A16	53.53	Leafhopper	0.0366	0.0082	22.4	0.0007
Midstream	A29	59.746	Leafhopper	0.0289	0.0082	28.4	0.0005
Midstream	B17	48.706	Leafhopper	0.0887	0.0247	27.8	0.0018
Midstream	B20	58.844	Leafhopper	0.0879	0.045	51.2	0.0015
Midstream	B26	52.802	Leafhopper	0.0466	0.0165	35.4	0.0009
Midstream	E09	41.47	Leafhopper	0.0213	0.0033	15.5	0.0005
Midstream	E18	71.552	Leafhopper	0.0367	0.01	27.2	0.0005
Midstream	E19	73.82	Leafhopper	0.0712	0.0061	8.6	0.0010
Downstream	A04	39.156	Leafhopper	0.0348	0.0054	15.5	0.0009
Downstream	A12	39.112	Leafhopper	0.0263	0.0061	23.2	0.0007
Downstream	A16	59.152	Leafhopper	0.0449	0.0037	8.2	0.0008
Downstream	C09	22.376	Leafhopper	0.036	0.0042	11.7	0.0016
Downstream	C35	26.96	Leafhopper	0.00913	0.002	21.9	0.0003
Downstream	C40	31.566	Leafhopper	0.191	0.0027	1.4	0.0061
Downstream	D22	13.032	Leafhopper	0.0209	0.0062	29.7	0.0016
Downstream	D33	4.712	Leafhopper	0.0371	0.0099	26.7	0.0079
Downstream	D44	30.762	Leafhopper	0.0897	0.0195	21.7	0.0029

[d] DOE. 2014b. Sampling and Analysis Plan for the Lower East Fork Poplar Creek Mercury Biouptake Study. Department of Energy, Oak Ridge, TN.

Table A3: Distances from each plot along Lower East Fork Poplar Creek (LEFPC) from the mercury origin source (Y-12 Complex) and from the contaminated creek (LEFPC).

Plot	*Distance from Y-12 (Km)*	*Distance From LEFPC (m)*
US-1	4	38
US-2	4	29
US-3	4	34
US-4	4.5	25
US-5	4.5	50
US-6	4.5	20
US-7	4.5	31
US-8	4.5	26
US-9	4.5	25
MS-1	8	53
MS-2	8	43
MS-3	8	22
MS-4	8	90
MS-5	8	103
MS-6	8	95
MS-7	8	21
MS-8	8	16
MS-9	8	17
DS-1	19	9
DS-2	19	23

Table A3 Continued

Plot	*Distance from Y-12 (Km)*	*Distance from LEFPC (m)*
DS-3	19	27
DS-4	23	58
DS-5	23	24
DS-6	23	19
DS-7	23	53
DS-8	23	61
DS-9	23	43

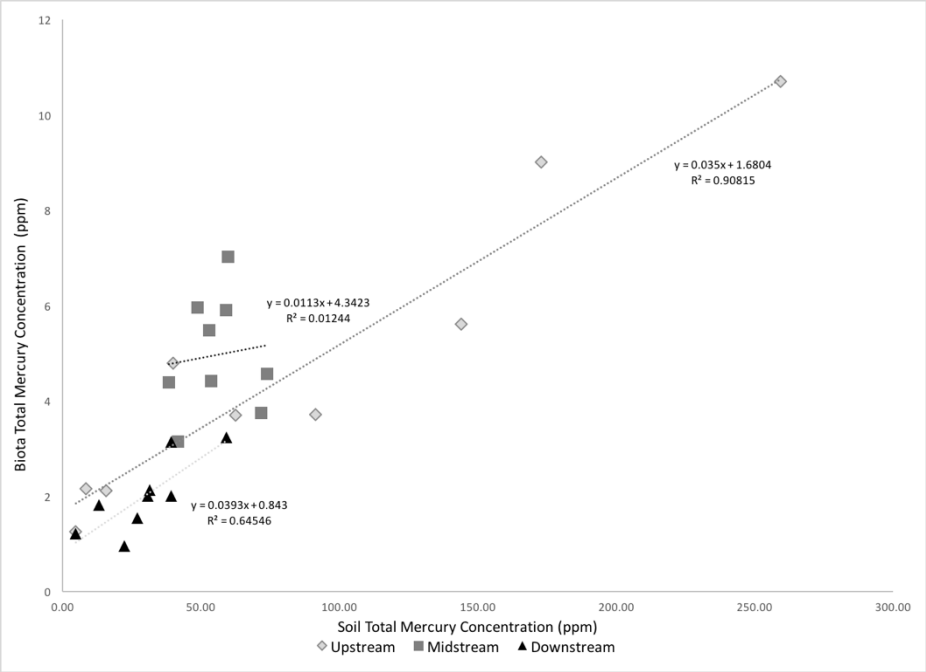

Figure A1: Isopod total mercury concentrations plotted against soil total mercury concentrations. Upstream, midstream, and downstream values are segmented with R^2 values reported, respectively.

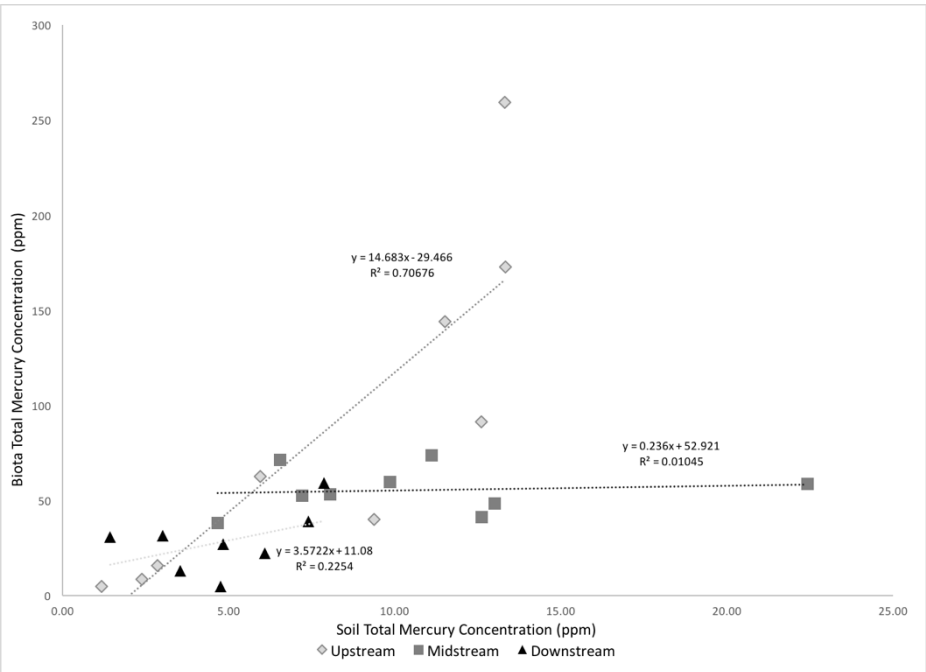

Figure A2: Earthworm total mercury concentrations plotted against soil total mercury concentrations. Upstream, midstream, and downstream values are segmented with R^2 values reported, respectively.

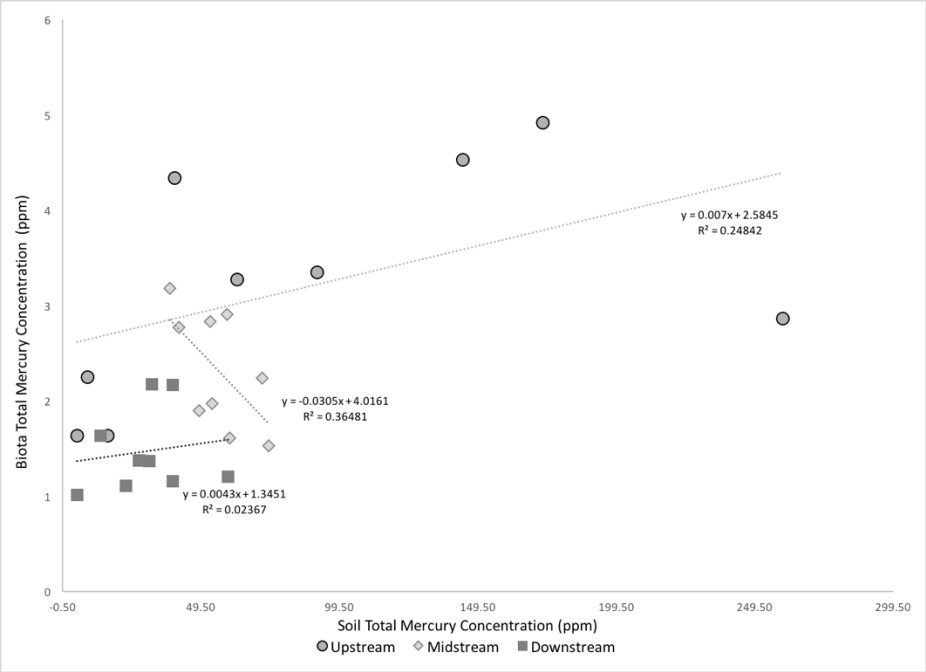

Figure A3: Wolf spider total mercury concentrations plotted against soil total mercury concentrations. Upstream, midstream, and downstream values are segmented with R^2 values reported, respectively.

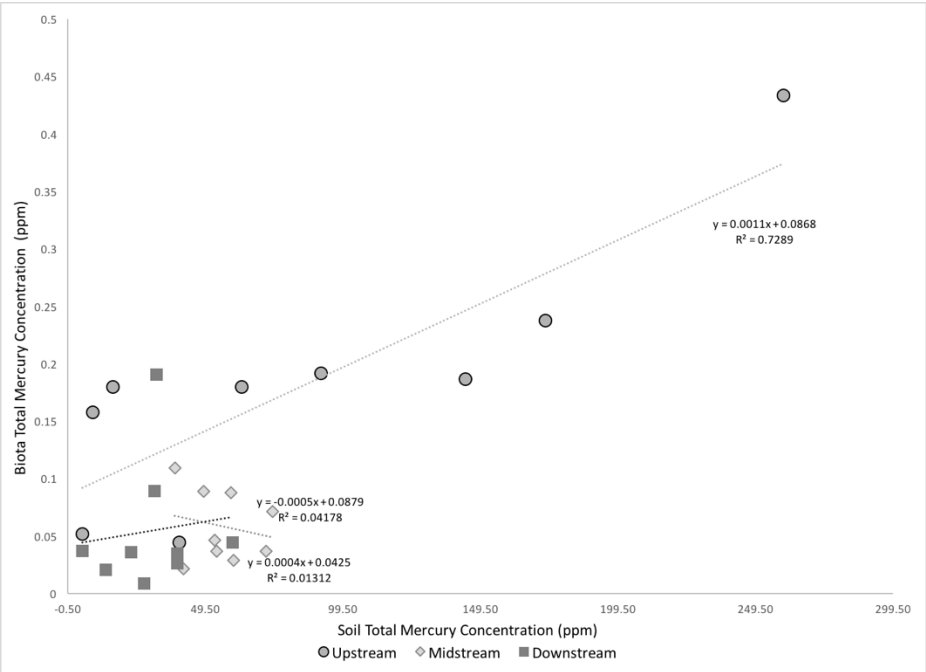

Figure A4: Leafhopper total mercury concentrations plotted against soil total mercury concentrations. Upstream, midstream, and downstream values are segmented with R^2 values reported, respectively.

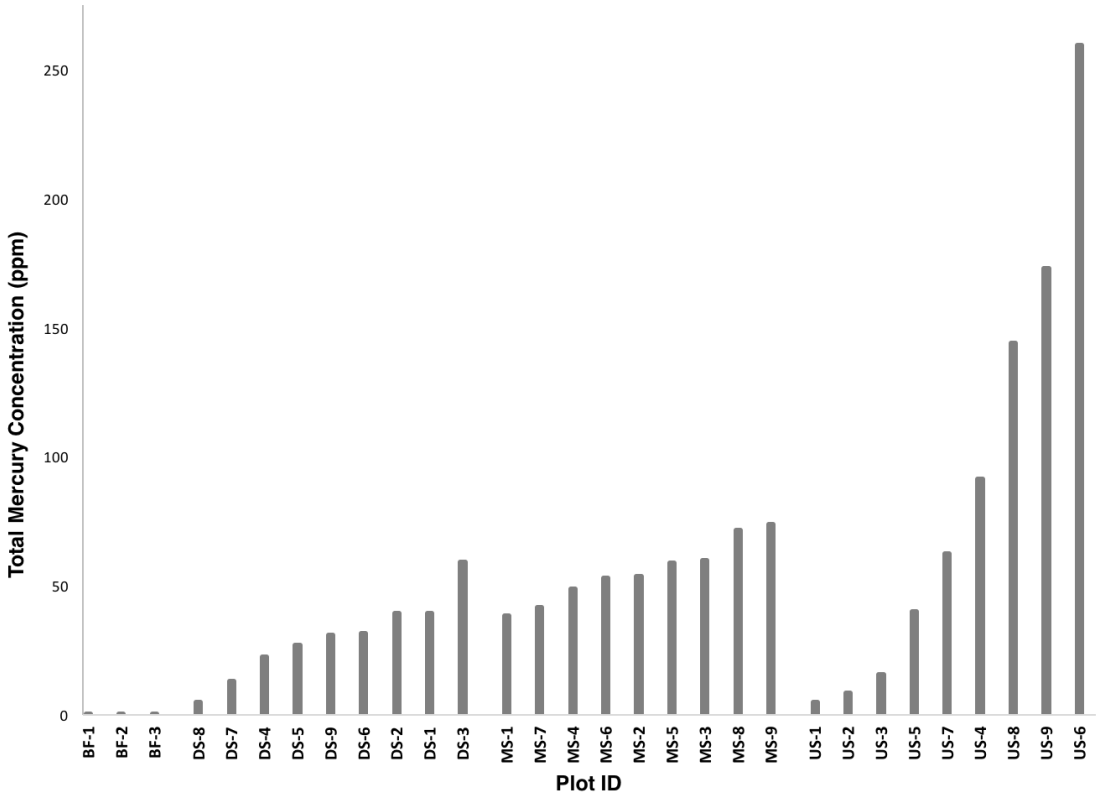

Figure A5: Soil total mercury concentrations for all 30 plots along Lower East Fork Poplar Creek where taxa were collected and analyzed. Plotted in order from left to right: reference site (BF), downstream (DS), midstream (MD), and upstream (US).

VITA

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