MERCURY BIOMAGNIFICATION IN AQUATIC FOOD WEBS OF GREAT SMOKY MOUNTAINS NATIONAL PARK

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ABSTRACT

Mercury is a widespread pollutant threatening human, fish, and ecosystem health on a global scale. Biomagnification concentrates mercury in upper trophic level organisms including predatory fishes, a primary route of dietary mercury exposure for humans. However, mercury biomagnification is not well understood in stream ecosystems, especially in places with no known point sources of contamination. A 2016 study revealed that Smallmouth Bass *Micropterus dolomeiu* mercury concentrations varied between three streams in Great Smoky Mountains National Park (GSMNP), Tennessee USA. However, the reason for this spatial variation in mercury concentrations is not understood. Our objectives were to (1) measure environmental and organismal concentrations of mercury, (2) describe and compare trophic pathways, (3) and evaluate evidence for mechanisms leading to differential mercury contamination, in three streams of GSMNP; and (4) to compare Smallmouth Bass mercury concentrations in 2016 and 2022. We analyzed stable isotope ratios and mercury concentrations of eight food web components of GSMNP streams: leaf detritus, periphyton, and filamentous algae to represent basal resources; mayfly nymphs (Heptageniidae), Central Stoneroller *Campostoma anomalum*, dragonfly nymphs (Odonata), and crayfish (Cambaridae) to represent intermediate consumers; and Smallmouth Bass, an apex predator. Stable isotope analyses revealed similar food web structure across all three streams. Total mercury (THg) concentrations were lowest in basal resources and highest in Smallmouth Bass and crayfish. Compared to a previous study in 2016, mean Smallmouth Bass THg concentrations were lower in 2022 in all three streams, however, many individuals from Abrams Creek still exceeded the EPA threshold for safe consumption of 0.3 mg/kg, ww. In both years, Smallmouth Bass mercury concentrations were highest in Abrams Creek and lower in Little River and Little Pigeon. Mean THg concentrations of basal resources did not differ between streams, but the spatial pattern of concentrations of intermediate consumers mirrored Smallmouth Bass. The slope of the relationship between log-transformed contaminant concentrations and trophic level was positive for all three streams, indicating biomagnification is occurring in GSMNP. Our findings indicate that differences in mercury contamination of top predators in these systems are driven by differences in food web dynamics rather than differences in basal resource THg concentrations.

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CHAPTER 1: INTRODUCTION

Mercury is a globally widespread pollutant that deleteriously affects human and ecosystem health. Methylmercury (MeHg), the organic form of mercury that is found in the environment, is a potent neurotoxin with devastating health effects (USEPA, 2001). Though MeHg exposure can affect people of all ages, exposure in utero is especially concerning as it causes permanent nervous system damage, blindness, ataxia, and other neurological harm to the developing fetus (ATSDR, 1999; Clarkson, 1997). In wildlife, effects of MeHg exposure span from individuals to ecosystems. MeHg biomagnification leads to dangerous mercury concentrations in top aquatic food web predators, causing adverse neurological, embryonic, teratogenic, and even demographic effects in fish and their mammalian and avian predators (Sorenson et al., 1991; Ward et al, 2010; Scheuhammer et al., 2007). Mercury dynamics in ecosystems may be modulated by abiotic factors such as water temperature, deposition (inputs), and pH (Greenfield, 2001), as well as microbial factors that influence the bioavailability of Hg (Hsu-Kim et al, 2013). Feeding relationships may also drive differences in biomagnification between systems (Lavoie et al, 2013). Despite the ubiquity of mercury in aquatic ecosystems and the potential danger posed to fish, wildlife, and people, there is limited understanding of the drivers of differential mercury contamination in the biota of pristine aquatic ecosystems at multiple trophic levels.

Mercury found in the environment is primarily inorganic and comes from a combination of current natural and anthropogenic sources, as well as re-emissions of historical natural and anthropogenic mercury inputs (UN Environment, 2019). Natural

sources of mercury include emissions from volcanic activity and the weathering of rocks; however, natural sources only account for 10% of present-day mercury emissions (UN Environment, 2019). Anthropogenic sources of mercury include a variety of sources and represent 30% of present-day emissions (UN Environment, 2019). More than half of present-day anthropogenic emissions come from fossil fuel combustion and artisanal gold mining, with other significant sources including cement and steel production and the incineration of consumer goods (electronics, light bulbs, batteries) and medical wastes that contain mercury (UN Environment, 2019, Pacyna et al., 2006). The remaining 60% of present-day emissions can be traced to environmental processes that recycle previously emitted mercury, including historical anthropogenic inputs or "legacy mercury" (UN Environment, 2019). Inorganic mercury is highly volatile and capable of global transport because of its long residence time in the atmosphere (Driscoll et al., 2013). Long-distance transport of inorganic mercury results in mercury exposure to ecosystems that are otherwise free of point-sources of mercury and many other anthropogenic contaminants (Driscoll et al. 2013, Selin, 2009, Valente et al. 2009, Fitzgerald et al., 1998).

Mercury in the environment is subjected to biological processes that change it from inorganic forms into its organic form, MeHg. This process, known as methylation, is driven by microbial communities and influenced by water quality variables such as pH, DOC, and nutrient input, as well as landscape-level variables such as the percentage of wetlands in the watershed (Ullrich et al., 2001, Tjerngren et al., 2012). Compared to inorganic mercury, MeHg is more toxic, highly bioavailable, and can reach high concentrations in organisms (Clarkson, 1997, Hsu-Kim et al., 2013).

Humans are primarily exposed to MeHg through diet; specifically, through consumption of fish and seafood (Choi and Grandjean, 2012). MeHg concentrations are typically highest in long-lived predatory fish, which are preferentially sought after and consumed by humans (NRC, 2000; Essington et al., 2005). Risks of MeHg exposure are elevated for recreational and subsistence fishers and their families, who are exposed to higher-than-average levels of MeHg (USEPA, 2001). Because fish consumption represents the most prominent pathway of human MeHg exposure, governmental and international organizations make efforts to monitor and communicate the risks of mercury contamination from fish consumption (Evers et al., 2016). In the U.S., the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) are tasked with publishing advice regarding fish consumption (USEPA, 2004). In addition to publishing federal advisories, the FDA and EPA provide guidance to state agencies that may choose to implement more restrictive fish consumption advisories for specific species or bodies of water within their jurisdiction. Despite the efforts of state and federal agencies, low awareness and even lower compliance with these advisories highlights the importance of increasing the body of knowledge regarding the environmental factors that modulate mercury exposure risk to humans (Ney and Ney, 2009; LePrevost, 2013).

Food web processes and trophic structure play an important role in the mercury dynamics of stream ecosystems, and these factors may drive significant variation in organismal mercury burdens among systems with similar mercury inputs. Higher densities of phytoplankton and zooplankton negatively correlate with mercury burdens in

their consumers through processes called “algal bloom dilution” and “zooplankton density dilution”; as such, differences in plankton density can drive variation in the mercury concentration of both herbivorous and predatory fish across similar systems (Chen and Folt, 2005). Increased algal density leads to a lower concentration of mercury per cell, ultimately resulting in lower dietary mercury exposure to primary consumers (Pickhardt et al., 2002). In addition to density-related mechanisms, mercury concentrations may also decrease through “growth dilution,” in which rapid, efficient growth reduces mercury burden in aquatic organisms (Karimi et al., 2007). In a field experiment, Atlantic Salmon (*Salmo sala*) that grew faster and larger had lower mercury concentrations than fish that grew slower, consistent with growth dilution (Ward et al., 2010b). Similarly, Smallmouth Bass (*Micropterus dolomieu*) in an Adirondack Lake had lower mercury concentrations following compensatory growth as a result of targeted removal (Taylor et al, 2020); however, despite the fact that they also grew faster post Smallmouth Bass removal, Lake Trout (*Salvelinus namaycush*) from the same study actually had higher mercury concentrations, likely due to a dietary shift toward prey of a higher trophic level. A study of long-term trends in mercury bioaccumulation in Lake Michigan found that mercury concentrations in fish have been substantially affected by changes in food web structure due to multiple species invasions (Lepak et al., 2019). Other food web variables, such as species diversity, may also influence trophic transfer of mercury (Kozak et al., 2021). Despite evidence that mercury dynamics are clearly influenced by these food web variables, research on this topic is sparse.

Physico-chemcial characteristics are also known to influence mercury dynamics in aquatic ecosystems, either directly or through mediation of biological processes. Water chemistry variables such as low pH and high dissolved organic carbon (DOC) may influence baseline concentrations of mercury due to higher rates of uptake and methylation (Jardine et al., 2013). This association may be due in part to the low pH and high DOC found in wetlands, which are a significant site of mercury methylation (Rudd, 1995); however, low pH may also be associated with slower fish growth, which could result in higher mercury concentrations in accordance with growth dilution (Watras et al., 1998). Temperature is another physical variable that may influence mercury dynamics through mediation of biological processes; in colder water, organisms undergo slower growth and excrete MeHg slower than organisms in warmer water, leading to higher levels of MeHg accumulation in organisms (Simoneau et al., 2005; Trudel and Rasmussen, 1997). Greater mercury deposition generally results in higher organismal concentrations of mercury (Hammerschmidt and Fitzgerald, 2006); however, systems with higher mercury deposition often paradoxically show decreased biomagnification rates (DeForest et al., 2007; Lavoie et al., 2013). The complex interplay between physico-chemical characteristics and biological processes is poorly understood, and much more investigation is required to determine the joint and individual effects of these variables on mercury dynamics in stream ecosystems (Clayden et al., 2013).

Mercury reaches high concentrations in organisms through processes known as bioaccumulation and biomagnification (Sorenson, 1991, Sandheinrich and Weiner, 2011, Kidd et al., 2012). Mercury bioaccumulation refers to an increase in mercury load or

concentration in organismal tissues over time, which occurs when an organism's rate of mercury intake exceeds the combined rates of mercury metabolism and excretion (Markich et al., 2001). Bioaccumulation can result in organismal mercury concentrations that exceed abiotic environmental concentrations by a factor of 10^7 (Kidd et al., 2012). Biomagnification of mercury refers to the increase of mercury concentration in a consumer relative to its diet. In aquatic food webs, biomagnification of mercury leads to tissue concentrations in predators equal to two-to-ten times that of their prey (Watras et al., 1998). A common approach for quantifying the degree of mercury biomagnification in food webs is to evaluate the Trophic Magnification Slope (TMS). TMS is the slope of the linear relationship between $\log_{10}$ transformed organismal mercury concentrations and trophic level (Lavoie et al., 2013). Factors that influence mercury biomagnification are unclear, and in some cases contradictory. In a worldwide meta-analysis, Lavoie et al. (2013) found that THg biomagnification increased with DOC and decreased with both total phosphorous and atmospheric mercury deposition. Conflictingly, Kidd et al. (2012) found that biomagnification increased with total phosphorous. Indeed, a review of the mechanisms underlying biomagnification reveals contrasting results at multiple scales, complicated greatly by differences in biological and physico-chemical variables, and by the complex interactions between the two that might jointly influence mercury dynamics in aquatic systems (Kozak et al., 2021).

Dietary exposure is the primary route of mercury bioaccumulation in fish, which is known to be deleterious to individual fish health (Spry and Weiner, 1991). It is estimated that wild fish obtain more than 90% of their MeHg from their diets

(Sandheinrich and Weiner, 2011). Some diet items may contribute more mercury than others; for example, crayfish total mercury (THg) explains 97% of variation in Smalmouth Bass THg concentrations in Ozark streams (Schmidt et al., 2013). Studies have shown that dietary MeHg exposure is associated with a suite of health effects in both laboratory and wild fish, such as reproductive impairment, slower growth, increased tissue parasite burdens, and abnormalities (Sandheinrich and Weiner, 2011; Drevnick and Sandheinrich, 2003; Drevnick et al., 2006; Friedmann et al, 1996; Blazer et al., 2023). Dietary MeHg exposure may also result in behavioral changes, such as decreases in predator avoidance, feeding efficiency, and competitive abilities (Webber and Haines, 2003; Fjeld et al., 1998).

Biomagnification of mercury through aquatic food webs drives mercury contamination in fish and the resultant risk to humans through consumption; therefore, it is critical to understand how mercury moves through food webs. Stable isotope analysis is a popular diet tracing technique used in food web ecology studies because it can provide information on long-term feeding habits of organisms (Peterson and Fry, 1987; Nielsen et al., 2017). Organisms assimilate the heavier isotopes of some elements from their foods at higher rates than lighter isotopes (McCutchan et al., 2003). Ratios of heavy to light stable isotopes of nitrogen ($\delta^{15}N$) are typically 3-4‰ higher in predators than prey, and as such can be used to estimate food web (trophic) position (Post, 2002). Ratios of heavy to light stable isotopes of carbon ($\delta^{13}C$), however, vary much less (usually <1‰) in consumers relative to their diet (McCutchan et al., 2003). This means that $\delta^{13}C$ values can be used to estimate energetic contribution of basal resources and differentiate

between C3 and C4 plants (Peterson and Fry, 1987), or allochthonous vs autochthonous production in a consumer's diet (Jardine et al., 2012). Because stable isotope ratios provide information on the long-term feeding habits of organisms (Peterson and Fry, 1987), they can be a useful tool for describing how mercury biomagnifies in food webs when paired with organismal mercury concentrations (van der Velden et al., 2013).

Among the pristine systems that are affected by mercury contamination are United States National Parks. Because National Park Service (NPS) units are federally protected and assumed to be free of significant point sources of mercury pollution, aquatic ecosystems found within the NPS system offer a unique opportunity to study mechanisms and patterns of bioaccumulation and biomagnification of non-point source mercury that ultimately drive human exposure risk (Eagles-Smith et al., 2020). A 2015-2016 nationwide monitoring effort of mercury in the fish of national parks revealed elevated mercury concentrations in the fillet tissue of Smallmouth Bass of Great Smoky Mountains National Park, Tennessee, United States. (GSMNP) (Eagles-Smith et al., in review). Smallmouth Bass represent an important apex fish predator of streams in GSMNP and a highly sought after game fish in the region, generating millions of dollars spent on gear and lodging by the fanatical anglers that pursue them (Etnier and Starnes, 1993). The high levels of mercury detected in GSMNP Smallmouth Bass prompted the Tennessee Department of Environment & Conservation (TDEC) to issue a precautionary fish consumption advisory for streams from two adjacent watersheds, Abrams Creek and Little River, because mercury concentrations above the EPA threshold for safe consumption (0.3 mg/kg, wet weight) were detected in some of the Smallmouth Bass of

both watersheds. In contrast, mercury concentrations in Smallmouth Bass from the nearby Middle Prong Little Pigeon River (hereafter "Little Pigeon") were not high enough to trigger an advisory (TDEC Division of Water Resources, 2022). Preliminary analyses of these data showed that total mercury (THg) concentrations increased with length of Smallmouth Bass at all three streams, which would indicate bioaccumulation of mercury was occurring. However, THg tissue concentrations appeared to differ between streams. Because of their geographical proximity and the fact that atmospheric deposition of THg in GSMNP is dominated by the global pool (Valente et al., 2007), regional and local differences in atmospheric THg inputs are unlikely to be driving the observed differences in Smallmouth Bass within GSMNP. One possible explanation for this phenomenon could be differences in rates of biomagnification (TMS) or basal mercury concentrations.

The dynamics of mercury in the aquatic food webs of GSMNP are poorly understood and represent an important knowledge gap with implications for the health of humans and wildlife. Our primary goal was to increase the understanding of how mercury moves through ecosystems by analyzing trophic transfer through the food web. Our objectives were to (1) describe and compare trophic pathways, (2) measure environmental and organismal concentrations of mercury, and (3) evaluate evidence for mechanisms leading to differential mercury contamination within and between three streams in Great Smoky Mountains National Park.

CHAPTER 2: METHODS

Study System

Great Smoky Mountains National Park (GSMNP) is the most visited of all U.S. national parks, with more than 14 million visits in 2021 (National Park Service, 2023). Located in the Appalachian Mountains of the Southeastern United States along the border of Tennessee and North Carolina, this park contains over 2,900 miles of freshwater streams, of which 800 miles are inhabited by a combined 80 species of fish (Fisher, 2022). Three of those 80 species are classified as either federally threatened or endangered. In addition to its high diversity of non-game fishes, five species of game fish occur in the park: Rainbow Trout, Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), Rock Bass (*Ambloplites rupestris*), and Smallmouth Bass.

Streams in GSMNP range from first-order to fifth-order and consist of cold-water and cool-water habitat (Fisher, 2022). Most stream substrates in the park, including those of both Little River and Little Pigeon, are composed of sandstone, which is not highly soluble and results in streams with low productivity, low fertility, and high acidity (Fisher, 2022). However, in certain areas of the park – like Cade's Cove, which contains the headwaters of Abrams Creek – the sandstone has been eroded significantly enough to expose underlying highly soluble limestone bedrock in the streambed, resulting in higher pH, nutrient input, and productivity compared to other streams in the park (Shaffer, 2004; Cole, 1983).

Samples were collected from Abrams Creek, Little River, and Middle Prong River during June-October 2022. Three sampling sites were selected within each stream:

an upstream site that represents the highest local elevation at which Smallmouth Bass are common and abundant, an intermediate site, and a downstream site (Figure 1, Table 1). All tables and figures are in the appendix. Sampling sites were chosen to represent the extent of fishable Smallmouth Bass populations within the park and for comparison to previous studies.

Eight representative food web components were sampled from each of the study streams for mercury and stable isotope analysis. Periphyton, detritus, and filamentous algae were selected to represent basal resources. Heptageniid mayfly larvae (hereafter referred to as 'mayfly larvae') and Central Stoneroller (*Campostoma anomalum*) were selected as representative primary consumers that are commonly found in all three systems. Crayfish (Cambaridae) were selected as an intermediate consumer, as well as a common prey item of Smallmouth Bass (Probst et al., 1984, Shaffer, 2004). Dragonfly larvae (Odonata) were selected as an intermediate consumer as well as for comparison with previous studies suggesting their utility as a bioindicator of environmental mercury contamination (Eagles-Smith, 2020). Smallmouth Bass, a prized sportfish in the region, were selected as the top aquatic fish predator, representing the primary Hg exposure risk for humans.

Field Methods

Food web components were collected with several common techniques. Smallmouth Bass were collected via angling from each site. Central Stoneroller were collected with pulsed-DC backpack electrofishing unit and a combination of seine and dip-netting techniques. Once collected, Smallmouth Bass and Central Stoneroller were held in aerated site water or flow-through minnow buckets instream until euthanized in tricaine

methanesulfonate according to IACUC protocols, at which point they were double-bagged in fresh whirl-pak or zip-seal bags and promptly placed on wet ice until processing. Mayfly larvae of genus Heptageniidae were hand-picked from instream cobbles with forceps and grouped into composite samples weighing 1-2g (wet weight, 0.1g) in Fisherbrand sterile cryogenic vials. Dragonfly larvae were collected by sweeping a D-net through root wads exposed by undercut banks, sandy substrates, and depositional areas, after which they were placed in clean Thermo Scientific Nalgene HDPE bottles with site water. Crayfish were collected by hand from underneath cobbles and by sweeping a D-net through depositional areas containing leaf detritus. Detritus and filamentous algae were collected by hand. Detritus samples came from in-stream depositional areas and consisted primarily of deciduous leaves. Filamentous algae was collected from colonies attached to large rocks. Periphyton was collected by removing 3-5 cobbles per sample and placing them in a clean plastic tray, where they were scrubbed with a stiff-bristled brush and rinsed with a squeeze-bottle filled with site water. The resulting slurry was decanted into a whirl pak. After collection, all component samples were double bagged in fresh whirl pack or zip seal bags and placed promptly on ice for transport back to the laboratory, where they were either processed further or frozen at -20 degrees Celsius until they could be processed for mercury and stable isotope analyses.

Laboratory Methods and Basic Computations

In the laboratory, Smallmouth Bass, Central Stoneroller, and crayfish were rinsed with deionized (DI) water and placed on a clean plastic liner for weighing (0.1g), measuring (total length, to the nearest millimeter), and dissection. Boneless, skinless axial muscle (fillet) tissue was removed from Smallmouth Bass using a filet knife or a scalpel, double

bagged in whirl-pak or zip-seal bags, and then frozen at -20° C until analysis for both mercury and stable isotopes (Scudder et al., 2008). After measurement, individual whole-body Central Stoneroller and crayfish were combined into multiple composite samples per site (6-10 individuals per composite; n=5-6 per stream) for mercury analysis. Skinless dorsal muscle tissue samples were removed from a subset of Central Stoneroller (n=14-16 per stream), and crayfish tail muscle (n= 12-13 per stream) was removed separated from the exoskeleton for stable isotope analysis. Muscle tissue samples were oven-dried at 60 degrees Celsius for at least 48 hours, ground into a fine powder with mortar and pestle, and shipped for stable isotope analysis. Prior to analysis, Central Stoneroller tissue was acidified to remove additional carbon from bony structures that were too small to remove by hand.

Dragonfly nymphs were grouped into composite samples (n=6-8 samples per stream) and placed into 50 ml centrifuge tubes. Dragonfly and mayfly nymph composites were frozen and then freeze-dried for at least 72 hours. Samples were homogenized in their containers using a stainless-steel weighing spatula. For stable isotopes, subsamples were weighed (0.001mg) into tin capsules and packed into 96-well polystyrene plates for shipment. Remaining sample mass was shipped for mercury analysis at PACE Analytical laboratory. Dragonfly nymphs were analyzed for THg exclusively. After a small subsample was weighed for stable isotope analysis, (n=5-9 per stream), mayfly nymph composite samples were determined to be too small for paired MeHg and THg sampling; so composite samples were analyzed for either THg (n=2-8 per stream) or MeHg (n=2-3 per stream) exclusively.

Detritus (n=9 per stream) and filamentous algae (n=3-8 per stream) samples were thawed, rinsed in DI water, and carefully picked through to remove coarse woody debris and invertebrates before oven-drying at 60 degrees Celsius for up to 72 hours. After drying, samples were ground to a fine powder using mortar and pestle and shipped to Boston University for stable isotope analysis. For mercury analysis, detritus samples were thawed, placed in centrifuge tubes, refrozen, and freeze-dried for at least 72 hours. In addition to THg analysis, three samples per stream were selected for MeHg analysis to determine percentage of THg as MeHg (Table 2). Filamentous algae was not analyzed for mercury.

For stable isotope and mercury analysis, frozen periphyton samples (n=6-9 per stream) were thawed in an upright position to allow periphyton to settle. Supernatant liquid was decanted and the remaining slurry was transferred into a 50 ml centrifuge tube, refrozen, and freeze-dried for at least 48 hours (McManamay et al., 2019). Samples were homogenized in their containers using plastic weighing spatulas. For stable isotopes, a subsample (~15mg) of each sample was weighed into glass vials and shipped to Boston University, where they were acidified before analysis to remove any carbonate material that might have been removed from rocks during the collection process. The remaining sample mass was shipped to Pace Analytical Laboratory for mercury analysis. All periphyton samples were analyzed for THg at Pace Analytical Laboratory. In addition to THg analysis, three to four samples per stream (Table 2) were selected for MeHg analysis to determine percentage of THg as MeHg.

Smallmouth Bass tissue samples (n=17-30 per stream) were analyzed for THg at Oak Ridge National Laboratory following EPA 1631 via cold vapor atomic absorption spectroscopy (U.S. EPA, 2001) using the LUMEX RA-915M Mercury Analyzer. A subsample (75-100 mg) of fillet was placed in a 40ml glass vial, weighed, and frozen until digestion in HNO_3/H_2SO_4. Following digestion, samples were reduced using stannous chloride (Mathews et al., 2013). Samples were analyzed in duplicate, and any samples with differences greater than 10% were rerun. For each digestion batch, blanks and standard reference materials (IAEA-407, IAEA-436) were prepared and analyzed for quality-assurance/quality control (QA/QC). Blanks showed no signs of contamination, and standard reference materials averaged >90% recovery. Calibration curves for the instrument were generated daily using laboratory standards.

Detritus, periphyton, mayfly larvae, dragonfly larvae, Central Stoneroller, and crayfish were analyzed for THg at Pace Analytical Laboratory following EPA 245.6 (U.S. EPA, 1991). A subset of samples (Table 2) was also analyzed for MeHg following a modification of EPA 1630 (U.S. EPA, 2001). QA/QC procedures included blanks, sample replicates, and laboratory standards. For analytes that were below the detection limit, a value equaling half the detection limit was substituted (Cope et al., 2021; Grieshaber et al., 2018; USEPA, 1991). Values were estimated for samples that cleared reagents.

Stable isotope analyses were conducted at the Boston University Stable Isotope Laboratory, the Cornell University Stable Isotope Laboratory, and the Arizona Climate and Ecosystems (ACE) Isotope Lab at Northern Arizona University. Samples were

weighed (0.001mg) into tins and combusted in a continuous-flow isotope ratio mass spectrometer. Once combusted, gases (CO_2, and N_2) from the sample were compared to standard gas reference materials the following formula was used to calculate isotopic ratios:

$$\delta X = (R_{standard}/R_{sample})$$

where X=^{15}N or ^{13}C, R_{sample}=^{15}N/^{14}N or ^{13}C/^{12}C in the sample, and $R_{standard}$=^{15}N/^{14}N or ^{13}C/^{12}C in the standard. Ratios of nitrogen and carbon isotopes were reported as per thousand (‰) differences. Quality assurance measures included in-house standard checks and sample replicates. Standard gas reference materials included Vienna Pee Dee Belemnite for carbon and air for nitrogen (Fry, 2006).

Trophic level (TL) of consumers in our study was calculated using a two-source model from Post (2002):

$$TL = \lambda + (\delta^{15}N_{consumer} - [\delta^{15}N_{base1} \times \alpha + \delta^{15}N_{base2} \times (1-\alpha)])/\Delta_n$$

where λ is the trophic position of basal resources (λ = 1 for detritus representing allochthonous production, and filamentous algae and periphyton representing autochthonous production), Δ_n is the amount of trophic fractionation per trophic level, and α is the proportion of nitrogen that the consumer derives from base one, which can be estimated using the following formula:

$$(\delta^{13}C_{consumer} - \delta^{13}C_{base2})/(\delta^{13}C_{base1} - \delta^{13}C_{base2})$$

The trophic fractionation value of 3.4‰ per trophic level was used for Δ_n (Post, 2002).

Trophic magnification slopes (TMS) and trophic magnification factor (TMF) were used to evaluate mercury biomagnification for each stream food web and calculated with the following equations (Borga et al., 2012):

$$\log_{10} \mathrm{Hg} = a + b * \mathrm{TL}$$

$$\mathrm{TMF} = 10^{b}$$

where Hg is the mean concentration of THg or estimated MeHg for each component in ng/g dry weight, per stream (Table 3, Table 7), and TL is stream-wide mean trophic level for each component (Table 8). MeHg estimation and dry weight concentration conversation calculations are described below.

Biomagnification factors (BMFs) were calculated using the following formula:

$$\mathrm{BMF} = \mathrm{THg}_{\mathrm{consumer}}/\mathrm{THg}_{\mathrm{food}},$$

where $\mathrm{THg}_{\mathrm{consumer}}$ is the stream-wide total mercury concentration of a consumer and $\mathrm{THg}_{\mathrm{food}}$ is the stream-wide total mercury concentration of the consumer's presumed diet item (Gray, 2002).

Smallmouth Bass, Central Stoneroller, and crayfish mercury concentrations were measured from the wet-weight of the sample and were converted to dry weights. The percent moisture of a subset of crayfish (n=11) was measured by weighing the wet sample, freeze-drying, and reweighing it. The average percent moisture of this subset (81.63%) was used to convert crayfish Hg concentrations from wet weight to dry weight. Percent moisture values for Smallmouth Bass and Central Stoneroller of 74.22% and 74.62%, respectively, were used to convert fish concentrations to dry weight (USEPA, 2016 & 2021; GEI, 2014).

A subset of samples (n=3-4 per item per stream) of periphyton, detritus, crayfish, and Central Stoneroller were analyzed for both THg and MeHg. We divided MeHg concentrations for each paired sample by its accompanying THg value to determine the percentage of THg as MeHg. Percent MeHg (%MeHg) values from paired samples were averaged by stream to determine the mean %MeHg for each component. Mean %MeHg values were then multiplied by THg values for samples which were only analyzed for THg to estimate the amount of MeHg in the sample. For components whose mean %MeHg estimates exceeded 100%, all THg was assumed to be MeHg.

Low sample volume for mayfly larvae did not allow for paired sampling, so a subset (n=2-3 per stream) of composite samples were selected for MeHg analysis exclusively. The rest (n=14) were analyzed for THg (Table 2). For MeHg estimation, mean MeHg values were divided by mean THg values on a per site basis as a surrogate for paired sampling. The resulting proportion was averaged across all three sites per stream and then multiplied by individual THg concentrations to estimate MeHg.

Because Smallmouth Bass are a high trophic level long-lived predator fish, all mercury was assumed to be MeHg (Bloom, 1992). Dragonfly larvae %MeHg was assumed to be 80% following results from Eagles-Smith et al. (2020).

Statistical analyses

All analyses were performed using R Statistical Software (v4.2.2; R Core Team 2022). The statistical analyses described in this section tested how THg differed with fish length, between years, and among streams. First, we constructed linear regressions where the response variable was THg concentration, and the predictor variables were fish length, year (two levels: 2016 and 2022), and an interaction between fish length and year.

Initially, separate models were run for each of the three streams. There was evidence for non-equal variances between years in Abrams Creek, which we accounted for using the varIdent function in the nlme package (v3.1-160, Pinheiro et al, 2022). We tested for a significant interaction between fish length and year at each stream (alpha = 0.05) to determine if relationships between fish length and THg differed between years. We detected a significant interaction between fish length and year in Little River ($F(1,51) = 10.185$, $p = 2.00e\text{-}3$), indicating that differences in Little River Smallmouth Bass THg between years depended on fish length. Therefore, we evaluated the among year differences in Smallmouth Bass THg at Little River for small, medium, and large fish, where we defined small, medium, and large as the 25^{th}, 50^{th}, and 75^{th} percentile of all Smallmouth Bass lengths collected from Little River in 2016 and 2022 (129mm, 172mm, and 203mm, respectively). Between year differences in Little River Smallmouth Bass THg at each size were evaluated using R package 'contrast' (v0.24.2, Kuhn et al. 2022). A significant interaction between fish length and year was not detected for Smallmouth Bass THg concentrations in Abrams Creek ($F(1,41) = 0.015$, $p = 0.9$) or Little Pigeon ($F(1,25) = 0.653$, $p = 0.427$). To account for non-equal variance between years in Smallmouth Bass THg in Abrams Creek, a generalized least squares (GLS) model in which variance structure was estimated using restricted maximum likelihood (REML) was used to compare differences between years. Little Pigeon Smallmouth Bass length-THg relationships met analysis of covariance (ANCOVA) assumptions, and length-adjusted differences were evaluated using estimated marginal means following Bonferroni correction using R package 'emmeans' (v1.8.3, Lenth 2022). For Abrams

Creek and Little Pigeon, differences in Smallmouth Bass THg between 2016 and 2022 were evaluated at stream-specific average lengths.

Second, we built linear regressions to compare how THg concentrations in 2022 varied among our three streams. The response variable in these models were THg concentration and the predictor variables were fish length, stream, and an interaction between fish length and stream. We performed pairwise testing for a significant interaction between fish length and year at each stream (alpha = 0.05) to determine if relationships between fish length and THg differed between streams. A significant interaction was detected between fish length and stream for Abrams Creek and Little River ($F(3,50) = 66.97$, $p = 0.002$), Abrams Creek and Little Pigeon ($F(3,43) = 50.84$, $p = 0.016$), but not for Little River and Little Pigeon ($F(3,37) = 16.95$, $p = 0.291$). Therefore, we evaluated the between-stream differences in Smallmouth THg for small, medium, and large fish, where we defined small, medium, and large as the 25^{th}, 50^{th}, and 75^{th} percentile of all Smallmouth Bass lengths collected in this study (167mm, 194mm, and 231mm, respectively). Between year differences in THg at each size were evaluated using R package 'contrast' (v0.24.2, Kuhn et al. 2022). Length-adjusted Smallmouth Bass THg concentration was calculated by averaging lengths of all Smallmouth Bass collected in this study and using the stream-specific length-THg regression slopes described above to calculate the expected THg concentration at that length.

Mean values and standard error for THg concentration, estimated and measured MeHg concentration, and trophic level for each component were calculated on a per-stream basis, including all samples from all three sites for each stream. Means values for

each component were compared across streams using analysis of variance (ANOVA). If a statistically significant difference was revealed between means of at least two streams, as indicated by a p-value of less than 0.05, a Tukey's Honest Significant Differences (HSD) Test was performed for pairwise comparisons between streams. Food web structure was analyzed visually using a biplot of mean values (error bars representing 95% confidence intervals) of carbon and nitrogen stable isotope ratios of each food web component, averaged across all samples from all sites at each stream.

Differences in TMS were evaluated through comparison of the slopes of the linear regression models between streams. The response variables in these models were mean $\log_{10}$THg or $\log_{10}$MeHg concentration and the predictor variables were mean trophic level, stream, and an interaction between mean trophic level and stream. We performed pairwise testing for a significant interaction between mean trophic level and stream (alpha = 0.05) to determine if relationships between log-transformed THg and MeHg concentrations and trophic position differed between streams. The relationship between Smallmouth Bass $\log_{10}$THg and δ^{15}N was evaluated using stream-specific linear regressions in which $\log_{10}$THg was the response variable and δ^{15}N was the predictor variable.

CHAPTER 3: RESULTS

Total Mercury

Mean THg concentration values followed similar patterns of increase from basal resource to top predator across all three streams studied (Table 3, Figure 2). Patterns of basal resource THg concentrations were similar between streams; mean periphyton THg concentrations (43.7 ng/g, dw) were, on average, ~2 times higher than concentrations in detritus (21 ng/g, dw) (Table 3). THg concentrations in lower trophic-level food web components did not differ between streams, but concentrations in certain intermediate and upper-level consumers did (Table 3). Mean THg concentrations in detritus, periphyton, and mayfly nymphs did not differ significantly between Abrams Creek, Little River, and Little Pigeon (Table 3). THg concentrations in Central Stoneroller were significantly higher in Abrams Creek (142 ng/g dw) than Little River (104 ng/g dw) and Little Pigeon (95 ng/g dw) (Table 3). Similarly, mean THg concentrations measured in Abrams Creek dragonfly nymphs (272 ng/g dw) and crayfish (399 ng/g dw) were more than twice the respective mean concentrations of Little River and Little Pigeon dragonfly nymphs (130 and 105 ng/g dw) and crayfish (177 and 148 ng/g dw) (Table 3).

Smallmouth Bass THg increased with length at all sites; however, this relationship differed between streams. Length explained more of the variation in Abrams Creek Smallmouth Bass THg (R^2=0.72) than Little River (R^2=0.35; Figure 3). Smallmouth Bass THg concentrations were significantly higher in Abrams Creek than in both Little River and Little Pigeon at all lengths, and this difference increased with length ($F(3,50) = 66.97$, $p<0.05$; Figure 4). Little River and Little Pigeon Smallmouth Bass THg

concentrations were not significantly different at any length ($F(3,37) = 16.95$, $p = 0.29$; Figure 4).

Mean Smallmouth Bass THg concentration were significantly lower in all three streams in 2022 than in 2016 (Figures 5, 6). This difference was greatest in Abrams Creek, where 2022 concentrations were 46% lower than 2016 (Table 4) and least pronounced in Little Pigeon at 27% (Table 4). All three length-categories of Smallmouth Bass from Little River had significantly lower mean THg concentrations ($p<0.05$) in 2022 than in 2016, with differences apparently increasing with length (Figure 6).

Similar patterns of biomagnification were observed in the taxa of all three streams. Relative to crayfish, the BMFs for THg in Smallmouth Bass ranged from 3.4-5.4 and were lowest in Abrams Creek and Little River and highest in Little Pigeon (Table 5). After adjusting for size, Smallmouth BMFs were more similar (3.3-4.2), but still lowest at Abrams Creek, followed by Little Pigeon and Little River (Table 5). BMFs for consumers of periphyton were highest in Abrams Creek. Periphyton-mayfly nymph BMFs averaged 2.2 across all three streams, ranging from 1.5-2.8, and Periphyton-Central Stoneroller BMFs ranged from 2.0-3.6 (Table 5). On average, length-adjusted THg in Smallmouth Bass was 5.4 times that of dragonfly nymphs; however, variation in this relationship was apparent between Abrams Creek and the other two streams (Table 5).

MeHg

%MeHg was, on average, nearly twice as high in detritus than periphyton; however, differences were not uniform and ranged from 1.3 fold (Little River) to 3.7 fold (Little Pigeon) (Table 6). %MeHg in detritus was highest in Abrams Creek (56%) and lowest in

Little Pigeon (22%) (Table 6). %MeHg in periphyton was highest in Little River (36%) and more than 6 times the value measured in Little Pigeon (6%) (Table 6). Central Stoneroller %MeHg values were similarly high across all three streams (96-114%) (Table 6). Crayfish %MeHg values in Abrams Creek and Little River exceeded 100%; however, like detritus and periphyton, the mean %MeHg value for Little Pigeon crayfish was lower than the other two streams (79%) (Table 6).

Estimated mean MeHg concentrations followed similar patterns to those observed for THg. Estimated mean MeHg concentrations were lower in Little Pigeon than Abrams Creek in all food web components after Smallmouth Bass concentrations were adjusted for length (Table 7). Similar to patterns observed in THg concentrations, estimated mean MeHg concentrations were lowest in basal resources at all sites, followed by mayfly nymphs, Central Stoneroller, dragonfly nymphs, crayfish, and Smallmouth Bass; however, one notable exception is that estimated Central Stoneroller MeHg concentrations were higher than those of dragonfly nymphs in Little Pigeon (Table 7). Estimated mean MeHg concentration of periphyton was lower in Little Pigeon River than the other two streams studied, observed values were on average, ~3 fold higher in Abrams Creek and ~5 fold higher in Little River (Table 7). Difference in estimated MeHg concentrations between basal resources were not as pronounced as in THg; for example, estimated MeHg concentrations of periphyton and detritus were equivalent in Abrams Creek, while THg concentrations were not (Tables 3, 7). Mean measured MeHg concentrations ranged from 4-10 ng/g dw in detritus, 2-17 ng/g dw in periphyton, and appeared lowest in Little Pigeon, as did concentrations in mayfly nymphs and crayfish

(Table 8). The magnitude of the difference in MeHg concentrations between Little Pigeon and Abrams Creek biota appeared to increase with food web position, from more ~3 fold in mayfly nymphs to ~5 fold in crayfish (Table 8).

Stable Isotopes and Trophic Position

Carbon and nitrogen stable isotopic ratios evidenced similar food web structure across the streams that were studied (Figure 7). Average $\delta^{13}C$ values for detritus were consistently most negative among all components, and basal resource $\delta^{15}N$ values were lower than that of other components (Figure 7). Mayfly nymph average $\delta^{13}C$ values reflected a reliance on periphyton, which follows the predicted feeding relationship associated with the 'scraper' guild (Figure 7). Smallmouth Bass average $\delta^{15}N$ values (and trophic positions) were higher than all other components, affirming their role as apex predator in these systems (Figure 7, Table 9). Notably, the average $\delta^{15}N$ and $\delta^{13}C$ average for Little Pigeon Smallmouth Bass had wider confidence intervals than those for Abrams Creek and Little River (Figure 7, Table 9), and Smallmouth Bass $\delta^{15}N$ values explained more of the THg variation in Abrams Creek (R^2=0.80) and Little River (R^2=0.87) than Little Pigeon (R^2=0.53; Figure 8). Average trophic position of dragonfly nymphs and Central Stoneroller did not differ between streams, but crayfish trophic position was higher in Abrams Creek and Little River than in Little Pigeon (Table 9). Despite this difference, Smallmouth Bass average trophic position was highest in Little Pigeon (Table 9).

Trophic Position and Mercury

TMS values for THg and estimated MeHg were positive for taxa of all three streams (Figures 9, 10). TMS values for THg ranged from 0.53-0.86 (Figure 9). Trophic position explained 83-87% of the variance in both THg and MeHg (Figures 9, 10). TMS values

for estimated MeHg were higher than THg, ranging from 0.82-1.1 (Figures 9, 10). Abrams Creek THg and estimated MeHg TMS values were higher than Little River and Little Pigeon (Figures 9, 10). TMS values did not differ between Abrams Creek and Little Pigeon ($F(1,10) = 0.332$; $p = .577$), Abrams Creek and Little River ($F(1,10) = 1.092$; $p = 0.321$), or Little River and Little Pigeon ($F(1,10) = 0.181$; $p = 0.679$).

CHAPTER 4: DISCUSSION

Our findings are evidence that THg contamination in Smallmouth Bass between years and between streams differ substantially in GSMNP. Between years, the magnitude of difference in mean THg concentrations ranged from 27% at Little Pigeon to 46% at Abrams Creek. There are several mechanisms that may explain the difference in Smallmouth Bass THg concentrations between 2016 and 2022. One possible explanation is that Smallmouth Bass THg concentrations are lower as a result of decreased atmospheric mercury deposition. Knightes et al. (2009) suggest that a 20-40% decrease in fish THg in a simulated river system may take 10-16 years following a 50% decrease in atmospheric deposition. Similarly, another study predicts that fish MeHg concentrations will decrease rapidly (within years) following emissions reductions (Harris et al, 2007). However, decreases in the ranges reported in our study (27-46% in six years) would be relatively rapid in comparison to the timescales for mercury reduction as predicted by Knightes et al. (2009) and Harris et al. (2007). Another potential explanation is that GSMNP Smallmouth Bass growth rates have increased since 2016, and therefore fish accumulate less mercury due to growth dilution. The magnitude of interannual differences in Smallmouth Bass mean THg levels that we report are consistent with those reported by Taylor et al., (2020), who found that mean Smallmouth Bass THg concentrations in an Adirondack Lake decreased from .349 mg/kg ww to .234 mg/kg ww as their growth rates increased; however, this decrease occurred over a 13-year period. Length-at-age and population data were not available for Smallmouth Bass collected in the Smokies in 2016, so it is uncertain whether growth rates due to population reduction or increased growth efficiency may explain the rapid decrease observed in the present

study. However, previous studies note that Smallmouth Bass in Abrams Creek and Little River rarely exceed five years in age (Shaffer, 2004), so it is possible that a new generation of Smallmouth Bass has been recruited and the current population may exhibit different growth characteristics than those collected in 2016. Finally, changes in water quality may explain some of differences we observed. Water acidity has been shown to explain some of the variation in Smallmouth Bass mercury concentrations between similar systems (McMurtry et al., 1989), with lower pH predictive of higher mercury concentrations. Recent declines in acid deposition in GSMNP lend credibility to this hypothesis (Schwartz et al., 2022); however, due to the complex interconnectedness of the variables discussed throughout, it is likely that several or all of them are at play simultaneously.

In relation to the 2016 data, similar trends appeared consistent with those observed in the present study. Abrams Creek Smallmouth Bass had the highest mercury concentrations among the three streams, followed by Little River and Little Pigeon. A significant and positive association between length and THg concentration was apparent across all streams for both years; however, it is notable that length explained more of the variation in Smallmouth Bass THg in 2016 than 2022 for Little River (Figure 3). Also of note, length-THg relationships were not equal between years for Little River as they were for Abrams Creek and Little Pigeon (Figure 3). This change could be due to an alteration of food web relationships, which will be discussed below; or it may be a result of fewer Smallmouth Bass greater than 200mm in our study than in the previous study.

Between streams in 2022, the length-adjusted mean Smallmouth Bass THg concentration in Abrams Creek was ~2.25 times the value in Little Pigeon. Though streams in GSMNP are considered pristine, the magnitude of this variation is comparable to the variation in THg concentrations in White Bass from a contaminated site versus an uncontaminated site in East Tennessee – which differed ~2.67 fold (Burger and Campbell, 2004). The ranges of Smallmouth Bass THg concentrations we report from GSMNP are comparable to those reported by Blazer et al. (2023) from the Chesapeake Bay watershed, which is subject to pollution from industrial effluents – signifying that despite its protected status and lack of point sources, mercury contamination in the Smallmouth Bass of GSMNP is still significant.

The proposed mechanisms for explanation of interannual mercury contamination differences may or may not also explain the differences we observed between systems. We did not observe differences in mean THg concentrations for detritus or periphyton when compared between streams, which, combined with the close geographical proximity of our systems, may reflect that depositional differences did not contribute to differences in THg observed higher in the food web (Desrosiers et al., 2006). Growth and biomass dilution may also be an insufficient explanation for between-stream differences in Smallmouth Bass THg concentrations. Smallmouth Bass from Abrams Creek have been reported to grow faster than those in Little River (Shaffer, 2004); additionally, the increased productivity in Abrams Creek should theoretically support more biomass, therefore leading to lower concentrations in biota (Shaffer, 2004; Cole, 1983). Little Pigeon Smallmouth Bass had the highest average trophic level of all three streams, yet

also had the lowest THg concentrations; therefore, it is unlikely that food chain length played a significant role in the differences observed between streams (Taylor et al., 2020). Given that average trophic level also did not differ between streams for dragonfly nymphs or Central Stoneroller, it is unlikely that this variable explains the difference in dragonfly nymph and Central Stoneroller THg concentrations between streams either. However, average trophic level for crayfish was highest in Abrams Creek, which could explain some of the variation in THg concentrations between streams. Crayfish THg concentrations may also vary by species and water quality variables such as conductivity (Allard and Stokes, 1989); given that we did not measure conductivity or identify crayfish to species, we cannot rule out the possibility that these factors may be driving variation in the mercury concentrations between streams.

Our findings support previous research that utilizes dragonfly nymphs as biosentinels and are comparable to other recent data collected in GSMNP by other personnel. The range of mean stream dragonfly nymph THg concentrations measured in this study (105-272 ng/g dw) fall within the range associated with 'moderate hazard' classification (100-300 ng/g dw) of an integrated impairment index created by Eagles-Smith et al. (2020), which considers ecological and human health risks associated with dragonfly mercury concentrations. Additionally, our finding of relatively high mean THg concentrations in Abrams Creek dragonfly nymphs (272 ng/g dw) and Smallmouth Bass (0.38 mg/kg ww) and substantially lower respective concentrations in taxa of the other two streams was consistent with Eagles-Smith et al.'s finding that the Aeshnid-equivalent dragonfly nymph THg concentrations in excess of 162 ng/g dw were associated with THg

concentrations in piscivorous fishes above the U.S. EPA criterion. The mean THg concentration in Abrams Creek dragonfly nymphs we report (272 ng/g dw) is comparable to other findings, falling within the range of concentrations reported from 2020-2022, though considerable variation between years is apparent (170.5-329.5 ng/g dw) (Eagles-Smith et al., 2018). The value we report for Little River dragonfly nymph THg (130 ng/g, dw) is substantially lower than the value reported in 2020 (226.5 ng/g dw); however, the significant yearly variation in Abrams Creek concentrations calls into question any ability to discern if concentrations are reliably decreasing, or if differences are merely a result of natural variation.

Very little historical data are available for fish Hg concentrations in GSMNP; however, some data do exist for comparison. Our study-wide average THg concentration of 0.029 mg/kg ww in Central Stoneroller is remarkably similar to the study-wide average of 0.033 mg/kg ww reported by Huckabee et al. in 1974. Our finding contrasts, however, with the more recently reported mean concentration values of 0.12 mg/kg ww in Little River and 0.37 mg/kg ww in East Fork Poplar Creek (EFPC) Central Stoneroller by Burger et al. (2012). EFPC is located on Oak Ridge Reservation in East Tennessee and was heavily impacted by direct point source Hg contamination during the Manhattan Project (Brooks and Southworth, 2011), so high mercury concentrations come as no surprise. However, the apparent difference between years for Little River Central Stoneroller, at ~4 fold, exceeds year-to-year variation in fish mercury concentration (0% to 28%) as reported by Braaten et al. (2014). This suggests that observed differences over time may be indicative of a longer-term trend, rather than just year-to-year variation.

Long-term, year-to-year monitoring is required to better understand dynamics of mercury contamination in GSMNP. As we have demonstrated, THg concentrations in intermediate consumers may indeed be driving differential concentrations in Smallmouth Bass, and consequently influencing human health risk across the park; therefore, more careful monitoring of food web components is clearly warranted.

Routes of mercury contamination to Smallmouth Bass may vary depending on life stage, season, prey availability, and study system. Smallmouth Bass are known to prey upon insects, crayfish, and fish throughout all life stages, but ontogenetic diet shifts from insects in early life to larger prey as adults (Dauwalter and Fisher, 2008) suggest that non-uniform mercury concentrations in prey items may result in non-uniform mercury exposure throughout the lifespan. Smallmouth Bass diet may also be influenced by seasonal shifts in prey availability (Angermeier, 1982), prey abundance and community structure, and habitat type (Dauwalter and Fisher, 2008; Probst et al., 1984; Etnier and Starnes, 1993). The abundance and of prey items in these systems were not explicitly measured in this study; however, notable mercury concentrations in GSMNP mayfly and dragonfly nymphs, Central Stoneroller, and crayfish indicate that mercury contamination in these components likely constitutes a significant pathway for bioaccumulation in top aquatic predators across all life stages. Crayfish THg concentrations in other systems have been reported to explain as much as 97% of the variation in Smallmouth Bass fillet tissue THg (Schmitt et al., 2013). In our study, observed mean crayfish and dragonfly THg concentrations from Abrams Creek were more than double that of Little River and Little Pigeon, suggesting higher contamination potential exists for multiple prey items

found in Abrams Creek, and serving as a potential explanation for the differential contamination observed in GSMNP Smallmouth Bass.

Our study is the first to show detailed evidence that biomagnification of mercury is occurring in the stream food webs of Great Smoky Mountains National Park. This finding is supported by positive and significant relationships between mercury and trophic position. Although slope differences between streams did not meet statistical significance, our results suggest that THg is biomagnifying at a higher rate in Abrams Creek than both Little River and Little Pigeon. As such, biomagnification rate differences may indeed be driving differential Smallmouth Bass THg concentrations. Previous studies posit that differential THg concentrations at the base of the food web, rather than different biomagnification rates, drive differential THg concentration in top aquatic predators (van der Velden, 2013). Our findings did not support this explanation. Detritus and periphyton THg concentrations did not differ between Abrams Creek, Little Pigeon, and Little River; however, as previously stated, Smallmouth Bass THg concentrations did (Table 1). Because our findings indicate similar THg inputs into the food web and different biomagnification rates, we conclude that food web dynamics are a key driver of Smallmouth Bass THg concentrations in this system.

Autochthonous production appears to be the predominant basal resource of trophic and contaminant pathways leading to Smallmouth Bass in Great Smoky Mountains National Park. $\delta^{13}C$ values for all components are more similar to those of periphyton and filamentous algae than those of detritus (Figure 6). This suggests that autochthonous production, rather than allochthonous production, serves as the

predominant basal energy input for the food web leading to Smallmouth Bass in Great Smoky Mountains National Park (Figure 6), and as such, periphyton represents the primary Hg influx to intermediate consumers that derive most of their Hg from food. Although THg concentrations in periphyton did not differ between streams, estimated mean MeHg concentrations did differ and were lowest in Little Pigeon. Consequently, mayfly nymph mean MeHg levels were also lowest in Little Pigeon, suggesting that grazing insects may be an important link in the trophic transfer of MeHg in these systems. Mean THg and estimated mean MeHg values for Central Stoneroller, another consumer of periphyton, were also highest in Abrams Creek, supporting the hypothesis that MeHg may be mobilized into food webs by consumers of periphyton. This hypothesis is further supported by research conducted in this region by McManamay et al. (2019) which demonstrated Stoneroller may mobilize, store in reservoirs, and transfer significant amounts of Hg. Since periphyton has been shown to be a major site of mercury methylation in aquatic ecosystems, it is likely that these factors could be driving some of the dynamics we observe in these systems (Rudd, 1995).

Our findings suggest that differential Smallmouth Bass mercury concentrations between three streams in GSMNP have highly relevant implications for human health. The boneless fillet tissue of nineteen of thirty (63.3%) Smallmouth Bass from Abrams Creek exceeded the US EPA threshold value of 0.3 mg/kg (ww) for recommendation for an advisory for consumption due to significant risk for sensitive groups (USEPA, 2001). Outside of Smallmouth Bass found in Abrams Creek, however, the only fish to exceed the EPA threshold value was a single individual from Little Pigeon, suggesting that

human exposure risk is not uniform across streams. Based on the findings of the previous report, a fish consumption advisory was instituted for Smallmouth Bass in Little River in response to tissue concentrations in excess of the EPA threshold value. Since we did not detect Smallmouth Bass in Little River exceeding the threshold value of .3 mg/kg ww, we suggest further monitoring to determine if a re-evaluation of the advisory is warranted. Recreational and sustenance fishing are both culturally valuable and important activities on lands protected by the National Park System. If future monitoring confirms our findings, re-evaluation of the advisory could represent a positive step towards the National Park Service's mission of conserving the natural and cultural values of Great Smoky Mountains National Park.

In addition to implications for human health, the Smallmouth Bass mercury concentrations measured in this study are ecologically and biologically relevant. Seven Smallmouth Bass from Abrams Creek had mercury concentrations in excess of 0.5 mg/kg (ww), a threshold associated with reduced reproductive success in piscivorous avian species (Barr, 1986; USFWS, 2003). In terms of the risk to Smallmouth Bass themselves, the concentrations of mercury that were measured in this study are consistent with those demonstrated to pose sublethal risk. The range of Smallmouth Bass mercury concentrations in Great Smoky Mountains National Park reported here were within a similar range to those reported by Blazer et al. (2023) from the Chesapeake Bay watershed (0.059 to 0.977 mg/kg ww), where THg concentrations were associated with a number of adverse health effects in Smallmouth Bass. It is important to note, however, that Smallmouth Bass in Chesapeake Bay are exposed to other stressors including PCBs,

pesticides, and estrogenic chemicals that may act synergistically with mercury contamination to drive observed adverse effects (Blazer et al., 2023). According to Lepak et al. (2016), there is evidence that whole body concentrations of 0.2 mg/kg ww in fish is a threshold value at which changes in gene expression occur, and concentrations of 0.3 mg/kg are evidenced to be a threshold for changes in reproduction, behavior, and histology. After estimating whole body THg concentrations as 74% of fillet THg concentration (Blazer et al, 2023), 38% of Great Smoky Mountains Smallmouth Bass were above the 0.2 mg/kg threshold, and 16.9% were above the 0.3 mg/kg threshold. Particularly concerning was Abrams Creek, where 70% of Smallmouth Bass sampled had concentrations above the 0.2 mg/kg threshold, and 40% had concentrations above 0.3 mg/kg. According to the concentration threshold values reported by Lepak et al. (2016), the THg concentrations measured here indicate that a substantial portion of Smallmouth Bass in GSMNP could be at risk for sublethal health effects.

CHAPTER 5: CONCLUSIONS

The findings of this thesis have direct implications on management of natural resources in GSMNP. The difference in Smallmouth Bass mercury concentrations that we have demonstrated suggests that mercury exposure risk to humans and wildlife is not uniform across Abrams Creek, Little River, and Little Pigeon. As such, future monitoring efforts should continue to incorporate an approach that considers risk assessment on a stream-by-stream basis. Additionally, the magnitude of the difference in Smallmouth Bass mercury concentrations across all three watersheds between years may warrant a re-evaluation of fish consumption advisories set in place in response to the 2016 data. However, since contaminant concentration data are highly variable, long-term monitoring is required to determine trends in mercury concentrations and human exposure risk in GSMNP.

Broadly, the work presented here supports the body of knowledge regarding biomagnification in aquatic ecosystems that are primarily afflicted with non-point source pollution of mercury and represents a contribution to better understanding the food web dynamics and pathways of mercury in natural systems. The role of lower trophic level consumers in the mobilization of mercury into upper trophic levels is a novel finding in these ecosystems and points to the utility of intermediate-level consumers as bioindicators of mercury contamination. The potential decrease in Smallmouth Bass mercury concentration over a six-year period contrasts with observations of increasing mercury concentrations in fish found elsewhere (Gandhi et al., 2014) and may represent a positive development with implications for wildlife and human health.

Our results open the door for future research directions. Further investigation is required to determine factors that underlie environmental flux of mercury in Abrams Creek, Little River, and Little Pigeon, and future investigations should explore the role and dynamics of mercury methylation that may be driving MeHg availability in these important ecosystems. Additionally, more research is needed to monitor growth rates of Smallmouth Bass and the potential relationship those may have to mercury tissue concentrations. If population size can influence growth rates, and growth rates modulate mercury concentrations in Smallmouth Bass in these systems, managers may be able to leverage this knowledge to further reduce risk of human exposure to mercury through contaminated Smallmouth Bass by reducing the population size of these top aquatic predators. Despite a potential decrease in mercury concentrations in GSMNP biota, the mercury concentrations reported in our study still fall within relevant ranges for significant ecological and biological harm. More research is required to determine if mercury contamination in Great Smoky Mountains National Park is indeed causing adverse health effects for these aquatic resources and other members of the food web, especially those that are piscivorous (such as ospreys and otters).

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APPENDIX

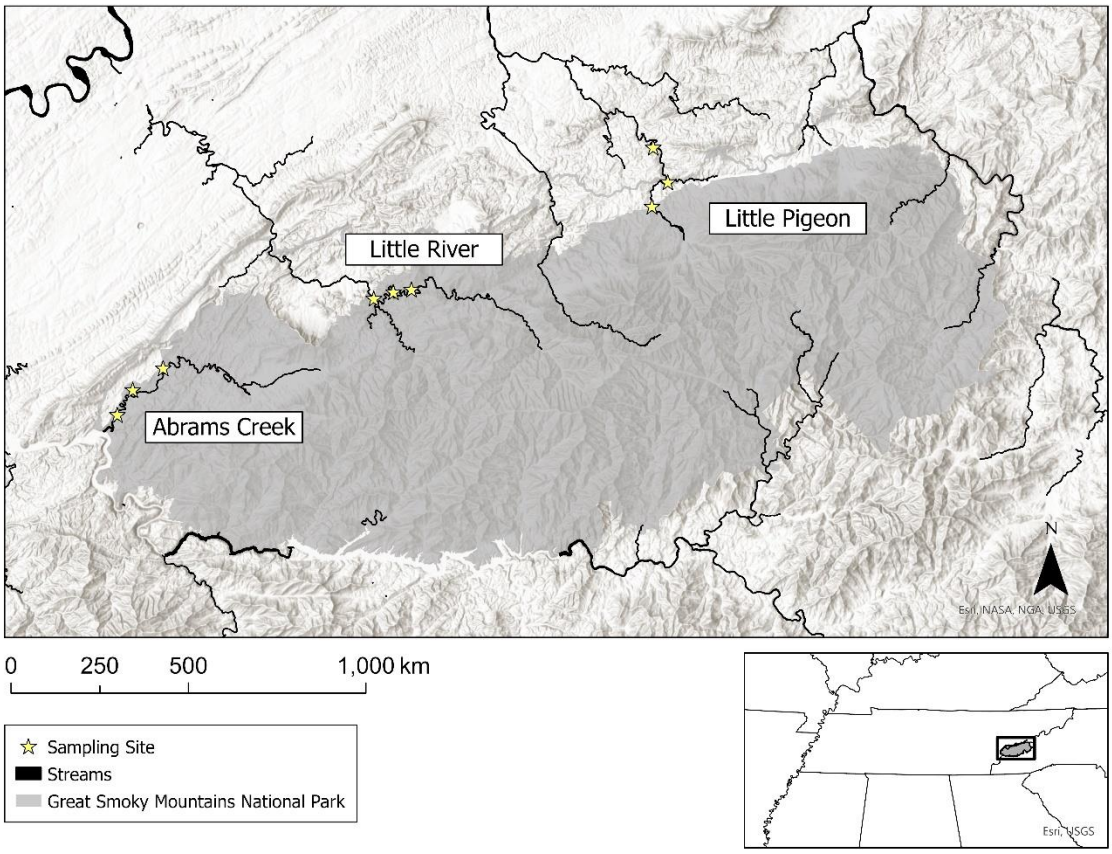


Figure 1. Map of study area. Star symbols denote sampling sites where food web components were collected from Abrams Creek, Little River, and Little Pigeon in GSMNP, Tennessee, USA.

Table 1. Latitude and longitude for collection sites for food web components.

Site	Latitude	Longitude
Abrams Creek		
Upstream	35.60897	-83.93497
Middle	35.59086	-83.96742
Downstream	35.56967	-83.98425
Little River		
Upstream	35.67186	-83.67142
Middle	35.66993	-83.69057
Downstream	35.66472	-83.71137
Little Pigeon		
Upstream	35.73803	-83.41558
Middle	35.75844	-83.3981
Downstream	35.78862	-83.41265

Table 2. Sample sizes of total mercury, (THg), methyl mercury (MeHg), and carbon and nitrogen stable isotope analysis of nitrogen and carbon (SIA).

	Sample Size			
	THg	MeHg	Paired	SIA
Little River				
Detritus	9	3	3	9
Filamentous Algae	-	-	-	8
Periphyton	9	3	3	9
Mayfly nymph	8	3	-	11
Central Stoneroller	5	3	3	14
Crayfish	5	3	3	13
Dragonfly nymph	8	-	-	8
Smallmouth Bass	24	-	-	23
Abrams Creek				
Detritus	9	3	3	9
Filamentous Algae	-	-	-	3
Periphyton	6	4	4	9
Mayfly nymph	4	2	-	7
Central Stoneroller	6	3	3	14
Crayfish	6	4	4	12
Dragonfly nymph	6	-	-	6
Smallmouth Bass	30	-	-	30
Little Pigeon				
Detritus	9	3	3	9
Filamentous Algae	-	-	-	3
Periphyton	9	3	3	6
Mayfly nymph	2	2	-	5
Central Stoneroller	6	3	3	16
Crayfish	6	3	3	12
Dragonfly nymph	6	-	-	6
Smallmouth Bass	17	-	-	17

Table 3. Mean total mercury concentrations (ng/g, dw) of food web components. Within each component, means followed by the same letter are not significantly different. Pairwise differences were evaluated using Tukey's HSD. Significance codes: * = <0.05; ** = <0.01; *** = <0.001.

	THg Dry Weight (ng/g)						
	Abrams Creek	**Little River**	**Little Pigeon**	**Average**	**Df**	**F-value**	**P-value**
Component							
Detritus	18±1 a	19± a	26±4 a	21	(2,24)	1.961	0.163
Periphyton	39±5 a	45±2 a	47±5 a	44	(2,21)	0.902	0.421
Mayfly nymph	111±16 a	66±13 a	114±17 a	97	(2,11)	3.035	0.089
Central Stoneroller	142±15 a	104±10 b	95±9 b	114	(2,14)	4.583	*
Dragonfly nymph	272±51 a	130±11 b	105±16 b	169	(2,17)	9.119	**
Crayfish	399±125 a	177±7 a	148±14 a	241	(2,14)	3.26	0.069
Smallmouth Bass	1,485±140 a	603±53 b	798±74 b	962	(2,68)	19.44	***
Smallmouth Bass Adjusted	1316±509 a	742±258 b	585±228 b	881	-	-	-

Mean±se

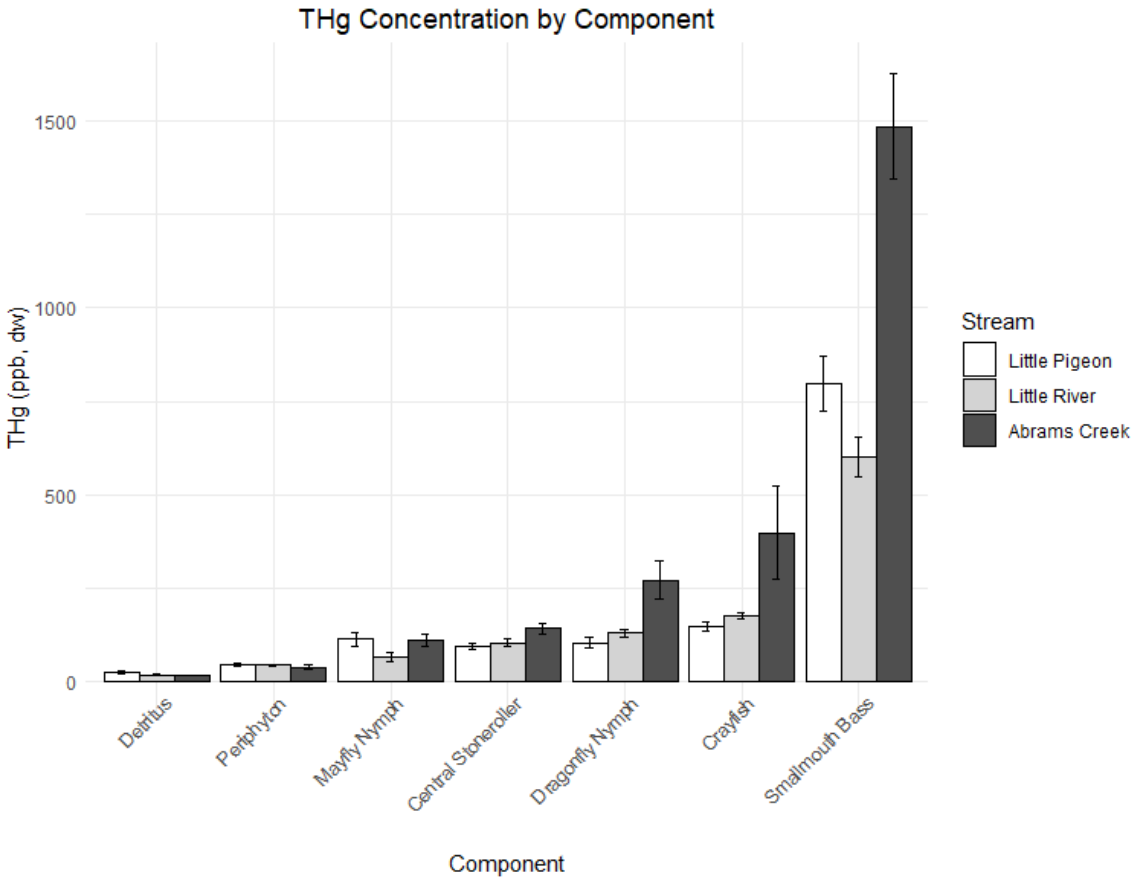


Figure 2. Mean total mercury (THg) concentration (+/- standard error) by food web component, by stream. Concentrations are reported in parts per billion, dry weight.

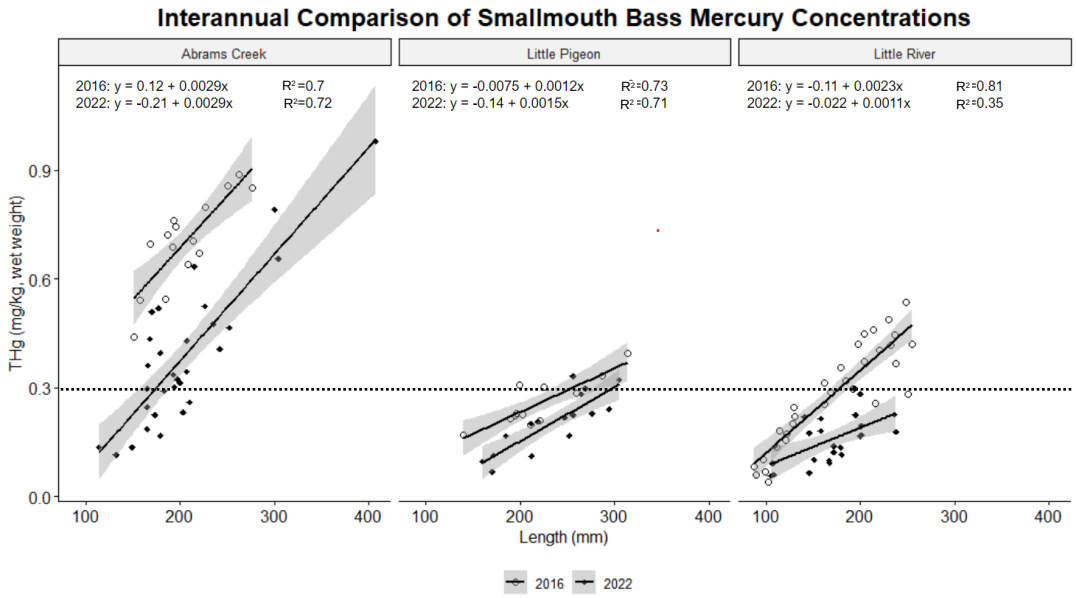


Figure 3. Linear regressions of total mercury (THg) concentration (mg/kg, wet weight) and length (mm) relationships between 2016 and 2022 for Smallmouth Bass in Abrams Creek, Little Pigeon, and Little River shown with standard error (shaded area). Data from 2016 and 2022 are indicated by white circles and black diamonds, respectively. Dotted line represents EPA threshold value for safe consumption (0.3 mg/kg).

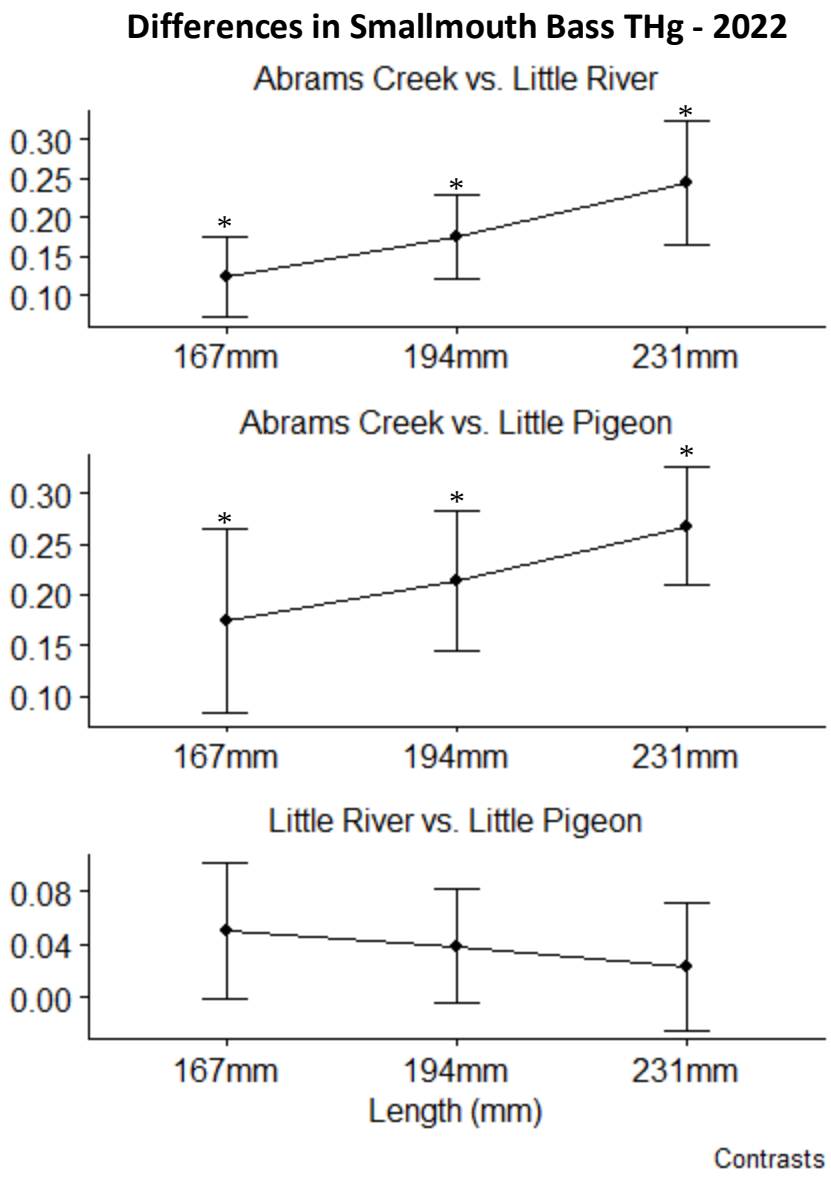


Figure 4. Pairwise comparison of total mercury (THg) tissue concentrations (mg/kg, ww) for Smallmouth Bass in Abrams Creek, Little River, and Little Pigeon. Shown are differences at the 25th, 50th, and 75th percentile of total length for all Smallmouth Bass sampled in 2022 (129mm, 172mm, and 203mm, respectively), calculated by linear regression via R package 'contrast'. Significant differences are indicated by asterisks ($p<0.05$).

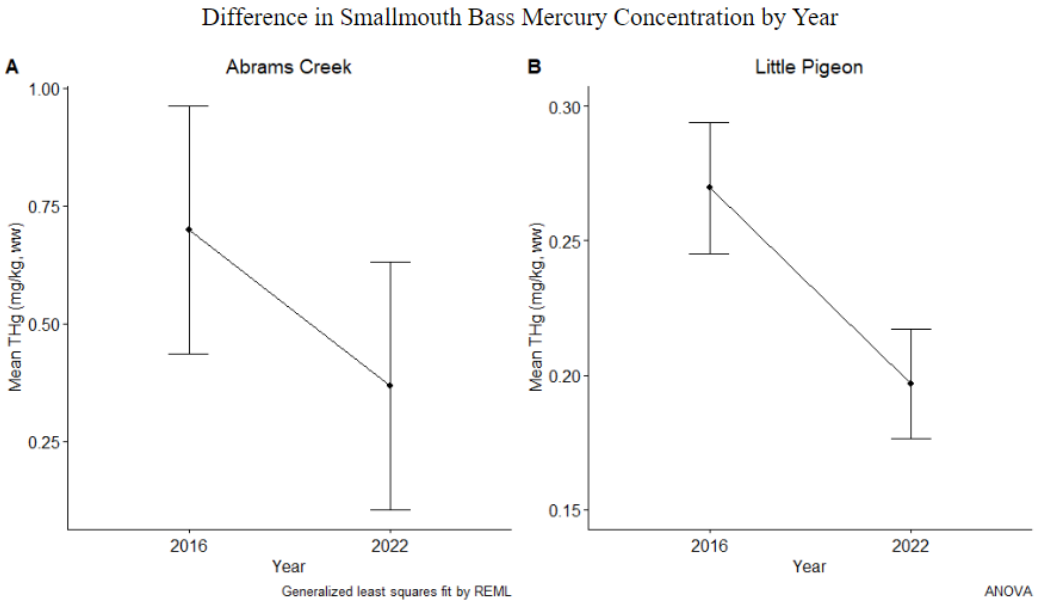


Figure 5. Difference in mean THg tissue concentrations for Smallmouth Bass between 2016 and 2022 in Abrams Creek and Little Pigeon. Shown are arithmetic means and 95% confidence intervals, with concentrations adjusted to stream-specific average Smallmouth Bass total length (Abrams Creek = 204mm, Little Pigeon = 229mm) via regressions using generalized least squares fit by restricted maximum likelihood (REML) for Abrams Creek and analysis of covariance (ANCOVA) for Little Pigeon. Differences were significant for both Abrams Creek ($F(1,41) = 103.864$; $p < 0.05$) and Little Pigeon ($F(1,42) = 106.36$; $p < 0.05$) .

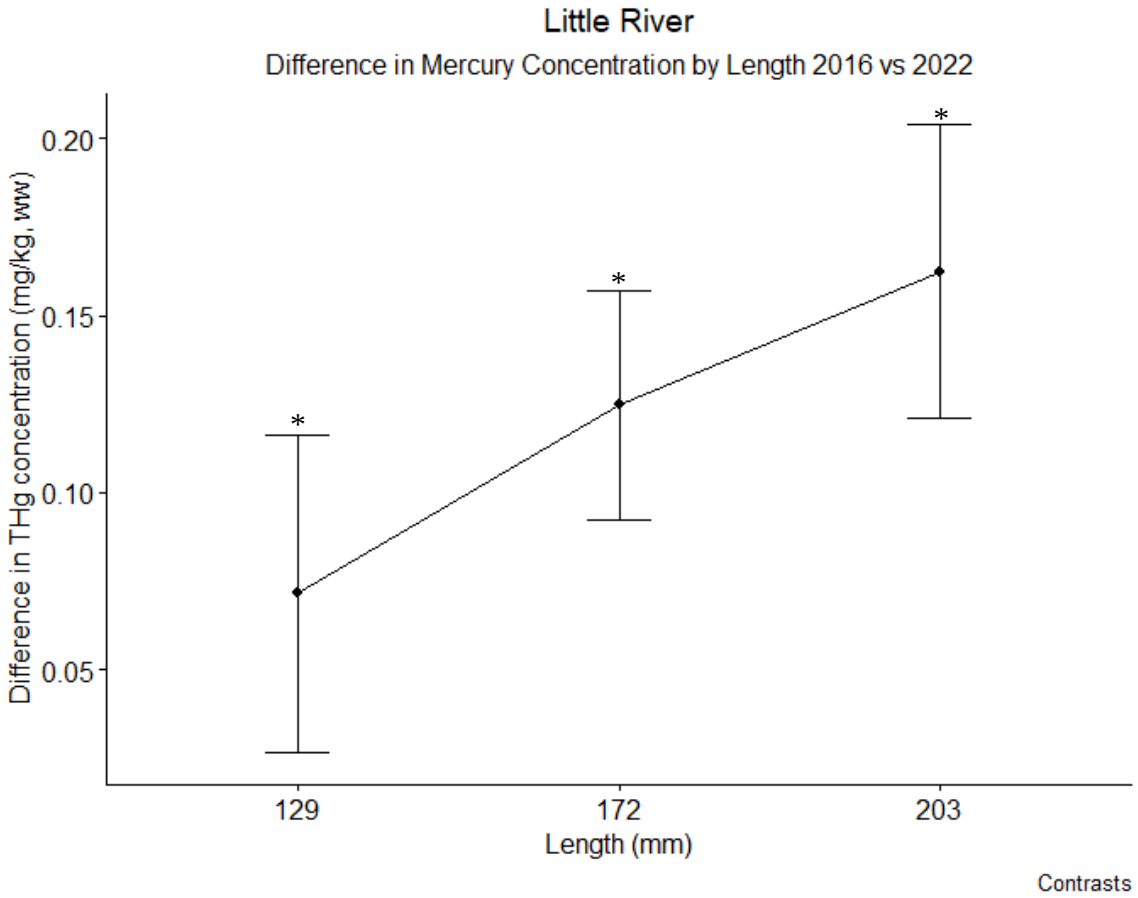


Figure 6. Difference in mean THg tissue concentrations (mg/kg, ww) for Smallmouth Bass between 2016 and 2022 for Little River. Shown are differences at the 25th, 50th, and 75th percentile of total length for all Little River Smallmouth Bass across both years (129mm, 172mm, and 203mm, respectively), calculated by linear regression via R package 'contrast'. Differences in concentrations between years were significant ($F(3,51) = 69.69$; $p < 0.05$) .

Table 4. Mean Smallmouth Bass length (mm) and total mercury fillet tissue concentration (mg/kg, ww) for 2016 and 2022. Also shown are the difference between years and percent decrease from 2016 to 2022.

	Mean Smallmouth THg (mg/kg, ww)				
	Length (mm)	**2016**	**2022**	**Difference**	**%decrease**
Stream					
Abrams Creek	204	0.71	0.38	0.33	46%
Little River	172	0.29	0.17	0.12	41%
Little Pigeon	229	0.27	0.20	0.07	27%

Table 5. Stream-specific total mercury (THg) biomagnification factors (BMFs) for various combinations of food web components. BMFs including length-adjusted values for Smallmouth Bass are denoted by the "adj" subscript.

THg Biomagnification Factors (BMFs)

	Abrams Creek	**Little River**	**Little Pigeon**	**Average**
Component				
Crayfish-Smallmouth Bass	3.4	3.7	5.4	4.2
Crayfish-Smallmouth $Bass_{adj}$	3.3	4.2	4.0	3.8
Periphyton-Mayfly nymph	2.8	1.5	2.4	2.2
Periphyton-Central Stoneroller	3.6	2.3	2.0	2.7
Dragonfly nymph-Smallmouth Bass	5.5	4.6	7.6	5.9
Dragonfly nymph-Smallmouth $Bass_{adj}$	4.8	5.7	5.6	5.4
Central Stoneroller-Smallmouth Bass	10.5	5.8	8.4	8.2
Central Stoneroller-Smallmouth $Bass_{adj}$	9.3	7.1	6.2	7.5

Table 6. Mean (+/- standard error) percent methylmercury by stream.

	%MeHg by Stream		
	Abrams Creek	**Little River**	**Little Pigeon**
Component			
Detritus	56±6	48±12	22±12
Periphyton	25±8	36±17	6±2
Central Stoneroller	114±9	96±3	98±16
Crayfish	116±10	114±25	79±15

Mean±sem

Table 7. Mean estimated MeHg concentrations (ng/g, dw) of food web components. Within each component, means followed by the same letter are not significantly different. Pairwise differences were evaluated using Tukey's HSD. Significance codes: * = <0.05; ** = <0.01; *** = <0.001.

Estimated MeHg (dw, ng/g)

	Abrams Creek	**Little River**	**Little Pigeon**	**Df**	**F-value**	**P-value**
Component						
Detritus	10±1 a	9±1 a	6±1 b	(2,24)	7.55	**
Periphyton	10±1 a	16±1 b	3±0 c	(2,21)	90.56	***
Mayfly Nymph	95±13 a	73±12 a,b	29±10 b	(2,6)	8.199	*
Central Stoneroller	142±15 a	100±10 a,b	93±8 b	(2,14)	5.233	*
Dragonfly Nymph	218±41 a	104±9 a,b	84±13 b	(2,17)	9.119	**
Crayfish	399±125 a	177±7 a,b	117±11 b	(2,14)	3.865	*
Smallmouth Bass	1,485±140 a	603±53 b	798±74 b	(2,68)	19.44	***
Smallmouth Bass Adjusted	1316±509 a	742±258 b	585±228 b	-	-	-

Mean±se

Table 8. Means of measured (+/- standard error) methymercury (MeHg) concentrations for food web components. All components are reported in ng/g dry weight, except those denoted by "(ww)", which are reported on a wet-weight basis.

	Mean MeHg Concentration (ng/g)		
	Abrams Creek	**Little River**	**Little Pigeon**
Component			
Detritus	10±1	10±4	4±3
Periphyton	9±2	17±8	2±0
Mayfly nymph	95±13	73±12	29±10
Central Stoneroller	135±7	91±15	89±11
Central Stoneroller (ww)	39±5	23±5	22±0
Crayfish	494±174	178±8	103±11
Crayfish (ww)	109±41	37±6	19±5

Mean±se

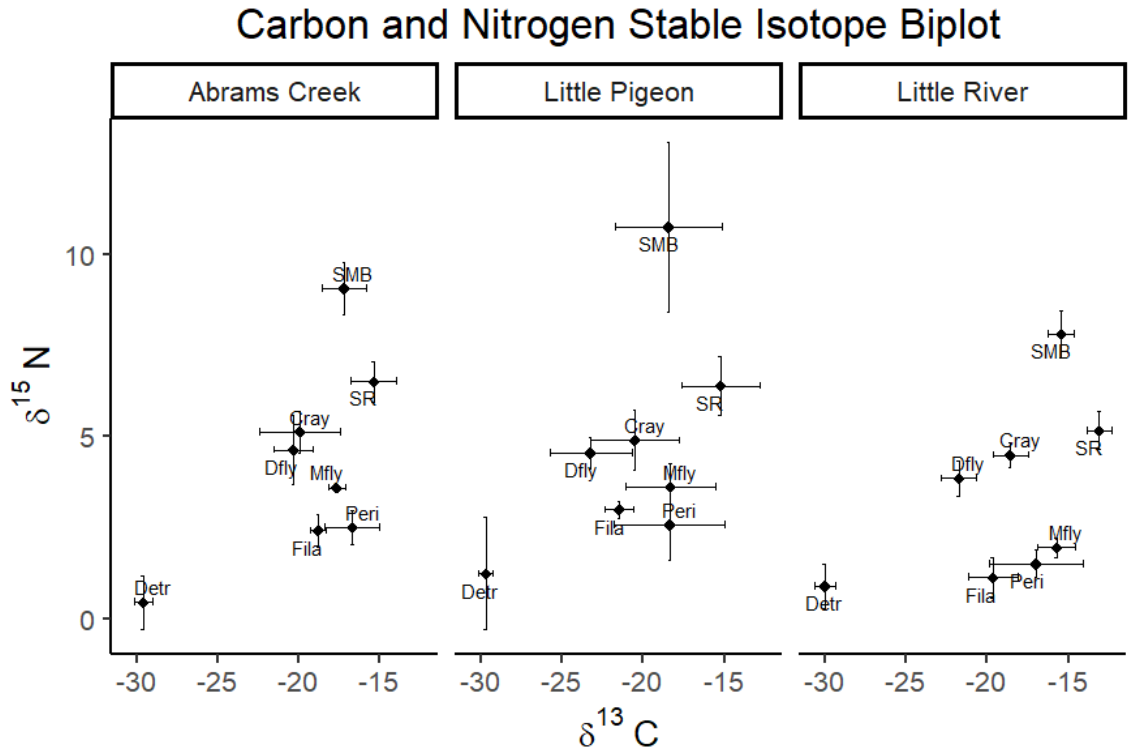


Figure 7. Food web structure, as shown by a biplot of stream-specific mean $\delta^{15}N$ and $\delta^{13}C$ stable isotope values (represented by black dots) for all food web components. Error bars show 95% confidence intervals around the mean. Components are abbreviated as follows: periphyton is shown as "Peri"; dragonfly nymphs as "Dfly"; filamentous algae as "Fila"; mayfly nymphs as "Mfly"; crayfish as "Cray"; Central Stoneroller as "SR"; Smallmouth Bass as "SMB."

Table 9. Average (+/- standard error) trophic position for consumers, calculated on a per-stream basis. Within each component, means followed by the same letter are not significantly different. Pairwise differences were evaluated using Tukey's HSD. Significance codes: * = <0.05; ** = <0.01; *** = <0.001.

Average Trophic Position

	Abrams Creek	**Little River**	**Little Pigeon**	**Df**	**F-value**	**P-value**
Component						
Mayfly Nymph	1.33±0.02 a	1.17±0.02 b	1.24±0.07 a,b	(2,21)	6.604	**
Dragonfly Nymph	1.77±0.10 a	1.79±0.05 a	1.70±0.06 a	(2,17)	0.484	0.625
Central Stoneroller	2.08±0.05 a	2.08±0.04 a	1.94±0.04 a	(2,41)	3.466	*
Crayfish	1.90±0.06 a	1.94±0.03 a	1.70±0.07 b	(2,34)	6.233	**
Smallmouth Bass	2.92±0.05 a	2.88±0.04 a	3.34±0.19 b	(2,67)	6.237	**

Mean±se

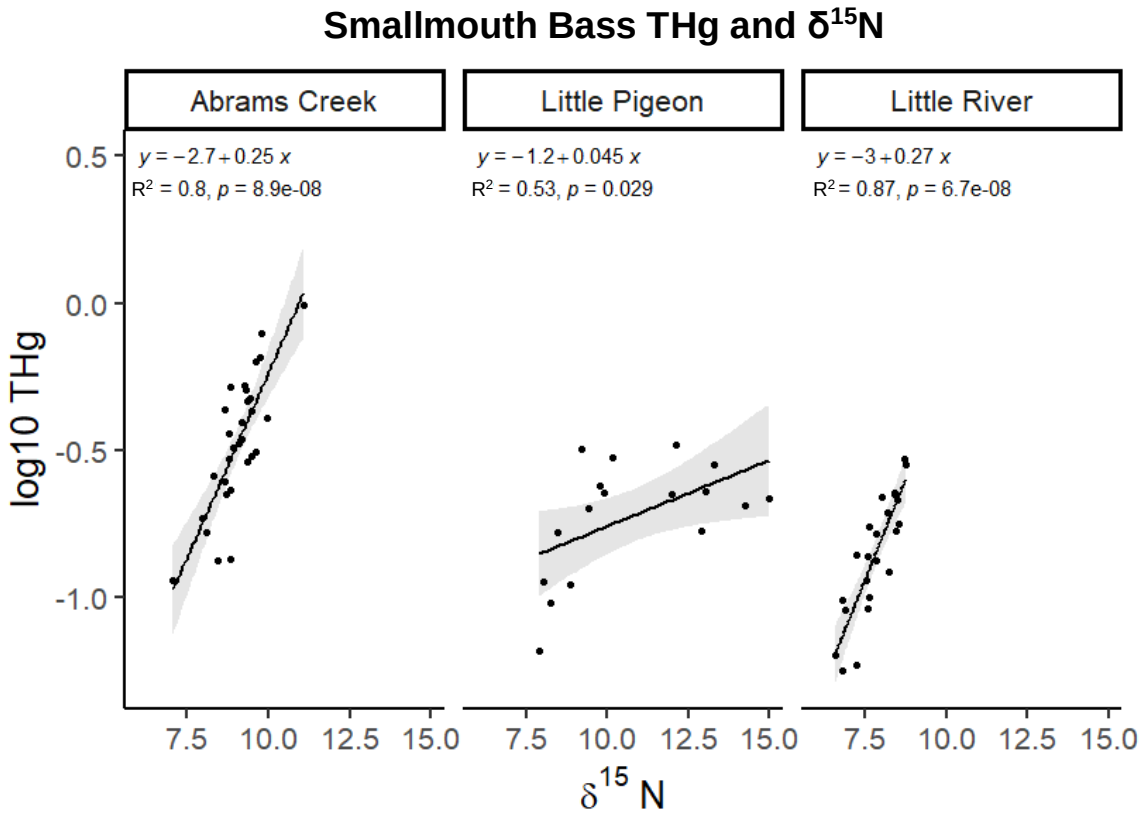


Figure 8. Linear regression between $\log_{10}$-transformed total mercury fillet tissue concentration and $\delta^{15}N$ value, shown with standard error (shaded area) for Smallmouth Bass from Abrams Creek, Little Pigeon, and Little River.

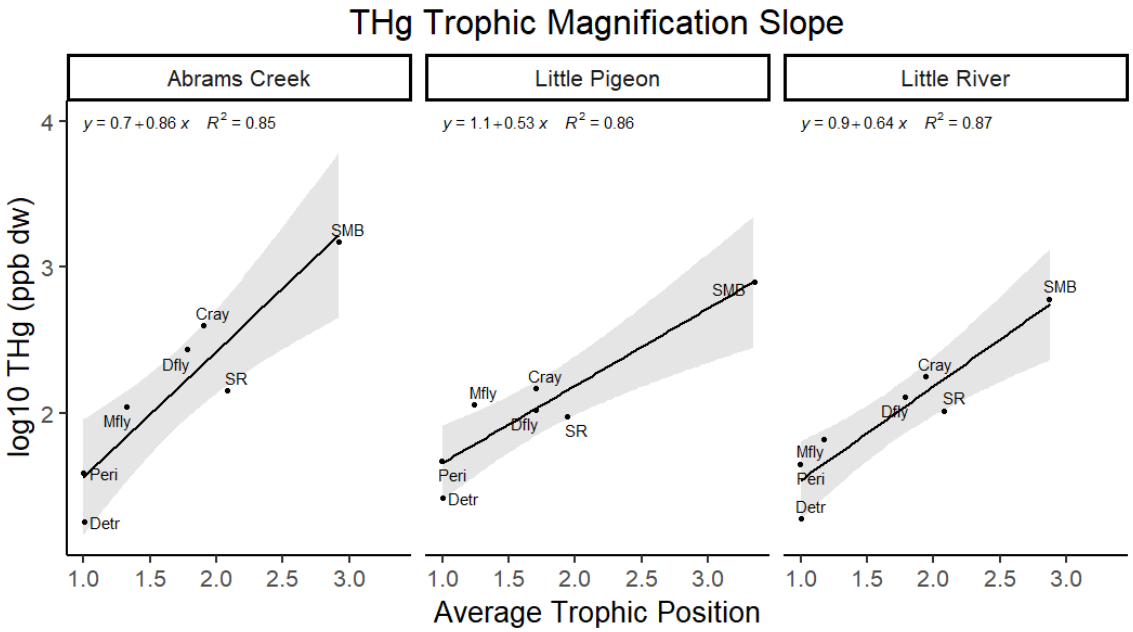


Figure 9. Linear regression of log_{10}-transformed THg concentration (parts per billion, dry weight) and trophic position for each food web component, shown with standard error (shaded area). Dots represent stream-wide averages for each component.

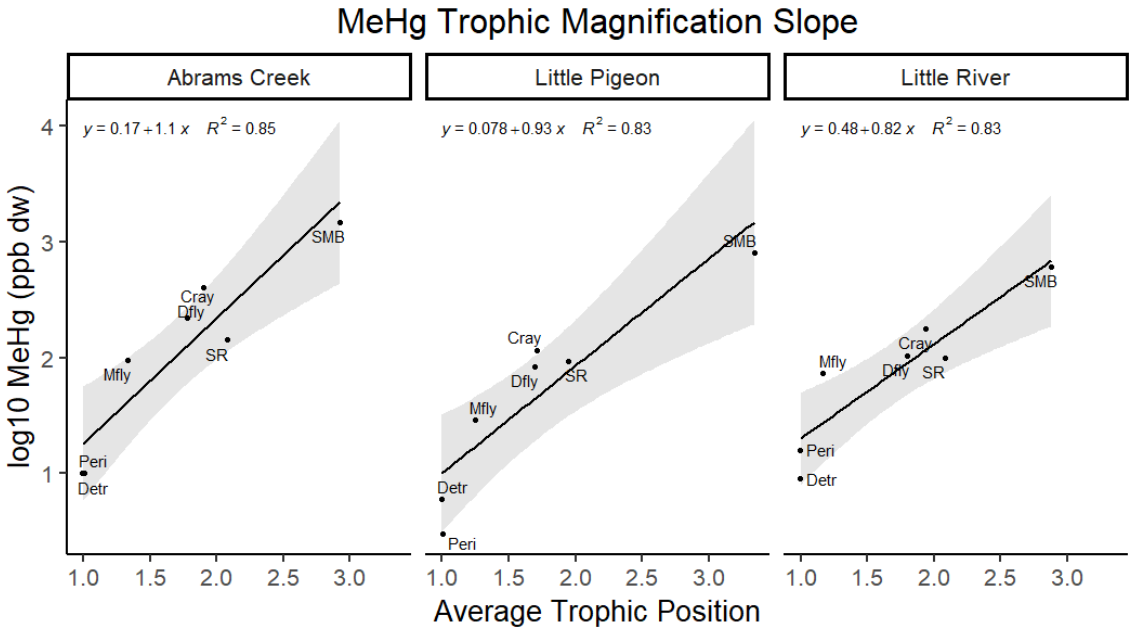


Figure 10. Linear regression of log_{10}-transformed THg concentration (parts per billion, dry weight) and trophic position for each food web component, shown with error bars (shaded area). Dots represent stream-wide averages for each component.

VITA

Zachary “Winston” Clark was born in New Haven, Connecticut on December 2nd, 1997. Winston graduated from Northwest Cabarrus High School in 2016 and received his B.S. in Fisheries, Wildlife, and Conservation Biology four years later in 2020. He was accepted to graduate school at the University of Tennessee, Knoxville in January 2021 and is a candidate for the Master of Science degree in Wildlife and Fisheries Science.