MERCURY DYNAMICS IN SOUTHERN APPALACHIAN MOUNTAIN AQUATIC AND RIPARIAN FOOD WEBS

by

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ABSTRACT

The understanding of how community level trophic ecology affects the bioaccumulation/biomagnification of contaminants, and more specifically Hg in streams, remains largely unstudied. The current paradigm was built largely on the backs of studies that investigated lowland lakes and wetlands with an industrial point source. Growing evidence suggests that in river and stream systems, where the source of Hg is atmospheric, the old paradigm must be reevaluated. The chapters of this dissertation examined the biomagnification potential of mercury (Hg) and the role of community level trophic ecology (CLTE) in six southern Appalachian Mountain headwater aquatic and riparian food webs. Utilizing ecological tracers like naturally abundant stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), this research determined that the community standard ellipse area (SEA_c) influenced THg biomagnification, and that carbon normalized to the trout baseline ($\delta^{13}C_E$) was the primary driver of total mercury (THg) bioaccumulation in trout. Although the SEA_c of each respective reach's neighboring aquatic and riparian communities had a high degree of trophic overlap in $\delta^{13}C$ and $\delta^{15}N$ biplot space, when the trophic level of riparian taxa was used to test the predictive function of aquatic THg biomagnification, the aquatic biomagnification model consistently underestimated concentrations in tetragnathids, araneids, and striders. These high Hg concentrations in spiders could cause harm to adult and nestling passerine birds inhabiting the southern Appalachian Mountains. This research sought to understand how riparian spiders can be better utilized with applied research (e,g,, ecological risk assessments) and the assumptions that researchers make when sampling. This research demonstrated that the selection of

which spider to use as a surrogate for "all spiders" can influence the characterization of risk to passerine birds, and that tetragnathids provide a more conservative estimation of risk than araneids. Additionally, this research sought to understand the assumptions of field collected tetragnathids. The research suggests that spiders could be sampled without regard for sex: female tetragnathids were significantly larger than male spiders and represented a larger proportion of spiders collected at all sites; however, no differences in growth dynamics, isotopic signature ($\delta^{13}C$ and $\delta^{15}N$), or THg concentrations were observed. Additionally, this research demonstrated that the leg of a tetragnathid can accurately represent the stable isotope signature of an entire spider.

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INTRODUCTION

Mercury (Hg) is a potent neurotoxin, nephrotoxin, and immunotoxin, and its ability to cross the blood-brain and placental barriers makes it a powerful teratogen and a significant threat to human and wildlife health (Tchounwou et al. 2003; Clarkson and Magos 2006; Scheuhammer et al. 2007; Driscoll et al. 2013; Eagles-Smith et al. 2018; Ralston and Raymond 2018). The most famous example of Hg poisoning occurred in the late 1940s/early 1950s when the Chisso chemical company discharged its industrial wastewater into the Minamata River, and the Hg-laden effluent eventually settled downstream in Minamata Bay and accumulated in the tissues of fish and shellfish (Hachiya 2006). The villagers and wildlife inhabiting Minamata that consumed the contaminated fish and shellfish exhibited severe neurological distress, and the Hg induced neurological syndrome was later termed "Minamata disease" (Kurland et al. 1960).

Almost 80 years later, Hg remains a contaminant of concern because it is naturally occurring (e.g.,, volcanic activity), anthropogenically enriched (e.g.,, artisanal gold mining and coal combustion), and capable of biomagnifying in aquatic habitats (Selin 2009; Lavoie *et al.* 2013; Streets et al. 2017). Gaseous Hg, produced via natural and anthropogenic processes, can travel long distances and deposit in remote areas (Selin 2009; Risch *et al.* 2012; Weiss-Penzias et al. 2016; Risch *et al.* 2017, Streets et al. 2018). Atmospherically deposited inorganic Hg may make its way into the adjacent aquatic habitat where it can be methylated by microorganisms (e.g.,, sulfate- and iron-reducing bacteria) that reside in anoxic sediment (Ullrich *et al.* 2001; Hsu-Kim et al. 2013). The result of this process is a less polar form of Hg, methyl-mercury (MeHg). MeHg more efficiently crosses

membranes than Hg; therefore, the tissues of primary producers (e.g., algae) reach higher concentrations than that of the aquatic environment, a process referred to as bioconcentration (Morel et al. 1998). Once integrated into the tissues of these primary producers, they serve as a dietary source of MeHg to primary consumers; because dietary MeHg is integrated into the tissues of primary consumers, this process is referred to as bioaccumulation (Borgå et al. 2012). Additionally, because the dietary absorption of MeHg is greater than its elimination, the concentration of MeHg increases with each successive step in the food chain, a process referred to as biomagnification (Kidd *et al.* 2012).

It is important to note, MeHg is often referred to as the "bioaccumulative and toxic form of Hg." However, MeHg analysis is expensive and specialized, so it is often more convenient and less cost prohibitive to analyze all Hg species (inorganic Hg + MeHg) at once and report total mercury (THg) concentrations. "THg" is often distinguished from "Hg" when specifying analysis and/or methodology. In writing, "Hg" connotes a theoretical and/or holistic viewpoint; whereas, "THg" implies a specific type of analysis.

Methyl-mercury's capacity to biomagnify and its neurotoxicity are inherently linked (Bridges and Zalups 2005; Hoffenmeyer 2006; Clarkson and Magos 2006; Ralston and Raymond 2018). For instance, when MeHg binds to the thiol (SH) group on the amino acid cysteine (Cys), the resulting MeHg-Cys complex mimics the molecular structure of the essential amino acid methionine (Figure 1; Simmons-Willis et al. 2002; Bridges and Zalups 2005; Hoffenmeyer 2006). This enables MeHg-Cys to cross cellular membranes by transport proteins like the L-type neutral amino acid carrier transport (LAT) system, and it

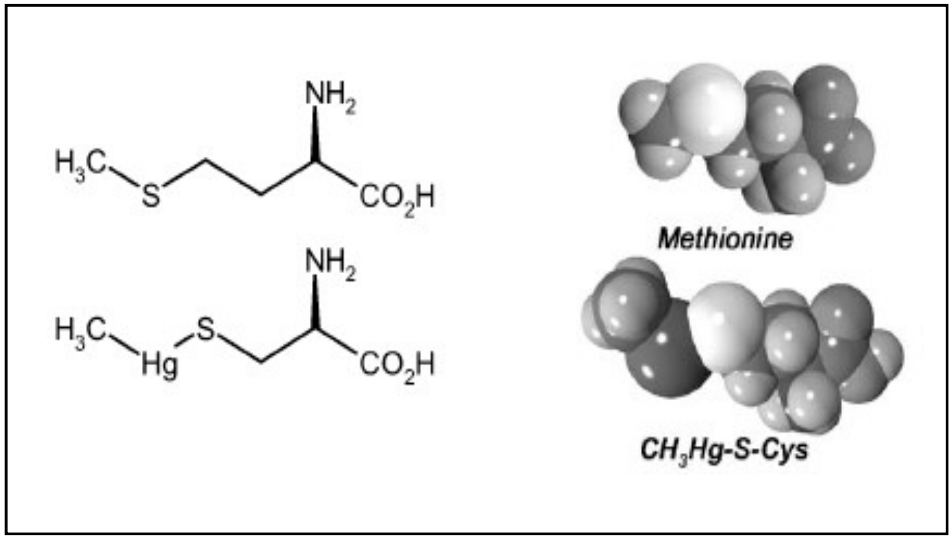


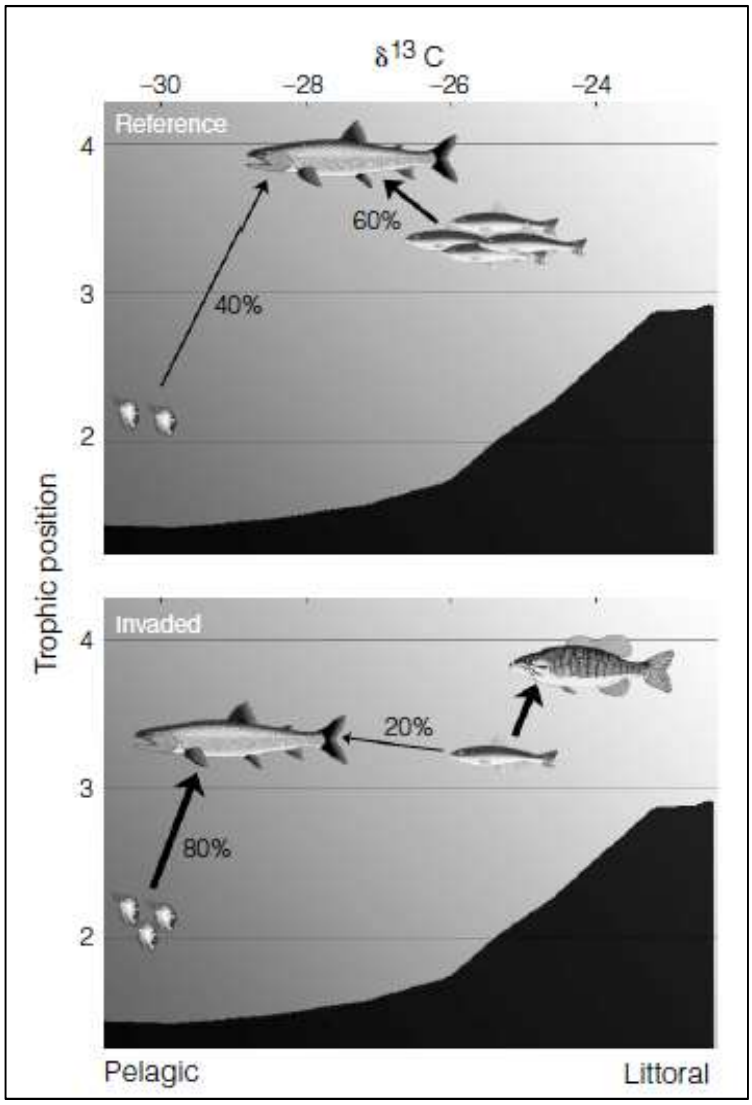
Figure 1. The structural model (top left) and space-filled model (top right) of the amino acid methionine and the structural model (bottom left) and space-filled model of the cysteine bound MeHg conjugate (modified from Bridges and Zallups 2005 and Hoffenmeyer et al. 2006).

also enables MeHg-Cys to cross the blood-brain and placental barriers (Zimmerman et al. 2014).

The exact mechanism of neurotoxicity is less well understood, but the general scientific consensus is that once MeHg-Cys crosses the blood-brain or placental barrier, it is exposed and depresses selenium dependent enzymes, like glutathione peroxidase (GPX), that protect the brain from oxidative stress (Ralston et al. 2007; Khan and Wang 2009; Korbas et al. 2010; Ralston and Raymond 2018). The ensuing oxidative stress has led to varying degrees of toxicity associated with neuronal cell loss in the brain; toxicity is characterized by broad neurological symptoms that include paresthesia (numbing and tingling of skin and extremities), ataxia (loss of coordination), dysarthria (gross motor speech disfunction), vision loss, coma, and death (Clarkson and Magos 2006; Ralston and

Raymond 2018). Concerning for humans, this neurotoxic molecular mimic, MeHg-Cys, is the primary form of MeHg in fish (Harris et al. 2003; Lemes and Wang 2009).

To track MeHg as it biomagnifies through aquatic food chains, the use of ecological tracers is often necessary. Two of the most common tracers are naturally abundant carbon stable isotope ratios ($^{13}C/^{12}C$, hereafter referred to as $\delta^{13}C$) and nitrogen stable isotope ratios ($^{15}N/^{14}N$, hereafter referred to as $\delta^{15}N$). Traditionally, $\delta^{13}C$ and $\delta^{15}N$ have been used in tandem to inform the contribution of different energy sources (e.g.,, benthic or pelagic) to fish diets (Vander Zanden and Vadeboncoeur 2002) and relational information about trophic position (Vander Zanden and Rasmussen 1999; Post 2002; Layman et al. 2007). Often, the data is illustrated as one or a series of $\delta^{13}C$-$\delta^{15}N$ bi-plots, where $\delta^{13}C$ is plotted on the x-axis and represents the energy



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source, and $\delta^{15}N$ (or a derivative trophic position) is plotted on the y-axis (Figure 2; Vander Zanden *et al.* 1999).

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OBJECTIVES

The objectives of this dissertation were:

1) to investigate the biomagnification potential of nonpoint source (atmospheric) Hg and the role trophic dynamics play in the movement of Hg through southern Appalachian Mountain headwater streams food webs.
2) to better understand the relationship of Hg trophic magnification in the aquatic food web with the Hg concentrations of neighboring riparian predators in southern Appalachian Mountain headwater streams.
3) to characterize the potential risk that Hg in wildlife poses to humans, piscivorous wildlife, and arachnivorous birds in southern Appalachian Mountain headwater streams.
4) to better understand the effects of behavior and physiology on tetragnathid spiders as bioindicators of aquatic Hg contamination

CHAPTER I: THE EFFECT OF COMMUNITY LEVEL TROPHIC ECOLOGY ON MERCURY BIOMAGNIFICATION IN SOUTHERN APPALACHIAN MOUNTAIN HEADWATER STREAMS

Introduction

Mercury (Hg) is a ubiquitous global contaminant. When in its gaseous form, Hg is capable of traveling long distances and depositing in and along streams, including those typically thought of as pristine (Selin 2009). Atmospherically deposited inorganic Hg may make its way into the adjacent aquatic habitat where Hg can be methylated by microorganisms (e.g., sulfate- and iron-reducing bacteria) that reside in anoxic sediment (Ullrich et al. 2001; Hsu-Kim et al. 2013). Once methylated, Hg may bioaccumulate in primary producers and biomagnify as it moves up successive links in a food chain. The biomagnification of Hg can lead to concentrations in aquatic predators that are over a million times higher than that found in water (Kidd et al. 2012). There are many cases where Hg tissue concentrations from remote fish populations have exceeded a threshold of ecological concern (Driscoll et al. 2007; Walters et al. 2015).

Food web structure is one of the main ecological mechanisms expected to control the bioaccumulation and biomagnification of contaminants, like Hg, in aquatic systems (Eagles-Smith et al. 2018). Considering biomagnification studies are concerned with contaminant movement, trophic magnification factors (TMFs), a value that represents the average biomagnification of the contaminant as it moves up each successive trophic level (TL), are typically calculated (Borgå et al. 2012). The same ecological tracer used to

calculate the TL of a consumer (stable nitrogen isotopes [$\delta^{15}N$]) (Post et al. 2002; Post et al. 2007) can also be used to calculate food-chain length (FCL). Perhaps naturally, biomagnification studies have primarily described "the effect of food web structure" as "the effect of FCL," while other ecological metrics, like "the effect of energy flow" (using stable carbon isotopes [$\delta^{13}C$]), have been considered less frequently (Won et al. 2017).

The effect of energy flow, inferred from $\delta^{13}C$ signatures, has been integrated into the analysis of food web structure by providing information about the organism(s) isotopic niche (Newsome et al. 2007). This has been traditionally accomplished by establishing the carbon resource pool and the baseline TL of primary consumers. This information is used to determine other organism's relative reliance on the resource pool as well as TL (Vander Zanden & Rasmussen 1999). A few studies have used TL and FCL to inform contaminant bioaccumulation/biomagnification dynamics (Ouedraogo et al. 2015; Azevedo-Silva et al. 2016; Mazzoni et al. 2018). However, a more comprehensive view of food web structure is obtainable by using $\delta^{13}C$ and $\delta^{15}N$ to calculate not only FCL and resource pool contributions, but also other metrics that measure trophic diversity, food web structure, and biodiversity (Layman et al. 2007). These metrics permit a quantitative assessment of trophic structure and may better inform contaminant bioaccumulation/biomagnification dynamics. Only recently have researchers explored these connections, for instance Corsolini & Sara (2017) characterized the trophic diversity of polar food web communities using $\delta^{13}C$ and $\delta^{15}N$ signatures that were also contaminated with persistent organic pollutants.

Increases in biodiversity may promote a more diverse diet that increases tissue turnover and leads to the biodilution of contaminants; however, the understanding of how community level trophic ecology affects the bioaccumulation and biomagnification of contaminants, and more specifically Hg in streams, remains largely unstudied (Borgå et al. 2012). A recent worldwide investigation into Hg bioaccumulation and biomagnification yielded only 74 stream sites with viable data (THg + MeHg) for inclusion in the meta-analysis (Lavioe et al. 2013). Only 50 of those stream sites were unique, and only 10 streams were from the USA (21 were in New Brunswick Canada, Jardine et al. 2013). Notably, of those 10 sites, none were in the southern Appalachian Mountains, a recognized hot-spot for atmospheric Hg deposition (Risch et al. 2017), and none of these studies used community level ecological metrics to describe the trophic ecology of the stream.

The purpose of this research was to investigate the biomagnification potential of Hg and the role that community level trophic ecology (CLTE) has on Hg dynamics in southern Appalachian Mountain headwater streams. The specific objectives of this research were to (1) describe the trophic structure of Appalachian Mountain headwater stream food webs in isotopic space (2) assess Hg tissue concentrations for the members of these food webs, (3) characterize Hg biomagnification at each reach (4) assess the effect of CLTE metrics on Hg tissue concentrations and Hg biomagnification.

Materials and Methods

Site Description

Six 100 m study reaches were selected among four headwater streams (Figure 1) that fall within the Unaka Range of Tennessee's Appalachian Mountains (Blue Ridge

Mountains, ecoregion 66; Omernik 1995), an area prone to high levels of atmospheric mercury deposition (Risch et al. 2017). Although as much as 98% of southern Appalachia has been altered directly by human disturbances (Gragson & Bolstad 2006), these four study streams are afforded some level of state or federal protection and have no documented upstream mining (USGS 2005). Additionally, these study streams either directly overlap or are in close proximity to streams recently characterized as part of the Tennessee's Ecologically At-Risk Streams (TEARS) project conducted by the Tennessee Wildlife Resources Agency (Olson et al. 2019A). Briefly, the TEARS project characterized fish contaminant burdens in regards to Hg, polychlorinated biphenyls (PCBs), phthalate esters, organochlorine (OC) pesticides, dioxins, and furans. Olson et al. (2019A) reported low variability in stream specific environmental factors expected to affect Hg biomagnification such as pH (Jardine et al. 2013), dissolved organic carbon (Chasar et al. 2009) and wetland density within the catchment (Greenfield et al. 2001; Brigham et al. 2009; Marvin-Pasquala et al. 2009). Additionally, PCBs and phthalates were below their respective method detection limits, and all OC pesticides, dioxins, and furans were below their respective quantitation limits, indicating that none of these organic contaminants were a current concern at these study reaches (Olson et al. 2019A).

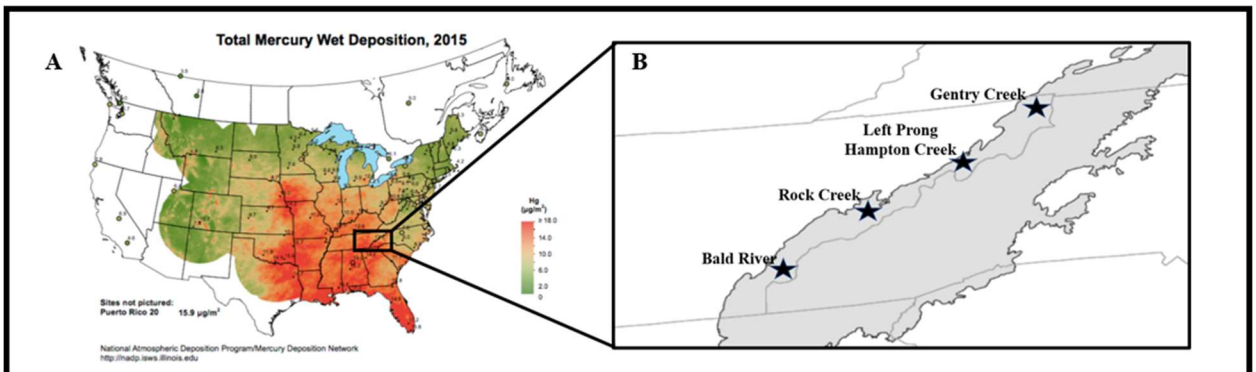
Two study reaches were established at Bald River in Monroe County, TN within Cherokee National Forest (CNF). Bald River has a natural water fall that prevents Rainbow Trout (*Oncorhyncus mykiss*) from encroaching on the upstream allopatric Eastern Brook Trout (*Salvelinus fontinalis*) populations. The study reach named Bald River Upstream was approximately 20 m upstream of the waterfall. The study reach named Bald River

Downstream was established ~200 meters downstream of the waterfall, in an area dominated by Rainbow Trout.

The study reach named Rock Creek is in Cocke County, TN. Rock Creek is within the boundaries of the Great Smoky Mountain National Park (GSMNP). The reach was established ~800 meters upstream of a natural barrier that prevent Rainbow Trout from encroaching on the established population of Eastern Brook Trout.

Two study reaches were established at Left Prong Hampton Creek (LPHC), Carter County, TN within CNF and the Hampton Creek Cove Natural Area which is managed by the Tennessee Department of Environment and Conservation (TDEC). To prevent Rainbow Trout from encroaching on Eastern Brook Trout territory, TWRA and TDEC coordinated the construction of a man-made barrier/waterfall in LPHC. An Eastern Brook Trout dominated reach, named LPHC Upstream, was established ~1,300 meters upstream of this barrier. A Rainbow Trout dominated reach, named LPHC Downstream, was established ~900 meters downstream of the barrier.

The study reach named Gentry Creek was established in Gentry Creek, Johnson County, TN within CNF. Gentry Creek has a fish assemblage consisting of Eastern Brook Trout, Rainbow Trout, and Mottled Sculpin (*Cottus bairdii*).



C Reach	Latitude	Longitude	Elevation (m)	Catchment Area (km^2)
Gentry Creek	36° 33.568'N	81° 42.669'W	970	5.5
LPHC Upstream	36° 8.382'N	82° 2.787'W	946	4.4
LPHC Downstream	36° 9.037'N	82° 3.107'W	1,070	2.6
Rock Creek	35° 45.239'N	83° 12.845'W	721	3.4
Bald River Downstream	35° 16.380'N	83° 8.517'W	300	11.7
Bald River Upstream	35° 16.278'N	83° 8.295'W	688	6.2

Figure 1. Mercury deposition map of the United States (A) and inset of streams (B) with gray outline of the Blue Ridge Ecoregion (level III ecoregion 66). The downstream point of stream reach locations (GPS points provided in degrees, decimal minutes [WGS-84]) and reach-specific basin characteristics (C).

Sample Collection

All animals were collected from their respective reaches in August of 2015. Invertebrate and larval salamander taxa were selected because their geographic distribution covered the entire footprint of the study. Bald River Upstream Eastern Brook Trout, Rainbow Trout, and larval caddisfly collection, processing, and Hg concentrations were previously reported by Olson et al. (2019B). Trout and larval salamanders were approved by the MTSU Institutional Animal Care and Use Committee (Protocol ID: 15-013; Appendix A). Unless stated otherwise, biological samples were placed in aluminum foil for THg and polypropylene tubes for stable isotope analysis. Samples and then stored on wet ice during transport to the laboratory, where they were stored at -20°C to prior to chemical analysis.

All fish were collected via electrofishing, verified to species, and measured for total length. Adult trout within a specified size range (140–165 mm) were kept; of those, 10 were kept and euthanized (all others were released). Longnose Dace (*Rhinichthys cataractae*) at Bald River Downstream and LPHC Downstream and Mottle Sculpin (*Cottus bairdi*) at Gentry Creek were collected (n = 10/reach). Hereafter, Longnose Dace and Mottled Sculpin are collectively referred to as forage fish. Fish were euthanized in accordance with, they were wrapped in aluminum foil, placed into individually labeled plastic bags. Fish processing consisted of measuring total length and total weight, certifying fish species, and the removal of the gastrointestinal tract and caudal fin. Caudal fins were placed into individual polypropylene tubes for stable isotope analysis (SIA; n = 10). Trout composite samples were analyzed for THg. Due to the biomass requirements of

companion studies (Olson et al. 2019A) trout-specific composites of equal mass were created by pairing larger fish with smaller fish (n = 5/reach).

Crayfish were targeted bycatch during the electrofishing process and organized into separate buckets after capture. The largest 5 crayfish from each reach were selected, wrapped in tinfoil boats and euthanized. Each crayfish had a small (~20 mg) portion of their tail muscle removed for SIA. The gastrointestinal tract was excised and discarded. The remaining crayfish was homogenized and analyzed for THg.

Larval caddisflies (*Pycnopsyche spp.*) collected in the present study are primarily shredders (Morse & Holzenthal 2008). Larval caddisflies were hand-collected from rocks within each study reach, removed from their cases, and sorted into three polypropylene tubes (9–13 animals/tube) and analyzed for THg. Additionally, five animals were placed into individual polypropylene tubes for SIA.

Larval stoneflies (Family Perlidae) were hand-collected from the underside of rocks or were collected as bycatch during the fish collection process. Five animals were placed into individual polypropylene tubes for SIA.

Larval Black-bellied Salamanders (*Desmognathus quadramaculatus*) were targeted bycatch during the fish collection process. *D. quadramaculatus* have a relatively long larval period (up to 4 years), so older larvae were easily distinguished from congeners by having a snout vent length (SVL) > 38 mm (Petranka 1998; Niemiller & Reynold 2011). When SVL was < 38 mm, *D. quadramaculatus* were indistinguishable from *Desmognathus marmoratus* (Niemiller & Reynolds 2011). However, these sister taxa are closely related with no known functional feeding differences (Titus and Larson 1996; Petranka 1998;

Niemiller & Reynolds 2011). Hereafter the term "larval salamander" refers to the collection of these two taxa combined. Larval salamanders were identified, measured for SVL, and the largest 6 were euthanized. All other larval salamanders were returned to the stream reach from which they were collected. All euthanized larval salamanders were wrapped in aluminum foil and placed into individually labeled plastic bags. Larval salamander processing consisted of measuring total length, SVL, total weight, removing the gastrointestinal tract, and excising a portion of the liver. The liver samples were placed into individual polypropylene tubes for SIA.

Mercury Analysis

All larval caddisfly samples were homogenized and analyzed for THg according to U.S. EPA Method 1631 (U.S. EPA 2002). Briefly, composite samples were homogenized and stored frozen in acid-cleaned glass fluoropolymer jars. Samples were then transferred to a digestion vessel, digested with HNO_3 and H_2SO_4 on a 58°C hot block for one hour. Once cooled, samples were diluted with 0.02 N BrCl and left at room temperature for an additional 4 hours. Prior to analysis, an initial calibration verification was tested, and a continuing calibration verification was performed every ten samples. Analysis of total mercury was conducted utilizing an Analytik Jena (Jena, Germany) automated mercury analyzer. All samples were analyzed alongside method blanks (all reagents) and a laboratory control sample (LCS) and laboratory control sample duplicate (LCSD). Method blanks were undetectable (< 0.32 ng/g) and below the method reporting limit of 1.1 ng/g ww. Mean ± SD %-recovery for LCS and LCSD were 91 ± 4% and 94 ± 9%, respectively. The mean ± SD relative percent difference (RPD) of the paired LCS and LCSDs was 4 ±

3%, and all were within acceptable ranges. Sample quality assurance included the use of Tort-3, a standard reference material (SRM), that was used to make one SRM blank, and matrix spike (MS) and matrix spike duplicates (MSD). In all cases, the %-recovery of the SRM blank, MS, and MSDs were within the acceptable range. Mean ± %-recovery of MS and MSDs were 102 ± 3% and 104 ± 1%, respectively.

Trout and crayfish samples were analyzed for THg via oxidation, purge and trap, desorption, and cold vapor atomic absorption spectrometry in accordance with U.S. EPA Method 7473 (U.S. EPA 1998) utilizing a Milestone Direct Mercury Analyzer (DMA-80; Sorisole, Italy). All samples were analyzed alongside a method blank (all reagents). In one instance, a method blank was above the method detection limit of 0.032 mg/kg; this method blank was below the method reporting limit of 0.020 mg/kg ww. Sample quality assurance included the use of Tort-3, which was used to make SRM blanks, MS, and MSDs. In all cases SRM blanks, MS, and MSDs were within the acceptable range. Mean ± SD %-recovery for MS and MSD were 95 ± 3% and 98 ± 9%, respectively. Each paired MS and MSD was within an acceptable range for relative percent recovery.

Stable isotope analysis

All samples were dried, homogenized, filtered to pass through a 40-mesh screen, combusted to CO^2 and N^2, and analyzed using a NC 2500 elemental analyzer *(*Carlo Erba Milan, Italy*)* interfaced to a Delta Plus isotope ratio mass spectrometer *(*Thermo Finnigan Bremen, Germany*).* All isotope values are reported in δ-notation in part per thousand, or per mil (‰) and represent the heavy to light isotopic ratio, $\delta^{13}C$ ($^{13}C/^{12}C$) and $\delta^{15}N$

($^{15}N/^{14}N$) relative to reference standards (Vienna Peedee Belemnite carbonate and air, respectively). Precision was greater than 0.1‰ (1 SD) for both elements.

THg differences and relationships

The reach-specific distribution of taxon THg concentrations were tested for normality using the Shapiro-Wilk test. Log transformations of non-normal data were attempted to meet assumption of normality. The effect of reach on taxon THg was evaluated using either an Analysis of Variance (ANOVA) or a Kruskal-Wallis test, and if appropriate, a Tukey or Steel-Dwass post hoc test, respectively. To determine whether the differences in THg could be attributed to higher THg concentrations at the base of the food chain, the relationship between trout and primary consumer (larval caddisfly) THg concentrations were evaluated using linear regression.

Trophic Level calculations

The mean larval caddisfly $\delta^{15}N$ at each reach was used to calculate the trophic level (TL) of stonefly larvae, caddisfly larvae, salamander larvae, crayfish, forage fish, Rainbow Trout, and Eastern Brook Trout (Hobson & Welch 1992; Jardine et al. 2006) utilizing the formula:

$$TL = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \Delta\delta^{15}N + \lambda \quad \text{(Formula 1)}$$

Where:

$TL_{consumer}$ = the calculated trophic position of the consumer (e.g.,, crayfish, Eastern Brook Trout)

$\delta^{15}N_{consumer}$ = specific consumer $\delta^{15}N$

$\delta^{15}N_{baseline}$ = reach-specific mean larval caddisfly $\delta^{15}N$

$\Delta\delta^{15}N$ = trophic discrimination factor 3.4 ‰ (suggested by Jardine et al. 2006)

λ = the trophic position of larval caddisfly (primary consumer, λ=2)

THg Trophic Magnification Factors

At each reach, trophic magnification factors were calculated using the mean $\log_{10}$-transformed THg concentrations and the mean TL of organisms expected to range over 3 trophic levels (Borgå et al. 2012): trichopteran, crayfish, and Eastern Brook Trout. The best fit line is expressed:

$$\log_{10}[MeHg] = \delta^{15}N\ (b) + a \qquad \text{(Formula 2)}$$

where (b) equals the trophic magnification slope and 10^b = TMF.

$\delta^{13}C$ calculations

Fish $\delta^{13}C_{bulk}$ values were lipid-normalized utilizing the formula provided by Post et al. (2007):

$$\delta^{13}C_{LN} = \delta^{13}C_{bulk} - 3.32 + 0.99 \times (C:N) \qquad \text{(Formula 3)}$$

For all macroinvertebrates, lipid corrections of macroinvertebrate whole-body or muscle $\delta^{13}C_{LN}$ were calculated utilizing the formula provided by Logan et al. (2008):

$$\delta^{13}C_{LN} = \beta 0 + \beta 1 \times Ln(C:N) + \delta^{13}C_{bulk} \qquad \text{(Formula 4)}$$

The "all species invertebrate" conversion factors were used for larval caddisflies and crayfish, β_0 = -2.06 and β_1 = 1.91. A stonefly specific conversion factor was used for stoneflies, β_0 = -1.084 and β_1 = 1.26.

All $\delta^{13}C_{LN}$ values were transformed to account for 0.8‰ trophic fractionation (Vander Zanden & Rasmussen 2001):

$$\delta^{13}C = \delta^{13}C_{LN} - (TL-2) * 0.8‰ \qquad \text{(Formula 5)}$$

Food Web Analysis

The reach-specific distribution of taxa $\delta^{13}C$, $\delta^{15}N$, and TL was tested for normality using the Shapiro-Wilk test, respectively. The effect of reach on taxon $\delta^{13}C$, $\delta^{15}N$, and TL was evaluated using either an Analysis of Variance (ANOVA) or a Kruskal-Wallis test, and if appropriate, a Tukey or Steel-Dwass post hoc test, respectively.

To characterize CLTE, quantitative approaches developed by Layman et al. (2007) and Jackson et al. (2011) were used. Because no snails were found/collected at the Bald River Upstream or Downstream reaches, analysis was standardized to larval caddisflies as the primary consumer. In this analysis, food chain length ($\delta^{15}N$ range [NR]), $\delta^{13}C$ range (CR), mean distance to centroid (CD), mean nearest neighbor distance (NND), and standard deviation of NND (SDNND) were calculated using the reach-specific $\delta^{13}C$ and $\delta^{15}N$ of each sampled animal matrix. In this type of analysis, food chain length (referred to by Layman et al. (2007) as NR, but hereafter referred to as food chain length [FCL]) and CR is the distance between the two species with the most extreme (most depleted and most enriched) mean $\delta^{13}C$ and $\delta^{15}N$ values within a given community. CD is the mean Euclidian distance between each species and the overall mean $\delta^{13}C$ and $\delta^{15}N$ value for all species in the food web. CD represents the average degree of trophic diversity. NND is the mean Euclidian distance between each species and its nearest neighbor, and is a measure of trophic redundancy. SDNND is the standard deviation of NND and is a measure of the evenness of species packing. In addition to these five metrics, Layman et al. (2007) proposed that Total Area (TA), the area encompassed by a convex hull drawn around all of the mean species data, could be used as a measurement of overall trophic diversity. The

convex hull approach has been used, but has also been criticized because it doesn't incorporate the variability of mean values (standard deviation) and because it is sensitive to sample size. To more address this variance, overall trophic diversity was also calculated using standard ellipse area (SEA_c ; Jackson et al. 2011).

To determine if community ecology affected the biomagnification of THg, linear regression was used to determine the relationship between each CLTE metric and THg TMF. In this analysis, neither carbon range nor FCL had a significant relationship with THg TMF; however, a significant relationship was detected between SEA_c and THg TMF. Note, that all CLTE metrics were calculated using the $\delta^{13}C$ and $\delta^{15}N$ of taxa not included in the calculation of TMFs. One of these taxa (larval salamanders) had substantial within site variation of $\delta^{13}C$, and the enriched $\delta^{13}C$ signatures may have had a substantial effect on carbon range and the size of SEA_c. To further understand the effect of carbon source (inferred from $\delta^{13}C$) and trophic level (inferred from $\delta^{15}N$) on THg concentrations, two *A posteriori* hypotheses were developed:

(1) Carbon enrichment is negatively correlated with Trout THg concentrations

(2) Trout feeding at a higher trophic level will have higher THg concentrations.

To test these hypotheses, we used a stepwise multiple linear regression approach that included trout total length, TL, and carbon enrichment ($\delta^{13}C_E$) as explanatory variables. Here $\delta^{13}C_E$ was calculated relative to the reach's respective trophic baseline:

$$\delta^{13}C_E = \delta^{13}C_{LN}\,(\text{trout}) - \delta^{13}C_{LN}\,(\text{larval caddisflies}) \qquad \text{(Formula 6)}$$

Collinearity of the explanatory variables (TL, $\delta^{13}C_E$, and total length) was assessed using a multivariate correlation analysis and by calculating variance inflation factors (VIF).

The correlation of explanatory variables was not significant ($p < 0.05$) and each VIF was below values of concern (VIF < 5; Montgomery & Peck 1992). The best fit model was selected using Akaike information criteria corrected for small samples sizes (AICc; Burnham & Anderson 2002).

The analysis of community level metrics was calculated using the statistical package SIBER v 2.1.4 in R v 3.6.1 (R Development Core Team, 2019). All tests for normality, ANOVA, linear regressions, and stepwise linear regression analyses were conducted in JMP 14.0 ($\alpha = 0.05$).

Results

Total Hg concentrations varied among reaches (Figure 2; THg and $\log_{10}$THg reach averages by taxon [± SE] are provided in Table 1). Significant differences were observed for larval caddisflies ($H = 14.2398$, $p = 0.0142$; Figure 2A) and trout THg ($H = 25.3206$, $p = 0.0002$; Figure 2C); however, the post-hoc, pairwise comparison did not identify specific differences between reaches ($p > 0.05$). Crayfish THg was not significantly different among reaches ($H = 3.0941$, $p = 0.6855$; Figure 2B). The relationship between trout and larval caddisfly (primary consumer THg) concentrations were not significant ($p = 0.3969$; Figure 3). Trophic magnification factors ranged from 2.98 to 4.68 (Figure 4).

Among reach differences were observed for $\delta^{13}C$, $\delta^{15}N$, and TL ($\delta^{13}C$ and $\delta^{15}N$ biplots of each group are shown in Figure 5). Additionally, $\delta^{13}C$, $\delta^{15}N$, and TL (mean ± SE) values with connecting letters reports are provided in Table 2. Larval caddisfly $\delta^{13}C$ was not significantly different among reaches, but among reach differences were observed for $\delta^{15}N$ ($\delta^{13}C$: $H = 8.0606$, $p = 0.1529$; $\delta^{15}N$: $H = 23.2503$, $p = 0.0003$; Figure 5A). Tthe

post-hoc, pairwise comparison did not identify specific differences between reaches larval caddisfly $\delta^{15}N$ ($p > 0.05$). Among reach differences were observed in crayfish $\delta^{13}C$ and $\delta^{15}N$ ($\delta^{13}C$: $H = 17.1894$, $p = 0.0042$; $\delta^{15}N$: $H = 24.0857$, $p = 0.0002$; Figure 5B) and TL ($H = 20.1235$, $p = 0.0012$) however, the post-hoc, pairwise comparison did not identify specific differences between reaches ($p > 0.05$). Among reach differences were observed in trout $\delta^{13}C$ and $\delta^{15}N$ ($\delta^{13}C$: $F = 8.7357$, $p < 0.0001$; $\delta^{15}N$: $F = 56.8619$, $p < 0.0001$; Figure 5C), and TL ($F = 17.6876$, $p < 0.0001$). Specific differences among reaches were detected for $\delta^{13}C$, $\delta^{15}N$, and TL (Table 2. Connecting letters). Among reach differences were not observed for stonefly $\delta^{13}C$ or TL, but $\delta^{15}N$ was different among reaches ($\delta^{13}C$': $H = 10.1355$, $p = 0.0715$; $\delta^{15}N$: $H = 22.2387$, $p = 0.0005$; TL: $H = 9.1806$, $p = 0.1021$; Figure 5D); however, the post-hoc, pairwise comparison did not identify specific differences between reaches ($p > 0.05$). Among reach differences were observed in salamander larvae $\delta^{13}C$, $\delta^{15}N$ ($\delta^{13}C$: $H = 15.2079$, $p = 0.0095$; $\delta^{15}N$: $H = 26.2987$, $p < 0.0001$; Figure 5E), and TL ($H = 11.4429$, $p = 0.0433$). However, the post-hoc, pairwise comparison did not identify specific differences between reaches $\delta^{15}N$, $\delta^{13}C$, or TL ($p > 0.05$). Among reach differences were not observed for forage fish $\delta^{13}C$ ($F = 1.7125$, $p = 0.1994$) but were observed in forage fish $\delta^{15}N$ ($F = 19.4194$, $p < 0.0001$; Figure 5F) and TL ($F = 15.1432$, $p = 0.0005$), and specific differences among reaches were detected for $\delta^{15}N$ and TL (See Table 2. Connecting Letters). The average snail $\delta^{13}C$ and $\delta^{15}N$ ratios were -21.42 ± 0.43 and 4.32 ± 0.14, respectively.

Community Level Trophic Ecology

The metrics used to measure CLTE varied by reach (Table 4), and $\delta^{13}C$ – $\delta^{15}N$ biplots for each reach are provided: Bald River Upstream (Figure 6), Bald River Downstream (Figure 7), Rock Creek (Figure 8), LPHC Upstream (Figure 9), LPHC Downstream (Figure 10), Gentry Creek (Figure 11).When mean taxa $\delta^{15}N$ values were compared among reaches, each respective matrix was significantly different and spanned at least 2 ‰ (Table 2); however, the variability observed in FCL (6.11 ‰ to 7.24 ‰), only spanned 1.13 ‰ overall (Table 4). Among reach differences of $\delta^{13}C$ were significantly different in only half of the tested taxa, but the overall $\delta^{13}C$ range of the reaches spanned 2.81 ‰ overall (1.53 ‰ to 4.25‰). Total area was highest at Rock Creek (15.6 $‰^2$) and lowest at the Gentry Creek (3.8 $‰^2$) spanning 11.80 $‰^2$. Nearest neighbor distance was highest at LPHC Upstream (2.30 ‰) and lowest at Gentry Creek (1.13 ‰) spanning 1.17 ‰. Standard deviation of nearest neighbor distance was highest at Bald River Upstream (1.71‰) and lowest at Rock Creek (1.41 ‰) spanning 0.30 ‰. SEA_c was highest at Rock Creek (13.89 $‰^2$) and lowest at Gentry Creek (4.02 $‰^2$) spanning 9.87 $‰^2$.

When CLTE metrics were regressed against TMFs, the only significant relationship was detected with SEA_c (Table 5). SEA_c is a product of $\delta^{13}C$ and $\delta^{15}N$ variability, but neither $\delta^{13}C$ range nor FCL were significant.

The multiple linear regression model that best described Trout THg concentrations included only $\delta^{13}C_E$ and total length as explanatory variables, and excluded trophic level ($F_{2, 27} = 10.1810$; $p = 0.0005$; $R^2 = 0.43$). In this model $\delta^{13}C_E$ had a significant negative relationship with THg concentrations ($t = -2.65$; $p = 0.0134$) and total length had a positive relationship with THg concentrations ($t = 1.62$; $p = 0.1178$).

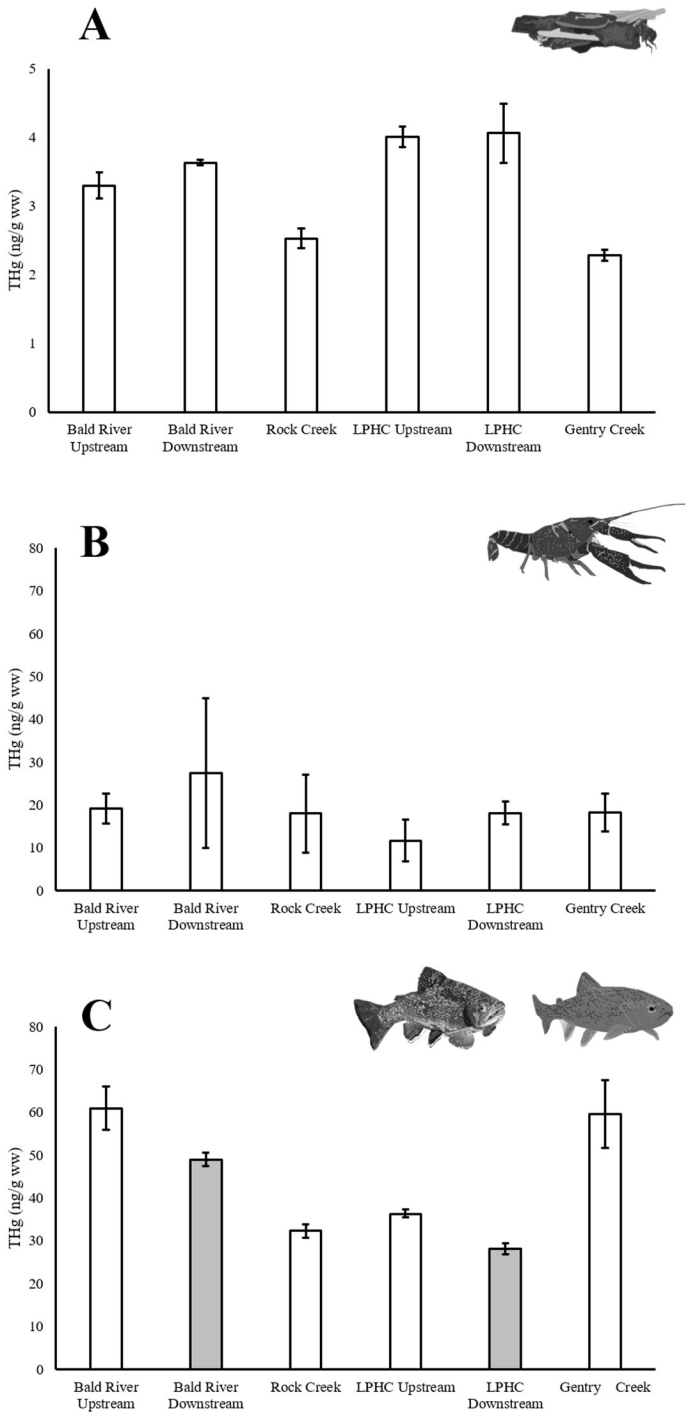


Figure 2. THg values (mean ± SE) for larval caddisflies (A), crayfish (B), and trout (C) by reach. In panel C, white bars represent Eastern Brook Trout and grey bars represent Rainbow Trout.

Table 1. Trout, crayfish, and caddisfly larvae reach-specific mean ± SE total mercury (THg) and Log_{10}THg. For trout, the species of trout is represented for Eastern Brook Trout (EBT) and Rainbow Trout (RT).

	THg	Log_{10}THG
Trout		
Bald River Usptream (EBT)	61.0 ± 2.4	1.78 ± 0.02
Bald River Downstream (RT)	49.0 ± 5.1	1.68 ± 0.00
Rock Creek (EBT)	32.4 ± 1.6	1.51 ± 0.02
Left Prong Hampton Creek Upstream (EBT)	36.4 ± 0.9	1.56 ± 0.01
Left Prong Hampton Creek Downstream (RT)	28.2 ± 1.2	1.45 ± 0.02
Gentry Creek (EBT)	59.6 ± 7.9	1.76 ± 0.06
Crayfish		
Bald River Usptream	19.2 ± 3.5	1.25 ± 0.08
Bald River Downstream	27.5 ± 17.4	1.18 ± 0.22
Rock Creek	18.1 ± 9.1	1.10 ± 0.21
LPHC Upstream	11.7 ± 4.9	0.98 ± 0.20
LPHC Downstream	18.2 ± 2.7	1.24 ± 0.07
Gentry Creek	18.3 ± 4.5	1.19 ± 0.13
Caddislfy Larvae		
Bald River Usptream	3.30 ± 0.19	0.52 ± 0.02
Bald River Downstream	3.63 ± 0.04	0.56 ± 0.00
Rock Creek	2.53 ± 0.14	0.40 ± 0.02
LPHC Upstream	4.01 ± 0.15	0.60 ± 0.02
LPHC Downstream	4.06 ± 0.43	0.60 ± 0.04
Gentry Creek	2.28 ± 0.08	0.36 ± 0.02

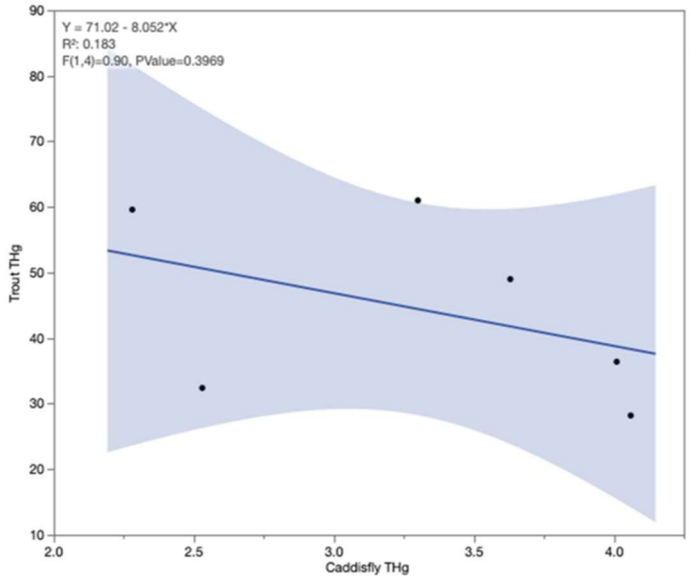


Figure 3. Regression of mean Trout THg and larval caddisfly THg. The best fit line is shown with a shaded 95% confidence interval.

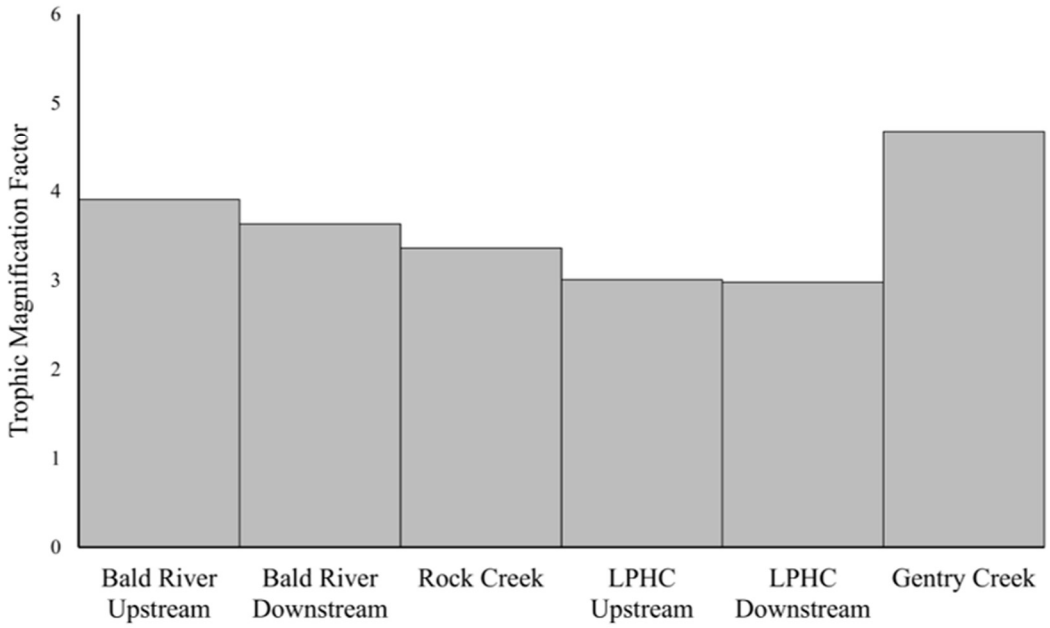


Figure 4. Total Hg Trophic Magnification Factor of the six study reaches.

Table 2. Trout, crayfish, caddisfly larvae, stonefly larvae, salamander larvae, and forage fish reach-specific mean ± SE stable isotope ratios for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as well as trophic level (TL). When present connecting letters represent taxon-specific significant differences among reaches for $\delta^{13}C$, $\delta^{15}N$ or TL. For trout, the species of trout is represented for Eastern Brook Trout (EBT) and Rainbow Trout (RT).

	$\delta^{13}C$	$\delta^{15}N$	TL
Trout			
Bald River Usptream (EBT)	-23.87 ± 0.08 ^{BC}	6.62 ± 0.06 ^{C}	4.13 ± 0.02 ^{A}
Bald River Downstream (RT)	-22.97 ± 0.20 ^{A}	6.04 ± 0.09 ^{D}	4.00 ± 0.08 ^{BCD}
Rock Creek (EBT)	-23.38 ± 0.12 ^{AB}	6.74 ± 0.09 ^{C}	4.11 ± 0.03 ^{AB}
Left Prong Hampton Creek Upstream (EBT)	-22.79 ± 0.21 ^{A}	8.57 ± 0.10 ^{A}	4.00 ± 0.03 ^{BC}
Left Prong Hampton Creek Downstream (RT)	-22.96 ± 0.28 ^{A}	7.20 ± 0.10 ^{B}	3.77 ± 0.03 ^{E}
Gentry Creek (EBT)	-24.24 ± 0.16 ^{C}	6.90 ± 0.13 ^{BC}	3.87 ± 0.04 ^{DE}
Gentry Creek (RT)	-23.65 ± 0.11 ^{ABC}	7.04 ± 0.17 ^{BC}	3.91 ± 0.05 ^{CDE}
Crayfish			
Bald River Usptream	-25.50 ± 0.08	3.03 ± 0.12	3.08 ± 0.04
Bald River Downstream	-25.27 ± 0.14	2.66 ± 0.09	3.00 ± 0.03
Rock Creek	-25.12 ± 0.10	4.00 ± 0.14	3.30 ± 0.04
Left Prong Hampton Creek Upstream	-24.90 ± 0.22	5.35 ± 0.23	3.05 ± 0.07
Left Prong Hampton Creek Downstream	-23.98 ± 0.26	5.89 ± 0.25	3.39 ± 0.07
Gentry Creek	-24.65 ± 0.20	4.67 ± 0.13	3.21 ± 0.04
Caddisfly Larvae			
Bald River Usptream	-25.00 ± 0.28	-0.63 ± 0.31	2.00 ± 0.09
Bald River Downstream	-24.49 ± 0.21	-0.75 ± 0.20	2.00 ± 0.06
Rock Creek	-24.77 ± 0.30	-0.43 ± 0.19	2.00 ± 0.05
Left Prong Hampton Creek Upstream	-24.68 ± 0.27	1.78 ± 0.16	2.00 ± 0.05
Left Prong Hampton Creek Downstream	-24.32 ± 0.16	1.17 ± 0.28	2.00 ± 0.08
Gentry Creek	-25.18 ± 0.09	0.55 ± 0.25	2.00 ± 0.07
Stonefly Larvae			
Bald River Usptream	-25.02 ± 0.24	4.77 ± 0.17	3.59 ± 0.05
Bald River Downstream	-24.56 ± 0.42	4.52 ± 0.22	3.55 ± 0.06
Rock Creek	-25.43 ± 0.16	5.38 ± 0.26	3.71 ± 0.08
Left Prong Hampton Creek Upstream	-24.41 ± 0.20	6.78 ± 0.22	3.47 ± 0.06
Left Prong Hampton Creek Downstream	-24.47 ± 0.29	6.39 ± 0.25	3.53 ± 0.07
Gentry Creek	-24.61 ± 0.88	5.38 ± 0.22	3.42 ± 0.06
Salamander Larvae			
Bald River Usptream	-23.50 ± 0.77	4.64 ± 0.18	3.55 ± 0.05
Bald River Downstream	-23.75 ± 0.32	4.75 ± 0.11	3.62 ± 0.03
Rock Creek	-21.18 ± 0.54	4.87 ± 0.12	3.56 ± 0.35
Left Prong Hampton Creek Upstream	-21.23 ± 0.82	6.64 ± 0.29	3.43 ± 0.09
Left Prong Hampton Creek Downstream	-22.04 ± 0.71	6.77 ± 0.12	3.65 ± 0.04
Gentry Creek	-23.67 ± 0.32	5.46 ± 0.22	3.45 ± 0.07
Forage Fish			
Bald River Downstream	-25.50 ± 0.37	5.59 ± 0.11 ^{A}	3.86 ± 0.03
Left Prong Hampton Creek Downstream	-24.46 ± 0.58	7.27 ± 0.08 ^{B}	3.79 ± 0.24
Gentry Creek	-24.68 ± 0.23	5.80 ± 0.17 ^{A}	3.54 ± 0.05

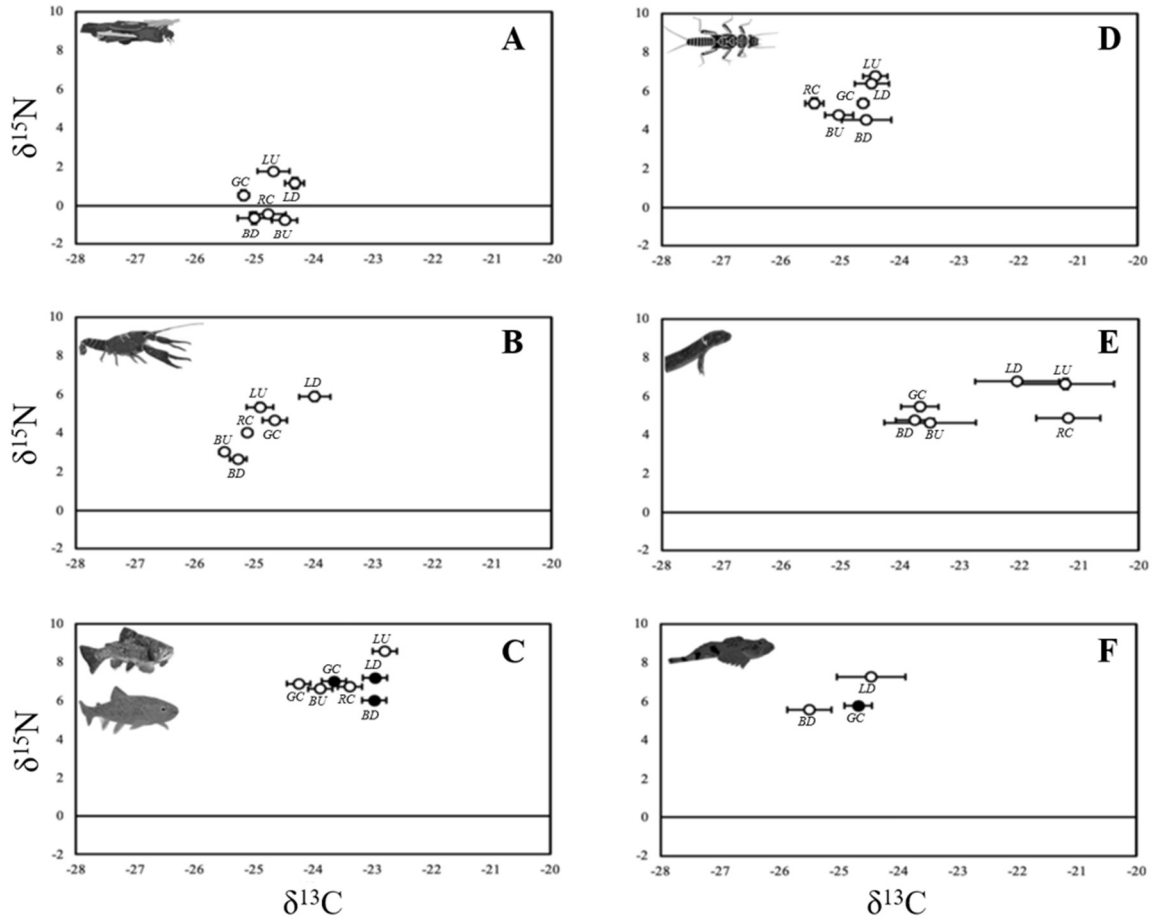
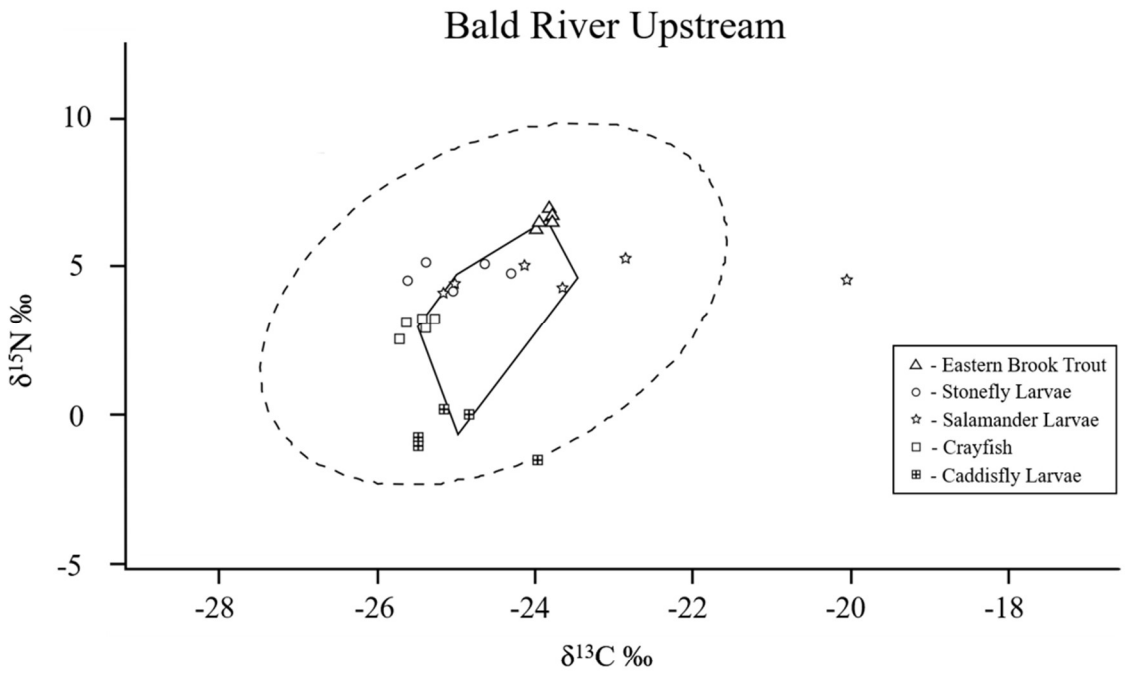
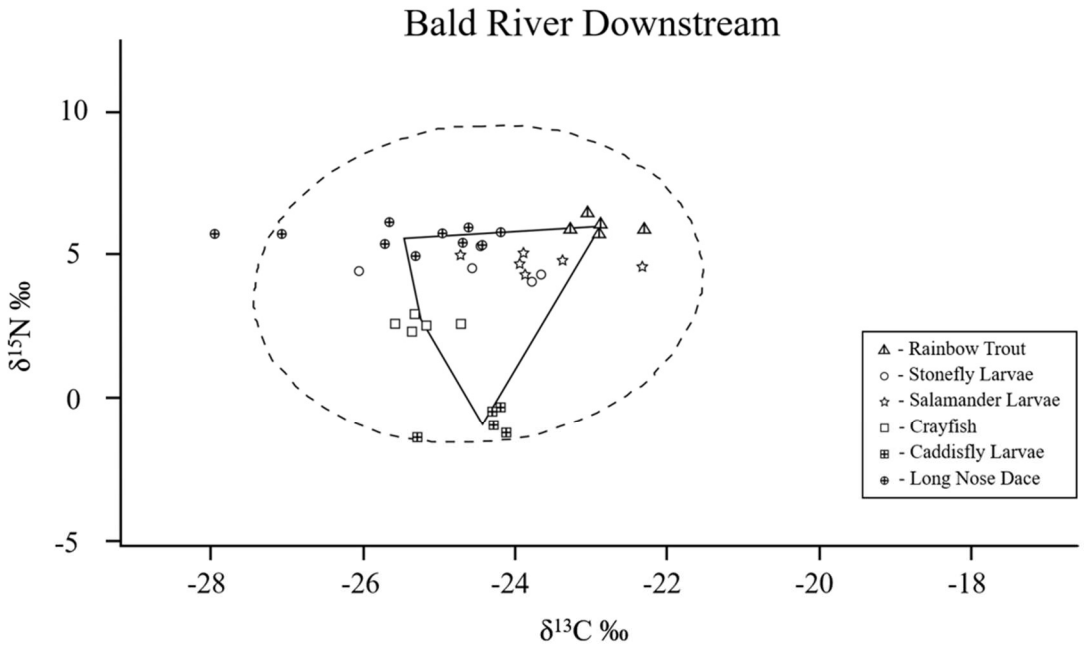


Figure 5. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SE) for larval caddisflies (A), crayfish (B), trout (C), larval stoneflies (D), larval salamanders (E), and forage fish (F). In panel C, open circles represent Eastern Brook Trout and filled circles represent Rainbow Trout. In panel F, open circle represent Longnose Dace and closed circles represent Mottled Sculpin.



Figures 6. Bald River Upstream stable isotope bi-plots based on the species collected. The convex hull (drawn in a solid line) is based on the mean $\delta^{13}C$ and $\delta^{15}N \pm SE$ of each respective group. The dashed line encircles the standard ellipse area corrected for small samples sizes (SEA_c).



Figures 7. Bald River Downstream stable isotope bi-plots based on the species collected. The convex hull (drawn in a solid line) is based on the mean $\delta^{13}C$ and $\delta^{15}N \pm SE$ of each respective group. The dashed line encircles the standard ellipse area corrected for small samples sizes (SEA_c).

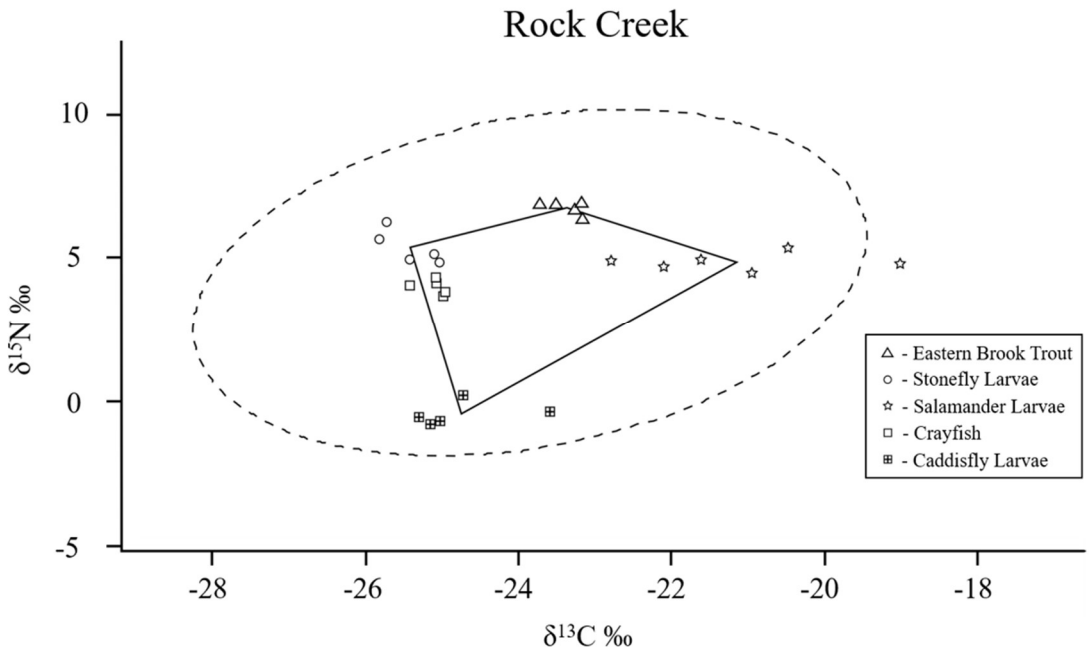
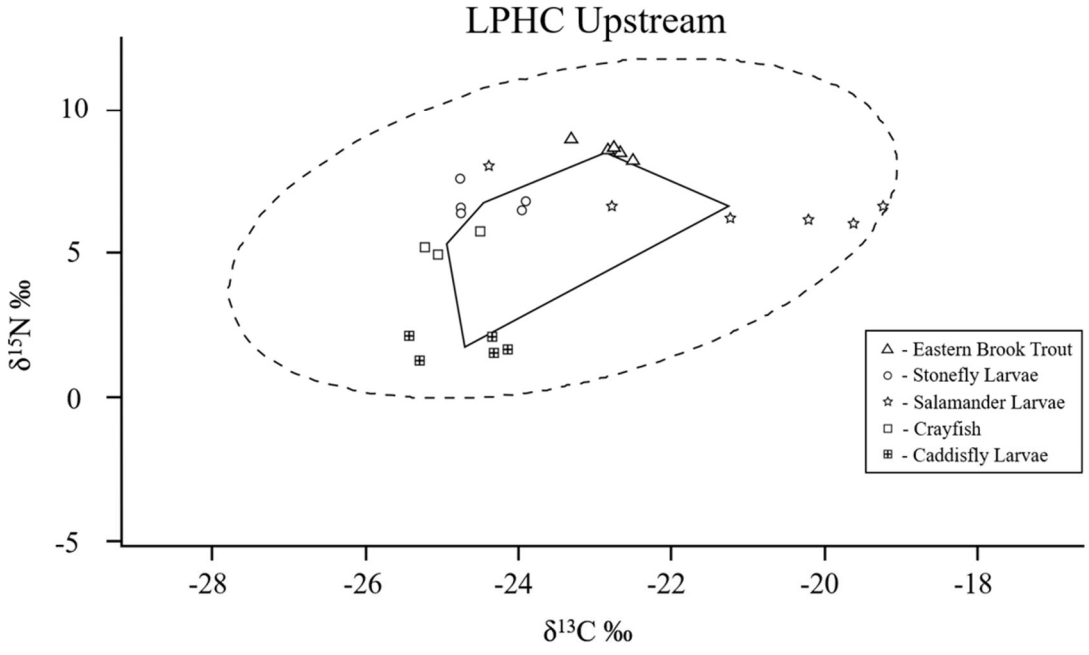
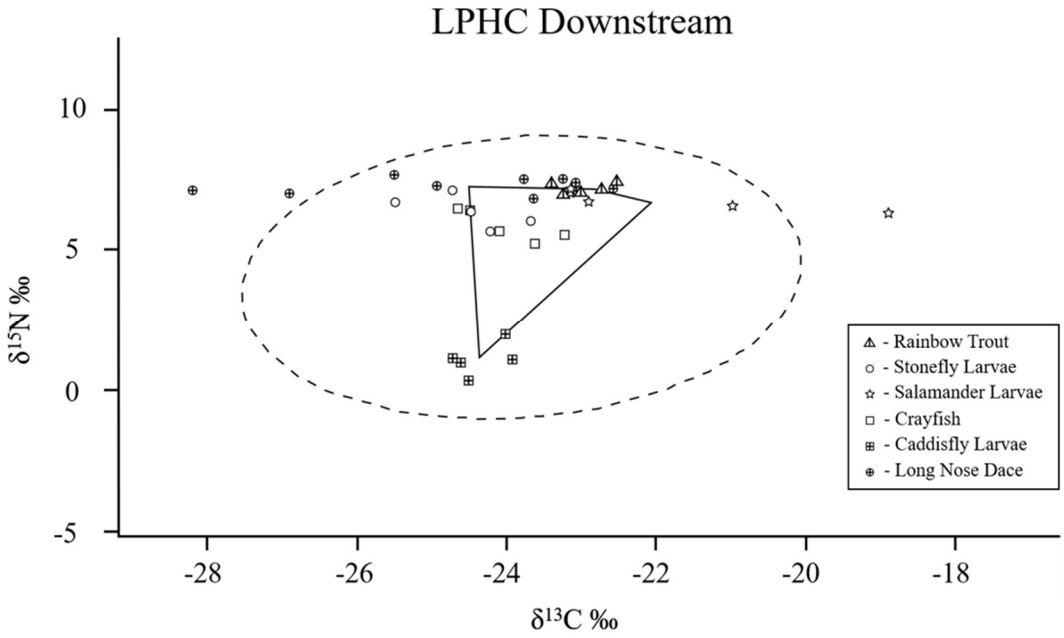


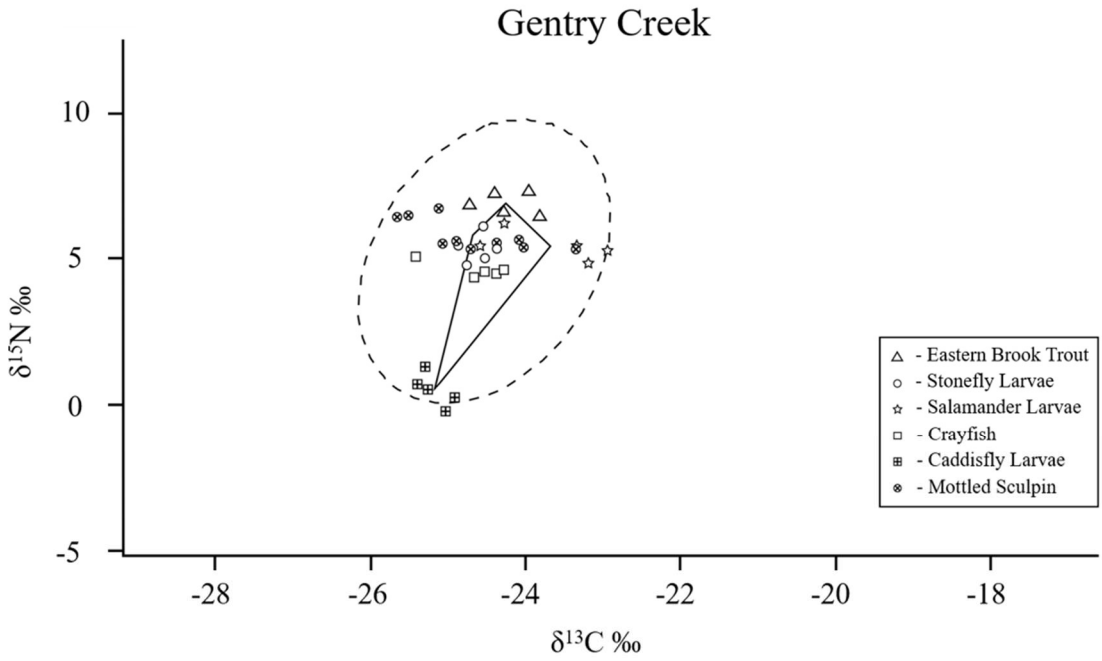
Figure 8. Rock Creek stable isotope bi-plots based on the species collected. The convex hull (drawn in a solid line) is based on the mean $\delta^{13}C$ and $\delta^{15}N \pm SE$ of each respective group. The dashed line encircles the standard ellipse area corrected for small samples sizes (SEA_c).



Figures 9. Left Prong Hampton Creek (LPHC) Upstream stable isotope bi-plots based on the species collected. The convex hull (drawn in a solid line) is based on the mean $\delta^{13}C$ and $\delta^{15}N \pm SE$ of each respective group. The dashed line encircles the standard ellipse area corrected for small samples sizes (SEA_c).



Figures 10. Left Prong Hampton Creek (LPHC) Downstream stable isotope bi-plots based on the species collected. The convex hull (drawn in a solid line) is based on the mean $\delta^{13}C$ and $\delta^{15}N \pm SE$ of each respective group. The dashed line encircles the standard ellipse area corrected for small samples sizes (SEA_c).



Figures 11. Gentry Creek stable isotope bi-plots based on the species collected. The convex hull (drawn in a solid line) is based on the mean $\delta^{13}C$ and $\delta^{15}N \pm SE$ of each respective group. The dashed line encircles the standard ellipse area corrected for small samples sizes (SEA_c).

Table 3. Reach-specific best fit line of Log_{10}THg vs. trophic level linear regression and trophic magnification factor (TMF) with results of linear regression.

Site	Best fit line (Log_{10}THg vs. TL)	TMF	*p*	r^2
Bald River Upstream	-0.692 + 0.591x	3.91	0.0518	0.99
Bald River Downstream	-0.578 + 0.5605x	3.64	0.0332	1.00
Rock Creek	-0.667 + 0.5137x	3.36	0.1046	0.97
Left Prong Hampton Creek Upstream	-0.264 + 0.486x	3.01	0.2476	0.86
Left Prong Hampton Creek Downstream	-0.398 + 0.5090x	2.98	0.1122	0.97
Gentry Creek	0.983 + 0.6709x	4.68	0.019	0.96

Table 4. Reach-specific community level trophic ecology metrics: δ^{13}C Range, food chain length (FCL), total area, nearest neighbor distance (NND), standard deviation of nearest neighbor distance (SDNDD), and standard elipse area corrected for small samples size (SEA_c).

Site	δ^{13}C Range (‰)	FCL (‰)	Total Area (‰^{2})	NND (‰)	SDNND (‰)	SEA_c (‰^{2})
Bald River Upstream	1.99	7.24	6.9	2.11	1.71	9.08
Bald River Downstream	2.53	6.79	9.0	1.68	1.64	8.54
Rock Creek	4.25	7.16	15.6	1.62	1.41	13.89
Left Prong Hampton Creek Upstream	3.67	6.80	12.0	2.30	1.55	12.13
Left Prong Hampton Creek Downstream	2.43	6.11	7.7	1.51	1.69	10.00
Gentry Creek	1.53	6.49	3.8	1.13	1.64	4.02

Table 5. Results of the linear regression between trophic magnification factor and each respecitive community level trophic ecology (CLTE) metric: δ^{13}C Range, food chain length (FCL), total area, nearest neighbor distance (NND), standard deviation of nearest neighbor distance (SDNDD), and standard elipse area corrected for small samples size (SEA_c).

CLTE metric	Best fit line	$F_{(1, 4)}$	*p*	r^2
δ^{13}C Range	y = -0.4258x + 4.76	3.54	0.1329	0.47
FCL	y = 0.1584x + 2.524	0.04	0.8427	0.01
Total Area	y = -0.1034x + 4.543	3.17	0.1497	0.44
NND	y = -0.7926x + 4.963	1.50	0.2877	0.27
SDNND	y = 1.573x + 1.068	0.32	0.6010	0.07
SEA_c	y = - 0.1562 + 5.097	8.62	0.0425	0.68

Discussion

Food web structure is one of the key mechanisms expected to directly affect contaminant bioaccumulation and biomagnification (Relyea & Hoverman 2006; Clements & Rohr 2009; Borgå et al. 2012; Clements et al. 2016; Eagles-Smith et al. 2018; Schiesari et al. 2018). Most studies that have approached the effect of food web structure on Hg bioaccumulation have primarily focused on lakes (Guildiford et al. 2008; Chumchal & Hambright 2009; Kidd et al. 2012; Verberg et al. 2014; Clayden et al. 2015; Ouédraogo et al. 2015; Poste et al. 2015; Finley et al. 2016), and only one known stream study has taken the analysis of trophic ecology beyond FCL (Willacker et al. 2019).

Trophic magnification factors (TMFs) calculated in this study (range 2.98 – 4.68) fell within the range expected for temperate freshwater systems (1.73 – 8.28; derived from Lavoie et al. 2013). TMFs were not correlated with the community level trophic ecology (CLTE) metrics: $\delta^{13}C$ range and food chain length (FCL). However, TMFs were correlated with the CLTE metric standard ellipse area (SEA_c, corrected for small sample sizes), a metric that provides a more holistic characterization of the community's isotopic niche width ($\delta^{13}C$ range) and height (FCL) (Jackson et al. 2011). In this analysis we recognized a notably larger carbon range in reaches (Figure 8, 9, and 10) that had high variability in larval salamander $\delta^{13}C$ (Figure 5). To further investigate the effects of $\delta^{13}C$, we modeled the effect of THg bioaccumulation in trout with respect to trophic level (TL), carbon enrichment ($\delta^{13}C_E$), and total length. Our results indicate the primary driver of THg bioaccumulation in trout was in fact $\delta^{13}C_E$, and the best fit model notably excluded trophic level (TL). These results indicate that in headwater streams, an increase in primary

production leads to a less contaminated and more enriched (positive) $\delta^{13}C$ signal associated with algae and grazing primary consumers. These results and interpretations support previous research that concluded trophic level was not driving the variability of top predator fish THg concentrations (Chasar et al. 2009).

The results and interpretations of this study conflict with the only other study that has similarly characterized headwater streams in regard to Hg bioaccumulation and CLTE. Willacker et al. (2019) evaluated the effects of timber harvest on food web structure in head water streams in Oregon and concluded that more leaf litter and less primary production led to the lower THg concentrations among the studied reaches. The discrepancy in this study's findings and that of Willacker et al. (2019) may be attributed to geographical and catchment level differences. Similarly, in lakes, differential reliance on pelagic and benthic carbon have found conflicting results. For instance, the effect of Hg bioaccumulation as a function of % pelagic carbon (as opposed to % benthic carbon) has been shown to have no effect (Kidd et al. 2012; Ouédraogo et al. 2015), a positive effect (Kidd et al. 2003; Clayden et al. 2015; Ouédraogo et al. 2015), and a negative effect (Eagles-Smith et al. 2008). Similarly, increasing temperatures and productivity have been associated with Hg concentration increases (Ahonen et al. 2018) and decreases (Braaten et al. 2019). It is important to note that neither this study nor Willacker et al. (2019) sampled periphyton or leaf litter directly, and that the conclusions of that study are largely based off a meta-comparison of algae and leaf litter dry weight Hg concentrations. An issue with this type of comparison is that algae has a higher consumption and production efficiency compared to leaf detritus (Vadeboncoeur & Power 2017), and a much higher moisture

content (as high as 95% wet-weight [Silva et al. 2008] vs. leaf litter: 30% [Risch et al. 2017]). In fact, the only study cited by Willacker et al. (2019) that has both algae and leaf litter concentrations (Tsui et al. 2012), shows 21-fold higher MeHg concentrations in periphyton compared to leaf litter, but the study itself found no difference in MeHg concentrations in the secondary consumers (grazers and shredders: Tsui et al. 2012).

The results of this study support the hypothesis that mercury bioaccumulation in food webs would decline with increasing primary production in headwater streams (the bloom dilution hypothesis). The bloom dilution hypothesis is empirically supported in estuaries (Luengen et al. 2009) lotic systems (Pickhardt et al. 2002; Chen & Folt 2005) and stream mesocosms (Walters et al. 2015).

In the current study, all sampled fish THg concentrations were below the U.S EPA screening values for human health (300 ng/g ww; U.S. EPA 2001). However, this does not mean that all fish in all southern Appalachian Mountain headwater streams are below the U.S. EPA screening value for human health. The negative correlations observed with TMF and SEA_c and trout THg and $\delta^{13}C_E$ indicate that future investigations may find higher concentrations in fish that inhabit headwater streams that are less reliant on detritus as a basal carbon resource and have greater wetland density (Greenfield et al. 2001; Brigham et al. 2009; Marvin-DiPasquala et al. 2009).

Notably, in our efforts to characterize the food webs in these headwater streams, the CLTE metrics were calculated presuming a detritus driven food web using an easy to identify larval caddisfly that was present and abundant among all reaches. These organisms are in a large woody case and are likely a “trophic cul de sac” because they are less

susceptible to predation than other unarmored benthic macroinvertebrates (Power 2006). Although trophic cul de sacs may not offer a direct link between production and predation, a strength of this study is the ease of characterizing the among reach differences and similarities by establishing higher trophic relationships relative to this same baseline. This type of design supports our inter- reach comparisons and allowed the integration of spatial variability in $\delta^{15}N$ (Elliott et al. 2007) while highlighting the relatively consistent $\delta^{13}C$ signature of larval caddisflies – likely due to the depleted (more negative) $\delta^{13}C$ associated with deciduous tree leaf litter (Finlay 2002). This research also highlights the difficulty in sampling multiple groups of organisms across a relatively large spatial scale (greatest distance between two streams was ~260 km). A more robust sampling regime at each reach (additional benthic macroinvertebrate groups) would allow more robust comparisons to be drawn about trophic interactions (Layman et al. 2007); however, because SEA_c is corrected for smaller sample sizes, the variability in each reach community's SEA_c is expected to be minimal and our conclusions would likely be unaffected (Jackson et al. 2011).

In this study no other primary consumers were sampled for $\delta^{13}C$ or $\delta^{15}N$, and the conclusion that carbon enrichment has led to lower THg concentrations assumes that tissues with enriched carbon have derived this carbon from a food web that does not reflect the nearly constant -28 ‰ $\delta^{13}C$ signature of terrestrial C3 plants (Brett et al. 2017). A possible explanation is that the epilithic algae and terrestrial $\delta^{13}C$ are distinct (Finlay 2002). Previous studies support that the $\delta^{13}C$ should not fluctuate due to the inter-reach similarities in dissolved oxygen, dissolved inorganic carbon, and carbonate concentrations, respectively (Finlay 2004; Olson et al. 2019A). Still, one study has attributed the variability

in phytoplankton $\delta^{13}C$ to the differences observed in higher TL fish (Poste et al. 2015) and has prevented another from making more robust conclusions (Ouédraogo et al. 2015). In this study we sought snails for the scraper baseline, and our efforts were unfruitful at all but two of the reaches. Although not necessarily intended, the prioritization of larval caddisflies may have led to the unintentional but subsequent marginalization of the scraper food web. Although this approach is superficially supported by the river continuum concept (RCC), a core stream ecology principle that hypothesizes a substantial influx of leaf litter and the reduction of photosynthetically active radiation in headwater streams (Vannote et al. 1980), algae has a higher consumption and production efficiency than detritus, and multiple studies have shown the importance of algae in headwater streams (Finlay 2001; Brett et al. 2017; Vadeboncoeur and Power 2017). For instance, even in headwater streams where algae constitute < 2 % of organic matter, algae can still support ~75% of the energy needs of certain groups of grazing insects (Mayer and Likens 1987).

In these headwater streams, we hypothesize that the overhead canopy has differentially reduced the standing stock of algae; future studies should be careful to integrate the paradox of the RCC and algae assimilation. In headwater streams, algae should be viewed as a cryptic trophic base that is preferentially grazed on by generalist benthic primary consumers and some obligate algivores (Brett et al. 2017; Vadeboncoeur and Power 2017). Although sampling periphyton may prove to be logistically difficult, future studies can still characterize trophic baselines by including primary consumers with a high likelihood of integrating the algae carbon into their tissues. Certain groups of benthic invertebrates have the propensity to feed on algae irrespective of its quality, quantity, or

elevation (Tomanova 2006; Atkinson et al. 2018). If future researchers attempt to build upon this work in southern Appalachian mountain headwater streams, I suggest two candidate species be considered as potential trophic baselines. Both candidate species were present at all streams and are believed to preferentially feed on algae (Joe Bidwell – personal communication). The first candidate is riffle beetles (Order: Coleoptera, Family: Elmidae, Genus: *Optioservus*). Riffle beetles (*Optioservus spp.*) are presumed algivores (Elliot 2008) and are an ideal candidate because they've demonstrated the ability to maintain the algivore $\delta^{13}C$ signature even when faced with an environmental perturbation (invasive snails; Moore et al. 2012). However, some dietary studies indicate that their diet can be made of up of primarily detritus (Hall 1995), so additional groups should also be considered. The second candidate is flat headed mayflies (Order: Ephemeroptera, Family: Heptageniidae, Genus: *Epeorus*). Although there are fewer studies that directly inform the $\delta^{13}C$ of *Epeorus spp.*, they have been composited with other closely related mayflies and have shown an enriched $\delta^{13}C$ signature (Merritt & Cummins 1996; Finlay et al. 1999; McNeely et al. 2007). A fair expectation is that both of these candidate species would graze algae if it were only a marginal contributor to the carbon resource pool of headwater streams.

It is important to note that we analyzed all tissue residues for total mercury and not methyl-mercury. This is a typical logistical constraint due to the biomass required for MeHg analysis and the high analytical costs. Areas like the Appalachian Mountains, and specifically these stream reaches may be attractive to study mercury dynamics because there are no known co-contaminants (Olson et al. 2019A). However, mercury

concentrations are relatively low, so biomass requirements are high for THg and indeed MeHg. More recent research has produced THg and MeHg fractions that could be used once confirmed in a subset of sites (e.g. Kwon et al. 2015).

Conclusion

In summary, this study examined the biomagnification potential of Hg and the role of CLTE in mercury dynamics in southern Appalachian Mountain headwater streams. Here a quantitative approach to CLTE was used to describe the trophic structure of the food webs and revealed that the community standard ellipse area (SEA_c) influenced THg biomagnification. Additionally, $\delta^{13}C_E$ was the primary driver of THg bioaccumulation in trout. These results indicate that in headwater streams, an increase in primary production, or an increase in the reliance on algal resources compared to terrestrial detritus, can lead to a less contaminated and more enriched (positive) $\delta^{13}C$ signal associated with algae and grazing primary consumers.

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CHAPTER II: TROPHIC MAGNIFICATION OF MERCURY IN LINKED AQUATIC AND RIPARIAN SYSTEMS

Introduction

Mercury (Hg) has been traditionally viewed as an aquatic problem; however, Hg can flux from aquatic to terrestrial ecosystems using insects as a vehicle that exposes riparian predators to aquatic contaminants like Hg. Many larval aquatic insects have a complex life-cycle where they go through metamorphosis and become adult aquatic insects (Figure 1). Because Hg (1) bioaccumulates in larval aquatic insect tissue, (2) does not affect larval survival, (3) does not affect emergence, and (4) is retained post-metamorphosis, adult aquatic insects can transfer Hg to riparian predators like spiders and salamanders (Figure 2; Cristol et al. 2008; Kraus et al. 2014; Otter et al. 2020-*In Review*).

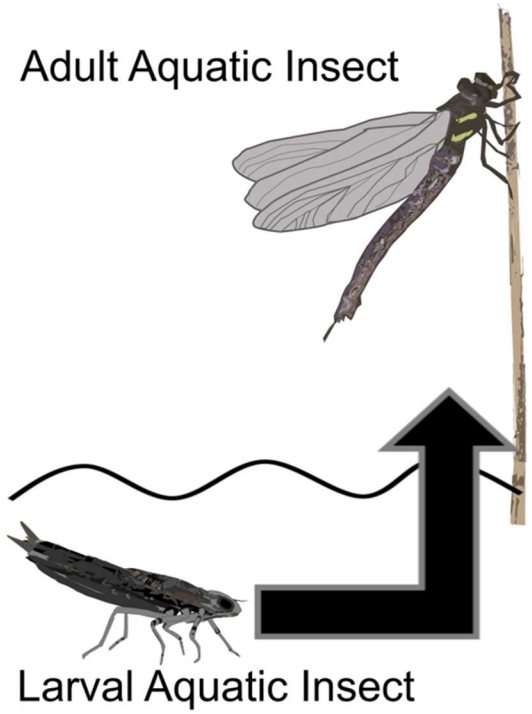

Figure 1. Most larval aquatic insects live much of their lives underwater and then undergo metamorphosis (arrow) to become flying adult aquatic insects (e.g. dragonflies). (from Otter et al. 2020).

Riparian predators rely on adult aquatic insects to varying degrees. Riparian spiders like the long-jawed orb-weaver (*Tetragnatha elongata*; hereafter referred to as tetragnathids) are considered land water interface specialists because they are prone to desiccation (Power 2004), spin a horizontal web directly

above the land-water interface (Levi 1981; Gillespie 1987; Aiken et al. 2000), and feed primarily on adult aquatic insects (Gillespie 1987; Chaves-Ulloa et al. 2016; Kautza & Sullivan, 2016). Other riparian spiders, like those belonging to the genus *Araneus* (hereafter referred to as araneids), are vertical web builders. When found in riparian zones, araneids have been shown to rely less on the aquatic dietary items than the more specialized tetragnathids (Kato et al. 2003). Water striders (Hemiptera, Gerridae), another type of riparian predator, are insects that walk (or glide) atop the water surface, and although like

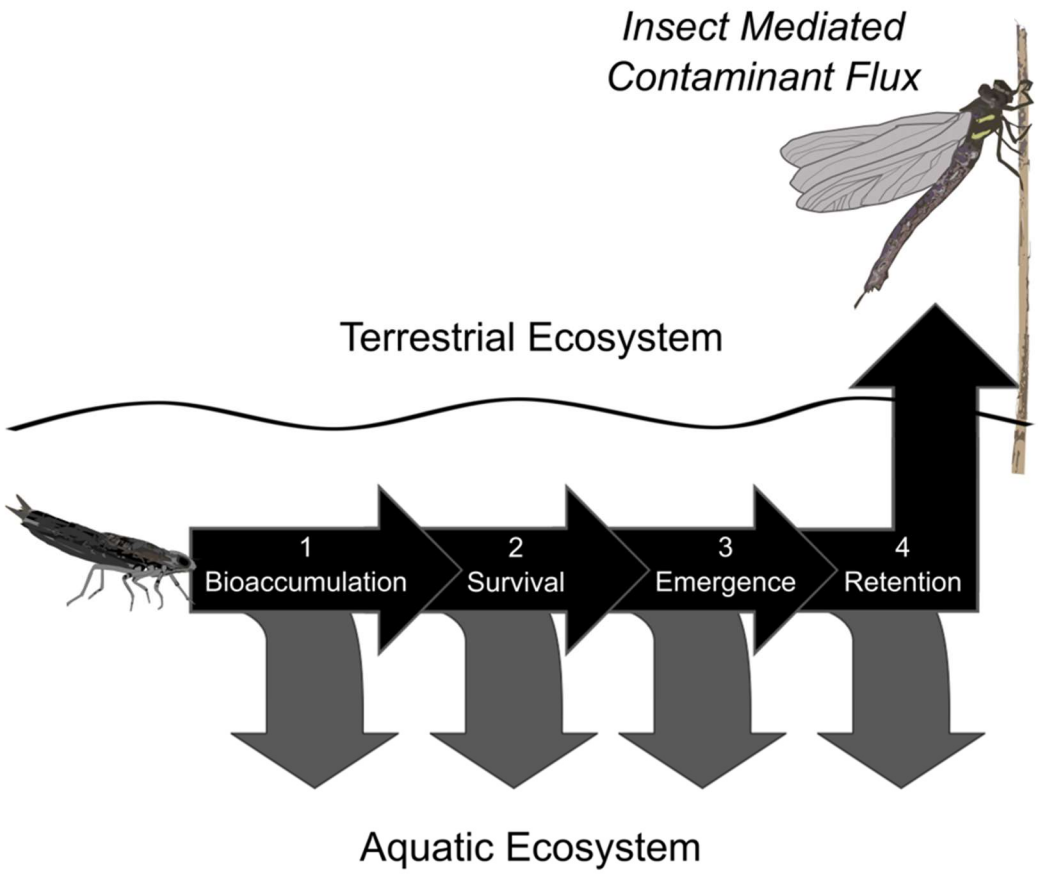

Figure 2. The Riparian Impact Test. A stepwise decision tool to assess if insect-mediated contaminant flux should be considered in an ecological risk assessment. Each numbered arrow represents a condition of the test. If the condition is not met, then the contaminant is retained in the aquatic ecosystem (gray arrows).

tetragnathids they are bound to the land water interface, they typically feed on terrestrial animals falling into the stream (Jardine *et al.* 2008).

Naturally abundant stable isotopes, like $\delta^{13}C$ and $\delta^{15}N$ have been used to infer the dietary reliance of riparian predators on adult aquatic insects and to estimate the degree of connectedness/trophic-overlap of neighboring aquatic and riparian communities. In the headwater streams of the southern Appalachian Mountains, the habitat along a stream edge and the assemblage of riparian predators would likely be tethered to each other in a constant feedback loop referred to as reciprocal subsidies (Nakano & Murakami 2001). In neighboring aquatic and riparian systems such as those studied here, the standard ellipse area corrected for sample size (SEA_c) of the riparian and aquatic food-webs would be expected to have a high percentage of overlap in $\delta^{13}C$ – $\delta^{15}N$ biplot space, although to the best of our knowledge, this has not be tested or published .

The dietary reliance of riparian predators on adult aquatic insects has been identified as the primary driver in the variable contaminant tissue concentrations observed among riparian predators (Walters et al. 2008; Jardine et al. 2009; Jardine et al. 2012; Walters et al. 2018; Ortega-Rodriguez et al. 2019). For instance, tetragnathid tissue concentrations were observed to be a product of THg trophic magnification in the aquatic system (Speir et al. 2014), and Ortega-Rodriguez et al. (2019) observed the highest Hg concentrations in the spiders with the highest % aquatic diet: fishing spiders and tetragnathids. Similarly, Walters et al. (2018) observed higher total polychlorinated biphenyl (PCB) concentrations in the presumably more aquatic feeding tetragnathids than the more terrestrial feeding araneids. Meanwhile, striders have been shown to be weakly

linked to aquatic primary production and have even been suggested as sentinels of terrestrial Hg contamination (Jardine *et al.* 2009; Jardine et al. 2012).

The effect aquatic Hg biomagnification has on riparian predator tissue concentrations in highly connected communities remains generally unexplored with no known studies expanding to even a regional level. The objective of this study was to better understand the relationship of THg trophic magnification in the aquatic food web with the THg concentrations of neighboring riparian predators at multiple Appalachian Mountain streams (Chapter 1). The hypotheses of this chapter are that:

(1) The variability in $\delta^{13}C$ and $\delta^{15}N$ in riparian predators will be spatially integrated with regards to the $\delta^{13}C$ and $\delta^{15}N$ observed in larval caddisflies (chapter 1). The spatial integration will lead to a high degree of trophic overlap (overlap of SEA_c) between the riparian and aquatic communities, and no differences in trophic level within taxa, among reaches.

(2) within a reach, tetragnathid and araneid Hg concentrations will be greater than the more terrestrial feeding striders and all riparian taxa will have lower THg concentrations than the neighboring trout population.

(3) The Hg concentrations will vary among reaches and taxa, and that these differences will be explained by Hg biomagnification in the aquatic system.

Materials and Methods

Site Description

For site description. See chapter 1.

Sample Collection

Identical sampling methodology was used during all sampling events. Tetragnathids, araneids, and striders were collected at night, at least 1 h after sunset. Using headlamps, tetragnathids and araneids were collected by hand from overhanging vegetation and structures along the shoreline, no further than 1 m inland. Striders were collected from the water surface by hand or with the use of sweep nets. At each reach, 5 individual tetragnathids and striders were collected, placed into individual 2 mL polyethylene tubes, and stored at -20^{o}C until laboratory sampling. All other animals were placed into taxon-specific polypropylene tubes, placed in a cooler with dry ice, and transported to the lab where they were sorted into taxon-specific composite samples of multiple animals (n =3/reach) for Hg analysis. To preserve biomass for mercury analysis, the front left leg (L1) of each araneid in a composite sample was excised where the femur met the trochanter, combined into separate polyethylene tubes, and stored at -20^{o}C until analyzed for stable isotopes (Walters et al. 2008). The remaining biomass was stored at -20^{o}C until analyzed for mercury.

Mercury analysis

All strider composite samples as well as the tetragnathid and araneid samples with less than 2 g biomass (n = 12) were homogenized and analyzed for THg according to USEPA Method 1631 (U.S. EPA 2002). Briefly, composite samples were homogenized

and stored frozen in acid-cleaned glass fluoropolymer jars. Samples were then transferred to a digestion vessel, digested with HNO_3 and H_2SO_4 on a 58°C hot block for one hour. Once cooled, samples were diluted with 0.02 N BrCl and left at room temperature for an additional 4 hours. Prior to analysis, an initial calibration verification was tested, and a continuing calibration verification was performed every ten samples. Analysis of total mercury was conducted utilizing an Analytik Jena (Jena, Germany) automated mercury analyzer. All samples were analyzed alongside method blanks (all reagents) and a laboratory control sample (LCS) and laboratory control sample duplicate (LCSD). Method blanks were undetectable (< 0.32 ng/g) and below the method reporting limit of 1.1 ng/g ww. Mean ± SD %-recovery for LCS and LCSD were 92 ± 8% and 99 ± 8%, respectively. The mean ± SD relative percent difference (RPD) of the paired LCS and LCSDs was 7 ± 2%, and all were within acceptable ranges. Sample quality assurance included the use of Tort-3, a standard reference material (SRM), that was used to make one SRM blank, and Matrix Spike (MS) and Matrix Spike Duplicates (MSD). In all cases, the %-recovery of the SRM blank, MS, and MSDs were within the acceptable range. Mean ± %-recovery of MS and MSDs were 99 ± 5% and 99 ± 5%, respectively.

Tetragnathid and araneid samples with adequate biomass (> 2 g) were analyzed for THg according to USEPA Method 7473 (U.S. EPA 1998) utilizing a milestone Direct Mercury Analyzer (DMA-80; Sorisole, Italy). These samples were analyzed alongside a method blank (all reagents). The method blank was below the method detection limit of 0.032 mg/kg and below the method reporting limit of 0.020 mg/kg ww. Sample quality assurance included the use of Tort-3, which was used to make SRM blanks, MS, and

MSDs. Sample quality assurance included the use of Tort-3, which was used to make SRM blanks, MS, and MSDs. In all cases SRM blanks, MS, and MSDs were within the acceptable range. Mean ± SD %-recovery for MS and MSD were 89 ± 4% and 91 ± 2%, respectively. Each paired MS and MSD was within an acceptable range for relative percent difference.

Stable isotope analysis

All sample were dried, homogenized, filtered to pass through a 40-mesh screen, combusted to CO^2 and N^2, and analyzed using a NC 2500 elemental analyzer *(Carlo Erba Milan, Italy)* interfaced to a Delta Plus isotope ratio mass spectrometer *(Thermo Finnigan Bremen, Germany).* All isotope values were reported in δ-notation in part per thousand, or per mil (‰) and represent the heavy to light isotopic ratio, $\delta^{13}C$ ($^{13}C/^{12}C$) and $\delta^{15}N$ ($^{15}N/^{14}N$) relative to reference standards (Vienna Peedee Belemnite carbonate and air, respectively). Precision was greater than 0.1‰ (1 SD) for both elements.

Trophic Level and $\delta^{13}C$ calculations

The mean larval caddisfly $\delta^{15}N$ at each reach was used to calculate the trophic level (TL) of striders, araneids, and tetragnathids (Hobson & Welch 1992; Jardine et al. 2006). To more appropriately compare the riparian consumers to adult aquatic insects, the trophic discrimination factor of 3.4 ‰ (Jardine et al. 2006) was modified by adding the 0.8 ‰ fractionation expected to occur when larval aquatic insects metamorphose into adult aquatic insects (Wesner et al. 2017):

$$TL = (\delta^{15}N \text{ consumer} - \delta^{15}N \text{ baseline}) / \Delta\delta^{15}N + \lambda \qquad \text{(Formula 1)}$$

Where:

TL = the calculated trophic position of the consumer

$\delta^{15}N_{consumer}$ = specific consumer $\delta^{15}N$

$\delta^{15}N_{baseline}$ = reach -specific mean larval caddisfly $\delta^{15}N$

$\Delta\delta^{15}N$ = trophic discrimination factor (4.2 ‰)

λ = the trophic level of larval caddisfly (primary consumer, λ=2)

Strider, tetragnathid, and araneid $\delta^{13}C_{bulk}$ were lipid-normalized utilizing the formulas and conversion factors (β_0 = -2.06 and β_1 = 1.91) provided by Logan et al. (2008):

$$\delta^{13}C_{LN} = \beta_0 + \beta_1 \text{ x } Ln(C:N) + \delta^{13}C_{bulk} \quad \text{(Formula 2)}$$

$\delta^{13}C_{LN}$ values (Mean ± SE) are provided in Table 2.

All $\delta^{13}C_{LN}$ values were transformed to account for 0.8‰ trophic fractionation (Vander Zanden & Rasmussen 2001):

$$\delta^{13}C = \delta^{13}C_{LN} - (TL-2) * 0.8‰ \quad \text{(Formula 3)}$$

The reach-specific distribution of taxa $\delta^{13}C$, $\delta^{15}N$, and TL was tested for normality using Shapiro-Wilk tests. The effect of reach on taxa $\delta^{13}C$, $\delta^{15}N$, and TL was evaluated using either an Analysis of Variance (ANOVA) or a Kruskal-Wallis test, and if appropriate, a Tukey or Steel-Dwass post hoc test, respectively.

Food web analysis

Each riparian community was characterized using methods developed by Layman et al. (2007) and Jackson et al. (2011). Briefly, Layman et al (2007) developed a quantitative approach to characterizing community level interactions (described in chapter 1); however, the total area metric encircles the mean values of organism groups and is sensitive to sample size and outliers. Jackson et al. (2011) developed additional

multivariate ellipse-based metrics that allow among community comparisons. One standard ellipse metric calculates standard ellipse area and corrects for small samples sizes and allows an unbiased comparison among communities.

$$SEA_c = SEA * (n-1)/(n-2) \quad \text{(Formula 4)}$$

Reach-paired riparian and aquatic trophic spaces (represented as SEA_c) were calculated respectively, and the % trophic overlap was defined as the intersection area of riparian and aquatic communities over the total niche area of the riparian community (Swanson et al. 2015).

Hg differences

THg concentrations were tested for normality using a Shapiro-Wilk tests. All data passed the assumptions of normality, and inter-reach THg concentrations among taxa were evaluated using either an ANOVA, and if appropriate, a Tukey post hoc test, respectively.

To determine whether THg concentrations were different among taxa, a one-way blocked analysis of variance (ANOVA) was used a Tukey HSD. THg data from aquatic taxa (Chapter 1) were integrated into the analysis. Here, taxon was considered a treatment, and reaches were treated as blocks with fixed effects. This analysis was conducted in JMP 14.0 ($\alpha = 0.05$).

Aquatic vs. Riparian Hg Dynamics

To assess whether mercury dynamics in the aquatic food web affect Hg tissue concentrations in riparian predators, the reach-specific best fit model for mercury biomagnification (Table 1), expressed as:

$$\log_{10}[THg] = \delta^{15}N\ (b) + a \quad \text{(Formula 5)}$$

was used to calculate predicted THg concentrations in riparian taxa. To determine whether the predicted values were affected by taxon, a ratio of the mean actual and mean predicted Hg values (A:P) was calculated for each reach and taxon, respectively. The ratios were then pooled by taxon, and compared using a one-way ANOVA, with a Tukey's post-hoc test ($p = 0.05$).

Reach and taxon mean THg concentrations and TL were pooled and a linear regression was performed to calculate the overall model for THg biomagnification in riparian taxa and aquatic taxa, respectively. Analysis of covariance (ANCOVA) to test the effect of community (aquatic or riparian) on $\log_{10}$THg using trophic level (TL) as a covariate.

Table 1. Reach-specific trophic magnifcation factors and best fit lines for THg biomagnifcation (from chapter 1).

	Aquatic THg Best Fit Line
Bald River Usptream	-0.692 + 0.5912X
Bald River Downstream	-0.578 + 0.5605X
Rock Creek	-0.667 + 0.5137X
LPHC Upstream	-0.264 + 0.4865X
LPHC Downstream	-0.398 + 0.5090X
Gentry Creek	-0.983 + 0.6709X

Results

Stable Isotope ratios

Tetragnathid $\delta^{13}C$ and $\delta^{15}N$ (Figure 3A) were significantly different among reaches ($\delta^{13}C$: $H = 18.0168$, $p = 0.0029$; $\delta^{15}N$: $F_{5, 29} = 19.6282$, $p < 0.0001$). Araneid $\delta^{13}C$ and $\delta^{15}N$ (Figure 3B) were significantly different among reaches ($\delta^{13}C$: $H = 12.7427$, $p = 0.0259$; $\delta^{15}N$: $F_{5, 17} = 51.1711$, $p < 0.0001$). Strider $\delta^{13}C$ and $\delta^{15}N$ (Figure 3C) were significantly different among reaches ($\delta^{13}C$*:* $F_{5, 29} = 10.9112$, $p < 0.0001$; $\delta^{15}N$: $F_{5, 29} = 21.6494$, $p < 0.0001$). Tetragnathid TL was significantly different among reaches ($F_{5, 29} = 3.9812$, $p < 0.009$; Table 2). Araneid TL was significantly different among reaches ($F_{5, 17} = 8.5147$, $p < 0.0012$; Table 2). Strider TL was significantly different ($F_{5, 29} = 7.0848$, $p < 0.0003$) among reaches (Table 2). Tetragnathid $\delta^{13}C_{LN}$ was significantly among reach es ($H = 17.5265$, $p = 0.0036$). Araneid $\delta^{13}C_{LN}$ was significantly different among reach es ($H = 10.7778$, $p = 0.0560$) Strider $\delta^{13}C_{LN}$ was significantly among reach es ($F_{5, 25} = 10.5146$, $p < 0.0001$). All $\delta^{13}C_{LN}$, $\delta^{13}C$, $\delta^{15}N$, and TL mean ± SE are reported by reach in Table 1.

Community Comparisons

The Bald River Upstream riparian SEA_c (1.93 ‰^{2}) was fully encompassed by the aquatic SEA_c (9.08 ‰^{2}) with 100% overlap (Figure 4A). The Bald River Downstream riparian SEA_c (8.06 ‰^{2}) and aquatic SEA_c (8.54 ‰^{2}) had the least amount of overlap (64%) (Figure 4B). The Rock Creek riparian SEA_c (1.41 ‰^{2}) was fully encompassed by the aquatic SEA_c (13.90 ‰^{2}) with 100% overlap (Figure 4C). The LPHC Upstream riparian SEA_c (12.14 ‰^{2}) was fully encompassed by the aquatic SEA_c (1.58 ‰^{2}) with 100% overlap (Figure 5A). The LPHC Downstream riparian SEA_c (10.00 ‰^{2}) was almost entirely

encompassed by the aquatic SEA_c (2.34 ‰^{2}) with 99% overlap (Figure 5B). The Gentry Creek riparian SEA_c (4.02 ‰^{2}) was almost entirely encompassed by the aquatic SEA_c (1.39 ‰^{2}) with 95% overlap (Figure 5C).

Hg differences

Total Hg concentrations were significantly different among taxa ($F_{6, 128}$, $p <$ 0.0001). THg concentrations in tetragnathids were significantly higher than araneids and all other taxa. Araneid THg concentrations were higher than all other taxa except Eastern Brook Trout. Total Hg concentrations varied among reaches depending on taxa (Figure 6; Table 3). The LPHC Upstream tetragnathids had significantly higher average THg concentrations than all other reaches ($F_{4, 14}$ = 22.5262, $p <$ 0.0001), and had the highest average THg concentration among all reported taxa (Figure 6A). Araneids THg concentrations were not significantly different among reaches ($F_{5, 17}$ = 2.5793, p = 0.0830; Figure 6B). Striders were significantly different among reaches ($F_{5, 17}$ = 6.6564, p = 0.0035; Figure 6C), and the highest average THg concentration in striders 41.2 ± 1.2 (Bald River Upstream) was below the lowest reported average spider THg concentration 43.7 ± 2.6 (Gentry Creek araneids).

Actual and expected THg concentrations

Expected THg concentrations in riparian predators were calculated using the best fit lines for trophic magnification from the aquatic system (Table 1). Actual riparian predator THg concentrations were higher than those predicted for all taxa at all reaches (Figure 7; Table 4). The actual : predicted ratio (A : P) were different among taxa ($F_{2, 14}$ = 5.9587, p = 0.0134). The tetragnathid A : P ratio (9.30) was significantly greater than strider

(p = 0.0110) but not araneid (p = 0.0870). There was no difference in the araneid and strider A : P ratios (p = 0.5001)

Ecosystem linear regressions showed a strong correlation between THg concentration and TL (Figure 8). Both regressions were statistically significant (aquatic: $F_{1, 16} = 288.97$, $p < 0.0001$; riparian: $F_{1, 15} = 27.79$, $p < 0.0001$). The TMF for the riparian ecosystem was 8.95 and for the aquatic ecosystem was 3.6. ANCOVA was used to determine if there was a difference in trophic magnification in the two ecosystems. There was a significant interactive effect between TL and ecosystem on $\log_{10}$THg indicating trophic magnification was different between the ecosystems ($F_{1, 31} = 6.8232$, $p = 0.0137$) and y-intercepts were different (p = 0.0021). The main effect of TL significantly and positively correlated with $\log_{10}$THg ($F_{1, 31} = 99.7952$; $p < 0.0001$), indicating that THg biomagnification occurred in taxa irrespective of ecosystem. There was a significant effect of ecosystem on $\log_{10}$THg ($F_{1, 31} = 230.4952$, $p < 0.0001$); the least square mean (LSM) ± SE of riparian taxa THg (1.95 ± 0.04) was greater than the that of aquatic taxa (1.06 ± 0.04).

Table 2. Tetragnathid, araneid, and strider site-specific mean ± SE lipid-normalized carbon ($\delta^{13}C_{LN}$), carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) stable isotope ratios as well as trophic level (TL). When present, connecting letters indicate taxon-specific significant differences among reaches for $\delta^{13}C_{LN}$, $\delta^{13}C$, $\delta^{15}N$, and TL.

	$\delta^{13}C_{LN}$	$\delta^{13}C$	$\delta^{15}N$	TL
Tetragnathids				
Bald River Upstream	-25.17 ± 0.27 ^{AB}	-25.26 ± 0.27 ^{AB}	4.16 ± 0.12 ^{B}	3.14 ± 0.03 ^{AB}
Bald River Downstream	-26.09 ± 0.94 ^{B}	-26.18 ± 0.94 ^{B}	3.96 ± 0.53 ^{B}	3.12 ± 0.13 ^{AB}
Rock Creek	-24.29 ± 0.35 ^{AB}	-24.40 ± 0.35 ^{AB}	4.88 ± 0.15 ^{B}	3.26 ± 0.04 ^{AB}
LPHC Upstream	-23.68 ± 0.19 ^{A}	-23.78 ± 0.19 ^{A}	6.90 ± 0.24 ^{A}	3.22 ± 0.06 ^{AB}
LPHC Downstream	-24.19 ± 0.05 ^{AB}	-24.30 ± 0.05 ^{AB}	7.14 ± 0.36 ^{A}	3.42 ± 0.08 ^{A}
Gentry Creek	-24.33 ± 0.28 ^{AB}	-24.41 ± 0.27 ^{AB}	4.67 ± 0.28 ^{B}	2.98 ± 0.07 ^{B}
Araneids				
Bald River Upstream	-24.80 ± 0.12	-24.88 ± 0.12	3.66 ± 0.17 ^{C}	3.02 ± 0.04 ^{A}
Bald River Downstream	-26.70 ± 1.17	-26.77 ± 1.17	2.99 ± 0.14 ^{C}	2.89 ± 0.03 ^{AB}
Rock Creek	-24.60 ± 0.06	-24.68 ± 0.06	3.75 ± 0.20 ^{C}	3.00 ± 0.05 ^{A}
LPHC Upstream	-24.59 ± 0.11	-24.68 ± 0.11	6.19 ± 0.14 ^{A}	3.05 ± 0.03 ^{A}
LPHC Downstream	-24.41 ± 0.01	-24.49 ± 0.14	5.13 ± 0.24 ^{B}	2.94 ± 0.06 ^{A}
Gentry Creek	-24.65 ± 0.07	-24.71 ± 0.07	3.63 ± 0.06 ^{C}	2.73 ± 0.01 ^{B}
Striders				
Bald River Upstream	-25.41 ± 0.22 ^{BC}	-25.46 ± 0.21 ^{BC}	1.73 ± 0.25 ^{C}	2.56 ± 0.06 ^{B}
Bald River Downstream	-26.09 ± 0.18 ^{C}	-26.12 ± 0.19 ^{C}	0.90 ± 0.17 ^{C}	2.39 ± 0.04 ^{B}
Rock Creek	-24.35 ± 0.16 ^{A}	-24.43 ± 0.16 ^{A}	3.37 ± 0.23 ^{AB}	2.9 ± 0.06 ^{A}
LPHC Upstream	-24.36 ± 0.14 ^{A}	-24.42 ± 0.14 ^{A}	4.69 ± 0.13 ^{A}	2.69 ± 0.03 ^{AB}
LPHC Downstream	-25.10 ± 0.30 ^{AB}	-25.16 ± 0.29 ^{AB}	3.99 ± 0.58 ^{A}	2.67 ± 0.14 ^{AB}
Gentry Creek	-25.17 ± 0.18 ^{AB}	-25.20 ± 0.18 ^{AB}	2.20 ± 0.28 ^{BC}	2.39 ± 0.07 ^{B}

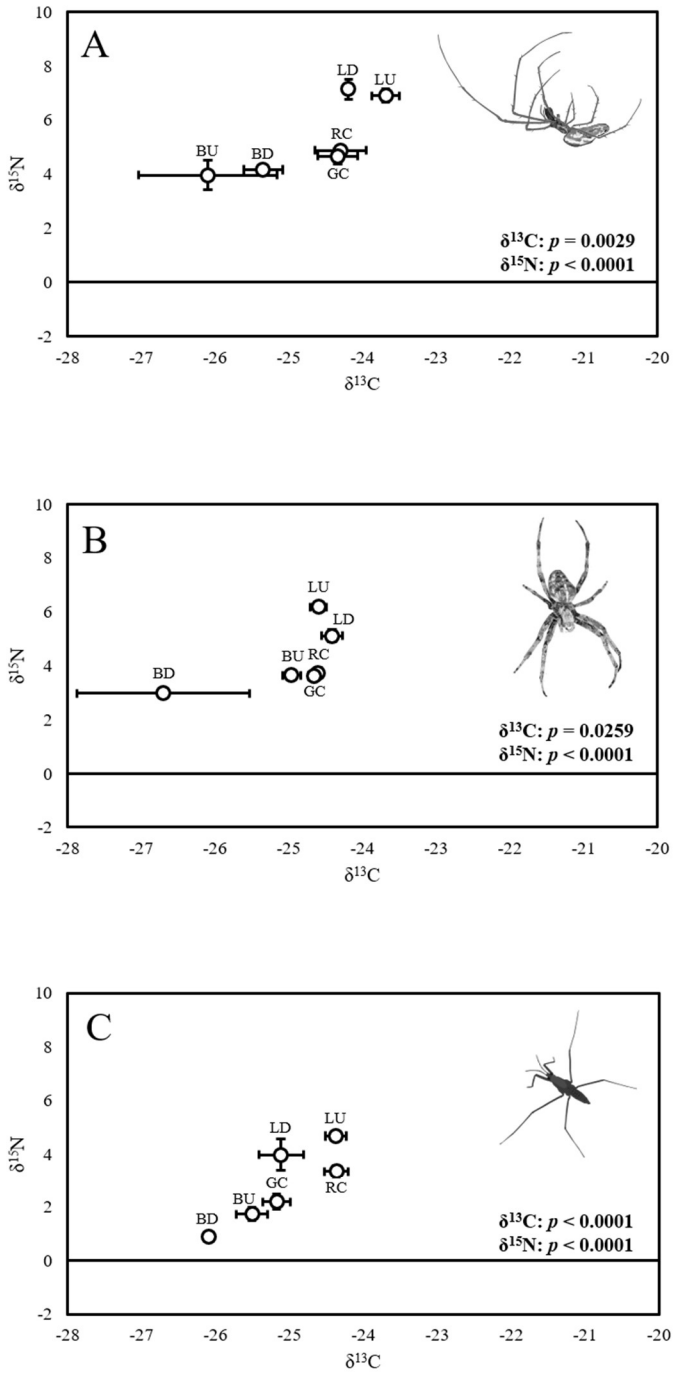

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ (mean ± SE) for (A) tetragnathids, (B) araneids, and (C) striders. The p-values provided within each respective sub panel represent $\delta^{13}C$ and $\delta^{15}N$ differences among sites: Bald River Upstream (BU), Bald River Downstream (BD), Rock Creek (RC), Left Prong Hampton Creek Upstream (LU), Left Prong Hampton Creek Downstream (LD), and Gentry Creek (GC).

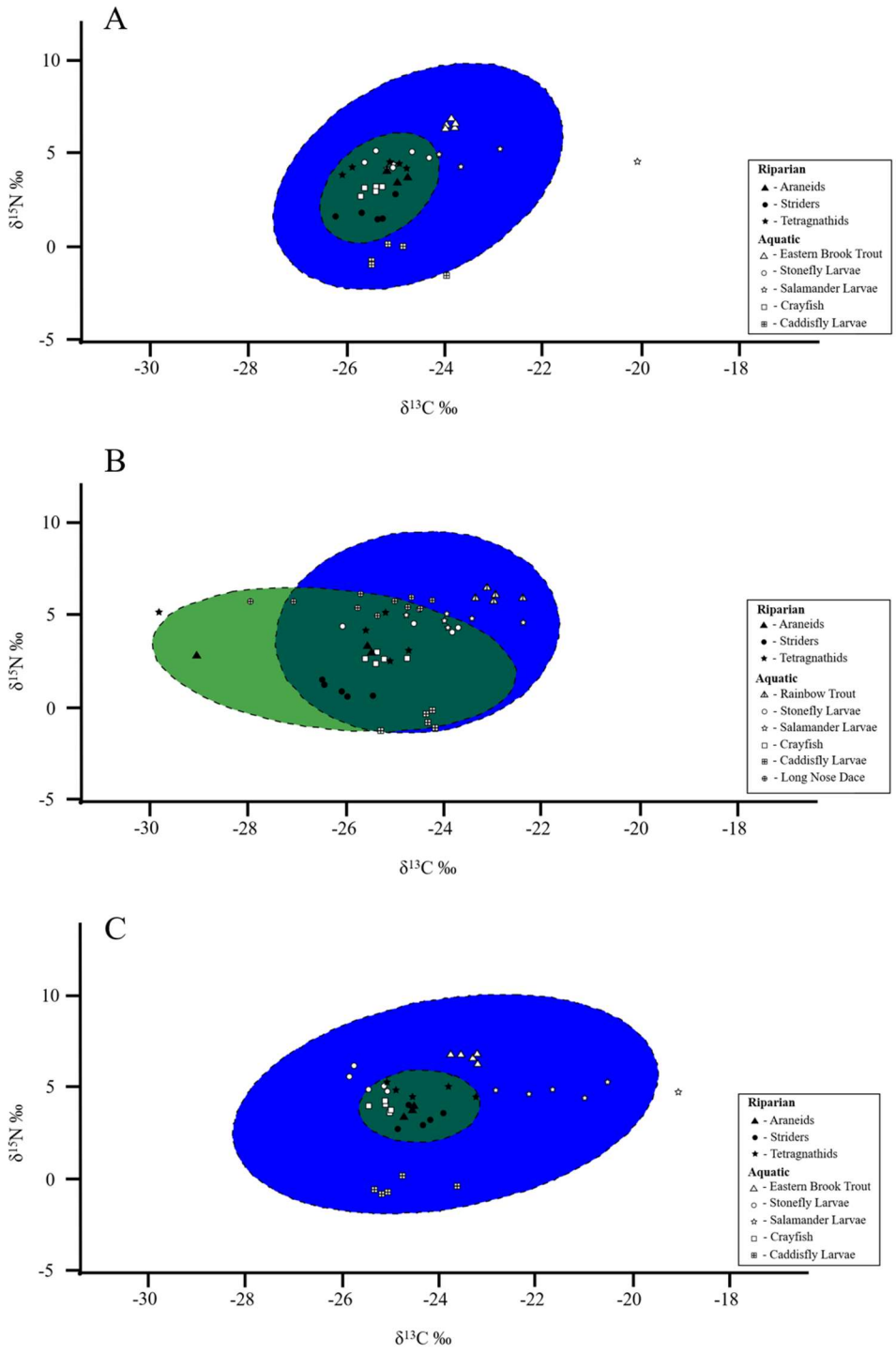

Figure 4. Stable isotope bi-plots for Bald River Upstream (A), Bald River Downstream (B), and Rock Creek (C) where the standard ellipse area (SEA_c) of the aquatic community is encircled and filled (blue) and the riparian community is encircled and filled (green). A legend with the riparian and aquatic taxa collected is provided with each respective reach.

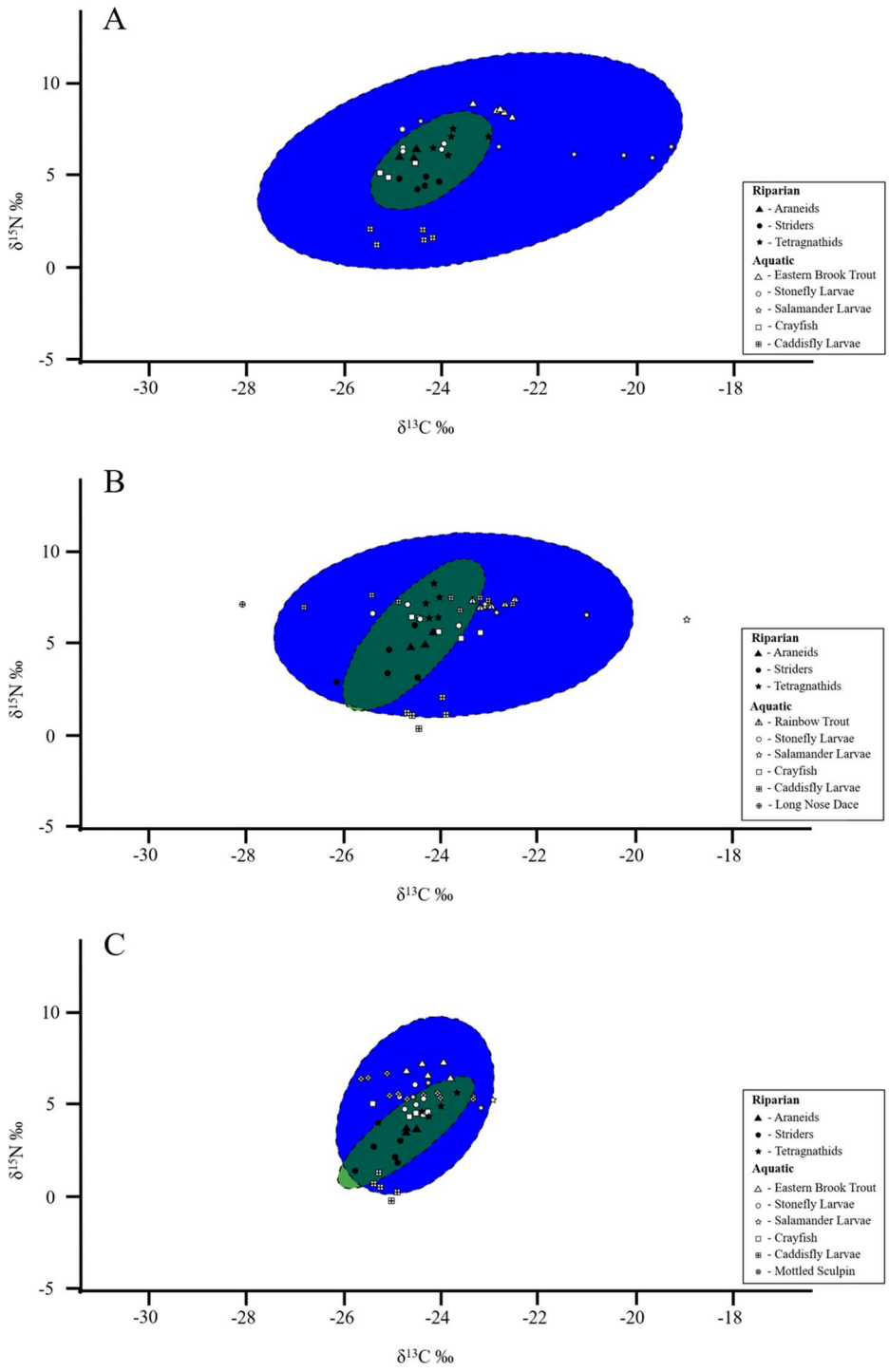

Figure 5. Stable isotope bi-plots for Left Prong Hampton Creek Upstream (A), Left Prong Hampton Creek Downstream (B), and Gentry Creek (C) where the standard ellipse area (SEA_c) of the aquatic community is encircled and filled (blue) and the riparian community is encircled and filled (green). A legend with the riparian and aquatic taxa collected is provided with each respective reach.

Table 3. Tetragnathid, araneid, and strider reach-specific mean ± SE total mercury (THg) and Log_{10}THg concentrations.

	THg	Log_{10}THg
Tetragnathids		
Bald River Upstream	144.7 ± 23.6	2.15 ± 0.07
Bald River Downstream	208.3 ± 33.8	2.31 ± 0.07
Rock Creek	146.7 ± 13.3	2.16 ± 0.42
LPHC Upstream	416.7 ± 29.4	2.62 ± 0.03
LPHC Downstream	185.0 ± 9.9	2.27 ± 0.23
Gentry Creek	*not collected*	*not collected*
Araneids		
Bald River Upstream	88.9 ± 34.3	1.88 ± 0.18
Bald River Downstream	141.4 ± 35.4	2.12 ± 0.13
Rock Creek	60.0 ± 3.2	1.78 ± 0.23
LPHC Upstream	99.0 ± 11.0	1.99 ± 0.05
LPHC Downstream	78.7 ± 10.7	1.89 ± 0.06
Gentry Creek	43.7 ± 2.6	1.64 ± 0.03
Striders		
Bald River Upstream	41.2 ± 1.2	1.61 ± 0.01
Bald River Downstream	38.6 ± 0.1	1.59 ± 0.00
Rock Creek	26.2 ± 2.4	1.42 ± 0.04
LPHC Upstream	31.2 ± 1.5	1.49 ± 0.02
LPHC Downstream	33.6 ± 4.2	1.52 ± 0.06
Gentry Creek	30.1 ± 0.8	1.48 ± 0.01

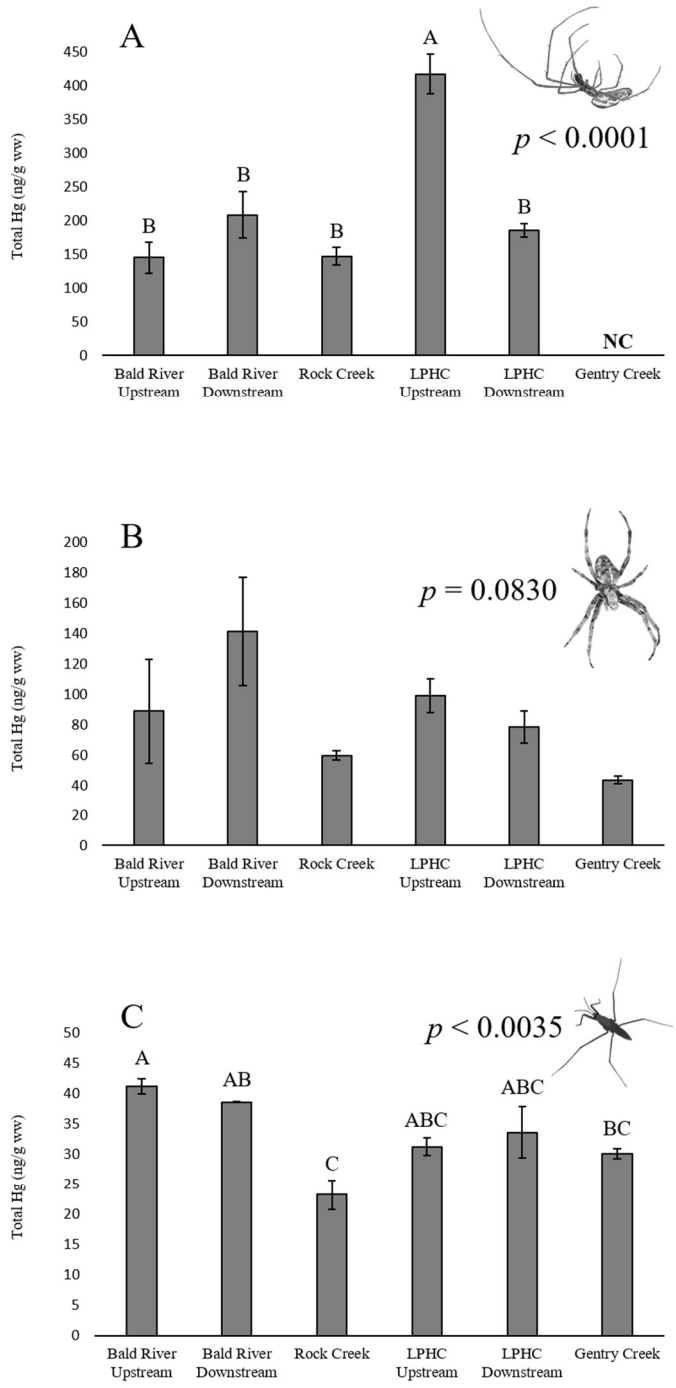

Figure 6. Total Hg (mean ± SE) for (A) tetragnathids, (B) araneids, and (C) striders. The p-values provided within each respective sub panel represent THg differences among reaches for the respective biota. Connecting letters indicate differences between reaches for each respective taxon. When insufficient biomass was collected for THg analysis, an "**NC**" is represented in place of the respective bar.

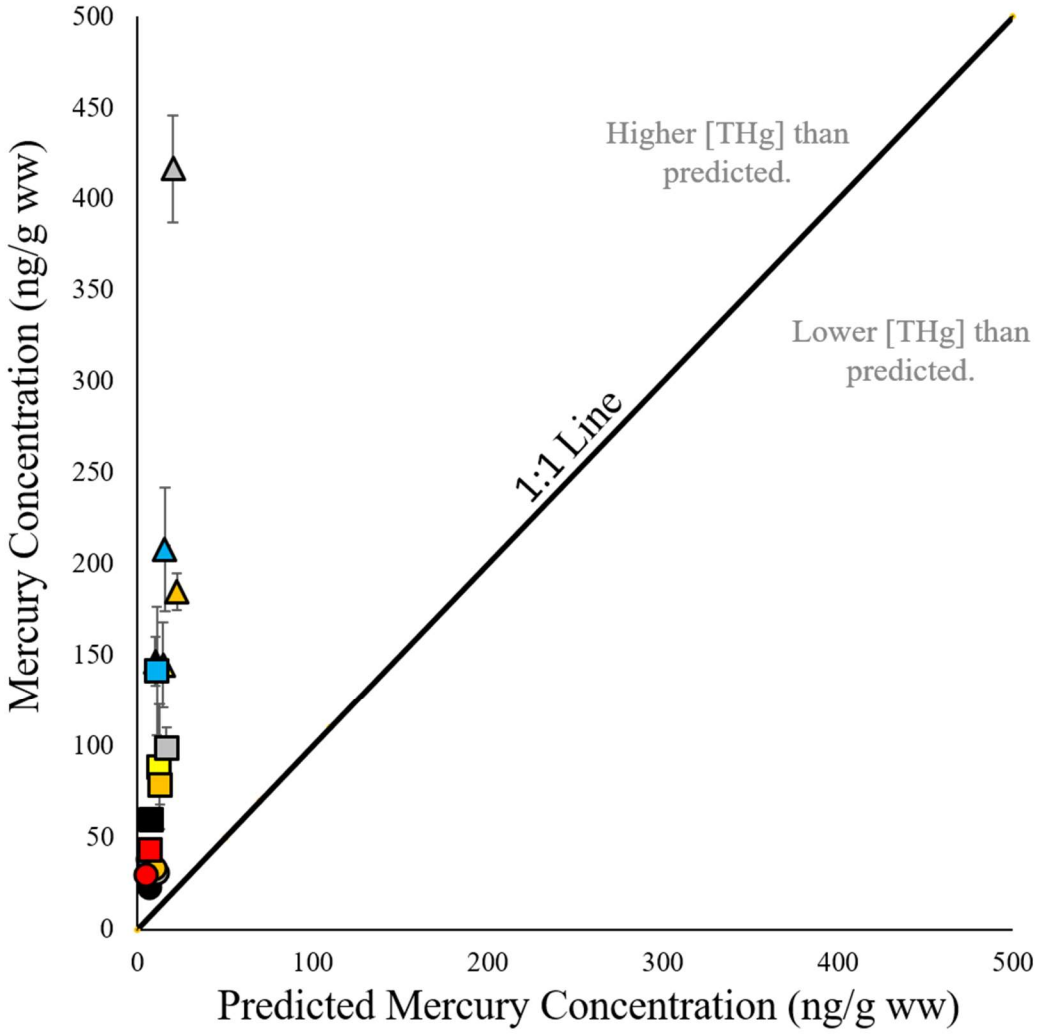

Figure 7. Riparian biota total mercury concentrations v. predicted mercury concentrations in riparian biota. Triangles represent tetragnathids, squares represent araneids, and circles represent striders. The color fill of each marker represents the site, where orange: Bald River Upstream, blue: Bald River downstream, yellow: Rock Creek, gray: LPHC upstream, black: LPHC downstream, and red: Gentry Creek

Table 4. Tetragnathid, araneid, and strider reach-specific mean ± SE predicted total mercury (THg) concentrations as a function of aquatic mercury biomagnification.

	THg
Tetragnathids	
Bald River Upstream	14.7 ± 0.6
Bald River Downstream	15.6 ± 2.4
Rock Creek	10.3 ± 0.4
LPHC Upstream	20.2 ± 1.3
LPHC Downstream	22.5 ± 2.3
Gentry Creek	10.6 ± 1.2
Araneids	
Bald River Upstream	12.5 ± 0.7
Bald River Downstream	11.0 ± 0.5
Rock Creek	7.5 ± 0.4
LPHC Upstream	16.6 ± 0.6
LPHC Downstream	12.6 ± 0.9
Gentry Creek	7.1 ± 0.1
Striders	
Bald River Upstream	6.7 ± 0.6
Bald River Downstream	5.8 ± 0.3
Rock Creek	6.7 ± 0.5
LPHC Upstream	11.1 ± 0.4
LPHC Downstream	9.7 ± 1.7
Gentry Creek	4.3 ± 0.5

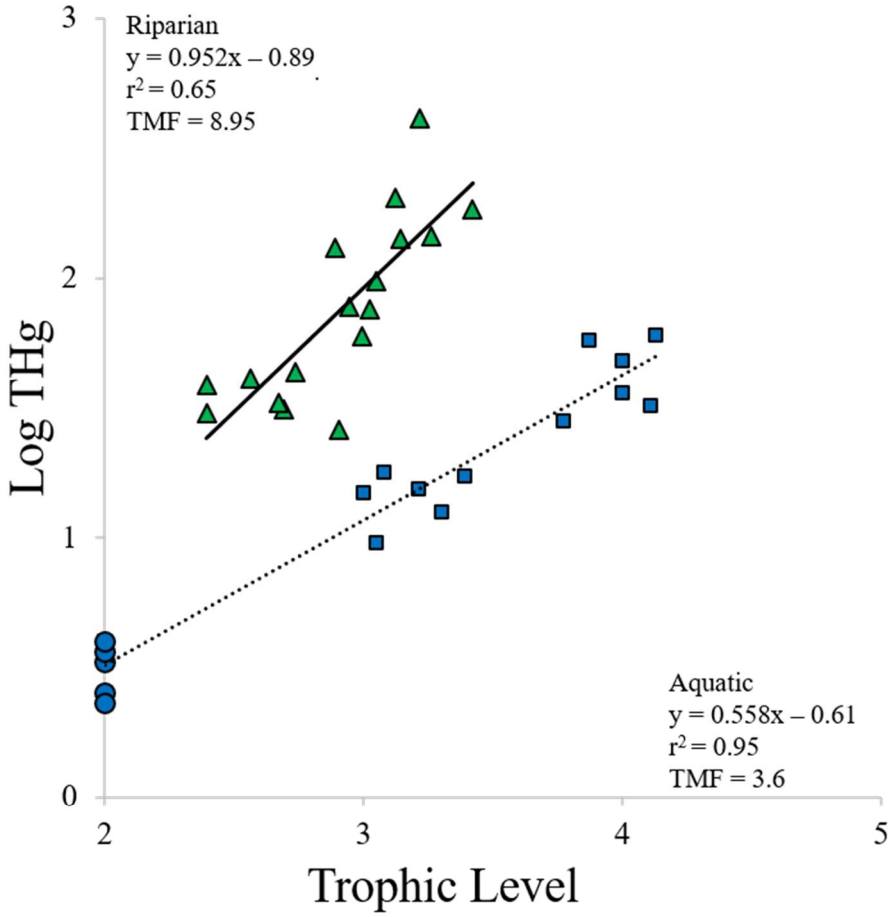

Figure 8. Log_{10}Total Mercury vs. Trophic Level of aquatic (blue squares) and riparian (green triangles) taxa collected at six sites in the Appalachian Mountains. Aquatic (dashed) and riparian (solid) best fit lines represent the overall model for THg trophic magnification in the respective systems.

Discussion

Terrestrial and aquatic food webs are linked together via a feedback loop often referred to as reciprocal subsidies (Nakano & Murakami 2001). In headwater streams – like those studied here – leaf litter would expect to be the primary carbon source and thus affect the benthic macroinvertebrate assemblages (Vannote et al. 1980). A large proportion of these insects are expected to emerge as adult aquatic insects and subsidize the riparian/terrestrial systems as prey (Ballinger & Lake 2006; Baxter et al. 2005). The exchange or flux of contaminants via these adult aquatic insects – often referred to as the dark side of subsidies – is expected to be the mechanism that moves the contaminants from aquatic food webs to riparian predators (Walters et al. 2008). The results of this study indicates that Hg dynamics in and around Southern Appalachian Mountain headwater streams are not solely the function of aquatic processes, but are also affected by terrestrial mechanisms/processes, such as terrestrial deposition and methylation, which are only now starting to be understood (Tsui et al. 2012; Bartrons et al. 2015; Kwon et al. 2015; Sullivan et al. 2016; Howie et al. 2019; Rodenhouse et al. 2019; Tsui et al. 2019).

Tetragnathids, araneids, and striders living adjacent to southern Appalachian Mountain headwater streams not only had variable $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios, but also variable trophic levels (TLs). In chapter 1, there was no difference in larval caddisfly $\delta^{13}C$, but there was a significant difference in $\delta^{15}N$. I hypothesized that (1) there would be a similar lack of $\delta^{13}C$ variability in riparian predators as there was at the base of the aquatic food web, and that (2) the significant differences in $\delta^{15}N$ would be spatially integrated – leading to a difference in $\delta^{15}N$ but no difference in the $\delta^{15}N$-based trophic level. However,

significant inter- reach differences for $\delta^{13}C$, $\delta^{15}N$, and TL were observed among each of the tested riparian taxa.

It's possible that the $\delta^{13}C$ in riparian predators is the product of a prominent terrestrial dietary item that wasn't tested. Raikow et al. (2011) utilized canopy traps to collect terrestrial arthropods and adult aquatic insects. The addition of canopy traps in the present study would have informed the terrestrial baseline $\delta^{13}C$ and would have allowed the characterization of the $\delta^{13}C$ and $\delta^{15}N$ in adult caddisflies and other adult aquatic insects. In the current study, tetragnathids and araneids at the Bald River Downstream had the greatest $\delta^{13}C$ variability, and striders had the most depleted (negative) $\delta^{13}C$ among all reaches. The more depleted $\delta^{13}C$ dragged the Bald River Downstream riparian SEA_c towards a more negative signature. This reach had the SEA_c with the highest observed riparian area and also had the lowest observed trophic overlap (64 %) of the aquatic and riparian communities (Figure 2B). The more negative $\delta^{13}C$ signatures observed are typically associated with terrestrial (C3) photosynthetic pathways (Brett et al. 2017). Future studies should characterize the $\delta^{13}C$ and $\delta^{15}N$ signatures of flying insects to better contextualize the $\delta^{13}C$ and $\delta^{15}N$ variability observed in riparian predators.

Spider contaminant concentrations can vary substantially based on the contaminant of concern and the species of the spider (Otter et al. 2013; Yang et al. 2016; Walters et al. 2018; Ortega-Rodriguez et al. 2019). For instance, Walters et al. (2018) found that total polychlorinated biphenyl (PCB) concentrations were higher in tetragnathids than araneids, and a positive correlation existed between the two taxa. Consistent with those findings, I observed higher THg concentrations in tetragnathids relative to araneids. However, in this

study there was no consistent relationship between the two taxa: the highest tetragnathid THg concentration (416.7 ± 29.4) was found at LPHC Upstream (Figure 4A), and the highest araneid THg concentration (141.4 ± 35.4) was found at Bald River Downstream (Figure 4B). With the high degree of overlap between the aquatic and riparian ecosystems (Figures 3 and 4), the inconsistent relationship appears to primarily be a function of dietary difference that affect trophic level – not resource pool - and thus THg concentrations (Figure 8).

A limitation of this study is the independence of $\delta^{13}C$ & $\delta^{15}N$ signatures and THg concentrations with the tetragnathids and striders. The data analysis of riparian predator tissue residues was thus limited by the way the data was collected. Future studies could excise the front left leg of tetragnathids designated for contaminant analysis, and (1) preserve biomass and (2) increase statistical power (Beaubien et al. 2019; Chapter 4).

The finding that aquatic and riparian communities had a high degree of trophic overlap, but that riparian predators THg concentrations were higher than expected, contradicts the traditional viewpoint that Hg is primarily an aquatic problem. The traditional viewpoint aligns with the recent work of Ortega-Rodriguez et al. (2019) who concluded that a higher proportion of aquatic dietary items led to higher concentrations in fishing spiders (Pisauridae, *Dolomedes*) and tetragnathids. The results of this study both support and contradict this dynamic – THg concentrations in tetragnathids exceed other riparian taxa; however, they also exceed all aquatic taxa. If THg concentrations in spiders were truly a product of the aquatic system, aquatic THg trophic magnification should model the maximum concentrations possible in riparian biota (e.g., Speir et al. 2014).

However, in the current study, THg concentrations in tetragnathids, araneids, and striders all exceeded expected concentrations.

The unconventional viewpoint – now gaining more traction and support – is that the terrestrial food web is contaminated with Hg at the same or higher levels than that of the aquatic food web. Bartrons et al. (2015) proposed that a possible mechanism that could lead to the high concentrations in spiders is a more terrestrial diet. Bartrons et al (2015) described this hypothesis as a "trophic bypass" – a process where spiders feeding more terrestrially, feed at a higher position on the food chain than those feeding aquatically and are thus exposed to higher concentrations of MeHg. If a more terrestrial diet was the primary factor leading to the high THg concentrations observed in riparian predators, we would expect that striders, not tetragnathids or araneids, would have the highest Hg concentrations, and that the a more depleted $\delta^{13}C$ signature would be associated with the highest concentrations within taxa. However, in the current study we observed the highest concentrations in tetragnathids (presumably most aquatic), and among reaches, we found the highest tetragnathid concentrations at the LPHC Upstream reach, not at the reach with the more terrestrial (negative) $\delta^{13}C$, Bald River Downstream.

This study demonstrates that THg biomagnification in the riparian food web occurs at a higher rate than the aquatic food web (Figure 8). The results of this study are not consistent with that of Tsui et al. (2019) who found that MeHg biomagnification in terrestrial food webs was occuring at the same rate as aquatic food webs (Tsui et al. 2019). Because Tsui et al. (2019) was focused on MeHg biomagnification and not THg biomagnification – like the research presented in this chapter – a direct comparison among

the studies is not appropriate. However, Tsui et al. (2019) found that aquatic and riparian TMFs fell within the range expected for temperate freshwater systems (1.73 – 8.28; derived from Lavoie et al. 2013), and in the present study the aquatic TMF fell within the expected range and the riparian did not (aquatic TMF = 3.6, riparian TMF = 8.95).

A possible explanation for the high tetragnathid concentrations and the inconsistent relationship with the araneid THg concentrations could be differential Hg deposition on the webs. Tetragnathids and araneids most often spin and ingest their web nightly (Gillespie 1987). However, whereas araneids spin a vertical orb higher in the riparian canopy, tetragnathids spin a horizontal web directly above the land-water interface (Levi 1981; Gillespie 1987; Aiken & Coyle 2000). The webs of spiders are physically and molecularly sticky, and because tetragnathids position themselves closer to the land-water interface their web may not only be susceptible to incoming Hg deposition, but also the Hg that has revolatilized off the water's surface (Selin 2009). If araneid web deposition is primarily unidirectional and tetragnathid web deposition is in fact bidirectional, the effect could be more than additive. Some researchers have found that PCBs bind to the webs of tetragnathids (Cindy Lee personal communication), but to date, there is no published research for Hg or other bioaccumulative contaminants. This phenomenon could be further complicated by the differences in web surface area and silk type. Future research could address this potential interference by collecting tetragnathid and araneid webs alongside their normal collections.

Conclusion

In this study, southern Appalachian Mountain tetragnathids, araneids, and striders living adjacent to headwater streams had variable $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios and TLs. The SEA_c of each respective reaches' neighboring aquatic and riparian communities had a high degree of trophic overlap in $\delta^{13}C$ and $\delta^{15}N$ biplot space (Figures 3 and 4). I hypothesized that the trophic overlap of the two communities would lead to tissue concentrations that were reflective of THg biomagnification in the aquatic system. I hypothesized that the more aquatic feeding tetragnathids would have higher THg concentrations than araneids, and both would have higher THg concentrations than the more terrestrial feeding striders, and in these systems, that was found to be true (THg: tetragnathid > araneids > striders). However, when the TL of riparian taxa was used to test the predictive function of aquatic THg biomagnification, the model consistently underestimated concentrations in tetragnathids, araneids, and striders, respectively (A:P was > 1; Figure 7). With regards to other riparian taxa, the higher concentrations in tetragnathids appear to be a product of their higher respective TL; however, tetragnathids had the highest average THg concentrations of all taxa – riparian and aquatic. THg appears to be moving through the riparian food chain at a higher rate than the aquatic. The higher concentrations observed in tetragnathids relative to aquatic taxa, maybe related to an unmeasured terrestrial input. The findings of this study contradict the theory that Hg concentrations in riparian taxa are solely a function of dietary Hg exposure via adult aquatic insects (Speir et al. 2014; Ortega-Rodriquez et al. 2019) and adds to a growing body of evidence that indicates terrestrial processes may be contributing to elevated Hg concentrations in riparian and terrestrial predators (Tsui et al. 2012; Tsui et al. 2014;

Bartrons et al. 2015; Sullivan et al. 2016; Tavshunsky et al. 2017; Howie et al. 2019; Rodenhouse et al. 2019; Tsui et al. 2019). However, these results do not implicate a more contaminated terrestrial food web otherwise the more terrestrial feeding striders and araneids would have higher concentrations than tetragnathids. Given the high concentrations of THg observed in riparian predators, THg exposure to riparian and terrestrial predators, like passerine birds, may be higher than previously expected is likely and warrants further investigation.

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CHAPTER III: THE POTENTIAL RISK OF MERCURY IN THE TISSUES OF SOUTHERN APPALACHIAN MOUNTAIN FISH AND SPIDERS.

Introduction

The southern Appalachian Mountains support high biodiversity in plants (Braun 1950; Stein et al. 2000), crayfish (Crandall and Buhay 2007) mussels (Parmalee and Bogan 1998; Graf and Cummings 2007), amphibians (Kozak et al. 2009), fishes (Lundberg et al. 2000; Abell et al. 2008), and birds (Quintero and Jetz 2018). The high levels of species and genetic diversity observed in southern Appalachian Mountain plants and animals has largely been attributed to two factors, (1) elevation gradients and the associated habitat diversity (Spehn et al. 2011; Quintero and Jetz 2018) and (2) the lack of glaciation events associated with higher latitudes (Hewitt 2000; Walker et al. 2009). Paradoxically, the location and elevation of the southern Appalachian Mountains are the primary factors attributed to the high rates of atmospheric mercury deposition (Selin 2009; Risch et al. 2017). Even in remote areas where the only known source of mercury was atmospheric deposition, the bioaccumulative properties of Hg have led to concentrations in fish that have exceeded thresholds of ecological concern for piscivorous birds and mammals (Lazorchak et al. 2003; Cristol et al. 2008; Evers et al. 2008; Jackson et al. 2013; Walters et al. 2015; Ackerman et al. 2016; Perkins et al. 2016).

Mercury has traditionally been viewed as an aquatic problem (see chapter 1); however, the emergence of adult aquatic insects has also led to high concentrations of Hg in riparian predators (see chapter 2), like spiders and birds (Cristol et al. 2008; Williams et al. 2017). Riparian spiders can be especially concerning because they extend the aquatic

food web into the riparian zone and provide an additional opportunity for the contaminants to biomagnify before the spiders themselves are preyed upon by other predators, like birds (Walters et al. 2009; Speir et al. 2014; Otter et al. 2020-*In Review*). Passerine nestlings may be particularly susceptible to Hg contamination because of the high proportion of spiders in their diet (Gunnarsson 2007). In fact, not only are spiders fed to nestlings, several studies have also demonstrated that parents provision a higher proportion of spiders to the youngest nestlings (Royama 1970; Ramsay and Houston 2003; Arnold et al. 2007; Gunnarsson 2007; Radford 2008; Browning et al. 2012).

To date, few studies have addressed the potential risk that contaminated spider tissues pose to arachnivorous birds. Those that have, have typically evaluated the potential risk of consuming spiders by calculating a spider Hg concentration threshold (spider-based avian wildlife values [SBAWV]), above which birds would be considered to be exposed to a physiologically significant concentrations of Hg if spiders are consumed in normal proportions of the expected diet (Cristol et al. 2008; Gann et al. 2015). Within the ecological risk assessment paradigm, potential risk to organisms of concern are often characterized using risk quotients (RQ). RQs are calculated by comparing a SBAWV, or some other calculated toxic threshold (TOX), to exposure (EXP), the concentration of the contaminant in the prey item: RQ = EXP/TOX. In this type of calculation, adverse effects are expected if the RQ is > 1.

The calculation of spider-based avian wildlife values are useful because they present a proverbial red-line, above which there is a concern; however, to facilitate this type of comparison, spiders must be collected and analyzed, and the question becomes

"which spider taxa should be collected and analyzed?". Two different taxa of riparian spiders have been sampled, analyzed, and used as surrogates to represent "all spiders" in the calculation of RQs: araneids (Walters et al. 2009) and Tetragnathids (Gann et al. 2015). Although few comparisons of tetragnathids and araneid Hg concentrations exist, other research has demonstrated that Hg concentrations can vary significantly among riparian spider taxa (Ortega-Rodriguez et al. 2019).

The objective of this chapter is to characterize the potential risk that MeHg in wildlife poses to human (consuming fish), piscivorous wildlife, and arachnivorous birds and to determine the effect of taxa selection on SBAWV based risk characterization. This chapter generates previously unpublished Hg SBAWVs for adult Brown Creepers (*Certhia americana*), adult and nestling Prairie Warblers (*Setophaga discolor*), adult and nestling Red-cockaded Woodpeckers (*Picoides borealis*), nestling Field Sparrows (*Spizella pusilla*), nestling House Wrens (*Trogolodytes aedon*), nestling Eastern Bluebirds (*Sialia sialis*), and American Robins (*Turdus migratorius*). Additionally, this chapter uses more current allometric scaling formulas (Nagy et al. 1999) that updates previously SBAWVs calculated by Walters et al. (2009) and Gann et al. (2015) for adult and nestling American Kestrels (*Falco sparverius*), adult House Wrens (*Trogolodytes aedon*), adult Carolina Wrens (*Thryothorus lucdovicianis*), adult Marsh Wrens (*Cisothorus palustris*), adult Eastern Bluebirds, and nestling Chickadees (*Poecile spp.*). It is worth noting that the output of these calculations also enables the derivation of other contaminant specific SBAWVs (e.g., PCBs, DDT).

I hypothesize that:

1. Nonpoint source Hg has led to high Hg concentrations in spiders that if ingested could cause harm to passerine bird nestlings inhabiting the Appalachian Mountains.
2. Tetragnathid MeHg concentrations will be significantly higher than araneid concentrations and that the characterization of risk will be different depending on which taxa is used as a surrogate for "all spiders."

Materials and Methods

At each reach, the potential risks to humans, piscivorous mammals (otter and mink), piscivorous birds (Belted Kingfishers), and arachnivorous birds were characterized using a deterministic approach. Risk quotients (RQ) were calculated using the prey MeHg concentration as an estimate of exposure (EXP), and referenced or calculated screening values/wildlife values were used to estimate the effects of toxicity (TOX):

$$RQ = EXP/TOX \quad \text{(Formula 1)}$$

In this type of analysis MeHg concentrations in fish or spiders are expected to cause adverse effects when RQ > 1.

Trout whole-body (WB) THg composites (from chapter 1) were assumed to be 100% MeHg (USEPA 2000). At each reach, EXP to mink, otter, and belted king fishers was defined as the mean trout WB MeHg concentration (Table 1).

To calculate EXP to humans, WB MeHg concentration were transformed into filet equivalent MeHg concentrations using the regression analysis developed by Peterson et al. (2007):

$$\log_{10}(\text{filet [Hg]}) = 0.2545 + 1.0623 * \log_{10}(\text{whole fish [Hg]}) \quad \text{(Formula 2)}$$

Human EXP was defined as the reach-specific mean filet equivalent MeHg concentration (Table 1). For humans, TOX was defined as the USEPA (2001) human health screening value, and for otter, mink, and Belted Kingfisher, TOX was defined as the wildlife values calculated by Lazorcak et al (2003) (Table 2).

Risk to passerines

To assess exposure to arachnivorous birds, spider THg composite samples were converted to MeHg assuming MeHg was 70% of THg for tetragnathid composites (Otter et al. 2013; Tweedy et al. 2013) and 37% for araneid composites (Wyman et al. 2011; Rodenhouse et al. 2019) (Table 1). At each reach, EXP was defined as the mean MeHg concentration for taxa-specific composite samples.

For birds, TOX was defined as the calculated spider-based avian wildlife value (SBAWV). Although spiders are not the only dietary source of mercury, SBAWVs are calculated with only consideration for the contaminant exposure expected from spiders. SBAWVs were calculated similarly to the WV calculations for mink, otter, and Belted Kingfishers (Lazorchak et al. 2003) and previously calculated SBAWV (Walters et al. 2009; Gann et al. 2015) and odonate-based avian wildlife values (Williams et al. 2017). Here, the SBAWV is slightly modified from the original formula published in Walters et al. (2009); this original formula was not incorrect, but it was unnecessarily redundant because it included the body weight (BW) of the bird three times, (two of which cancel each other out). SBAWVs were calculated using the following equation:

$$SBAWV = [TD * (UF_a * UF_s * UF_l)^{-1}] * [(IR) * \%\text{-}S]^{-1} \quad \text{(Formula 3)}$$

Table 1. Methyl mercury (MeHg) mean ± SE for trout and spiders (Tetragnathid and Araneid) across all reachees. Trout species are annotated as reach subscripts, eastern brook trout (EBT); rainbow trout (RT). Insufficent spider mass was collected for the analysis of tetragnathid MeHg at the Gentry Creek site.

Site	Trout Filet MeHg (ng/g ww)	Trout Whole Body MeHg (ng/g ww)	Tetragnathid MeHg (ng/g ww)	Araneid MeHg (ng/g ww)
Bald River Upstream (EBT)	142 ± 6	61 ± 2	101 ± 16	33 ± 13
Bald River Downstream (RT	112 ± 12	49 ± 5	146 ± 24	52 ± 13
Rock Creek (EBT)	72 ± 4	32 ± 2	103 ± 9	22 ± 1
LPHC Upstream (EBT)	82 ± 2	36 ± 1	292 ± 21	37 ± 4
LPHC Downstream (RT)	62 ± 3	28 ± 1	130 ± 7	29 ± 4
Gentry Creek (EBT)	138 ± 20	60 ± 8	-	16 ± 1

Table 2. Reported mercury human health screening values (USEPA 2003) and piscivorous wildlife values (Lazorchak et al. 2003).

Organism	Human Health Screening Value (ng/g ww)
Human	300
	Wildlife Value (ng/g ww)
North American River Otter (*Lotra canadensis*)	100
American Mink (*Neovison vison*)	70
Belted Kingfisher (*Ceryle alcyon*)	30

where SBAWV is the concentration in spiders that could cause a physiologically significant effect (mg/kg or ng/g); TD is a referenced test dose (mg/kg/d); UFa, UFs, and UF_1 are uncertainty factors used to ensure the test dose is conservative (unitless); BW is the species-specific and age-specific body weight (g); IR is the wet mass of prey ingested per day normalized to 1 gram of body weight; and %-S is the % of spiders normally found within the diet of the given species and the specific age. Additional notes on each of the input variables follow:

Test Dose (TD) and Uncertainty Factors (UF_a, UF_s, UF_l)

The TD for MeHg (0.078 mg/kg/d) used in SBAWV calculation was the value recommended for the derivation of avian wildlife values (WVs) by the Great Lakes Water Quality Initiative Criteria Documents for the Protection of Wildlife (hereafter referred to as GLWQI; USEPA 1995). The test dose recommended by the GLWQI was referenced from reproductive effects using the mallard duck (Heinz 1974; Heinz 1975; Heinz 1976a; Heinz 1976b; Heinz 1979) and then uncertainty factors are applied to the value to ensure that it is properly conservative. Uncertainty factors used in this study were for extrapolating 1) across different species ($UF_a = 3$), 2) from subchronic to chronic ($UF_s = 1$), and 3) from the lowest observable adverse effect level (LOAEL) to the no observable adverse effect level (NOAEL) ($UF_1 = 2$). For this calculation, $TD/(UF_a * UFs * UF_1) = 0.013$ mg/kg/d. The 0.013 mg/kg/d threshold is also referred to as the toxic reference value (TRV; Fuchsman et al. 2017). The 0.013 mg/kg/d value has been disputed by Fuchsman et al. (2017) as being overly conservative; however, the TRV suggested by Fuchsman et al. (2017) for small to medium sized birds (12 – 423 g) was 0.05 mg/kg/d and if the uncertainty

factor for extrapolating across species were applied ($UF_a = 3$), the resultant TRV (0.017 mg/kg/d) is comparable to the value (0.013 mg/kg/d) used in this study.

Ingestion rate (IR)

Ingestion rate (IR; g/g/d) was calculated using the formula:

IR = [(FMR / DM) * [100%/(100% - %-moisture)]]/BW (Formula 4)

Field Metabolic Rates (FMR; kJ/d) were referenced from literature or calculated using the referenced species- and age-specific body weights and allometric equations provided by Nagy et al. (1999). Dry mass (DM; kJ/g) represents the amount of metabolizable energy provided by each gram of prey. In each calculation, the suggested value of 18.0 kJ/g for insectivorous birds and reptiles was used (Nagy et al. 1999). To account for the difference of water in the food, the 69 %-moisture in spiders (Gann et al. 2015) was used to calculate the amount of fresh matter ingested per day. The referenced species- and age-specific BW used to calculate FMR is used to normalize ingestion to 1 gram of body weight.

Percent spider diet (%-S)

The percentage (by volume) of the bird's diet that is expected to be spiders, was calculated using the best available data for each respective life stage. When a range of % spider diet was available, a range of SBAWVs were calculated for the appropriate life stages.

Species analyzed

To calculate species-specific SBAWVs, mass, ingestion rate, and dietary information were required. Because bird ingestion rate can be estimated using mean body

mass and allometric scaling (see section on ingestion rate), the primary limitation in the derivation of new SBAWVs was quantifiable dietary data (by volume or mass) that can be transformed into a relative percentage. A preliminary search for dietary data on birds that breed or reside in the Appalachian Mountain was conducted utilizing The Cornell Lab of Ornithology, Birds of North America (BNA) online data base. The original literature cited in BNA was then referenced and vetted to ensure it could be used in the development of new SBAWVs. New SBAWVs were calculated for Brown Creeper (*Certhia* americana) adults, Marsh Wren (*Cisothorus palustris*) adults, Prairie Warbler (*Setophaga discolor*) adults and nestlings (1 d, 3 – 4 d, and 12d), Field Sparrow (*Spizella pusilla*) nestlings (1 d, 2 d, 3 d, 4 d, and 5 d), House Wren (*Trogolodytes aedon*) adults and nestlings (10 d), Eastern Bluebird (*Sialia sialis*) nestlings (2 d, 5 d, 8 d, and 14 d), American Robin (*Turdus migratorius*) nestlings (2 d, 4 d, 8 d, 10 d, and 14 d), and Red-cockaded Woodpecker (*Picoides borealis*) adults and nestlings (9 – 12 d). Utilizing updated allometric equations (Nagy et al. 1999), updated SBAWVs were calculated for American Kestrel (*Falco sparverius*) adults and nestlings (1 -3 d and 7 – 10 d), Carolina Wren (*Thryothorus ludovicianus*) adults, Eastern Bluebird adults, and Chickadee (*Poecile spp.*) nestlings (1 d and 12 d).

Data Analysis

To determine whether tetragnathid and araneid MeHg concentrations were different, a one-way blocked analysis of variance (ANOVA) was performed. In this analysis spider type was considered a treatment, and reaches were treated as blocks with fixed effects. This analysis was conducted in JMP 14.0 ($\alpha = 0.05$).

Results

Human Health and Piscivorous Wildlife

At all reaches, trout filet MeHg concentrations were below the 300 ng/g USEPA (2003) human health screening value. All risk quotients were < 1 (range: 0.21 – 0.46) and the RQ combined average (± SE) was 0.34 ± 05 (Figure 1).

At all reaches, trout whole-body (WB) MeHg concentrations were below the otter and mink wildlife values (100 ng/g and 70 ng/g respectively; Lazorchak et al. 2003) with RQs < 1 at all reaches (Figure 2). RQs for otter and mink ranged from (0.28 – 0.61) and (0.40 – 0.87). The otter and mink RQ combined averages (± SE) were 0.44 ± 0.06 and 0.63 ± 0.08, respectively. With the exception of LPHC Downstream, trout WB MeHg concentrations exceeded the Belted Kingfishers (BKF) wildlife value (30 ng/g; Lazorchak et al. 2003) with RQs > 1 at 5 of the 6 reaches (Figure 2). BKF RQs ranged from (0.93 – 2.03) and the all reaches combined average (± SE) was 1.48 ± 0.20.

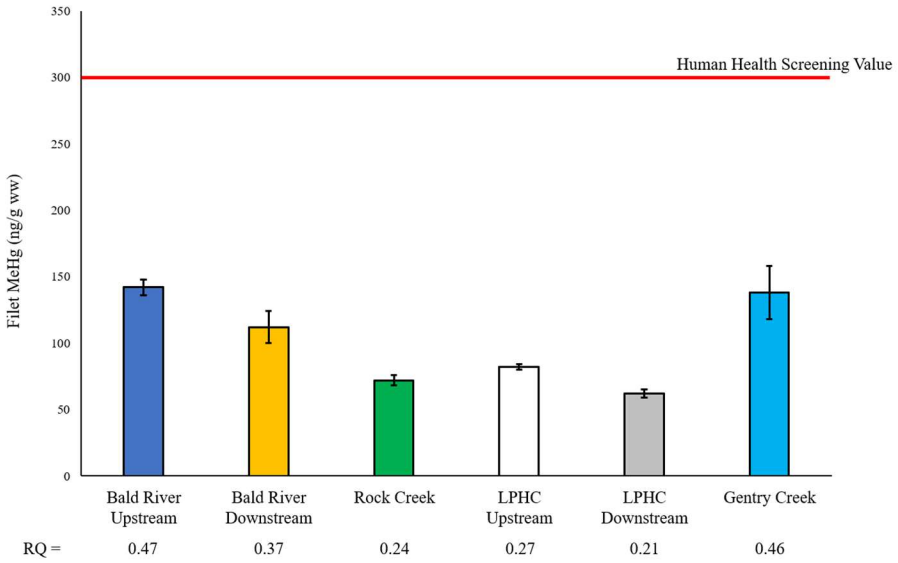
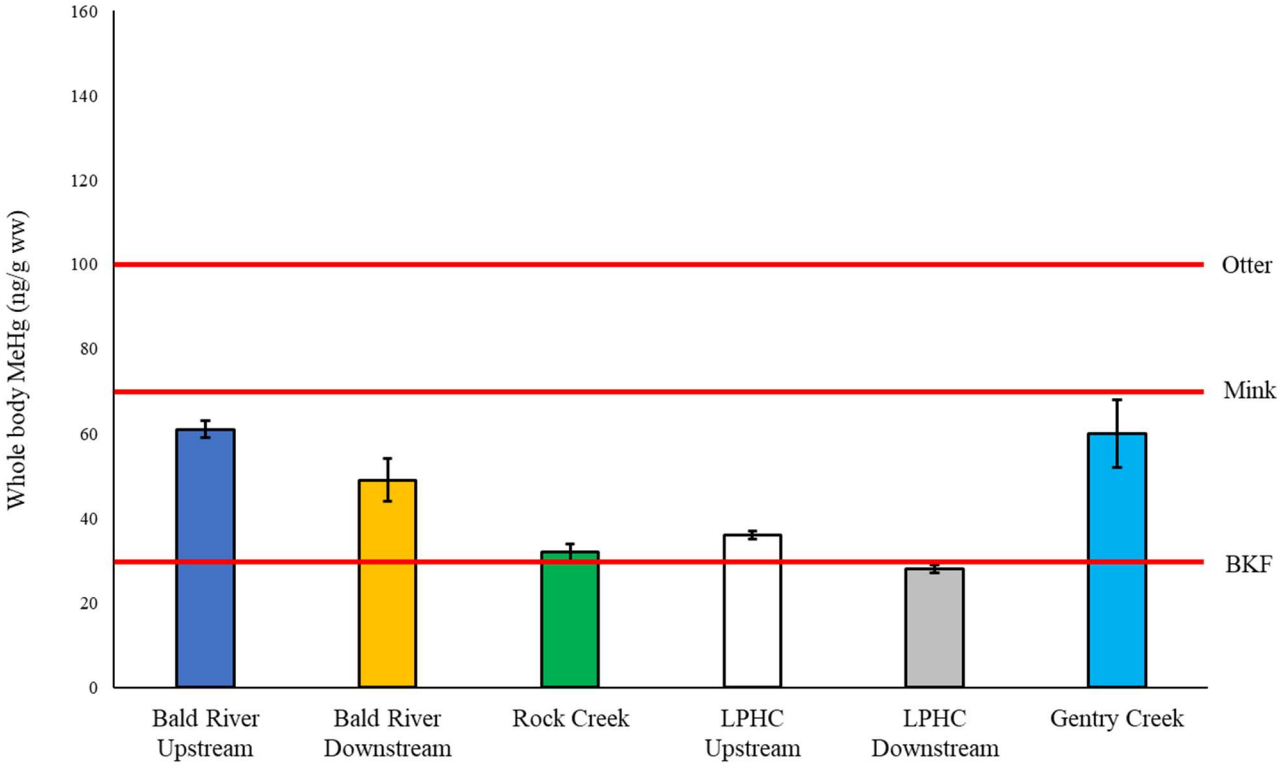


Figure 1. Average ± SE MeHg concentrations for trout filets from each respective reach. The USEPA (2003) human health screening value of 300 ng/g ww is indicated by the red line. The site specific RQ (risk quotient) is displayed below the reach name.



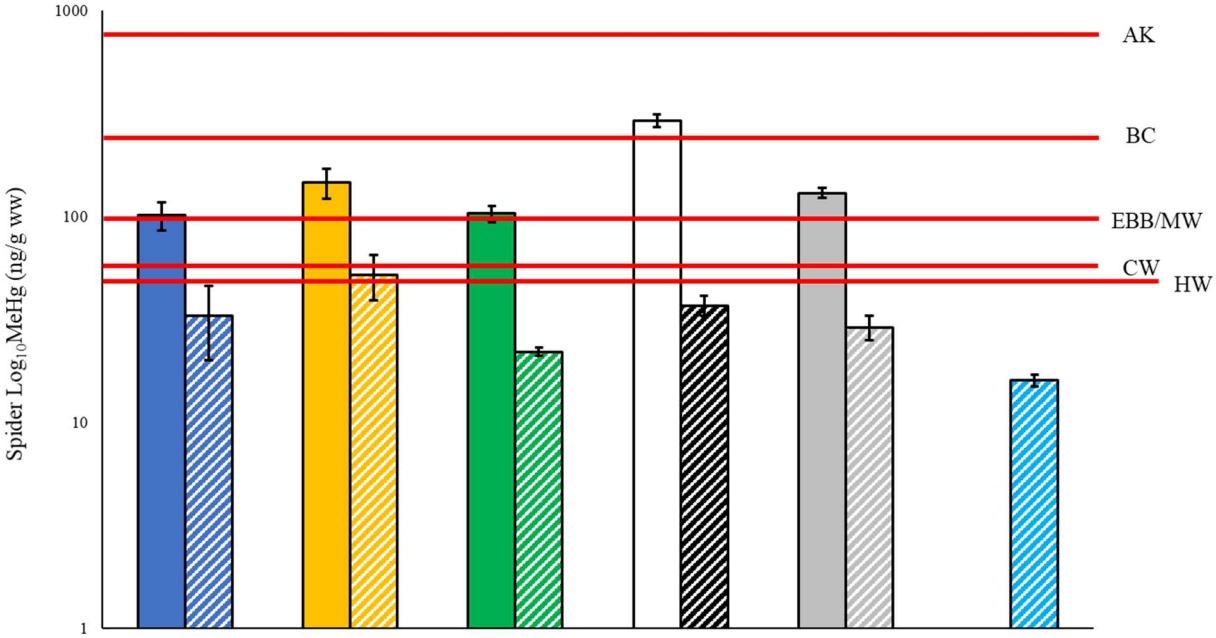
	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
Otter RQ =	0.61	0.49	0.32	0.36	0.28	0.60
Mink RQ =	0.87	0.70	0.46	0.51	0.40	0.86
BKF RQ =	**2.03**	**1.63**	**1.07**	**1.20**	0.93	**2.00**

Figure 2. Average ± SE MeHg concentrations for whole body trout at Appalachian Mountain streams. Otter, mink, and Belted Kingfisher (BKF) wildlife values (Lazorchak et al. 2003) are indicated by red lines. For each piscivore, the site-specific RQ (risk quotient) is displayed below the stream name. RQs > 1 are **bold.**

Adult SBAWVs

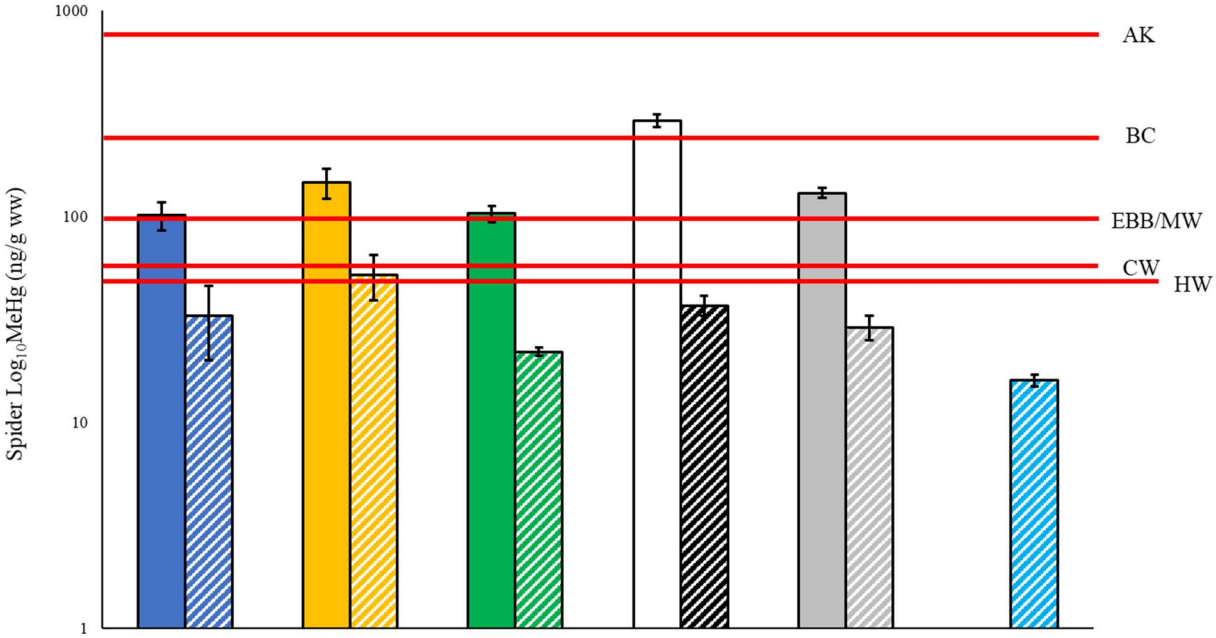
Adult bird, body weights (BW), field metabolic rates (FMR), ingestion rates (IR), percent spider diet (% SD) and the calculated spider based avian wildlife values (SBAWV) are provided in Table 1. Adult SBAWVs ranged from 48 – 1,188 ng/g. The SBAWV of the American Kestrel, Brown Creeper, House Wren, Carolina Wren, Marsh Wren, and Eastern Bluebird were calculated with respect to only one % SD. Of those birds, all but the American kestrel had at least one instance of an RQ > 1 (Figure 3). With the exception of the Bald River Downstream araneids, all RQ exceedances were calculated using tetragnathids as a surrogate for all spiders.

At all reaches where tetragnathids were collected, adult Prairie Warblers had a RQ > 1 for the high %-S (low SBAWV), and in one instance the RQ was > 1 with the low %-S (high SBAWV); there were no calculated exceedances when araneids were used as a surrogate for all spiders (Figure 4). For adult Red-cockaded Woodpeckers, no RQ were > 1 for the low or medium spider diets, 2 % S and 8 % S respectively. When spider diet was calculated as higher % of the diet (15 %-S), an RQ > 1 was detected at the reach with the highest tetragnathid MeHg concentrations (Figure 5).



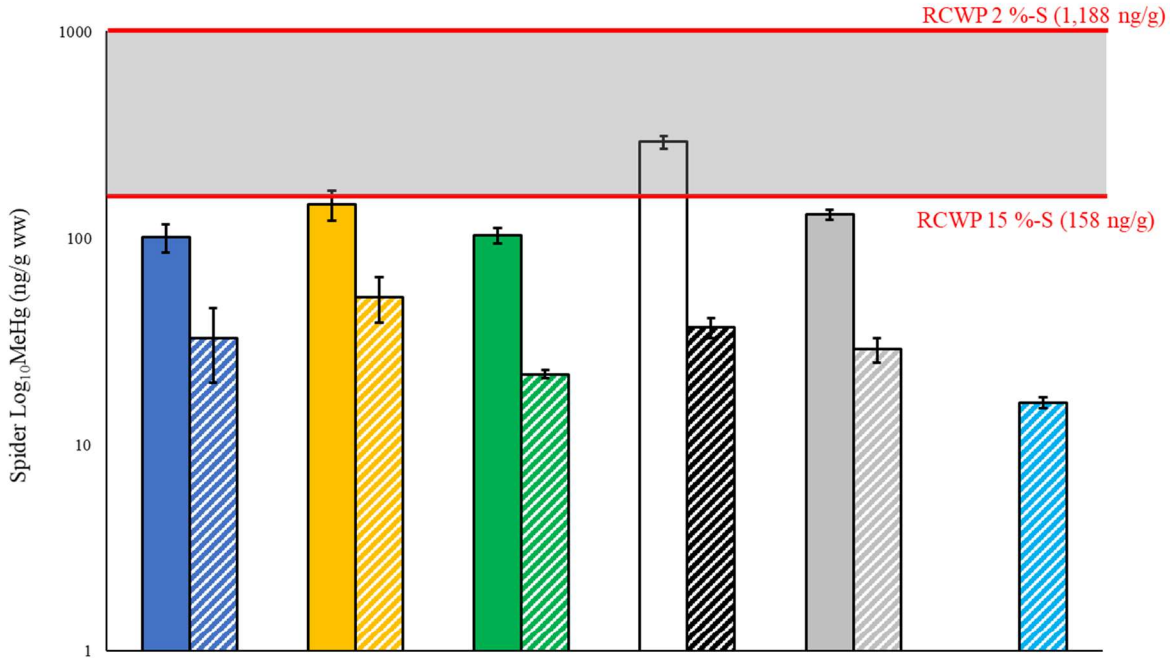
	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
AK RQ =	0.13, 0.04	0.19, 0.07	0.14, 0.03	0.38, 0.05	0.17, 0.04	- , 0.02
BC RQ =	0.42, 0.14	0.61, 0.22	0.43, 0.09	**1.22**, 0.15	0.54, 0.12	- , 0.07
EBB RQ =	**1.04**, 0.34	**1.51**, 0.54	**1.06**, 0.23	**3.01**, 0.38	**1.34**, 0.30	- , 0.16
HW RQ =	**2.10**, 0.69	**3.04**, **1.08**	**2.15**, 0.46	**6.08**, 0.77	**2.71**, 0.60	- , 0.33
CW RQ =	**1.74**, 0.57	**2.52**, 0.90	**1.78**, 0.38	**5.03**, 0.64	**2.24**, 0.50	- , 0.28
MW RQ =	**1.06**, 0.35	**1.54**, 0.55	**1.08**, 0.23	**3.07**, 0.39	**1.37**, 0.31	- , 0.17

Figure 3. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. American Kestrel (AK), Brown Creeper (BC), Eastern Bluebird (EBB), House Wren (HW), Carolina Wren (CW), and Marsh Wren (MW) SBAWVs are indicated by red lines. For each species, the site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name. RQs > 1 are **bold.**



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
AK RQ =	0.13, 0.04	0.19, 0.07	0.14, 0.03	0.38, 0.05	0.17, 0.04	- , 0.02
BC RQ =	0.42, 0.14	0.61, 0.22	0.43, 0.09	**1.22**, 0.15	0.54, 0.12	- , 0.07
EBB RQ =	**1.04**, 0.34	**1.51**, 0.54	**1.06**, 0.23	**3.01**, 0.38	**1.34**, 0.30	- , 0.16
HW RQ =	**2.10**, 0.69	**3.04**, **1.08**	**2.15**, 0.46	**6.08**, 0.77	**2.71**, 0.60	- , 0.33
CW RQ =	**1.74**, 0.57	**2.52**, 0.90	**1.78**, 0.38	**5.03**, 0.64	**2.24**, 0.50	- , 0.28
MW RQ =	**1.06**, 0.35	**1.54**, 0.55	**1.08**, 0.23	**3.07**, 0.39	**1.37**, 0.31	- , 0.17

Figure 3. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. American Kestrel (AK), Brown Creeper (BC), Eastern Bluebird (EBB), House Wren (HW), Carolina Wren (CW), and Marsh Wren (MW) SBAWVs are indicated by red lines. For each species, the site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name. RQs > 1 are **bold.**



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
RCWP (2 %-S) RQ =	0.09, 0.03	0.12, 0.04	0.09, 0.02	0.25, 0.03	0.11, 0.02	- , 0.01
RCWP (8 %-S) RQ =	0.34, 0.11	0.49, 0.18	0.35, 0.07	0.98, 0.12	0.44, 0.10	- , 0.05
RCWP (15 %-S) RQ =	0.64, 0.21	0.92, 0.33	0.65, 0.14	**1.85**, 0.23	0.82, 0.18	- , 0.10

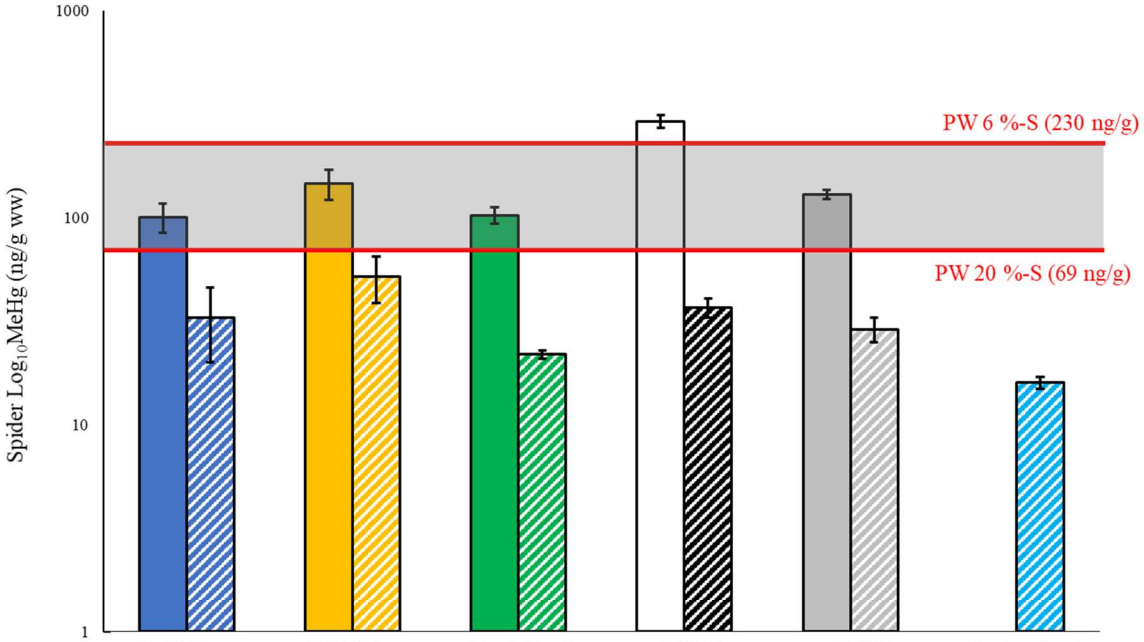
Figure 4. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. Red-Cockaded Woodpecker (RCWP) high and low SBAWVs, 2 % spider diet (%-S) and 15 %-S, are indicated with red lines and red font; the range between the two values is shaded gray. The site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name for the 2 %, 8 %, and 15 %-S SBAWVs. RQs > 1 are **bold.**

Table 3. Species specific adult body weights (BW), field metabolic rates (FMR), and ingestion rates (IR), and % spider diet (%SD) used in the calculation of MeHg spider-based avian wildlife values (SBAWV). Lower-case superscipts indicate where the data was referenced. Upper-case subscripts represent the low(L), medium (M), and high (H) reported averages and the associated SBAWV.

Order - Family **Common name (*Species*)**	**BW (g)**	**FMR (kJ/d)**	**IR (g/g/d)**	**% SD**	**SBAWV (ng/g)**
Falconiformes - Falconidae					
American Kestrel (*Falco sparverius*)	109[a]	256.3	0.43	4 %[h]	761
Passeriformes - Certhiidae					
Brown Creeper (*Certhia americana*)	8.55[b]	44.8[b]	0.95	5.7 %[i]	240
Passeriformes - Parulidae					
Prarie Warbler (*Setophaga discolor*)	8.78[c]	45.6	0.94	6 %$_L$, 20 %$_H$ [c]	230$_L$, 69$_H$
Passeriformes - Troglodytidae					
House Wren (*Troglodytes aedon*)	10.6[d]	60.8	1.04	9 %[h]	48
Carolina Wren (*Thryothorus ludovicianis*)	20[e]	79.8	0.72	31 %[e]	58
Marsh Wren (*Cisothorus palustrus*)	10[f]	49.8	0.90	15 %[f]	95
Passeriformes - Turdidae					
Eastern Bluebird (*Sialia sialis*)	18[e]	105.1	0.64	21 %[h]	97
Piciformes - Picidae					
Red-cockaded Woodpecker (*Picoides borealis*)	48[g]	144.6	0.55	2 %$_L$, 8 %$_M$, 15 %$_H$ [j]	1,188$_L$, 297$_M$, 158$_H$

∗ Unless indicated, Field metabolic rates (FMR) were calculated using allometric equations provided by Nagy et al. (1999). For Falconiformes and Piciformes, FMR was calculated using the "all birds" equation: $10.5 * BW^{0.681}$. For Passeriformes, FMR was calculated using the "passerine" equation: $10.4 * BW^{0.68}$.

[a] Bloom 1973 [c] Nolan 1978 [e] Cristol et al. 2008 [g] Koenig et al. 2005 [i] Otvos and Stark 1985
[b] Dunning 1984 [d] Dykstra and Karasov 1993 [f] USEPA 1993 [h] Walters et al. 2009 [j] Hess and James 1998



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
PW (6 %-S) RQ =	0.44, 0.14	0.63, 0.23	0.45, 0.10	**1.27**, 0.16	0.57, 0.13	- , 0.07
PW (20 %-S) RQ =	**1.46**, 0.48	**2.12**, 0.75	**1.49**, 0.32	**4.23**, 0.54	**1.88**, 0.42	- , 0.23

Figure 5. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. Prairie Warbler (PW) high and low SBAWVs, 6 %-spider diet (%-S) and 20 %-S, are indicated with red lines and red font; the range between the two values is shaded gray. The site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name for the 6% and 20%-S SBAWVs. RQs > 1 are **bold.**

Nestling SBAWVs

Nestling bird, age, BW, FMR, IR, % SD and the calculated SBAWVs are provided in Table 4. Nestling SBAWVs ranged from 28 – 1, 251 ng/g.

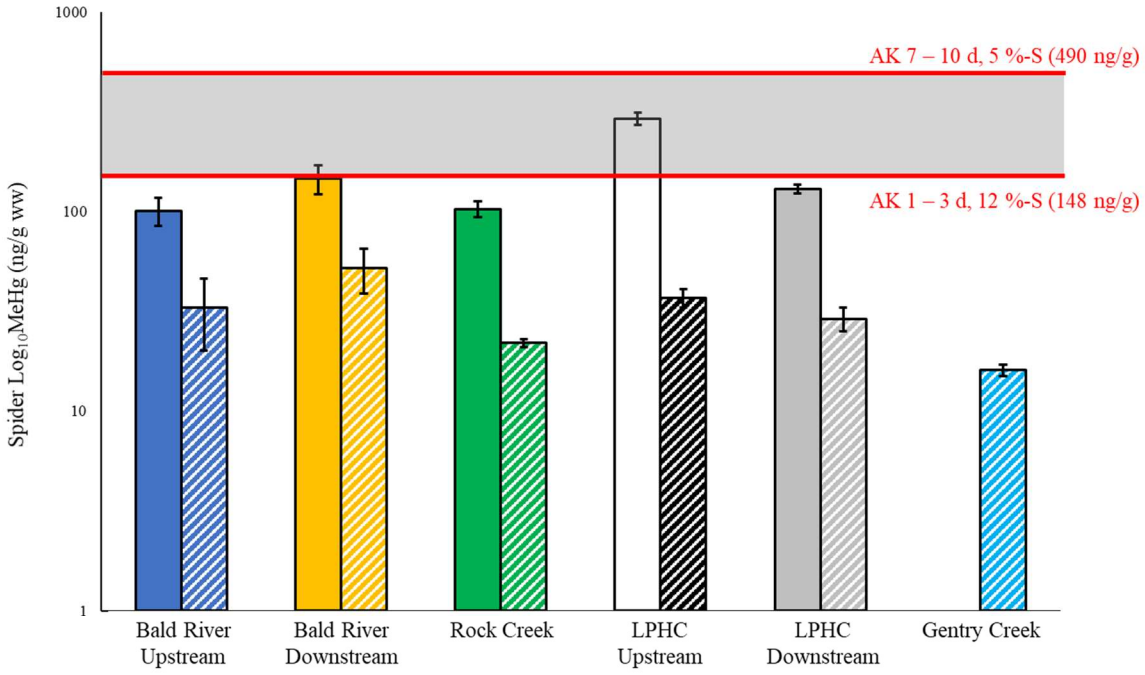
American Kestrel nestlings had an RQ > 1 at the LPHC Upstream reach for the 1 - 3 d and 7 – 10 d nestlings, but only when spiders were a high percentage of the diet, and only when tetragnathids were used as a surrogate for all spiders (Figure 6). Chickadee 1 d and 12 d nestlings had RQs > 1 at all reaches where tetragnathids were used as surrogate for all spiders (Figure 7). At all but two reaches, 1 d nestlings had RQs > 1 when araneids were used as a surrogate for all spiders, and at two of the reaches RQs were > 1 for 1 d and 12 d nestlings regardless of whether tetragnathids or araneids were used as a surrogate.

Prairie Warbler 1 d nestlings had an RQ > 1 at all reaches where tetragnathids were used as surrogate and spider diet was calculated at 9% (Figure 7). At two of the reaches, Bald River Downstream and LPHC Upstream, all nestling age groups (1 d, 3 – 4 d, and 12 d) had RQs > 1 when tetragnathids were used as a surrogate. All RQs were < 1 for all age groups and %-S when araneids were used as a surrogate. Similarly, Field Sparrow RQs were < 1 in all cases where araneids were used as a surrogate, but across all nestling age groups (1 d, 2 d, 3 d, 4 d, and 5 d) RQs were > 1 at LPHC Upstream when tetragnathids were used as a surrogate (Figure 8). At all reaches where tetragnathids were collected, RQs > 1 was calculated for field sparrows (Figure 9).

House wren RQs were < 1 at all reaches except LPHC Upstream (Figure 10). Eastern Bluebird RQs were > 1 for 1 d nestlings at all reaches when tetragnathids were used as a surrogate, and at two of the reaches, 1 d nestlings RQs were > 1 when araneids

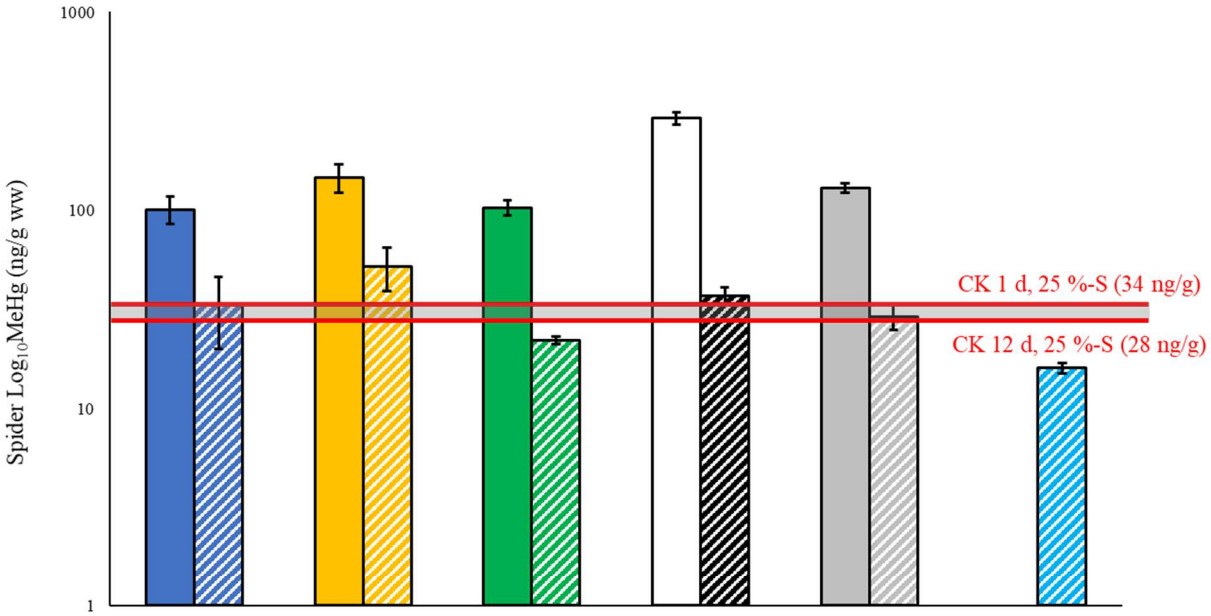
were used as a surrogate. (Figure 11). At LPHC Upstream, all eastern bluebird nestlings groups (1 d, 5 d, 8 d, and 14 d) had RQs > 1 when tetragnathids were used as a surrogate.

American Robin RQs were < 1 across all age groups regardless of the surrogate (Figure 12). Red-cockaded Woodpeckers calculated at a 15%-S had an RQ > 1 at all reaches (Figure 13). For 15 %-S, exceedances were calculated each time tetragnathids were used as a surrogate, as well as two reaches where araneids were used. LPHC Upstream RQs were > 1 for 5, 11, and 15 %-S when tetragnathids were used as a surrogate.



	T, A	T, A	T, A	T, A	T, A	T, A
AK 1 – 3 d (5 %-S) RQ =	0.28, 0.09	0.41, 0.15	0.29, 0.06	0.82, 0.10	0.37, 0.08	- , 0.05
AK 1 – 3 d (12 %-S) RQ =	0.68, 0.22	0.99, 0.35	0.70, 0.15	**1.97**, 0.25	0.88, 0.22	- , 0.11
AK 7 – 10 d (5 %-S) RQ =	0.21, 0.07	0.30, 0.11	0.21, 0.04	0.60, 0.08	0.27, 0.06	- , 0.08
AK 7 – 10 d (12 %-S) RQ =	0.50, 0.16	0.72, 0.25	0.50, 0.11	**1.43**, 0.18	0.64, 0.14	- , 0.08

Figure 6. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. American Kestrel (AK) nestlings high and low SBAWVs, 6 – 10 d, 5 %-spider diet (%-S) and 1 -3 d, 12 %-S, are indicated with red lines and red font; the range between the two values is shaded gray. The site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name for the 1 -3 d and 7 – 10 d nestlings for 5 % and 12 %-S SBAWVs. RQs > 1 are **bold.**



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
CK 1 d RQ =	**3.61, 1.18**	**5.21, 1.86**	**3.68**, 0.79	**10.43, 1.32**	**4.64, 1.04**	- , 0.57
CK 12 d RQ =	**2.97**, 0.97	**4.29, 1.53**	**3.03**, 0.65	**8.59**, **1.09**	**3.82**, 0.85	- , 0.47

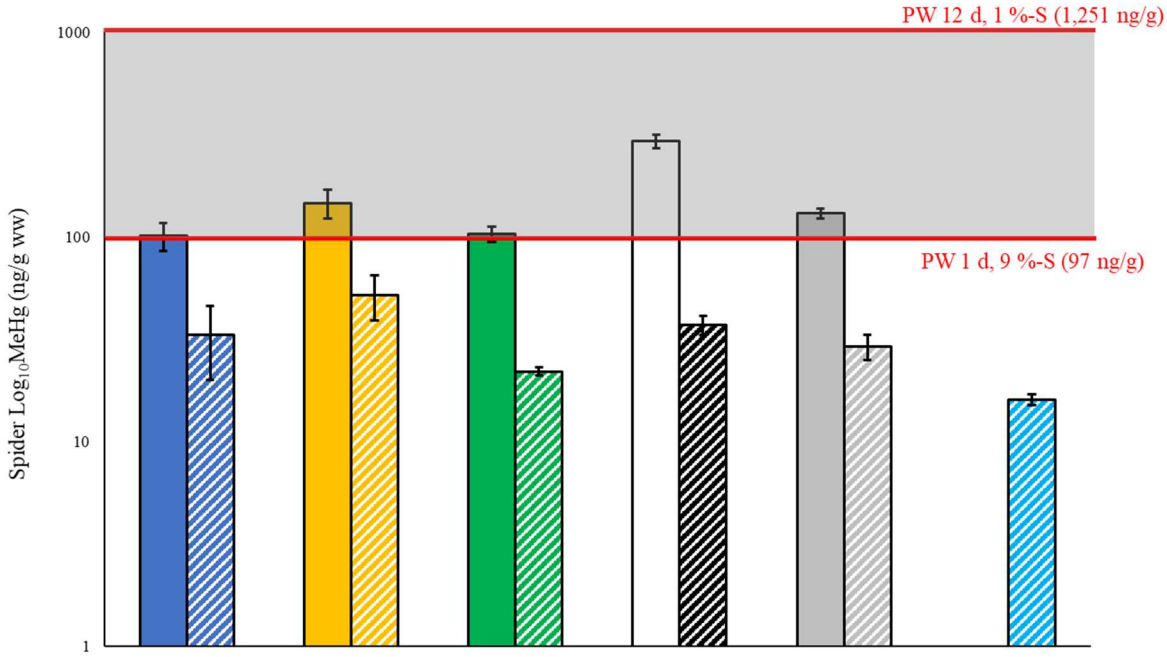
Figure 7. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. Chickadee nestlings (1 d and 12 d) are indicated with red lines and red font; the range between the two values is shaded gray. The site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name for the 1 d and 12 d nestlings. SBAWVs. RQs > 1 are **bold.**

Table 4. Species and age specific nestling body weights (BW), field metabolic rates (FMR), and ingestion rates (IR), and % spider diet (%-S) used in the calculation of MeHg spider-based avian wildlife values (SBAWV). Lower-case superscipts indicate where the data was referenced. Upper-case subscripts represent the low($_L$), medium ($_M$), and high ($_H$) reported averages and the associated SBAWV.

Order - Family **Common name** ***Species***	**Age**	**BW (g)**	**FMR (kJ/d)***	**IR (g/g/d)**	**%-S**	**SBAWV (ng/g)**
Falconiformes - Falconidae						
American Kestrel	1 - 3 d	20[a]	80.8	0.73	5 %$_L$[a], 12 %$_H$[h **]	355$_L$, 148$_H$
Falco sparverius	7 - 10 d	55[a]	160.8	0.53	5 %$_L$[a], 12 %$_H$[h **]	490$_L$, 204$_H$
Passeriformes - Paridae						
Chickadee	1 d	1[a]	10.4	1.89	25 %[a **]	28
Poecile spp.	12 d	2[a]	16.7	1.51	25 %[a **]	34
Passeriformes - Parulidae						
Prarie Warbler	1 d	2.11[b]	17.3	1.49	1 %$_L$[b], 9 %$_H$[b **]	874$_L$, 97$_H$
Setophaga discolor	3 -4 d	3.61[b]	24.9	1.25	1 %$_L$[b], 9 %$_H$[b **]	1,038$_L$, 115$_H$
	12 d	6.46[b]	37.0	1.04	1 %$_L$[b], 9 %$_H$[b **]	1,251$_L$, 139$_H$
Passeriformes - Passerellidae						
Field Sparrow	1 d	2.28[c]	18.2	1.45	6 %$_L$[c], 12 %$_H$[c **]	149$_L$, 75$_H$
Spizella pusilla	2 d	3.52[c]	24.5	1.26	6 %$_L$[c], 12 %$_H$[c **]	172$_L$, 86$_H$
	3 d	5.05[c]	31.3	1.13	6 %$_L$[c], 12 %$_H$[c **]	193$_L$, 96$_H$
	4 d	6.53[c]	37.3	1.04	6 %$_L$[c], 12 %$_H$[c **]	209$_L$, 105$_H$
	5 d	7.87[c]	42.3	0.98	6 %$_L$[c], 12 %$_H$[c **]	222$_L$, 111$_H$
Passeriformes - Troglodytidae						
House Wren *Troglodytes aedon*	10 d	10.6[d]	49.8	0.90	9 %[i]	160
Passeriformes - Turdidae						
Eastern Bluebird	2 d	3.9[e]	26.2	1.22	31 %[j]	34
Sialia sialis	5 d	10.75[e]	52.3	0.88	31 %[j]	48
	8 d	18.45[e]	75.5	0.74	12 %[j]	152
	14 d	26.8[e]	97.3	0.66	7 %[j]	290
American Robin	2 d	12.6[f]	58.3	0.84	2 %[f **]	673
Turdus migratorius	4 d	24.3[f]	91.0	0.68	2 %[f **]	830
	8 d	50.9[f]	150.5	0.54	2 %[f **]	1,053
	10 d	55.2[f]	159.1	0.52	2 %[f **]	1,080
	14 d	55.0[f]	158.7	0.52	2 %[f **]	1,080
Piciformes - Picidae						
Red-cockaded Woodpecker *Picoides borealis*	9 - 12 d	33.8[g]	115.5	0.62	5 %$_L$[l], 11 %$_M$[l],15 %$_H$[m]	184$_L$, 140$_M$, 35$_H$

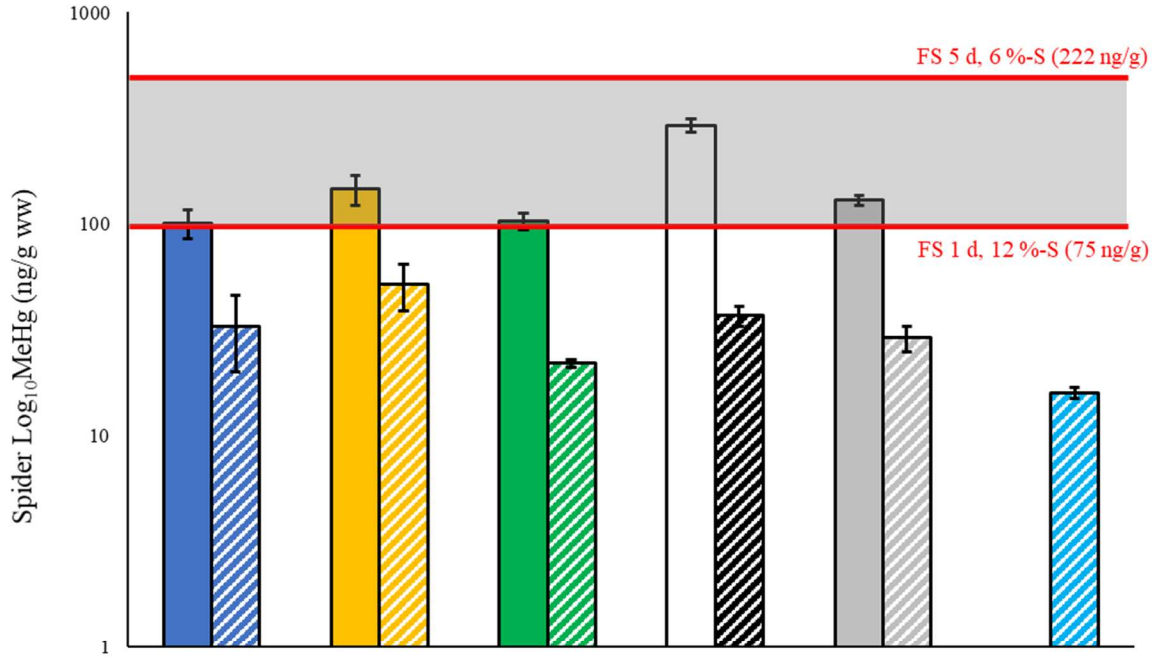
* Unless indicated, FMRs were calculated using allometric equations provided by Nagy et al. (1999). For Falconiformes and Piciformes, FMR was calculated using the "all birds" equation: $10.5 * BW^{0.681}$. For Passeriformes, FMRs were calculated using the "passerine" equation: $10.4 * BW^{0.68}$.

[a] Walters et al. 2009
[b] Nolan 1978
[c] Best 1977
[d] Fredricks et al. 2011a
[e] Roby et al. 1992
[f] Howell 1942
[g] Stangel and Lenartz 1988
[h] McDermot 2016
[i] Fredricks et al. 2001b
[j] Pinkowski 1978
[k] McDermot 2016
[l] Hanula Engstrom 2000
[m] Hess and James 1998



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
PW 1 d, 1 %-S RQ =	0.12, 0.04	0.17, 0.06	0.12, 0.03	0.33, 0.04	0.15, 0.03	- , 0.02
PW 1 d, 9 %-S RQ =	**1.04**, 0.34	**1.51**, 0.54	**1.06**, 0.23	**3.01**, 0.38	**1.34**, 0.30	- , 0.16
PW 3 – 4 d 1 %-S RQ =	0.10, 0.03	0.14, 0.05	0.10, 0.02	0.28, 0.04	0.13, 0.03	- , 0.02
PW 3 – 4 d, 9 %-S RQ =	0.88, 0.29	**1.27**, 0.45	0.90, 0.19	**2.54**, 0.32	**1.13**, 0.25	- , 0.14
PW 12 d, 1 %-S RQ =	0.08, 0.03	0.12, 0.04	0.08, 0.02	0.23, 0.03	0.10, 0.02	- , 0.01
PW 12 d, 9 %-S RQ =	0.73, 0.24	**1.05**, 0.37	0.74, 0.16	**2.10**, 0.27	0.94, 0.31	- , 0.12

Figure 8. Average ± SE MeHg concentrations for tetragnathid and araneid spiders at Appalachian Mountain streams. Prairie warbler (PW) high and low SBAWVs, 12 d, 1 %-spider diet (%-S) and 1 d, 9 %-S, are indicated with red lines and red font. For each species, the site-specific RQ (risk quotient) using Tetragnathid (T) and Araneids (A) as surrogates for "all spiders" are displayed below the stream name. RQs > 1 are **bold.**



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
FS 1 d, 6 %-S RQ =	0.68, 0.22	0.98, 0.35	0.69, 0.15	**1.96**, 0.25	0.87, 0.19	-, 0.11
FS 1 d, 12 %-S RQ =	**1.35**, 0.44	**1.95**, 0.69	**1.37**, 0.29	**3.89**, 0.49	**1.73**, 0.39	-, 0.21
FS 2 d, 6 %-S RQ =	0.59, 0.19	0.85, 0.30	0.60, 0.13	**1.70**, 0.22	0.76, 0.17	-, 0.09
FS 2 d, 12 %-S RQ =	**1.17**, 0.38	**1.70**, 0.60	**1.20**, 0.26	**3.40**, 0.43	**1.51**, 0.34	-, 0.19
FS 3 d, 6 %-S RQ =	0.52, 0.17	0.76, 0.27	0.53, 0.11	**1.51**, 0.19	0.67, 0.15	-, 0.08
FS 3 d, 12 %-S RQ =	**1.05**, 0.34	**1.52**, 0.54	**1.07**, 0.23	**3.04**, 0.39	**1.35**, 0.30	-, 0.17
FS 4 d, 6 %-S RQ =	0.48, 0.16	0.70, 0.25	0.49, 0.11	**1.40**, 0.18	0.62, 0.14	-, 0.08
FS 4 d, 12 %-S RQ =	0.96, 0.31	**1.39**, 0.50	0.98, 0.21	**2.78**, 0.35	**1.24**, 0.28	-, 0.15
FS 5 d, 6 %-S RQ =	0.45, 0.15	0.66, 0.23	0.46, 0.10	**1.32**, 0.17	0.59, 0.13	-, 0.07
FS 5 d, 12 %-S RQ =	0.91, 0.30	**1.32**, 0.17	0.93, 0.20	**2.63**, 0.33	**1.17**, 0.26	-, 0.14

Figure 9. Average ± SE MeHg concentrations for tetragnathid (solid bars) and araneid (hatched bars) spiders for each respective reach. Field Sparrow (FS) high and low SBAWVs, 5 d, 6 %-spider diet (%-S) and 1 d, 12 %-S, are indicated with red lines and red font. For each age and %-S, the reach-specific RQ (risk quotient) using tetragnathid (T) and araneids (A) as surrogates for "all spiders" are displayed below the reach name. RQs > 1 are **bold.**

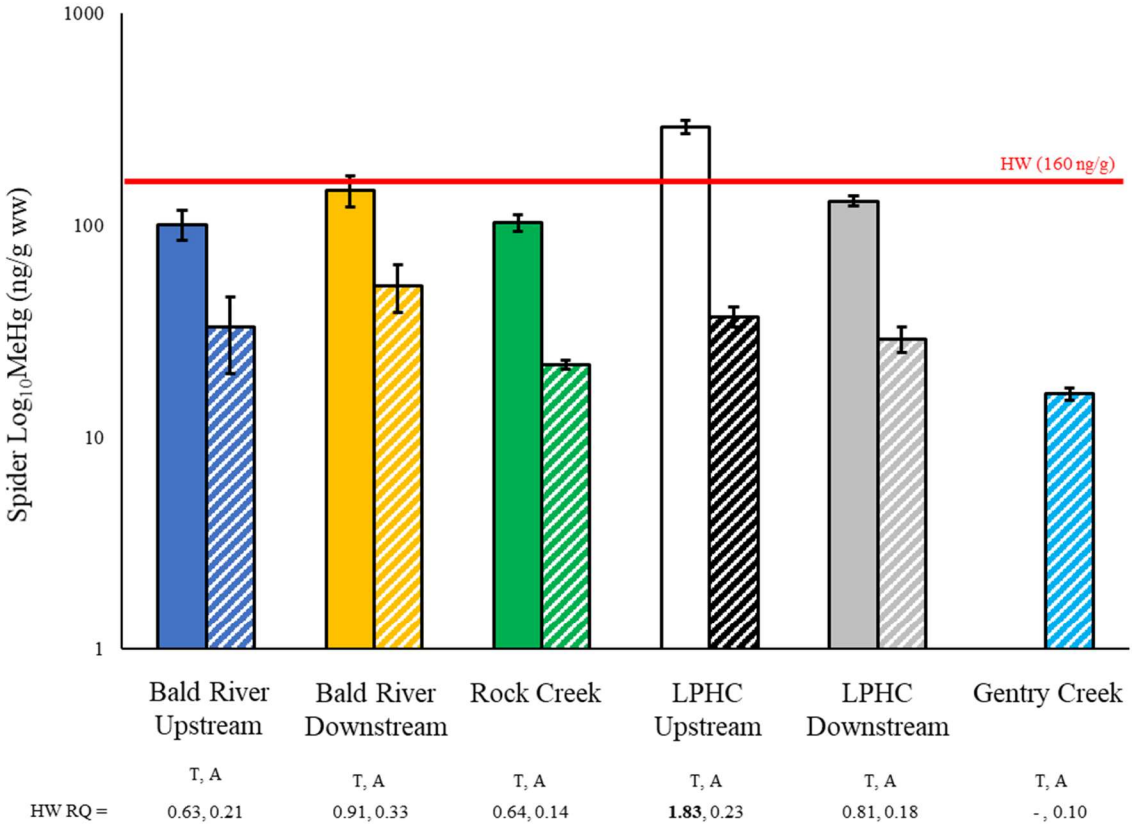
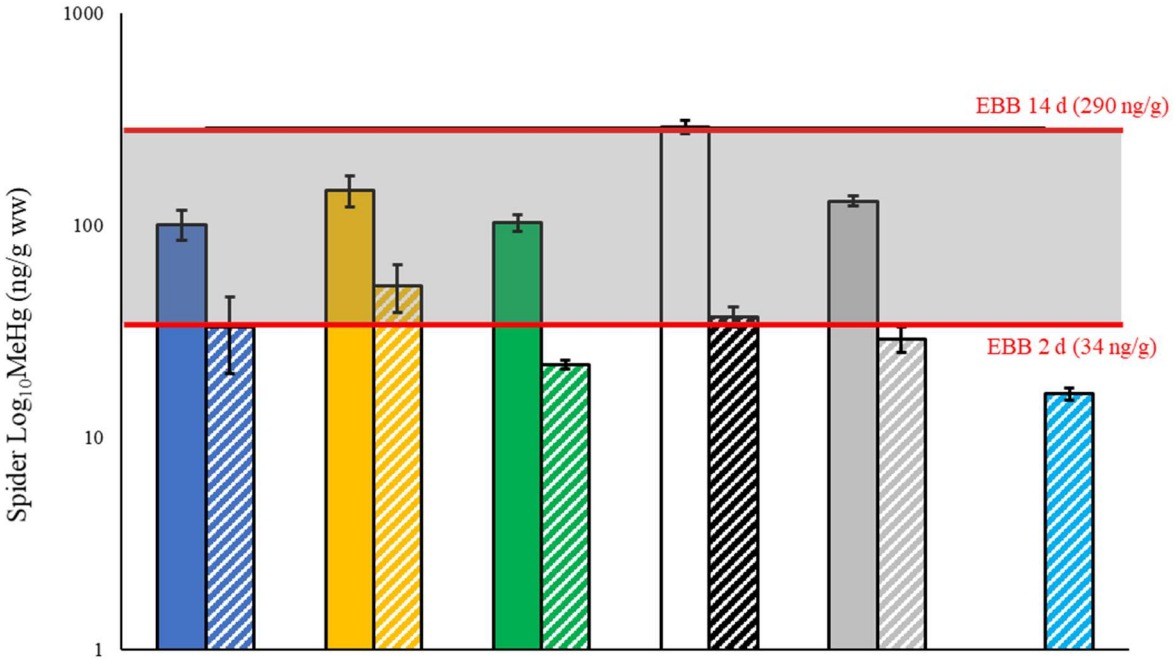
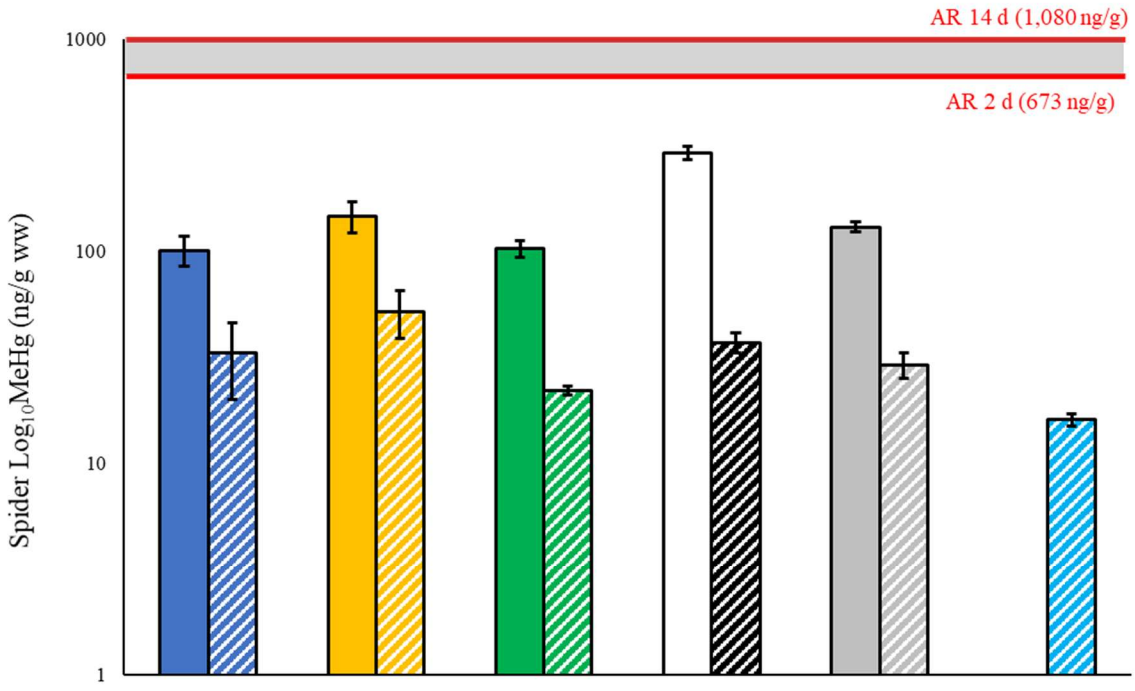


Figure 10. Average ± SE MeHg concentrations for tetragnathid (solid bars) and araneid (hatched bars) spiders for each respective reach. House Wren (HW) SBAWV (160 ng/g) is indicated with a red line. Each reach-specific RQ (risk quotient) using tetragnathid (T) and araneids (A) as surrogates for "all spiders" are displayed below the reach name. RQs > 1 are **bold.**



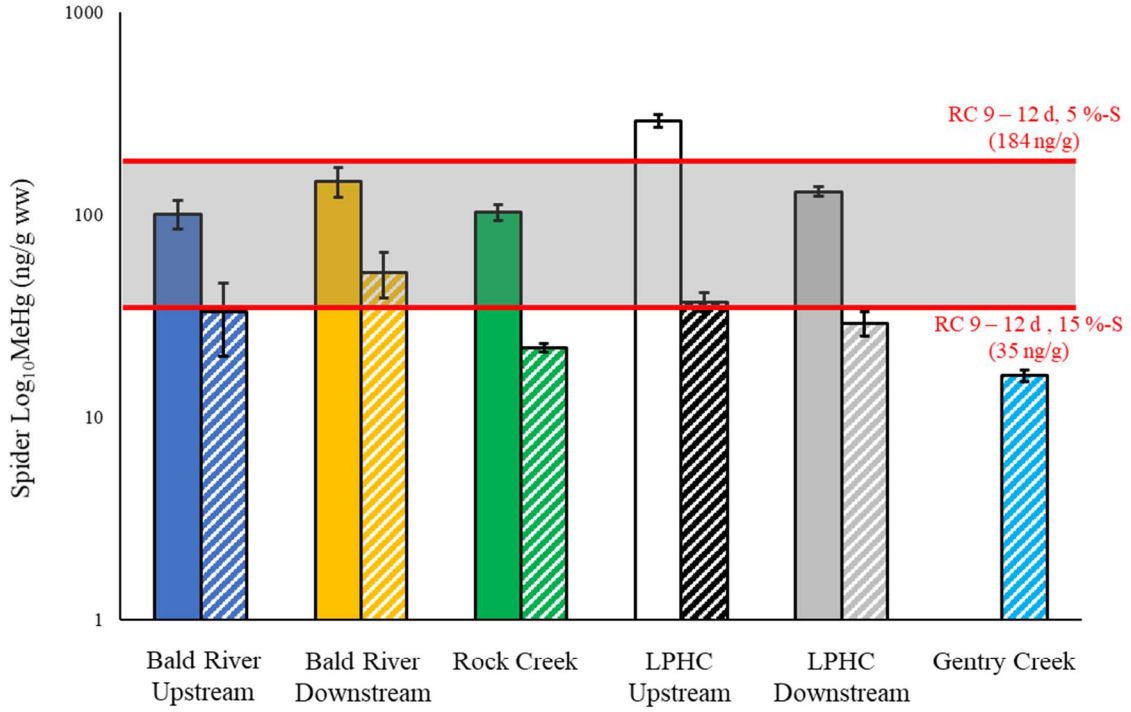
	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
EBB 1 d, 31 %-S RQ =	**2.97**, 0.97	**4.29**, **1.53**	**3.03**, 0.65	**8.59**, **1.09**	**3.82**, 0.85	-, 0.47
EBB 5 d, 31 %-S RQ =	**2.10**, 0.69	**3.04**, 1.08	**2.15**, 0.46	**6.08**, 0.77	**2.71**, 0.60	-, 0.33
EBB 8 d, 12 %-S RQ =	0.66, 0.22	0.96, 0.34	0.68, 0.14	**1.92**, 0.24	0.86, 0.19	-, 0.11
EBB 14 d, 7 %-S RQ =	0.35, 0.11	0.50, 0.18	0.36, 0.08	**1.01**, 0.13	0.45, 0.10	-, 0.06

Figure 11. Average ± SE MeHg concentrations for tetragnathid (solid bars) and araneid (hatched bars) spiders for each respective reach. Eastern Bluebird (EBB) high and low SBAWVs, 14 d, 7 %-spider diet (%-S) and 1 d, 31 %-S, are indicated with red lines and red font. For each age and %-S, the reach-specific RQ (risk quotient) using tetragnathid (T) and araneids (A) as surrogates for "all spiders" are displayed below the reach name. RQs > 1 are **bold.**



	Bald River Upstream	Bald River Downstream	Rock Creek	LPHC Upstream	LPHC Downstream	Gentry Creek
	T, A	T, A	T, A	T, A	T, A	T, A
AR 2 d, 2 %-S RQ =	0.15, 0.05	0.22, 0.08	0.15, 0.03	0.43, 0.05	0.19, 0.04	-, 0.02
AR 4 d, 2 %-S RQ =	0.12, 0.04	0.18, 0.06	0.12, 0.03	0.35, 0.04	0.16, 0.03	-, 0.02
AR 8 d, 2 %-S RQ =	0.10, 0.02	0.14, 0.05	0.10, 0.02	0.28, 0.04	0.12, 0.03	-, 0.02
AR 10 d, 2 %-S RQ =	0.09, 0.03	0.14, 0.05	0.10, 0.02	0.28, 0.04	0.12, 0.03	-, 0.01
AR 14 d, 2 %-S RQ =	0.09, 0.03	0.14, 0.05	0.10, 0.02	0.27, 0.03	0.12, 0.03	-, 0.01

Figure 12. Average ± SE MeHg concentrations for tetragnathid (solid bars) and araneid (hatched bars) spiders for each respective reach. American Robin (AR) high and low SBAWVs, 14 d and 2d, both 2 %-spider diet (%-S), are indicated with red lines and red font. For each age and %-S, the reach-specific RQ (risk quotient) using tetragnathid (T) and araneids (A) as surrogates for "all spiders" are displayed below the reach name. RQs > 1 are **bold.**



	T, A	T, A	T, A	T, A	T, A	T, A
RCWP 9 - 12 d, 5 %-S RQ =	0.55, 0.18	0.79, 0.28	0.56, 0.12	**1.59**, 0.20	0.71, 0.16	- , 0.09
RCWP 9 – 12 d, 11 %-S RQ =	0.72, 0.24	**1.04**, 0.37	0.74, 0.16	**2.09**, 0.26	**0.93**, 0.21	- , 0.11
RCWP 9 – 12 d, 15 %-S RQ =	**2.89**, 0.94	**4.17**, **1.49**	**2.94**, 0.63	**8.34**, **1.06**	**3.71**, 0.83	- , 0.46

Figure 13 Average ± SE MeHg concentrations for tetragnathid (solid bars) and araneid (hatched bars) spiders for each respective reach. Red-cockaded Woodpeckers (RCWP) high and low SBAWVs, 9 – 12 d, 6 %-spider diet (%-S) and 15 %-S, are indicated with red lines and red font. For each %-S, the reach-specific RQ (risk quotient) using tetragnathid (T) and araneids (A) as surrogates for "all spiders" are displayed below the reach name. RQs > 1 are **bold.**

The range of RQs for humans, otter, mink, Belted Kingfishers, and the most susceptible life stage and %-S of adult and nestling arachnivorous birds are displayed in Figure 14. Tetragnathid MeHg concentrations were significantly higher than araneids ($F_{1, 29} = 56.1189$, $p < 0.0001$). For arachnivorous adult birds, tetragnathids had a higher proportion of threshold exceedances than araneids (tetragnathids: 27/40; araneids: 1/48). For arachnivorous nestling birds, tetragnathids had a higher proportion of threshold exceedances than araneids (tetragnathids: 27/40; araneids: 1/48) (Figure 14).

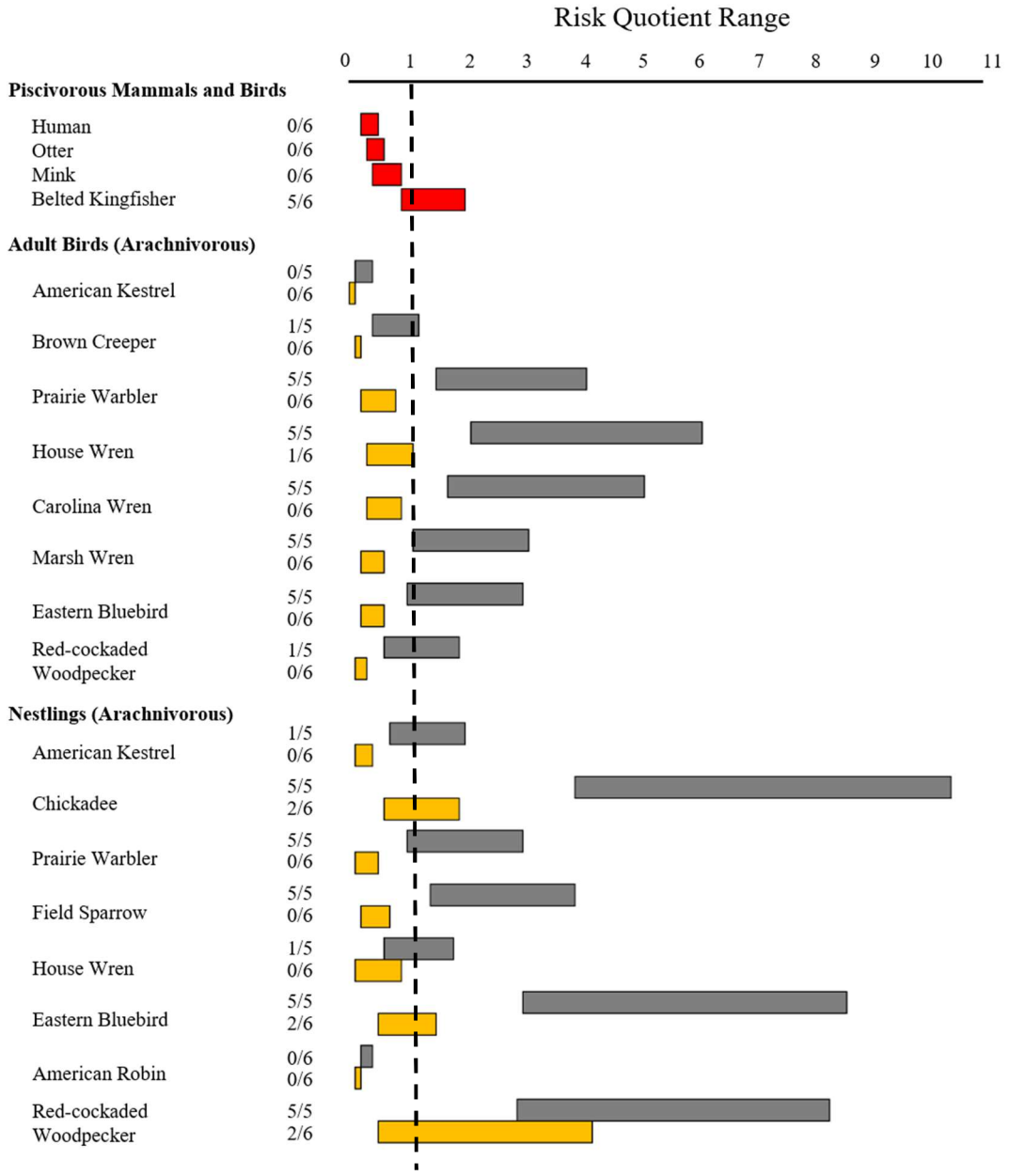


Figure 14. Risk Quotient (RQ) ranges across sites by animal group. Colored bars indicate the prey item used to calculate the RQ, where red = trout, gray = tetragnathid spiders, and orange = araneid spiders. The proportion of RQ exceedances to the number of sites tested is shown to the left of each bar.

Discussion

This research demonstrates that the risk to piscivorous mammals is not necessarily indicative of the potential risk to passerine birds, and that the selection of spider taxa can influence risk characterization. At these six southern Appalachian Mountain reaches, the average MeHg concentrations in fish exceeded neither the human health screening value (300 ng/g; USEPA 2003) nor the mink and otter wildlife values (WV), 100 ng/g and 70 ng/g, respectively (Lazorchak et al. 2003) (All risk quotients [RQs] < 1; Figures 1 and 2). Although humans, mink, and otter are expected to have no adverse effects due to the dietary exposure of MeHg via fish, the concentrations may be physiologically significant for piscivorous birds, like Belted Kingfishers, who exceeded the 30 ng/g WV at all but one reach (All RQs but one > 1; Figure 3). Similarly, adult and nestling birds exceeded spider based avian wildlife values (SBAWV) at multiple reaches and may be at risk. In almost all cases, the highest risk quotients were calculated when tetragnathids were used as a surrogate for all spiders (Figure 14), and when the ingestion rate (youngest at the youngest life stage) and % of spiders in the diet (%-S) were highest (Table 4).

Riparian spider MeHg concentrations can vary by taxa (Ortega-Rodriguez et al. 2019). Consistent with those findings, we found that tetragnathids had significantly higher Hg concentrations than araneids, and that the risk characterization of adult and nestling birds was heavily influenced by which of these taxa were integrated into the sampling design. In the current study, nestling RQs were > 1 for 68 % of tetragnathid and 13% of araneid based RQs. Adult RQs were > 1 for 68 % of tetragnathid and 2 % of araneid based RQs (Figure 14). It is unlikely that any of the birds selected here feed exclusively on either

taxa, and the use of tetragnathids or araneids as a surrogate for "all spiders" may lead to overestimations or underestimations of potential risk, respectively

Study Limitations and Considerations

The %-S and the type of spiders ingested is likely influenced by a suite of factors like spider availability, life stage, and other species-specific life history traits that influence foraging strategy (Gajdoš, P. and Krištšn 1997). In the current study, SBAWVs (1) assumed that all other dietary items were free of MeHg and (2) did not always consider the increased provisioning of spiders to younger life-stage nestlings. On the other hand, SBAWVs did not consider (1) the potential benefits of nestlings feeding on spiders or (2) the potential ameliorating effects of selenium (Ralston et. al 2007).

Taxa-specific WVs like SBAWVs assume all other dietary items in the organisms of concern's diet (other than that being used in the formula) are free of MeHg (Walters et al. 2009; Gann et al. 2014; Williams et al. 2017). Many of birds in this study also prey on adult aquatic insects (e.g., mayflies and odonates) and terrestrial insects (e.g., caterpillars and grasshoppers). Because Hg has traditionally been considered an aquatic concern, a natural criticism of this study would be the potential underestimation of dietary MeHg exposure for birds that supplement their diet with Hg contaminated adult aquatic insects. However, I contend that a more robust underestimation may occur if the remaining diet is primarily terrestrial. Recent research indicates that terrestrial food webs adjacent to aquatic systems are contaminated with MeHg (Bartrons et al. 2014; Kwon et al. 2015; Howie *et al.* 2018). Bartrons et al. (2014) found that the terrestrial food web was more contaminated than the aquatic food web and hypothesized that in terrestrial food webs, MeHg could take

a “trophic bypass” where fewer links in the food chain lead to less trophic dilution and higher Hg concentrations in terrestrial animals. The use of Hg and MeHg isotopes has led others to support this theory (Tsui et al. 2012; Kwon et al. 2015). Kwon et al. (2015) asserts that even when insect-mediated contaminant flux (IMCF) is high, the dominant MeHg source in riparian food webs is likely terrestrial.

Increased spider provisioning to younger life-stage nestling has been reported with multiple bird species (Royama 1970; Pinkowski 1978; Cowie and Hinsely 1988; Arnold et al. 2007; Radford 2008; Browning et al. 2012). In the current study, the %-S data was often the average %-S across multiple life stages (e.g., American Kestrels and American Robins). Thus, the younger life-stage nestlings %-S and MeHg exposure may have been underestimated and led to less protective SBAWVs.

Paradoxically, no consideration was given to the increased nutritional value that these spiders may provide young nestlings. In humans, increased prenatal and postnatal fish consumption has been associated with a higher exposure to dietary MeHg, but the nutritional benefits have also been shown to offset the risk and positively influence developmental scores (Davidson et al. 1998; Daniels et al. 2004; Clarkson and Magos 2006; Khan and Wang 2009; Zhang et al. 2014). Hg ingestion via spiders may demonstrate a similar paradox. Spiders have a higher fresh mass (80 kJ/g; Norberg 1978) than insects (60 kJ/g; Nagy et al. 1999) and may provide additional nutritional value relative to other arthropods because they have higher concentrations of calcium and the essential amino acid taurine (Royama 1970; Ramsay and Houston 2003). A positive relationship between the percentage of spiders in nestling diets and tarsus length was found in Blue Tits

(*Cyanistes caeruleus*) (García-Navas et al. 2013; Serrano-Davies and Sanz 2017), Great Tits (*Parus major*) (García-Navas et al. 2013), and Pied Flycatchers (*Ficedula hypoleuca*) (Samplonius et al. 2016). Although riparian spiders in the southern Appalachian Mountains had high Hg concentrations, SBAWVs did not consider the benefits of a high spider diet.

The risk-benefit analysis of consuming food items high in MeHg is further complicated by the potential ameliorating effects of selenium (Se). In humans, evidence supports that the mechanism of MeHg neurotoxicity is inherent in its ability to bind to selenoenzymes (Ralston and Raymond 2018). Risk calculations proposed by Khan and Wang (2009), Ralston *et al.* (2007 and 2016), and Zhang *et al.* (2014) incorporate these effects and essentially reduce the complexity of MeHg toxicity to a Hg:Se molar ratio (Khan and Wang 2009; Zhang et al. 2014; Ralston *et al.* 2016; Eagles-Smith *et al.* 2018). The spiders collected were not analyzed for Se, and the calculated SBAWVs did not consider Hg-Se antagonism. Only one study I know of analyzed spider tissues for Hg and Se (Otter et al. 2013). The spider MeHg concentrations reported by Otter et al. (2013) exceeded the chickadee and eastern bluebird nestling SBAWVs presented in this study. However, if potential risk was calculated using methods proposed by Ralston et al. (2016), the MeHg concentrations would not be expected to have an adverse effect in the same nestlings. I agree with Zhang et al. (2014) and Eagles-Smith et al. (2018), who support the integration of Hg-Se risk benefit values to study the range of possible outcomes in non-human based risk assessments, but that previous risk calculations should not be prematurely replaced prior to a better understanding of the epidemiology.

Implications

Hg deposition in the southeastern US has decreased over the last several years (Weiss-Penzias et al. 2017), and this decrease has largely been attributed to the Mercury Air and Toxic Standards (MATS) policy (USEPA 2011; Risch and Kenski 2018). MATS regulated the release of Hg, a hazardous air pollutant (HAP), from coal- and oil-fired power plants (USEPA 2011). However, in 2015, the U.S. Supreme Court ruled that the U.S. EPA must consider the costs of reducing HAPs (U.S. Supreme Court, Michigan v. EPA, 2015), and in 2018, the MATS policy was revised to no longer regulate the release of mercury and other HAPs due to the high costs and lack of quantifiable benefits (USEPA 2018). The monetary benefit of releasing HAPs has primarily been viewed though a human-health prism, and benefits to wildlife have largely been unquantified (Giang and Selin 2016; Sunderland et al. 2016; USEPA 2018; Budnik and Casteleyn 2019). In this study, fish consumption from streams in a high mercury deposition area is not expected to cause adverse health effects in humans and increased medical costs. However, if mercury in spiders leads to a decrease in bird diversity, ecotourism (a quantifiable benefit to Tennesseans) may eventually suffer. U.S Fish and Wildlife Service (USFWS) estimates that $76 billion dollars was generated by wildlife watchers (mostly bird watchers) in 2016 (USFWS 2018). Nationally, food and lodging alone accounted for $6.1 billion, and USFWS estimates that of the 23.7 million people that that left their home to watch, feed, or photograph wildlife, 17 million watched birds, and 79% visited public land (USFWS 2018).

A potentially attractive species to bird watchers might be the federally endangered Red-cockaded Woodpeckers (USFWS 2019). This species is listed as near threatened by the International Union for Conservation of Nature's Red List of threatened species (IUCN Red List) and is no longer found in Tennessee's range of the Appalachian Mountains (BirdLife international 2017). Although the historical decline and reduction in range of the red-cockaded woodpecker is outside the scope of this study, it is worth noting that population declines have largely been attributed to the decrease in old growth pine forests (Jackson 1994), and it has been suggested that pine/coniferous forests have an amplifying effect on Hg deposition (Drenner et al. 2013; Eagles-Smith et al. 2016). Red-cockaded Woodpecker reintroduction efforts primarily take place among in their native range of the southeastern U.S., a regional Hg deposition hotspot, and areas like the North Carolina Sandhills and northern Florida Red Hills, likely subregions hotspots due to the high density of pines (USFWS 2019; Cox et al. 2016; Smith et al. 2018). Since Hg emissions are no longer regulated under MATS (USEPA 2018), local Hg emission and deposition rates are expected to increase (Sunderland et al. 2016; Perlinger et al. 2018). Considering the high %-S, dietary MeHg exposure to adult and nestling Red-cockaded Woodpeckers is a pathway that warrants future consideration at targeted reintroduction areas.

Conclusion

In summary, I found that nonpoint source Hg deposition has led to MeHg concentrations in spiders that if ingested could cause harm to passerine bird nestlings inhabiting the southern Appalachian Mountains. Tetragnathid MeHg concentrations were significantly higher than araneids and the selection of which spider to use as a surrogate

for “all spiders” impacted the risk characterization of adult and nestling birds. I suggest the use of tetragnathid spiders if future investigations are seeking to use riparian spiders as an initial screening value. Tetragnathids are relatively easy to sample (Beaubien et al. 2019) and present a more conservative and likely more appropriate approach.

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CHAPTER IV: THE ROLE OF SEXUAL DIMORPHISM AND TISSUE SELECTION IN ECOTOXICOLOGICAL STUDIES USING THE RIPARIAN SPIDER *TETRAGNATHA ELONGATA*

Beaubien, G. B., Olson, C. I., & Otter, R. R. (2019). The Role of Sexual Dimorphism and Tissue Selection in Ecotoxicological Studies Using the Riparian Spider Tetragnatha elongata. *Bulletin of environmental contamination and toxicology*, 1-8.

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Introduction

Spiders of the genus *Tetragnatha* (Araneae, Tetragnathidae) are globally distributed and abundant predators, with most species residing near water (Levi 1981). Riparian spiders of this genus (hereafter referred to as "tetragnathids") spin generally horizontal webs above the land-water interface and feed on emergent aquatic insects (e.g., midges) (Gillespie 1987; Sanzone et al. 2003). This feeding behavior has linked tetragnathids to the insect-mediated contaminant flux (the transfer of contaminants that occurs when aquatic insects emerge and become terrestrial) of polychlorinated biphenyls (PCBs), methyl-mercury, and selenium, prompting investigations into their use as bioindicators (Cristol et al. 2008; Walters et al. 2008, Otter et al. 2013, Tweedy et al. 2013). Tetragnathids also have a small home range; Walters et al. (2009) demonstrated how this life history trait can inform ecotoxicological studies, revealing that tetragnathids integrated the sediment-PCB signal at the 100-meter reach scale. Additionally, tetragnathids can be collected by hand, making sampling simple and inexpensive. Currently, the USEPA's

Office of Research and Development uses tetragnathids as one line of evidence to assess remedy effectiveness (Kraus et al. 2017, Walters et al. 2018).

The use of tetragnathids as a bioindicator of aquatic contamination is directly tied to their dietary reliance on emergent aquatic insects; however, the dependence of tetragnathids on the adjacent aquatic food web can fluctuate based on aquatic insect emergence (Nakano and Murakami 2001). For example, ecological studies have demonstrated, through stable isotopes analysis (carbon [$\delta^{13}C$], nitrogen [$\delta^{15}N$]), that tetragnathid dietary reliance on aquatic subsidies can vary based on seasonal insect emergence (Akamatsu et al. 2004). Additionally, Gergs et al. (2014) showed that when the density of a fully aquatic invasive predator increased, the biomass of emergent aquatic insects decreased, and resulted in the diet of neighboring tetragnathid populations to shift from primarily aquatic to primarily terrestrial. These studies emphasize the necessity of contextualizing the diet of tetragnathids when they are being used as a bioindicator.

To date, researchers investigating tetragnathids diets (via $\delta^{13}C$ and $\delta^{15}N$) alongside contaminant concentrations, have used the entire spider (body + legs) to perform isotope analysis (Walters et al. 2008, Speir et al. 2014). One drawback to this approach is the small size of an individual spider. In fact, at sites where population densities of tetragnathids are low, reaching adequate biomass requirements for contaminant detection and quantitation limits can be difficult and has inhibited study designs in the past (Kraus et al. 2017; Walters et al. 2018). To reach analytical biomass and provide dietary context, researchers working with the riparian spider *Dolomedes*, used the legs of spiders to represent the isotope signature of the entire individual (Walters et al. 2008). To date, no studies have investigated

the stable isotope signatures of tetragnathid legs and whether they accurately reflect the signature of the entire spider.

Tetragnathids are sexually dimorphic in both size and behavior. The smaller, wandering males court the openly-positioned females by outcompeting rival males on the periphery of the female webs (Danielson-François et al. 2002). These differences make it more likely for females to be noticed during field collections and likely result in composite samples with large female proportions. If differences in mass or physiology between sexes affect diet or contaminant burdens, these sampling biases could lead to spurious conclusions. Past researchers have designed field studies specifically avoiding the collection of male tetragnathids to eliminate the confounding potential that sex may have on diet and mercury accumulation (Chaves-Ulloa et al. 2016); however, to date, no study has investigated whether these differences exist in tetragnathids.

To better understand the effects of behavior and physiology on tetragnathids as bioindicators, this study investigated four populations of tetragnathids in the southern United States. The objectives of this study were to determine (1) the variability of tetragnathid body conditions among different tetragnathid populations in the southern Appalachian Mountains, (2) the effect sex has on spider stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) and mercury concentrations, and (3) whether stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ were different between tetragnathid legs and whole-body samples.

Materials and Methods

Four sites across Tennessee (one forested pond, two forested streams, and one suburban stream) were utilized in this study. A description of each site is provided below.

Whigg Meadow Pond (35º 18.558'N, 84º 2.313'W) is a permanent and constructed fishless pond located within the Cherokee National Forest. Whigg Meadow Pond was sampled twice during this study, once in fall of 2017 and again in spring of 2018. The site is at an elevation of ~1,489 m, where an open grassland transitions to a northern hardwood forest. The riparian habitat is characterized by a mix of herbaceous species and rhododendron (*Rhododendron spp.*).

Left Prong Hampton Creek is a forested stream located within Cherokee National Forest and the Hampton Creek Cove Natural Area and was sampled during spring of 2018. The most downstream point of the 130 m reach (36º 8.382'N, 82º 2.787'W) was at an elevation of ~1,070 m. The riparian habitat of Left Prong Hampton Creek consists primarily of an herbaceous understory (absent of rhododendron) and an immediate overstory consistent with the neighboring rich cove forest: yellow birch (*Betula allegheniensis*), sugar maple (*Acer saccharum*), yellow buckeye (*Aesculus flava*), American ash (*Fraxinus americana*), black cherry (Prunus serotina), and basswood (*Tilia americana*).

Gentry Creek is a forested stream located within the Cherokee National Forest and was sampled during spring of 2018. The most downstream point of the 130 m reach (36º 33.568'N, 81º 42.669'W) was at an elevation of ~970 m. The riparian habitat transitions immediately from a sparse riparian under-canopy mixed with rhododendron, various shrubs and herbaceous species to an acid cove forest consisting primarily of yellow birch, tulip poplar (*Liriodendron tulipifera*), northern red oak (*Quercus rubra*), basswood, and eastern hemlock (*Tsuga canadensis*).

Bushman Creek is a suburban stream that was sampled in fall of 2018. This site (35º 53.715'N, 86º 20.887'W) is at an elevation of ~186 m. The riparian habitat transitions from a suburban subdivision to a 3 m forested riparian edge consisting primarily of eastern hemlock, tulip poplar, and sugar maple. The riparian understory consisted primarily of grasses and other herbaceous species.

Identical sampling methodology was used during all sampling events. Tetragnathids were collected at night, at least 1 hour after sunset. Using headlamps, tetragnathids were collected by hand from riparian vegetation and structures along the shoreline, no further than 1 m inland. Only tetragnathids larger than ~7 mm total length (visual approximation; total length ≥ the width of a standard #2 field pencil) were collected. In the field, individuals were sexed by visually inspecting pedipalps; the tarsus of the male pedipalp is swollen giving an appearance of "boxing gloves" (Figure 1). After capture, each spider was placed into an individual 15 mL polypropylene tube. Samples were then placed in a cooler filled with dry ice and transported to the laboratory where they were stored at -20o C until laboratory processing.

To compare body condition measurements between sexes and sites, spiders were collected during spring 2018 (Whigg Meadow Pond: n=44; Left Prong Hampton Creek: n=58; Gentry Creek: n=66). Laboratory processing of spiders consisted of confirming each individual spider for sex and species (T. elongata) by examining the eye layout, chelicera size, and the ornamentation of the pedipalp conductor tip of a subset of male spiders (Levi 1981; Figure 1). Total mass (TM) for each individual spider was measured using a 0.1 mg analytical balance (Mettler Toledo, New Classic ML104). Total length (TL), prosoma

length (PL), opisthosoma length (OL), and the length of the front left leg (L1) was measured for each individual spider using calipers with 0.1 mm precision (Vinen, DCLA-0605). Total length (TL) was defined as the distance between the clypeus and the most posterior point of the opisthosoma. Prosoma length (PL) was defined as the distance between the clypeus and pedicel. Opisthosoma length (OL) was defined as the distance between the most anterior and posterior points of the Opisthosoma. L1 Leg length was measured from the tip of the L1 tarsus to the most proximal point of the trochanter (Figure 1). To compare stable isotope signatures and mercury concentration between male and female spiders, 40 individuals (25 females and 15 males) were collected during fall 2018 at Whigg Meadow Pond. Sex-specific composite samples (Female: n=7; Male: n=4) of 3-4 individual spiders were made to meet biomass requirements for mercury analysis. The L1 of each spider in a replicate was excised where the femur met the trochanter, combined into separate polyethylene tubes, and stored at -20o C until analyzed for stable isotopes. The remaining biomass (prosoma, opisthosoma, and 7 legs) of all spiders in a replicate were placed into separate polyethylene tubes and stored at -20o C until analyzed for mercury.

To compare the stable isotope signatures of tetragnathid legs (L1) to their respective whole-body values, a subset of spiders from each sampling event was used (Whigg Meadow Pond-fall: n=7; Whigg Meadow Pond-spring: n=5; Left Prong Hampton Creek: n=6; Gentry Creek: n=6; Bushman Creek: n=7). Laboratory processing consisted of excising the L1 leg of each spider where the femur met the trochanter and placing the L1 leg into an individual polyethylene tube. The remaining biomass (prosoma, opisthosoma,

and 7 legs) was then placed into a separate polyethylene tube and defined as a “whole-body” sample. All samples were stored at -20o C prior to stable isotope analysis.

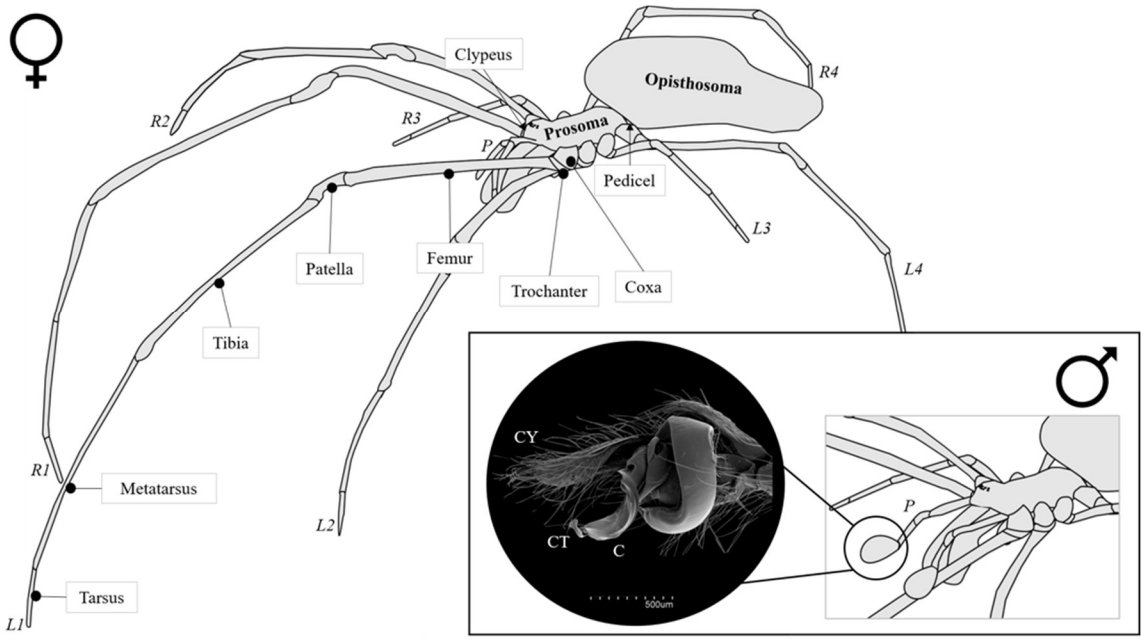

Figure 1. Pictured are the external features of a female *Tetragnatha elongata* (Tetragnathidae, Araneae). The two major body segments, the prosoma (cephalothorax) and opisthosoma (abdomen) are connected by the pedicel (a barely visible narrow stalk). The clypeus is the most anterior point of the prosoma. The right pedipalp is labeled with a *P*. Left and right legs are labeled with an *L* or *R*, and then numbered *1-4* from front to back. The major segments of the leg are labeled on the front left leg (*L1*). Total length (TL) was defined as the distance between the clypeus and the most posterior point of the opisthosoma. Prosoma length (PL) was defined as the distance between the clypeus and pedicel. Opisthosoma length (OL) was defined as the distance between the most anterior and posterior points of the Opisthosoma. Leg length was measured from the tip of the L1 tarsus to the most proximal point of the trochanter. *Inset:* male *Tetragnatha elongata* with larger copulatory structures at the pedipalp terminus relative to females. *Detail:* Scanning electron micrograph (SEM) of a male *Tetragnatha elongata* pedipalp collected in Rutherford County, TN. The conductor (C) tapers towards the conductor tip (CT) and bends towards the cymbium (CY). Scale bar represents 500 µm, dashes represent 50 µm intervals. (The background of the SEM was removed using Inkscape version 0.92.4. SEM photo credit: Connor I. Olson)

Stable isotope analysis (whole-body and L1) samples were dried, homogenized, filtered to pass through a 40-mesh screen, combusted to CO2 and N2, and analyzed using a NC 2500 elemental analyzer (Carlo Erba Milan, Italy) interfaced to a Delta Plus isotope ratio mass spectrometer (Thermo Finnigan Bremen, Germany). All isotope values are reported in δ-notation in part per thousand, or per mil (‰) and represent the heavy to light isotopic ratio, $\delta^{13}C$ (13C/12C) and $\delta^{15}N$ (15N/14N) relative to reference standards (Vienna Peedee Belemnite carbonate and air). Precision was greater than 0.1‰ (1 SD) for both elements.

Samples were analyzed for mercury via oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry in accordance with USEPA Method 1631 (USEPA 2002). Briefly, composite samples were homogenized and stored frozen in acid-cleaned glass fluoropolymer jars. Samples were then transferred to a digestion vessel, digested with HNO3 and H2SO4 on a 58oC hot block for one hour. Once cooled, samples were diluted with 0.02 N BrCl and left at room temperature for an additional 4 hours. Prior to analysis, an initial calibration verification was tested, and a continuing calibration verification was ran every ten samples. Analysis of total mercury was conducted utilizing an Analytik Jena automated mercury analyzer.

All samples were analyzed as a single batch that included two method blanks (all reagents) and a Laboratory Control Sample (LCS) and Laboratory Control Sample Duplicate (LCSD). Both method blanks were below the method reporting limit of 1.1 ng/g. The LCS and LCSD were within acceptable limits (93% and 95% respectively). Sample quality assurance included the use of Tort-3, a Standard Reference Material (SRM), that

was used to make one SRM Blank, and two sets of Matrix Spike (MS) and Matrix Spike Duplicates (MSD). The %-recovery of the SRM Blank was 98%. The average recovery of MS and MSDs was 113% (range, 106-116%; n =4).

To compare body condition, measurements (TM, TL, PL, OL, L1) were pooled by sex and analyzed for normality using a Shapiro-Wilk test. Log transformations of non-normal data were attempted to meet assumption of normality. Differences in body condition measurements between sexes within each site were then investigated using either a Student's t-test or a non-parametric Wilcoxon signed-rank test. To determine the effects of site on male and female body conditions, we used a one-way analysis of variance (ANOVA) or the nonparametric Kruskal-Wallis test (one-way ANOVA on ranks) and when appropriate a Tukey HSD or non-parametric Steel-Dwass post-hoc test, respectively. Sex-specific biomass and sample size data from each site were pooled and overall differences between sexes were analyzed using a Wilcoxon signed-rank test. To determine whether sex- and site-specific body condition measurements were different, linear regression analysis was used to determine the relationship between TL and spider TM among sex, site, and sex*site interaction groups; spider TM was log-transformed prior to regression to meet assumption of normality. We used analysis of covariance (ANCOVA) to test the effect of sex, site, and the interaction of sex*site on TM using TL as a covariate. Whole-body and leg $\delta^{13}C$ and $\delta^{15}N$ differences were investigated using a Kruskal-Wallis test and a Steel-Dwass post hoc test. No significant differences were found within sites, so data were pooled and the strength of the relationship between L1 and whole-body δ13C & δ15N was tested using the nonparametric Spearman's rho correlation coefficient. The

effect of sex on $\delta^{13}C$, $\delta^{15}N$, and mercury at Whigg Meadow Pond was analyzed using a nonparametric Kruskal-Wallis test. All analyses were completed using JMP13.0 software. Significance was defined as $\alpha = 0.05$.

Results and Discussion

The search methods used in this study (collecting spiders by hand) were similar to those used by other researchers (Walters et al. 2008; Otter et al. 2013) and were not intended to be biased; however, the spider sampling that investigated body condition measurements resulted in collections with a disproportionate number of females. Across sites females represented 73%, 86%, and 76% of the spiders collected and 83%, 93%, and 88% of the total biomass collected at Whigg Meadow Pond, Left Prong Hampton Creek and Gentry Creek, respectively (Table 1). Likewise, average TM, TL, PL, and OL were significantly greater in females at all sites and when sites were combined. The measurement of L1, however, was significantly longer in male spiders compared to females at Gentry Creek and when all sites were combined. Male tetragnathids are expected to have longer front legs than females; it's hypothesized that longer leg length evolved in male tetragnathids to deter sexual cannibalism, where the female eats the male post copulation (Elgar et al. 1990).

When female and male spiders were compared independently across the three sites, spiders from Gentry Creek where the smallest, on average, for all body condition measurements (TM, TL, PL, OL, L1), even if not significantly different (Table 1). These results demonstrate that the average size of tetragnathids can differ even when comparing two forested streams (Left Prong Hampton Creek and Gentry Creek) in the same region.

These site differences in mass and total length are likely explained by prey availability, temperature, and humidity (Gillespie 1987, Elgar et al. 1990) whereas L1 length is likely genetically determined. To examine these site-specific differences further we compared the relationship between TM and TL using site- and sex-specific data sets. When investigating the impact of sex on log TM female and male log TM was strongly correlated with TL (female $r2 = 0.89$, $p < 0.0001$; male $r2 = 0.94$, $p < 0.0001$) and no differences were detected between sexes (ANCOVA, $F1, 161 = 2.1355$, $p = 0.1459$). When investigating the impact of site on log TM, log TM was strongly correlated with TL (Left Prong Hampton Creek, $r2 = 0.93$, $p < 0.0001$; Gentry Creek, $r2 = 0.90$, $p < 0.0001$; Whigg Meadow Pond $r2 = 0.93$, $p < 0.0001$) and no differences were detected among sites (ANCOVA, $F2, 159 = 1.6228$, $p = 0.2006$). When the sex*site interaction was included as a factor, log TM was strongly correlated with TL and the slopes of the regressions were not significantly different (all correlations $p < 0.001$; ANCOVA, $F5, 153 = 0.4633$, $p = 0.8031$; Figure 2). These results indicate that TM is generally a function of TL, and although both are expected to be affected by the environment, in the present study this relationship is independent of sex and site (Figure 2; Overall $r2 = 0.92$, $y = 2.0099e0.2988x$)

To determine if sex-specific dietary differences existed in spiders we compared the isotope signatures ($\delta^{13}C$ & $\delta^{15}N$) of spider legs from the fall sampling event at Whigg Meadow Pond. No differences were observed between female and male $\delta^{13}C$ values ($H = 0.036$, $p = 0.850$) (Figure 3, left), and no differences were observed between female and male $\delta^{15}N$ signatures ($H = 0.5714$, $p = 0.450$) (Figure 3, right). These results imply that male and females were feeding in the same food web and at the same trophic position.

These results differ from those found by Sanzone et al. (2003), which used a 15N stream enrichment method and found that female spiders inhabiting riparian vegetation were consuming a higher proportion of their diet from aquatic insects than males. Similar to isotopic signature, mercury concentrations were not impacted by sex, with no differences between female (124.7 ± 9.6 ng/g ww) and male (121.3 ± 9.8 ng/g ww) composite samples ($H = 0.144$, $p = 0.748$) (Figure 4). In fact, when mercury concentrations of sex-specific composites were pooled, a normal distribution among the samples was observed ($W = 0.964$, $p = 0.815$) (Figure 4). Sex differences have been shown to influence how many organisms accumulate and rid the body of contaminants, including mercury (Burger 2007). The present study is the first to investigate sex-related differences in mercury accumulation in tetragnathids and supports previous researcher's methodology of compositing spiders without regard to sex (Walters et al. 2009, Otter et al. 2013, Tweedy et al. 2013, Gann et al. 2015, Kraus et al. 2017, Walters et al. 2018). Spiders were pooled by site, irrespective of sex, to determine if a spider leg (L1) could act as an accurate surrogate for the spider's whole-body stable isotope signature. Subsamples were taken from all sampling events and showed no significant differences between whole-body and L1 $\delta^{13}C$ or $\delta^{15}N$ signatures for any event (Figure 5A). When all data were pooled significant correlations were found between whole-body and L1 $\delta^{13}C$ ($R = 0.97$, $P < 0.0001$; Figure 5B) and $\delta^{15}N$ ($R = 0.87$, $P < 0.0001$; Figure 5C) reinforcing that L1 legs of tetragnathids were an accurate surrogate for whole-body dietary signatures of both $\delta^{13}C$ and $\delta^{15}N$. These results were similar to those of Collier et al. (2002) that found no significant differences in $\delta^{13}C$ between the legs and cephalothorax of the fishing spider *Dolomedes*. The implication of these results is that

researchers investigating the bioaccumulation or biomagnification of a contaminant in tetragnathids may provide dietary context without sacrificing much of the critical biomass needed to reach contaminant detection limits.

In summary, female tetragnathids were significantly larger than male spiders and represented a larger proportion of spiders collected at all sites. However, despite the difference in size between tetragnathids sexes, no differences in growth dynamics, isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) or how much mercury they accumulated were observed. It was determined that the leg of a tetragnathid can accurately represent the stable isotope signature of an entire spider.

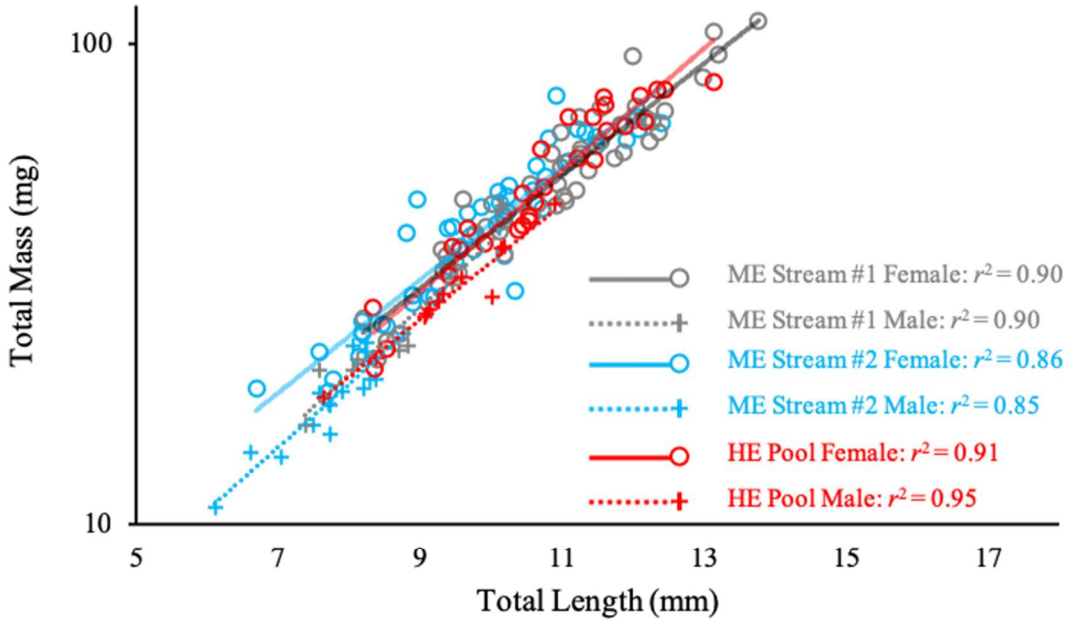

Figure 2. Regressions of total mass and total length of tetragnathids by sex, and site. Total mass is presented on log10 scale. Females (+) and males (o) are separated by site. Left Prong Hampton Creek (LPHC) is shown in gray (female, n = 50; male, n = 8); Gentry Creek in blue (female, n = 50; male, n = 16), and Whigg Meadow Pond (WM Pond) in red (female, n = 32; male, n = 12). LPHC). The overall best fit line is shown in black (Overall n = 168).

Table 1. Site-specific and pooled body condition measurements of male and female tetragnathids. P-values represent comparisons between male and female body conditions via a student's t-test or non-parametric Wilcoxon signed-rank test. Connecting letters represent the effect of site of body conditions within sexes via a one-way ANOVA or non-parametric Kruskal-Wallis test. TM = total mass, TL = total length, PL = prosomal length, OL = opisthosoma length, L1 = left front leg.

	Whigg Meadow			
	Total	Female	Male	p
n	44	32	12	N/A
% of Total Spiders		73%	27%	-
Total Biomass (g)	2.101	1.734 (1.569)	0.367	N/A
% of Total Biomass		83%	17%	-
TM (mg)		54 ± 3^{A}	31 ± 2^{A}	< 0.0001*
TL (mm)		10.7 ± 0.2^{A}	9.4 ± 0.2^{A}	0.0004*
PL (mm)		3.2 ± 0.1^{A}	3.0 ± 0.1^{A}	0.0469*
OL (mm)		8.0 ± 0.2^{AB}	6.4 ± 0.2^{A}	< 0.0001*
L1 (mm)		30.0 ± 0.9^{A}	30.9 ± 1.6^{A}	0.3105
	Left Prong Hampton Creek			
	Total	Female	Male	p
n	58	50	8	N/A
% of Total Spiders		86%	14%	-
Total Biomass (g)	2.977	2.777	0.199	N/A
% of Total Biomass		93%	7%	-
TM (mg)		56 ± 3^{A}	25 ± 3^{AB}	< 0.0001*
TL (mm)		10.9 ± 0.2^{A}	8.5 ± 0.5^{AB}	< 0.0001*
PL (mm)		3.2 ± 0.0^{A}	3.0 ± 0.1^{A}	0.0216*
OL (mm)		8.3 ± 0.2^{A}	6.0 ± 0.4^{A}	< 0.0001*
L1 (mm)		29.5 ± 0.6^{A}	30.9 ± 1.3^{A}	0.1744
	Gentry Creek			
	Total	Female	Male	p
n	66	50	16	N/A
% of Total Spiders		76%	24%	-
Total Biomass (g)	2.467	2.169	0.298	N/A
% of Total Biomass		88%	12%	-
TM (mg)		43 ± 2.2^{B}	18.6 ± 1.0^{B}	< 0.001*
TL (mm)		9.8 ± 0.2^{B}	7.7 ± 0.2^{B}	< 0.001*
PL (mm)		2.8 ± 0.0^{B}	2.7 ± 0.1^{B}	0.0493*
OL (mm)		7.3 ± 0.2^{B}	5.4 ± 0.1^{B}	< 0.001*
L1 (mm)		27.4 ± 0.5^{B}	30.5 ± 0.9^{A}	0.0021*
	Combined Site Averages			
	Total	Female	Male	p
n	168	44 ± 6	12 ± 2	0.0463*
% of Total Spiders		78 ± 4%	22 ± 4%	0.0495*
Total Biomass (g)	7.545	2.227 ± 0.302	0.288 ± 0.049	0.0495*
% of Total Biomass		87 ± 3%	13 ± 3%	0.0495*
TM (mg)		50 ± 2	23 ± 1	<0.0001*
TL (mm)		10.4 ± 0.1	8.4 ± 0.2	<0.0001*
PL (mm)		3.1 ± 0.0	2.9 ± 0.1	0.0014*
OL (mm)		7.9 ± 0.1	5.8 ± 0.1	<0.0001*
L1 (mm)		28.8 ± 0.4	30.7 ± 0.7	0.0087*

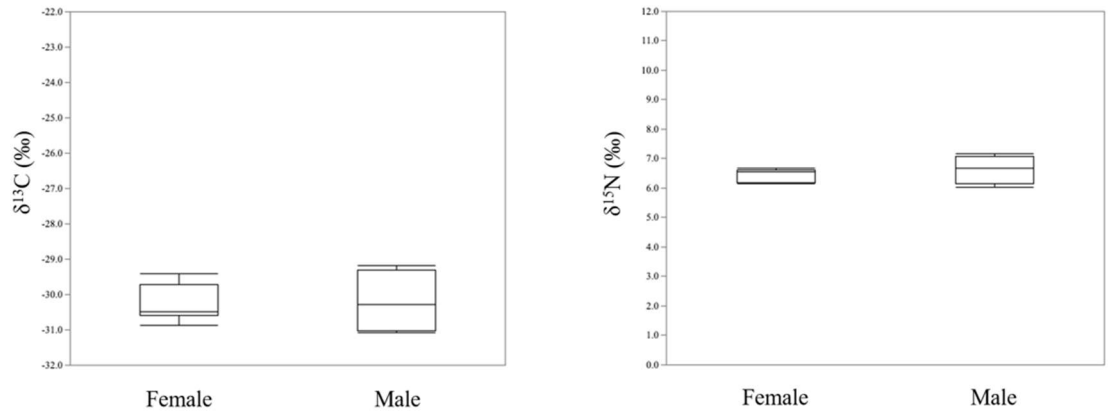

Figure 3. Boxplots of $\delta^{13}C$ (left) and $\delta^{15}N$ (right) in female (n = 7) and male (n = 4) tetragnathid spiders. Boxes represent interquartile ranges and middle bars represents the medians. Whiskers indicate the highest and lowest values within 1.5x interquartile range of the 25th and 75th percentile.

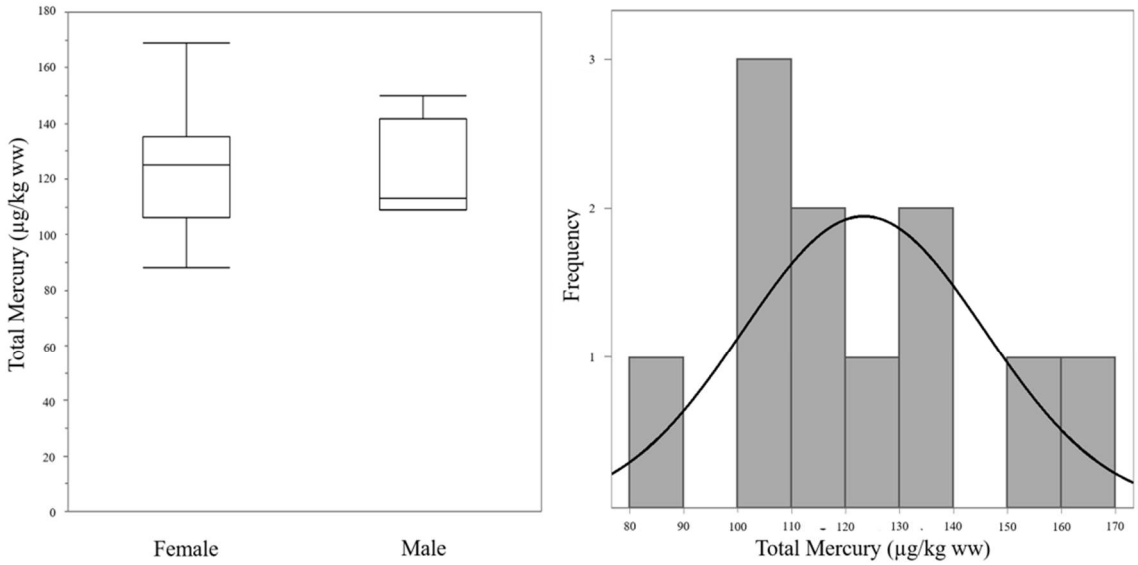

Figure 4. Boxplots of Total mercury (µg/kg ww) distributions in female (n = 4) and male (n = 7) tetragnathid spiders. Boxes represent interquartile ranges and middle bars represents the medians. Whiskers indicate the highest and lowest values within 1.5x interquartile range of the 25th and 75th percentile (left). Histogram of total mercury concentrations in tetragnathids (n = 11) with a fitted normal distribution (right).

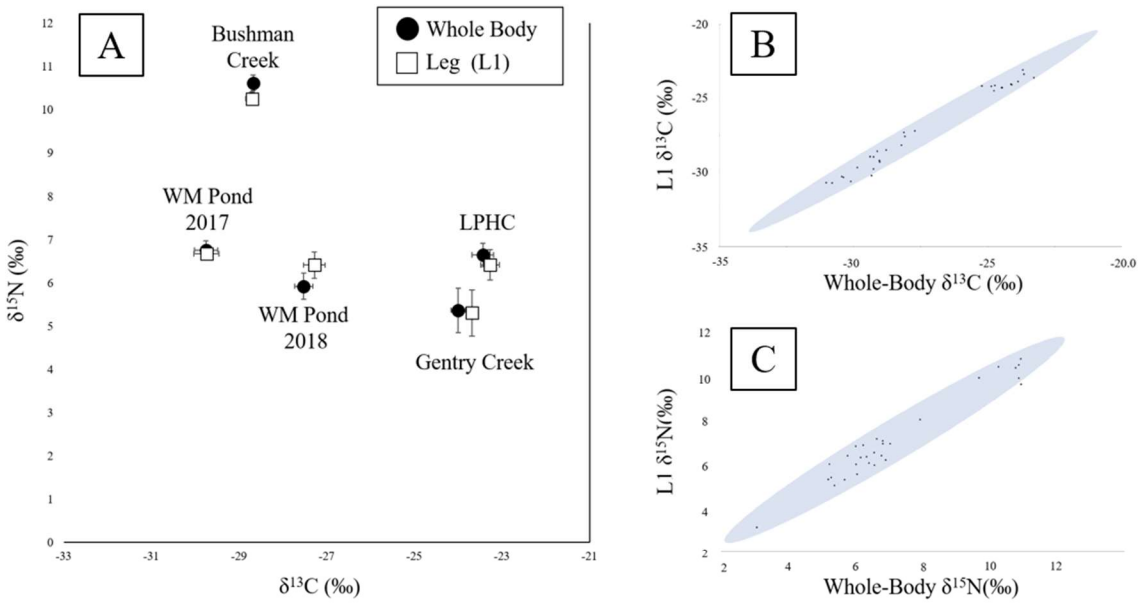

Figure 5. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of tetragnathid whole-body and leg tissue (n = 31). Site-specific averages of $\delta^{13}C$ and $\delta^{15}N$ (A) in tetragnathid whole-body (circle) and leg (square) tissues. Bars represent standard error. Correlation of $\delta^{13}C$ (B) and $\delta^{15}N$ (C) in tetragnathid whole-body and leg (L1) tissues surrounded by a 95% confidence interval. (Left Prong Hampton Creek = LPHC; Whigg Meadow Pond = WM Pond)

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DISSERTATION CONCLUSION

This study examined the biomagnification potential of Hg and the role of community level trophic ecology (CLTE) in mercury dynamics in southern Appalachian Mountain headwater streams and the adjacent riparian habitat.

In Chapter I, a quantitative approach to CLTE was used to describe the trophic structure of the food webs and revealed that the community standard ellipse area (SEA_c) influenced THg biomagnification, and that $\delta^{13}C_E$ was the primary driver of THg bioaccumulation in trout.

In Chapter II, a similar approach was used to discern the influence of aquatic food web biomagnification on riparian predator tissue concentrations. Although, the SEA_c of each respective site's neighboring aquatic and riparian communities had a high degree of trophic overlap in $\delta^{13}C$ and $\delta^{15}N$ biplot space, when the TL of riparian taxa was used to test the predictive function of aquatic THg biomagnification, the aquatic biomagnification model consistently underestimated concentrations in tetragnathids, araneids, and striders. With regards to other riparian taxa, the higher concentrations in tetragnathids appear to be a product of their higher respective TL; however, tetragnathids had the highest average THg concentrations of all taxa – riparian and aquatic.

In Chapter III, I found that these high Hg concentrations in spiders could cause harm to adult and nestling passerine birds nestlings inhabiting the southern Appalachian Mountains, and that the selection of which spider to use as a surrogate for "all spiders" would influence the risk characterization of these streams, and suggest that tetragnathids – rather than araneids – are a more appropriate and conservative surrogate for "all spiders."

In Chapter IV, I sought to better understand the effects of behavior and physiology on tetragnathids as bioindicators and I found that female tetragnathids were significantly larger than male spiders and represented a larger proportion of spiders collected at all sites. However, despite the difference in size between tetragnathids sexes, no differences in growth dynamics, isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) or how much mercury they accumulated were observed. Additionally, it was determined that the leg of a tetragnathid can accurately represent the stable isotope signature of an entire spider.

APPENDICES

APPENDIX A: IACUC APPROVAL LETTER

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

PROTOCOL APPROVAL NOTICE

Monday, September 14, 2015

Investigator Name(s): Ryan Otter
Investigator Email(s): ryan.otter@mtsu.edu
Department/Unit: Biology

Protocol ID: **15-013**
Protocol Title: ***Tennessee ecologically at-risk streams (TEARS)***

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Full Member Review mechanism*** and has approved your protocol in accordance with PHS policy. This approval is effective for three (3) years from the date of this notice. Your study expires 9/14/2018. Investigator(s) MUST file a Progress Report annually regarding the status of the study and submit an end-of-project report.

MTSU Policy defines an investigator as someone who has contact with animals for research or teaching purposes. Anyone meeting this definition needs to be listed on your protocol and needs to complete IACUC training through the CITI program. Addition of investigators requires submission of an Addendum Approval to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918.

Also, all research materials must be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office
(On behalf of IACUC)
Middle Tennessee State University
Tel: 615 494 8918
Email: compliance@mtsu.edu