EFFECTS OF SPIDER TAXA AND BODY SIZE ON MERCURY CONTAMINATION OF RIPARIAN SPIDERS: IMPLICATIONS FOR THE USE OF SPIDERS AS SENTINELS

by

ANDREW CHARLES TODD
Bachelor of Science, 2018
Middle Tennessee State University
Murfreesboro, TN

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Dissertation approved:

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Major Professor

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For The College of Science and Engineering

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INTRODUCTION

All aquatic ecosystems have been contaminated with mercury (Hg) at levels that exceed historic baselines due to widespread atmospheric deposition of inorganic Hg (IHg) (Global Mercury Assessment 2018). Anthropogenic activities, such as artisanal gold mining and coal combustion, are responsible for the majority of IHg emissions to the atmosphere (Chen & Driscoll 2018). Inorganic Hg in the atmosphere can be deposited near the emission source or circulate around the globe before deposition (Global Mercury Assessment 2018). When IHg is deposited into aquatic ecosystems, anaerobic bacteria convert IHg into the more bioavailable methylmercury (MeHg) (Selin 2009). Methylmercury bioaccumulates in the tissues of organisms at the base of the food web and biomagnifies as it moves through the food web (Lavoie et al. 2013). Methylmercury is highly toxic and poses a potential health risk to humans as well as high trophic position predators such as piscivorous fish and arachnivorous birds (Scheuhammer et al. 2007, Mergler et al. 2007).

The hazard posed by MeHg in the environment to wildlife and human health is determined in part by its bioavailability; however, determining the bioavailability of MeHg from measurements of concentrations in water or sediment can be difficult (Chumchal et al. 2022, Phillips & Segar 1986, but see Lydyet et al. 2014). Monitoring MeHg in water and sediment can be challenging from an analytical perspective because MeHg occurs at low concentrations, is variable over space and time, and has the potential for matrix interference during analyses (Chumchal et al. 2022). In addition, the concentration of MeHg in water or sediment may not be reflective of its bioavailability, due to the complex factors affecting the rate of uptake of MeHg by an organism (Chumchal et al. 2022). Sentinel species are used to overcome some of these challenges and to characterize bioavailable chemical contaminants in food webs (Beeby 2001, Chumchal et al. 2022). Sentinels are organisms that are found in high abundance, are easy to sample, and are known to sequester contaminants without significant adverse effects (Beeby 2001, Rainbow & Phillips 1993, Chumchal et al. 2022).

Riparian spiders have been proposed as sentinels of aquatic MeHg contamination (Tweedy et al. 2013, Speir et al. 2014, Gann et al. 2015, Chumchal et al. 2022). Riparian spiders are exposed to MeHg when they consume adult emergent aquatic insects that are contaminated with MeHg as larvae in aquatic ecosystems (Tweedy et al. 2013, Speir et al. 2014, Ortega-Rodriguez et al. 2019). A recent review identified spiders in the families Tetragnathidae, Araneidae, and Lycosidae to be particularly well-suited as sentinels of aquatic contamination because they are known to accumulate aquatic contaminants and are abundant along the shorelines of aquatic environments (Chumchal et al. 2022). However, limited work has been done to assess how ecological factors, such as taxonomic identity, body size, and level of ecosystem contamination, might affect contaminant concentrations in spiders and their suitability as sentinels (but see Tweedy et al. 2013, Ortega Rodriguez et al. 2019, Hannappel et al. 2021, and Drenner et al. 2022). Here we assess the effect of body size on MeHg concentrations in four taxa of riparian spiders collected from two rivers with different levels of MeHg contamination.

METHODS

We collected riparian spiders from the Clear Fork of the Trinity River and West Fork of the Trinity River, in Fort Worth, Texas (hereafter referred to as the Clear Fork and West Fork). There are no contemporary point sources of Hg into the Clear Fork or West Fork, and we assumed that most of the Hg in the two forks is from atmospheric deposition of IHg into the rivers, reservoirs, and their watersheds (Drenner et al. 2022). The Clear Fork and the West Fork are channelized and leveed rivers located downstream from large impoundments (Drenner et al. 2022). The Clear Fork receives water from Benbrook Lake while the West Fork receives water from Lake Worth and Eagle Mountain Lake, located immediately upstream from Lake Worth (Figure 1) (Drenner et al. 2022).

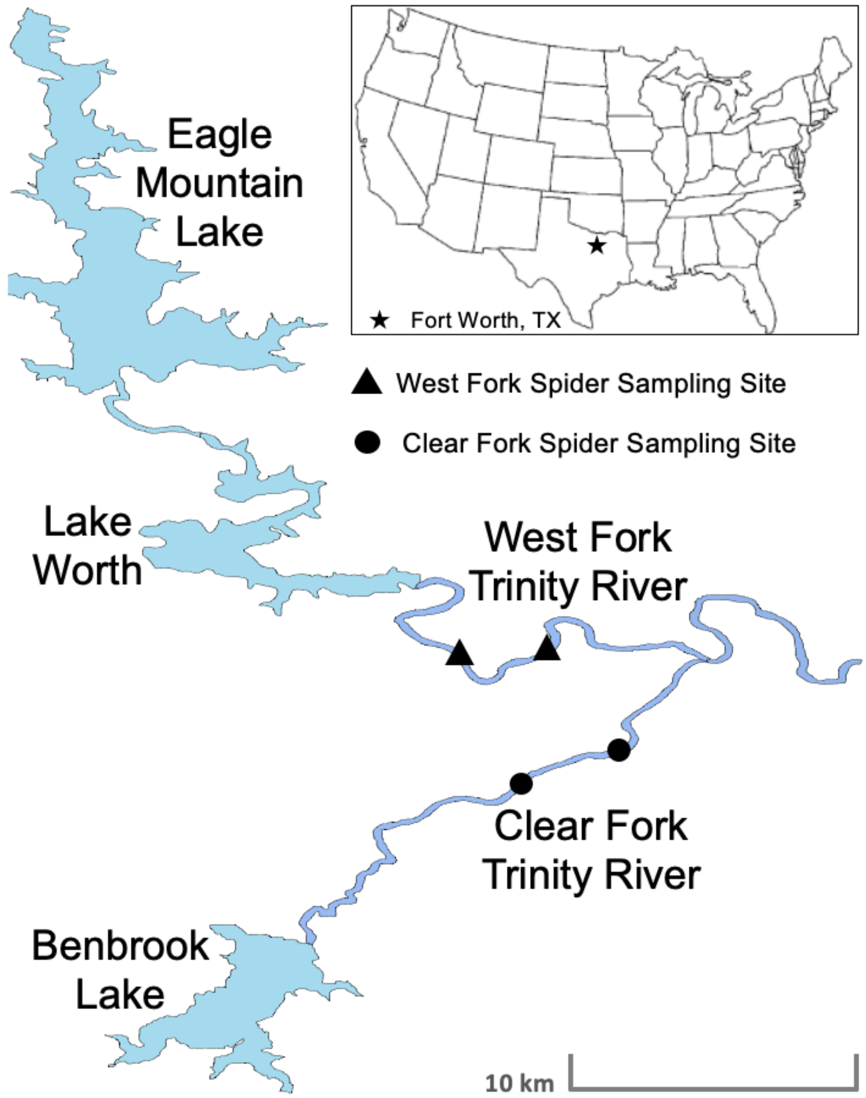


Figure 1. Location of sampling sites for spiders collected on the Clear Fork and West Fork of the Trinity River, Fort Worth, Texas, USA.

The Clear Fork is more contaminated with Hg than the West Fork (Drenner et al. 2022). Drenner et al. (2022) hypothesized that the Clear Fork is more contaminated with Hg than the

West Fork as a result of relatively large water-level fluctuations that occur in Benbrook Lake (the upstream impoundment), that create biogeochemical conditions more conducive for Hg methylation. In the present study, we selected two sampling sites on each river (Figure 1) with each containing diverse habitat types: grass, small shrubs, bare ground, and a bridge.

We collected spiders from the families Tetragnathidae (*Tetragnatha* sp.), Araneidae (*Larinioides* sp.), and Lycosidae (*Rabidosa* sp. and *Pardosa* sp.). Tetragnathid, araneid, and lycosid spiders are abundant, consume emergent aquatic insect prey (Sanzone et al. 2003, Kato et al. 2004, Du Laing et al. 2002), and these riparian taxa are hypothesized to reflect MeHg contamination of aquatic systems (Chumchal et al. 2022). All taxa of spiders were collected by hand from both forks from 5/25/2021 to 6/25/2021. *Larinioides* sp. were collected from the rails of bridges crossing over the river at each sampling site. *Tetragnatha* sp. and *Pardosa* sp. were collected within 2m of the shoreline from riparian vegetation (grass and shrubs) and on the bare ground, respectively. *Rabidosa* sp. were collected from the ground (grass and bare ground) within 20 m of the shoreline. Spiders were immediately placed into new polypropylene containers and preserved with 95% ethanol. Ethanol is commonly used to preserve invertebrates and ethanol preservation does not affect Hg concentrations (Hannappel et al. 2021). Spiders were identified to genus under a dissecting microscope, using a spider identification key (Ubick et al. 2017).

We used leg length (tibia + patella of the first leg) as a proxy for body length because leg length is correlated with other measures of body size in araneid, lycosid and tetragnathid spiders (e.g., carapace length and width) (Hannappel et al. 2021) and is representative of body size regardless of nutritional or reproductive state (Danielson-François et al. 2002). Leg length has also been positively correlated with Hg concentration in araneid, lycosid and tetragnathid spiders

(Hannappel et al. 2021, Drenner et al. 2022). Samples were separated by taxa and composited into 0.5mm leg length size classes prior to Hg analysis. In some cases (n = 5), samples were combined into 1mm size classes due to low sample size. Composite samples were dried in an oven at 60°C for 48 hours before being homogenized using a ball mill grinder.

Composite spider samples were analyzed for total Hg (THg; inorganic Hg + MeHg) using a Milestone DMA-80 Direct Hg Analyzer, which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy (US Environmental Protection Agency method 7473; 1998a). Quality assurance included reference standards (National Research Council of Canada Institute for National Measurement Standards), method blanks (empty quartz sample boats), and duplicate samples. Reference standards (DORM-4) were analyzed every 10 samples, and the average recovery percentage for DORM-4 was 98.42 ± 0.62 % (mean ± standard error [SE]; range 88.86% – 105.97%; n = 30). The mean mass of THg in blanks was 0.0029 ng (range 0.0007 – 0.0078 ng; n = 30). Duplicate samples were analyzed every 20 samples, and the average relative percent difference was 2.04 % (range 0.389 – 5.33 %; n = 14). All samples were above the method limit of detection of 0.26 ng THg calculated by adding the limit of blank to 1.645× the standard deviation of low concentration samples (Ambruster and Pry 2008).

Most THg in the four genera of spiders utilized in the present study is MeHg (50.9% in *Larinioides* sp., 105.9% in *Pardosa* sp., 70.8% in *Tetragnatha* sp., and 84.7% in *Rabidosa* sp.) Hannappel et al. (2022). Therefore, we use assumed that most THg was MeHg.

We used analysis of covariance (ANCOVA) models to determine the effect of river (categorical variable) and leg length (covariate) on MeHg concentrations in all four genera of spiders. We constructed an initial model that included the 3-way interaction between genera x river x leg length, as well as all possible 2-way interactions (genera x river, river x leg length, and genera x leg length) and main effects of genera, river and leg length. None of the interaction terms, except for genera x leg length, were significant. Therefore, we removed these non-significant interaction terms from the model and created a reduced model that contained the genera x leg length interaction and main effects of genera, river, and leg length. A significant genera x leg length interaction was also identified in the reduced model (indicating heterogeneous slopes), so we assessed the effect of leg length on THg concentrations within each river using simple linear regression, and the effect of river on THg concentrations using an F-test based on the linearly independent pairwise comparisons among estimated marginal means. Statistical significance was determined at $p < 0.05$ and statistical tests were performed using SPSS.

RESULTS/DISCUSSION

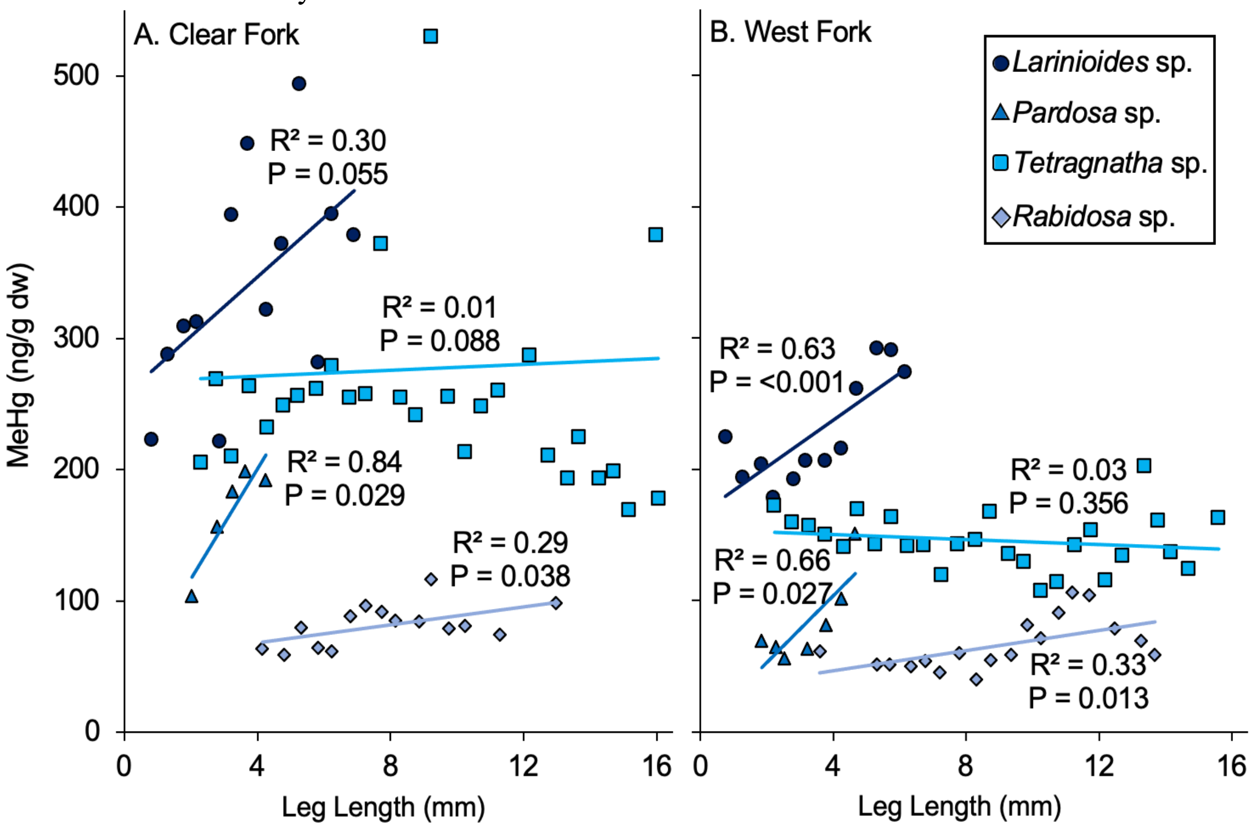
We detected a significant main effect of river on MeHg concentrations in spiders such that spiders from the Clear Fork had higher concentrations of MeHg than spiders from the West Fork (Table 1, Figure 2A-B). In a previous study, Drenner et al. (2022) found that tetragnathid spiders collected in 2016 and 2019 and bluegill (*Lepomis macrochirus*) collected in 2019 from

Table 1: Model parameters of Analysis of Covariance assessing the two-way interaction between genera x leg length and main effects of genera, leg length and river on MeHg concentrations in four genera of spiders.

Factor	df	F	P
Leg Length x Genera	3,118	2.740	0.046
Leg Length	1,118	5.053	0.026
Genera	3,118	5.958	<0.001
River	1,118	50.921	<0.001

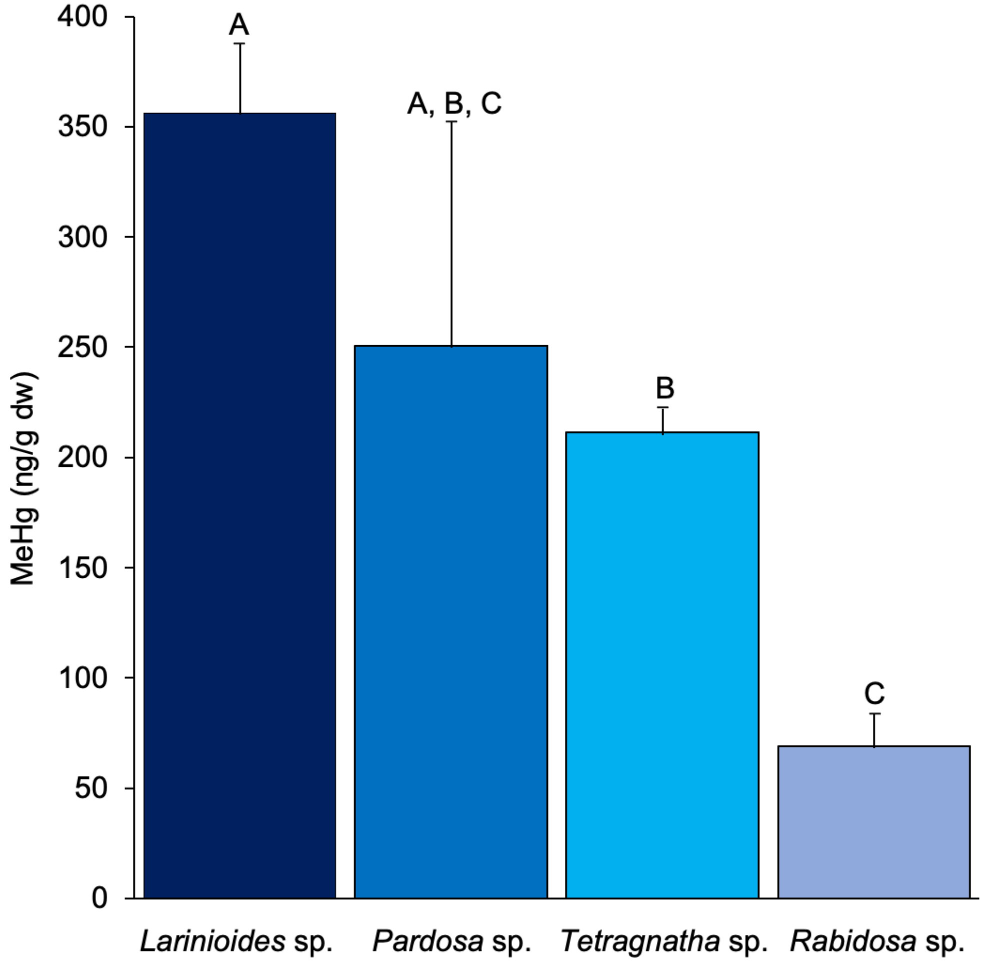
the Clear Fork had higher concentrations of THg compared to the West Fork. The results from the present study and Drenner et al. (2022) suggest that differences in the Hg concentrations of biota from the two forks persist between years.

Figure 2: Relationships between leg length and MeHg concentrations in *Larinioides* sp., *Rabidosa* sp., *Pardosa* sp., and *Tetragnatha* sp. on the A) Clear Fork of the Trinity River and B) West Fork of the Trinity River.



We detected significant main effects of genera and leg length on MeHg concentrations in spiders, but also a significant genera x leg length interaction, indicating that these factors were not independent (Table 1, Figure 2A-B). At a leg length of 7.26 mm, spider genera exhibited significantly different estimated marginal mean MeHg concentrations (Figure 3). Methylmercury concentrations were positively correlated with leg length in *Rabidosa* sp. and *Pardosa* sp. on both forks, and *Larinioides* sp. on the West Fork (Figure 2A-B).

Figure 3: Differences between estimated marginal mean MeHg concentrations between *Larinioides* sp., *Rabidosa* sp., *Pardosa* sp., and *Tetragnatha* sp. spiders, evaluated at a leg-length of 7.26mm. Significant differences in mean MeHg concentration among taxa are indicated by different upper-case letters.



We observed taxonomic differences in MeHg concentrations of riparian spiders in this study (Figure 3). Ortega-Rodriguez et al. (2019) demonstrated that differences in MeHg concentrations between spider taxa can largely be explained by the proportion of aquatic prey in their diet. There have been five other studies that have made similar comparisons between Hg concentrations of some of the taxa found in the present study (Abeysinghe et al. 2017, Bartrons et al. 2015, Beaubien et al. 2020, Hannappel et al. 2021, Ortega-Rodriguez et al. 2019). However, the rank order of Hg concentrations between families is not consistent within the literature. Two non-mutually exclusive hypotheses could explain the differences in rank order of

Hg concentrations between families observed in previous studies: 1) previous studies have not examined the same genera and 2) the proportion of aquatic insects in spider diets could vary with time and location. Most previous studies did not identify all taxa to genera, so the discrepancies in family level Hg concentrations between studies could be due to the fact that previous studies studied different genera. Most of these studies also did not determine the proportion of aquatic insects in the diets of the spiders they examined, so potential discrepancies between diet cannot be assessed. Further investigation is needed to determine why Hg concentrations differ at the family level in riparian spiders.

We observed taxonomic differences in the relationship between leg length and Hg concentrations in this study (Figure 2). One previous study has examined leg length and Hg concentrations in the spider families from the present study (Hannappel et al. 2021). Similar to the present study, Hannappel et al. (2021) found that araneid spiders and *Pardosa* sp. both had positive leg length and Hg relationships, but unlike the present study, found that *Tetragnatha* sp. had a positive relationship while *Rabidosa* sp. did not. A previous study conducted on the Clear Fork and West Fork (Drenner et al. 2022) found no effect of leg length on Hg concentration in tetragnathids on the West Fork, but unlike the present study, a positive relationship between leg length and Hg concentration on the Clear Fork. There have been few studies examining the effect of leg length on Hg concentrations in riparian spiders and the methodologies have not been consistent (e.g. spiders collected at different times of the year, in multiple locations, and composites of different genera). Additional studies with similar methodologies will be needed to determine if Hg contamination is consistently correlated with leg length in different spider families.

In a recent review, Chumchal et al. (2022) suggested riparian spiders have great potential as sentinels, but that there has been relatively little work into the factors that influence contaminant concentrations within riparian spiders. In the present study, we determined that MeHg concentrations differed between spider taxa and were impacted by body size. Although MeHg vs leg length relationships exhibited genera-specific patterns, similar patterns were observed on both forks, suggesting that riparian spiders have utility as sentinels but that in future studies taxa and body size should be taken into account.

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VITA

Personal Background	Andrew Charles Todd Murfreesboro, TN
Education	Master of Science, Biology, Texas Christian University, Fort Worth, TX 2022 Bachelor of Science, Biology, Middle Tennessee State University, Murfreesboro, TN, 2019 Honors Diploma, Siegel High School, Murfreesboro, TN, 2015
Experience	Graduate Teaching Assistantship, Texas Christian University Department of Biology, 2020-2022
Memberships	Society of Toxicology and Chemistry American Arachnological Society

ABSTRACT

EFFECTS OF SPIDER TAXA AND BODY SIZE ON MERCURY CONTAMINATION OF RIPARIAN SPIDERS: IMPLICATIONS FOR THE USE OF SPIDERS AS SENTINELS

by Andrew Charles Todd, M.S., 2022
Department of Biology
Texas Christian University

Thesis Advisors: Dr. Ray Drenner and Dr. Matt Chumchal, Professor of Biology

Riparian spiders have been proposed as sentinels of aquatic mercury (Hg) contamination since they accumulate Hg through the consumption of emergent aquatic insects. The objective of this study was to assess the effect of spider taxa and body size on Hg concentrations in riparian spiders collected from two rivers with different levels of Hg contamination. In this study, we determined the effect of spider taxa and body size on Hg concentrations in four taxa of riparian spiders (*Larinioides* sp., *Tetragnatha* sp., *Rabidosa* sp., and *Pardosa* sp.) in Fort Worth, Texas. Average concentrations of Methyl-Hg (MeHg) in riparian spiders were significantly different between rivers. We determined that MeHg concentrations differed between spider taxa and were impacted by body size. These findings suggest that future studies must take these factors into account when using riparian spiders as sentinels.