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Running Head: MEHG CONTAMINATION OF SHORELINE SPIDERS

Relationship Between Methyl Mercury Contamination and Proportion of Aquatic and Terrestrial Prey in Diets of Shoreline Spiders

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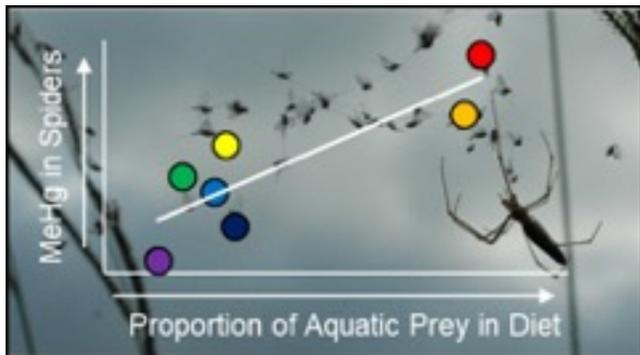
Accepted Article

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Abstract: Terrestrial organisms such as shoreline spiders that consume prey from aquatic food webs can be contaminated with methyl mercury (MeHg). However, no studies have examined the relationship between MeHg contamination of shoreline spider taxa and the proportion of aquatic and terrestrial prey in their diets. The present study had two objectives: 1) determine concentrations of MeHg in seven taxa of shoreline spiders, and 2) assess the relationship between concentrations of MeHg in spiders and the proportion of aquatic and terrestrial prey in spider diets. We collected shoreline spiders, emergent aquatic insects and terrestrial insects from in and around 10 experimental ponds. Methyl mercury concentrations were greatest in spiders, intermediate in aquatic insects and lowest in terrestrial insects. The elevated MeHg concentrations in spiders indicate that they were feeding, at least in part, on emergent aquatic insects. However, variability in MeHg concentration observed among spider taxa suggested that the proportion of aquatic and terrestrial prey in spider diets likely varied among taxa. We estimated the proportion of aquatic and terrestrial prey in the diet of each spider taxon from the nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope values of spiders and their potential aquatic and terrestrial prey items. The median proportion of aquatic prey in spider diets varied by almost 2-fold and MeHg concentrations in shoreline spiders were strongly correlated with the proportion of aquatic prey in their diet. In the present study, we demonstrate for the first time that the degree of connectivity to aquatic food webs determines MeHg contamination of shoreline spiders.

Graphical Abstract



Keywords: mercury, spiders, food webs, ponds

This article includes online-only Supplemental Data.

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INTRODUCTION

All landscapes are contaminated with inorganic mercury (IHg) from widespread atmospheric deposition (Chen and Driscoll 2018). In aquatic systems, IHg can be converted to highly toxic and accumulative methyl mercury (MeHg), that enters the food chain and results in aquatic organisms being contaminated with MeHg (Chen and Driscoll 2018). Organisms feeding near the top of aquatic food webs, like piscivorous fish and birds, can accumulate concentrations of MeHg high enough to pose a threat to their health (Scheuhammer et al. 2007; Wiener 2013; Gerstle et al. 2019).

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While the earliest research on MeHg in biota focused on fish and fish-eating wildlife (Wiener et al. 2003), recent studies have demonstrated that terrestrial organisms such as shoreline spiders can be contaminated with MeHg if they consume prey from aquatic food webs (Tweedy et al. 2013; Speir et al. 2014). We hypothesize that hunting strategy and habitat use by shoreline spiders may have important effects on the ratio of aquatic to terrestrial prey in their diets and their level of MeHg contamination. For example, shoreline spiders that build webs in vegetation over the water's surface, such as long-jawed orb weavers (Tetragnathidae: *Tetragnatha* sp.), feed primarily on emergent aquatic insects (i.e., have a high degree of connectivity to the aquatic food web) and can accumulate high concentrations of MeHg (Tweedy et al. 2013; Speir et al. 2014; Gann et al. 2015). However, shoreline spider taxa that hunt on the ground in terrestrial vegetation, such as wolf spiders (Lycosidae), may have diets with a low ratio of aquatic to terrestrial prey (i.e., have a low degree of connectivity to the aquatic food web) (Sanzone et al. 2003) and have relatively low concentrations of MeHg. No studies have examined the relationship between MeHg contamination of shoreline spider taxa and the proportion of aquatic and terrestrial prey in their diets.

The present study had two objectives: 1) determine concentrations of MeHg in shoreline spiders including one taxon of orb weaver and six taxa of hunting spiders, and 2) assess the relationship between concentrations of MeHg in spiders and the proportion of aquatic and terrestrial prey in spider diets using stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$). Here we demonstrate for the first time a strong correlation between MeHg concentration in shoreline spiders and proportion of aquatic prey in their diet, suggesting that

the degree of connectivity to aquatic food webs determines MeHg contamination of shoreline spiders.

METHODS

Study site

We conducted the present study in experimental ponds at the Eagle Mountain Fish Hatchery (32°52'32.95"N, 97°28'29.00"W) near Fort Worth, Texas, USA (Supplemental Data, Figure S1). The ponds are supplied with water from the limnetic zone of Eagle Mountain Lake, a large drinking water supply reservoir. Ponds range in size from 0.23 to 0.54 ha and have a mean depth of 0.8 meters. The ponds are whole ecosystems with earthen bottoms that contain complex communities of macrophytes and benthic invertebrates and some contain fish. Previous studies revealed that the ponds have food chains contaminated with Hg (Tweedy et al. 2013; Speir et al. 2014; Chumchal et al. 2017; Williams et al. 2017; Chumchal et al. 2018). The source of Hg in the ponds is atmospheric deposition to the pond surfaces and the watershed of Eagle Mountain Lake.

Approach

In the present study, we collected shoreline spiders, emergent aquatic insects and terrestrial insects from in and around 10 experimental ponds. For each spider and insect taxon, we attempted to collect one composite sample from each experimental pond but not all taxa were present at all ponds.

Collection of spiders

We collected spiders on June 30, July 6 and 17, August 6 and 13 and September 10, 2015. Spiders were collected from the ground and vegetation within 2 m of the water's edge with nets or by hand and preserved in 95% ethanol (Supplemental Data, Figure S1). Spider taxa included one taxon of orb weaver (long-jawed orb weaver) and six taxa of hunting spiders (crab spiders [Thomisidae], fishing spiders [Pisauridae: *Dolomedes* sp.], lynx spiders [Oxyopidae: *Peucetia viridans* (green lynx spiders) and *Oxyopes salticus* (striped lynx spiders)], jumping spiders [Salticidae] and wolf spiders [Lycosidae]) (Figure 1). We collected an average of 434 ± 43.2 (mean \pm S.E.) individual spiders from each pond (Supplemental Data, Table S1). Each taxon of spider was collected from an average of 7.4 ± 1.3 (mean \pm SE) ponds (Supplemental Data, Table S1). For each pond, spiders were pooled by taxon into composite samples and dried at 60°C for 72 h. Each composite spider sample was then homogenized to a fine powder using a clean mortar and pestle or a ball-mill grinder.

Collection of biota to assess the proportion of aquatic and terrestrial prey in spider diet

We used small-bodied emergent aquatic insects (midges [Chironomidae] and micro-caddisflies [Hydroptilidae]) to represent $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the aquatic food web. Emergent aquatic insects were collected from each pond using pyramid-shaped floating emergence traps during a continuous 7-month period beginning in February 2015. Each trap sampled an area of $0.53 \text{ m} \times 0.53 \text{ m}$ (0.28 m^2) (Supplemental Data, Figure S1). Two traps were deployed in each pond approximately 3 m from shore, where they typically floated between 50 and 75 cm above the sediment surface. Traps were tethered to a fence post with a 1-m length of twine. The traps funneled emerging insects into a collecting bottle containing 95% ethanol. Four taxonomic groups of emergent insects were captured in adequate numbers

for analyses: herbivorous/detritivorous chironomid midges (Chironomidae: Chironominae), herbivorous/detritivorous orthoclad midges (Chironomidae: Orthoclaadiinae), predatory midges (Chironomidae: Tanypodinae), and micro-caddisflies. We collected an average of $3,543 \pm 963$ (mean \pm SE) individual emergent insects from each pond over the sampling period (Supplemental Data, Table S1). Each taxon of emergent insect was collected from an average of 5.3 ± 1.8 (mean \pm SE) ponds (Supplemental Data, Table S1). For each pond, emergent insects collected over 7-months were pooled by taxon into composite samples and dried for at least 48 h at 60 °C. Each composite emergent-insect sample was then homogenized to a fine powder using a clean mortar and pestle or a ball-mill grinder.

We used terrestrial insects to represent $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the terrestrial food web. Terrestrial insects were collected on June 30, July 6, August 6 and 13 and September 10, 2015. Terrestrial insects were collected from the ground and vegetation within 2 m of the water's edge with nets or by hand and preserved in 95% ethanol (Supplemental Data, Figure S1). Terrestrial insects included caterpillars (Lepidoptera), crickets (Orthoptera: Gryllidae), grasshoppers (Orthoptera: Acrididae), katydids (Orthoptera: Tettigoniidae), leafhoppers (Hemiptera: Cicadellidae), sharpshooters (Hemiptera: Cicadellidae: *Draeculacephala* sp.) and shield bugs (Hemiptera: Pentatomidae). We collected an average of 159 ± 32.0 (mean \pm SE) individual terrestrial insects from each pond (Supplemental Data, Table S1). Each taxon of terrestrial insect was collected from an average of 6.4 ± 1.7 (mean \pm SE) ponds (Supplemental Data, Table S1). For each pond, terrestrial insects were pooled by taxon into composite samples and dried at 60°C for 72 h. Each terrestrial insect composite sample was then homogenized to a fine powder using a clean mortar and pestle or a ball-mill grinder.

Total Mercury Analysis

All samples collected in the present study were analyzed for total Hg (MeHg + inorganic Hg) using direct mercury analysis (Milestone DMA-80 Direct Hg Analyzer) which uses thermal decomposition, gold amalgamation, and atomic absorption spectroscopy (USEPA 1998a). Quality assurance included reference (National Research Council of Canada Institute for National Measurement Standards) and duplicate samples. Reference samples (DORM-4 and PACS-3) were analyzed every 10 samples, and the mean recovery percentage for DORM-4 was 94.6 (range, 88.8 – 98.7%; N= 27). Mean recovery percentage for PACS-3 was 95.9 (range, 91.9 – 99.7 %; N = 17). Duplicate samples were analyzed every 20 samples, and the mean relative difference percentage was 4.74% (range, 0.1 – 14.9%; N = 24). All samples were above the method detection limit of 0.48 ng total Hg calculated as 3.14 times the standard deviation of seven low level samples.

Methylmercury Analysis

A subset of samples were analyzed for MeHg using GC-CVAFS (Tekran® Series 2700, Tekran Instruments Corporation), which uses purge and trap, gas chromatography, and cold vapor atomic fluorescence spectroscopy (USEPA 1998b). Digestion procedures followed the methods described by Perkins et al. (2017). Samples were digested at ~130-140°C with 8 mL of 25% potassium hydroxide in methanol for 4 h. After cooling, digests were filled to 30 mL with methanol and then stored in a freezer (-20°C) until analysis. For quantification of MeHg, appropriate aliquots of the sample digests (estimated from the total Hg concentration) were transferred to individual glass vials containing ~25 mL of ultrapure water and acetate buffer to achieve a pH of 4.5. The total volume of each tube was adjusted

to 30 mL with ultrapure water before ethylation of the Hg species with 1% sodium tetraethylborate. Reference samples (SRM 2976 and DORM-4) were digested and analyzed in the same manner as the samples. Additional quality assurance included the analysis of duplicate samples, reagent blanks, and initial and ongoing precision recovery samples (0.5 ng/L MeHg). Reagent blanks consisted of potassium hydroxide and methanol without the addition of sample. Mean recovery percentages for SRM 2976 and DORM-4 were 103.2 (range, 100.6 – 105.3%; N=3) and 113.9 (range, 111.8 – 116.2%, N=4), respectively. The mean recovery percentage of MeHg from initial and ongoing recovery samples was 99.2 (range, 92.7 – 116.3; N=18). The mean concentration of MeHg in digestion blanks was 0.01 ng/L (range 0.01 – 0.04; N=3). The mean relative difference percentage between duplicates was 10.3 (range, 0.3 – 28.6; N=8). All samples were above the theoretical method detection limit of 1.2 ng MeHg calculated by adding the mean of reagent blanks to 3x the standard deviation of the same blanks.

Because of the high analytical costs, MeHg concentrations could not be measured directly in all samples. To estimate MeHg concentrations, we analyzed total and MeHg concentrations in 1-7 samples of each taxon and determined the percentage of total Hg that was MeHg (Supplemental Data, Table S1). We used these data to estimate MeHg concentrations from total Hg concentrations in any sample in which we did not directly measure MeHg concentration (Supplemental Data, Table S1). All estimated MeHg concentrations (hereafter MeHg concentrations) are presented as nanograms MeHg per gram of dry weight of invertebrate tissue.

Stable Isotope Analysis

The proportion of aquatic and terrestrial prey in spider diets was determined using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Dried, ground, and homogenized spider and insect samples were encased in tin capsules before analysis at the University of California–Davis Stable Isotope Facility using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer.

Carbon and N isotope values are given as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}} - 1)] \times 1000$$

where R is $^{13}\text{C}/^{12}\text{C}$ for $\delta^{13}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ for $\delta^{15}\text{N}$. Standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were Vienna Pee Dee Belemnite and air N_2 , respectively.

Stable Isotope Dietary Mixing Model

We utilized a Bayesian inference stable isotope mixing model to estimate the proportion of aquatic and terrestrial prey in the diet of each spider taxon. This modelling approach used: 1) the stable isotope values of spiders and their potential aquatic and terrestrial prey items determined in the present study, and 2) isotope trophic enrichment factors (TEFs) from the literature (Post 2002) to generate probability distributions of the proportion of aquatic and terrestrial prey in spider diets (Parnell et al. 2010). These probability distributions were used to generate the median proportion of aquatic prey in spider diets. Analyses were performed in Stable Isotope Analysis in R (SIAR, version 4). Herbivorous/detritivorous chironomid midges, herbivorous/detritivorous orthoclad midges, micro-caddisflies and predatory midges were classified as aquatic prey and caterpillars, crickets, grasshoppers, katydids, leafhoppers, sharpshooters and shield bugs were classified

as terrestrial prey in the model. Mean (± 1 SD) TEFs used in models were $0.4 \pm 1.3\%$ and $3.4 \pm 1\%$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Post 2002). Model fitting was conducted with uninformed priors via Markov Chain Monte Carlo simulations, which produces likely values of the proportional contribution of aquatic and terrestrial prey to each spider taxon. Each model was run for $n = 500,000$ iterations with an $n = 50,000$ burn-in.

Statistical Analysis

A mixed-model ANOVA was used to determine if MeHg concentrations differed among spider families. Spider taxa was a fixed factor and the pond from which spiders were collected was a random factor in the analysis. Concentrations of MeHg were \log_{10} -transformed before analysis to improve homogeneity of variance but untransformed data are presented in figures for ease of interpretation. Linear regression analysis was used to assess the relationship between the median proportion of aquatic prey in diet and mean MeHg concentration in spiders. All analyses were completed using SPSS Version 25, and statistical significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Spiders and aquatic and terrestrial insects varied in their level of MeHg contamination (Figure 2). Mean MeHg concentrations in spiders ranged from 59.3 to 200 ng/g in striped lynx spiders and fishing spiders, respectively. Mean MeHg concentrations were significantly different between spider taxa (Figure 2; mixed-model ANOVA, $F_{6,44} = 7.59$, $p < 0.0001$) but did not significantly differ among ponds (mixed-model ANOVA, $F_{9,44} = 0.64$, $p = 0.757$). Average MeHg concentrations (mean \pm SE) in aquatic and terrestrial insects were 63.0 ± 1.4 ng/g and 3.46 ± 0.7 ng/g, respectively (Figure 2; Supplemental Data, Table S1). The elevated

MeHg concentrations in spiders indicates that they were feeding, at least in part, on emergent aquatic insects. However, the difference in MeHg concentration among spider taxa suggests that the proportion of aquatic and terrestrial prey in spider diets likely varied among taxa.

To examine the proportion of aquatic and terrestrial prey in spider diets, we used a stable isotope mixing model based on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of spiders and their potential prey items collected in the present study (Supplemental Data, Figure S2). The stable isotope dietary mixing model indicated that the median proportion of aquatic prey in spider diets varied from 0.46 for striped lynx spiders to 0.81 for fishing spiders (Figure 3). The concentration of MeHg in spider taxa increased as a function of the estimated proportion of aquatic prey in their diet (Figure 4, linear regression, $F_{1,6} = 13.5$; $p = 0.014$, $r^2 = 0.73$). The strong correlation between MeHg concentration in spiders and proportion of aquatic prey in their diet suggests that the degree of connectivity to aquatic food webs determined MeHg contamination in shoreline spiders.

Differences in the proportion of aquatic prey in the diet of shoreline spider taxa were likely related to differences in hunting strategy and habitat use by spiders. The two spider taxa most closely associated with the aquatic environment (long-jawed orb weavers and fishing spiders, Figure 1), had the highest proportions of aquatic prey in their diets. Long-jawed orb weavers build their webs in vegetation near or above the water surface (Ubick et al. 2017) and feed primarily on small emergent aquatic insects (e.g., midges and micro-caddisflies) (Tweedy et al. 2013). Fishing spiders of the genus *Dolomedes* are semi-aquatic hunters, consuming mainly insects active at the water surface (Zimmermann and Spence 1989). The other hunting spiders in the present study (crab, jumping, lynx and wolf spiders)

also consumed aquatic prey and became contaminated with MeHg, although to a lesser degree than fishing spiders and long-jawed orb weavers. Crab, jumping, lynx and wolf spiders hunt insect prey on the ground or in terrestrial vegetation (Michalko and Pekár 2016; Ubick et al. 2017). We hypothesize that these opportunistic hunting spiders consumed a mix of terrestrial and emergent aquatic insects that would have been available along the shoreline.

Shoreline spiders have been proposed as biosentinels that could be used to monitor biomagnifying contaminants in aquatic ecosystems (Walters et al. 2008; Walters et al. 2009; Otter et al. 2013; Tweedy et al. 2013) and provide insight into the risk posed by contaminants to birds that feed on spiders (Walters et al. 2009). Most studies have focused on long-jawed orb weavers that have potential as biosentinels because they have a wide geographic distribution, occur at high densities and specialize in the consumption of emergent aquatic insects (Walters et al. 2008). However, long-jawed orb weavers cannot be used to monitor contaminants away from the shoreline because many taxa of long-jawed orb weavers are obligate shoreline species (Gillespie 1987). The present study demonstrates that other spider taxa, such as crab, jumping, lynx and wolf spiders, have potential as biosentinels because they utilize aquatic prey and were contaminated with MeHg. Unlike long-jawed orb weavers, the spatial distributions of these taxa are not limited to shorelines and they can be found across the terrestrial landscape. Because the degree of connectivity to aquatic food webs is a determinant of MeHg concentration in spiders and thus risk to arachnivorous birds, utilizing different species of spiders as biosentinels will require an understanding of the proportion of aquatic prey in spider diets.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data accessibility—Data are freely available from the TCU Scholarly Works repository (<http://doi.org/10.18776/tcu/data/26483>).

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FIGURES

Graphical Abstract. The concentration of MeHg in spider taxa increased as a function of the proportion of aquatic prey in their diet, demonstrating for the first time that the degree of connectivity to aquatic food webs determines MeHg contamination of shoreline spiders.

Figure 1. Representative photographs of the six spider families included in the present study:

A) crab spiders (Thomisidae), B) fishing spiders (Pisauridae), C) jumping spiders

(Salticidae), D) long-jawed orb weavers (Tetragnathidae), E) lynx spiders (Oxyopidae) and

F) wolf spiders (Lycosidae). Photographs depict the spider families included in the present

study but are not necessarily the species examined in the present study. Photo credits: A.

Arto Hakola, Shutterstock; B. Jukka Jantunen, Shutterstock; C. pong6400, iStock; D.

davemhuntphotography, Shutterstock; E. Bill Gozansky, agefotostock; F. Brett Hondow,

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Figure 1

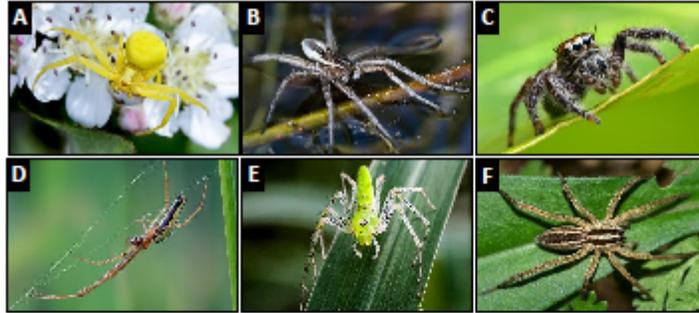


Figure 2. Average MeHg concentration (mean \pm SE) in seven taxa of spiders and aquatic and terrestrial insect taxa.

Figure 2

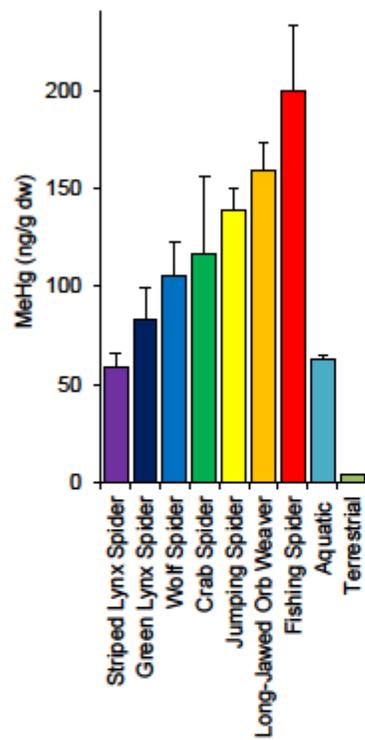


Figure 3. Proportion of aquatic prey in diets of seven taxa of spiders estimated from a $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ mixing model. Estimated proportional contribution of aquatic prey in spider diets are represented by the mean (point), median (horizontal line), 25th and 75th percentile (boxes), and the 10th and 90th percentile (whiskers) posterior probability values.

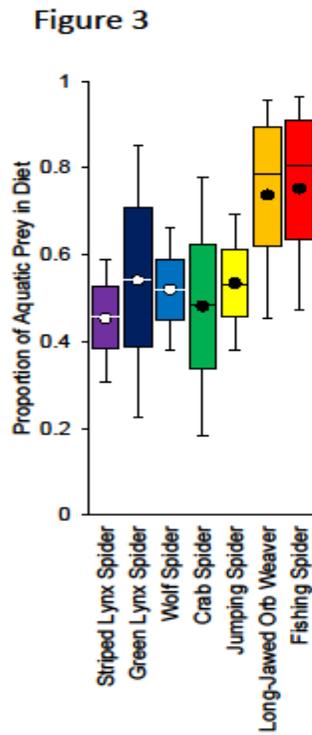


Figure 4. Mean MeHg concentration vs median proportion of aquatic prey in the diet of seven taxa of spiders.

