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Short Communication

METHYL MERCURY AND STABLE ISOTOPES OF NITROGEN REVEAL THAT A TERRESTRIAL SPIDER HAS A DIET OF EMERGENT AQUATIC INSECTS

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Abstract: Terrestrial spiders transfer methyl mercury (MeHg) to terrestrial consumers such as birds, but how spiders become contaminated with MeHg is not well understood. In the present study, the authors used stable isotopes of nitrogen in combination with MeHg to determine the source of MeHg to terrestrial long-jawed orb weaver spiders (*Tetragnatha* sp). The authors collected spiders and a variety of other aquatic and terrestrial taxa from 10 shallow ponds in north Texas, USA. Based on MeHg concentrations and stable nitrogen isotope ratios, the authors identified distinct aquatic- and terrestrial-based food chains. Long-jawed orb weaver spiders belonged to the aquatic-based food chain, indicating that they are exposed to MeHg through their consumption of emergent aquatic insects. Additionally, the present study suggests that ecologists can use stable isotopes of nitrogen ($\delta^{15}N$) in conjunction with MeHg speciation analysis to distinguish between aquatic and terrestrial food chains. *Environ Toxicol Chem* 2014;33:2506–2509. © 2014 SETAC

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INTRODUCTION

Methyl mercury (MeHg) is a highly toxic contaminant found at elevated concentrations in waterbodies throughout the world because of widespread atmospheric deposition [1,2]. Elemental forms of mercury (Hg) are emitted to the atmosphere primarily by the burning of fossil fuels and small-scale gold mining [1,3]. Elemental Hg can circulate around the world before it is deposited as inorganic Hg into watersheds [1,4]. In aquatic ecosystems, sulfate-reducing bacteria convert the inorganic form of Hg into its toxic and bioaccumulative methyl form [1,4]. Organisms at the base of the food web, such as periphyton, concentrate MeHg directly from the water [5], and consumers are exposed through their diets [6]. Methyl mercury can biomagnify in aquatic food webs, reaching high concentrations in top consumers, such as largemouth bass (*Micropterus salmoides*) [7].

Historically, studies of MeHg contamination of food webs have focused on aquatic organisms because inorganic Hg is converted to MeHg in aquatic ecosystems. However, elevated concentrations of MeHg have recently been found in the tissues of terrestrial consumers [8–11]. For example, Cristol et al. [9] found that songbirds were exposed to high concentrations of MeHg by consuming terrestrial spiders. Cristol et al. [9] proposed 2 alternative hypotheses to explain how terrestrial spiders, which are commonly found near aquatic ecosystems, became contaminated with MeHg: 1) emergent aquatic insects that are contaminated by MeHg during their aquatic larval stages transport MeHg to adjacent terrestrial ecosystems when they emerge from the water as adults [11–13] and are then eaten by spiders [9], or 2) terrestrial food webs that include spiders become contaminated by MeHg deposited onto the floodplain [9].

The purpose of the present study was to determine the source of MeHg in a common shoreline spider, the long-jawed orb weaver (*Tetragnatha* sp.), using stable isotopes of nitrogen ($\delta^{15}N$) in conjunction with MeHg speciation analysis. *Tetragnatha* may be one of the most widespread and abundant orb weaving spider genera in the world [14]. Long-jawed orb weavers have been proposed as biosentinels of contaminant concentrations in small emergent insect taxa and indicators of the overall level of food web contamination [11,12,15,16].

METHODS

We conducted the present study in 10 experimental ponds near Fort Worth, Texas, USA. An image of the experimental pond facility can be found in the Supplemental Data (Figure S1). The experimental ponds are whole ecosystems with earthen bottoms that contain complex communities of macrophytes, benthic invertebrates, and herptiles. The ponds are large and range from 0.23 ha to 0.54 ha, with maximum and average depths of 1.2 m and 0.6 m, respectively. A previous study revealed that the ponds had food chains contaminated with Hg [11]. The source of Hg in the ponds is atmospheric deposition to the pond surfaces and the watershed of a nearby reservoir, Eagle Mountain Lake, which is the source of water to the ponds. In 2009, 10 ponds were filled with water from Eagle Mountain Lake. In 2010, 5 ponds in the present study were stocked with fish, including bluegill (*Lepomis macrochirus*), hybrid sunfish (*Lepomis* sp.), and largemouth bass; the other 5 ponds did not contain fish.

We collected periphyton from each pond over a continuous 5-wk period beginning in May 2012, using floating periphyton samplers. Two samplers were placed in each pond at random locations, and samplers were held in place with a plastic-coated stake (1 cm diameter) pushed into the sediment by hand. Each sampler contained 8 glass slides on which periphyton could grow. Each week, all 8 glass slides were removed from each sampler and replaced, and the samplers were moved to new random locations in the ponds. After collection, slides covered in

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periphyton were frozen at –20 °C in the lab. Periphyton samples were scraped from slides and dried at 60 °C for 72 h. Periphyton collected from the 2 traps within a pond were pooled into a single composite sample. Periphyton samples were homogenized to a fine powder using a mortar and pestle or a ball-mill grinder.

Emergent aquatic insects were collected from each pond during a continuous 5-wk period beginning in May 2012. We used pyramid-shaped floating emergence traps to sample adult emerging insects (Supplemental Data, Figure S2). Each trap sampled an area of 0.53 m × 0.53 m (0.28 m^2). Four traps were deployed in each pond. Each trap was held in place with 2 plastic-coated stakes (1 cm-diameter) pushed into the sediment by hand. Traps were staked at random locations near each corner of the pond. The traps funneled the emerging insects (midges, microcaddisflies, and 27% of the damselflies) into a collecting bottle containing 85% nondenatured ethanol. All dragonflies and 73% of damselflies did not move into the sampling bottle and were captured by hand from the lower part of the traps, placed on ice, and then frozen at –20 °C in the laboratory. Collecting bottles were replaced, and traps were moved to new locations once each week.

Five taxonomic groups of insects accounted for most of the emergence in the present study. As in a previous study in the experimental ponds [11], the same insect taxa emerged from ponds with and without fish. Predatory taxa included dragonflies (Odonata: Anisoptera), damselflies (Odonata: Zygoptera), and predatory midges (Chironomidae: Tanypodinae). Herbivorous taxa included microcaddisflies (Trichoptera: Hydroptilidae) and herbivorous midges (Chironomidae: Chironominae and Chironomidae: Orthocladiinae). We collected an average of 1354 ± 298 (average ± standard error) individual emergent insects from each pond. Each taxa of insect was collected from 2 to 10 ponds. All individuals of each taxa collected from a given pond were counted and then pooled into a single composite sample for each taxa for each pond. All pooled samples were dried at 60 °C for 72 h. Whole bodies of insects were homogenized to a fine powder using a mortar and pestle or a ball-mill grinder.

Fish were collected in May and June 2012 using fyke nets set in 3 ponds for 24 h. We collected an average of 6.5 juvenile largemouth bass, 26.3 bluegill, and 15.5 hybrid sunfish with average total lengths of 8.2 ± 0.9 cm, 11.9 ± 0.4 cm, and 17.4 ± 0.2 cm, respectively, from each pond. Each species of fish was collected from 2 to 3 ponds. Immediately after capture, fish were euthanized in buffered MS-222 according to the manufacturer's guidelines, placed on ice in the field, and then frozen at –20 °C in the laboratory. Epaxial muscle samples were taken from individual fish and dried at 60 °C for 48 h. Muscle tissue was homogenized to a fine powder using a mortar and pestle or a ball-mill grinder. Equal weights of tissue from each individual were combined to create composites of each taxa.

Shoreline vegetation was collected on 6 June and 12 June 2012. We collected samples from those plant species that were most common along the shorelines of the study ponds, including Bermuda grass (*Cynodon dactylon*), lotus (*Nelumbo* sp.), paspalum (*Paspalum* sp.), smartweed (*Polygonum* sp.), and spikerush (*Eleocharis* sp.). Each taxon of plant was collected from 4 to 10 ponds. We also collected samples from several other species that were locally abundant and combined these samples into a terrestrial plant composite sample. Species included in the composite sample were: *Aristida purpurea, Bothriochloa ischaemum*, *Bromus catharticus*, *Bromus japonicus*, *Bromus secalinus*, *Bromus* sp., *Dichanthelium aciculare*, *Lolium perenne*, *Nelumbo* sp., *Paspalum* sp., *Schedonorus arundinaceus*, *Setaria pumila* and *Sorghum halepense*. Plant samples were collected near 2 corners of each pond and consisted of leaves and stems. Samples were dried at 60 °C for 72 h. Samples were homogenized to a fine powder using a mortar and pestle or a ball-mill grinder. Although some of the shoreline plant species collected in the present study were rooted in inundated soils, we classified them as terrestrial in our analyses because most of their biomass occurs outside of the water, and their leaves, stems, and flowers are directly used for food or habitat by terrestrial consumers.

We collected terrestrial spider and terrestrial insect samples from the shoreline vegetation surrounding each pond on 6 June and 12 June 2012. Species collected included long-jawed orb weaver spiders, ladybugs (Coccinellidae), grasshoppers (Caelifera), and leafhoppers (Cicadellidae). On each date, we collected sweep net samples from each pond. We collected an average of 20 ± 3 individual terrestrial insects and 91 ± 9 individual spiders from each pond. Each taxa of insect was collected from 5 to 10 ponds. Terrestrial insects were preserved in 85% nondenatured ethanol, separated by taxa, and dried at 60 °C for 72 h. Whole bodies of spiders and insects were homogenized to a fine powder using a mortar and pestle or a ball-mill grinder.

Total Hg (MeHg + inorganic Hg) analysis was conducted with a Milestone DMA-80 Direct Hg Analyzer, which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy [17]. Quality assurance included reference (National Research Council of Canada Institute for National Measurement Standards) and duplicate samples. Reference samples (DORM-3) were analyzed every 10 samples, and the average recovery percentage was 99.2% (range, 91.2–103%; $n = 17$). Duplicate samples were analyzed every 20 samples, and the average relative difference percentage was 1.64% (range, 0.25–3.08%; $n = 3$).

Analysis of MeHg was conducted at the Dartmouth College Trace Element Analysis Core Lab using a Brooks Rand MERX automated MeHg System interfaced with an Agilent 7500c inductively coupled plasma–mass spectrometer [18,19]. Samples of 2 certified reference materials (NIST Mussel 2976 and NIST Oyster 1566b) were analyzed for quality assurance. For NIST 2976, the average recovery percentages of MeHg and total Hg (determined as the sum of MeHg and inorganic Hg) were 92% (range, 85.7–103%; $n = 6$) and 103% (range, 83.9–128%; $n = 6$), respectively. For NIST Oyster 1566b, the average recovery percentages for MeHg and total Hg (determined as the sum of MeHg and inorganic Hg) were 114% (range, 88–106%; $n = 4$) and 93.9% (range, 101–123%; $n = 4$), respectively.

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 a few composite and individual samples of a given taxa 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. For periphyton and insect taxa, we determined the percentage of total Hg that was MeHg, as described previously. For shoreline plants, we determined the percentage of MeHg in a composite sample of several species of plants and used this value to estimate the percentage of MeHg in all plant taxa. We assumed that 100% of total Hg was MeHg in fish because Bloom [20] estimated that MeHg accounted for at least 95% of the total Hg in several species of fish, including largemouth bass, and the US Environmental Protection Agency [21] recommends

analyzing total Hg in fish tissues as a proxy for MeHg. All MeHg concentration data are presented as nanograms MeHg per gram of dry weight of plant or animal tissue.

Stable isotopes of nitrogen were used to estimate the relative trophic position of each organism. Stable nitrogen isotopes are used differentially in cellular processes [22], resulting in a predictable increase in the heavy isotope, ^{15}N, relative to ^{14}N with each increase in trophic level [23]. Tissue samples were analyzed at the University of California–Davis Stable Isotope Facility using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. Tank nitrogen gas calibrated with known standards was used as a working reference material in daily laboratory operation. Nitrogen isotope results are given as

$$\delta^{15}N = (R_{sample}/R_{standard-1}) \times 1000$$

where R is $^{15}N/^{14}N$. Standards for $\delta^{15}N$ were air N_2.

We used linear regression analysis to examine the relationships between log-transformed MeHg concentrations and $\delta^{15}N$ values for both aquatic and terrestrial taxa. We used analysis of covariance to compare the slopes of the relationship between MeHg concentrations and $\delta^{15}N$ values between the 2 habitats. A significant $\delta^{15}N \times$ habitat interaction indicates that the slopes of the relationship between MeHg concentrations and $\delta^{15}N$ values differ between habitats. Before log transformation, 1 was added to all MeHg concentrations to avoid negative log values. All analyses were completed using SPSS, and statistical significance was determined at $p < 0.05$.

RESULTS

The aquatic taxa exhibited a strong positive relationship between MeHg concentration and $\delta^{15}N$ values (Figure 1; linear regression, $p < 0.001$). As a primary producer, periphyton had the lowest MeHg concentration and $\delta^{15}N$ values. Low- and mid-level consumers, such as midges, microcaddisflies, damselflies, and dragonflies, had intermediate MeHg concentrations and $\delta^{15}N$ values. Top consumers, such as largemouth bass, bluegill, and hybrid sunfish, had the highest MeHg concentrations and $\delta^{15}N$ values.

The relationship between MeHg concentration and $\delta^{15}N$ values in the terrestrial taxa was not significant (Figure 1; Linear Regression, $P = 0.12$). Terrestrial taxa had lower MeHg concentrations than all aquatic consumers despite having a wide range of $\delta^{15}N$ values. The slope of the relationship between MeHg concentrations and $\delta^{15}N$ values in the terrestrial habitat was significantly different from the slope of the relationship in the aquatic habitat ($p < 0.001$ analysis of covariance, $F_{1,16} = 24.7$, $p < 0.001$).

The elevated MeHg concentrations and $\delta^{15}N$ values of the long-jawed orb weaver spider relative to other terrestrial organisms suggests that these spiders are connected to the aquatic food chain. Long-jawed orb weaver spiders were elevated above small emergent insects such as herbivorous midges by approximately 2‰ (Figure 1). In invertebrates, $\delta^{15}N$ is predicted to increase by 2.2‰ with each trophic level [22].

DISCUSSION

In the present study, shoreline long-jawed orb weaver spiders were connected to the aquatic food chain, as has been found in previous studies [12,16,24–27]. Furthermore, our data suggest that long-jawed orb weaver spiders in the present study were exposed to MeHg by consuming emergent aquatic insects. Corroborating our isotope and Hg data, long-jawed orb weaver

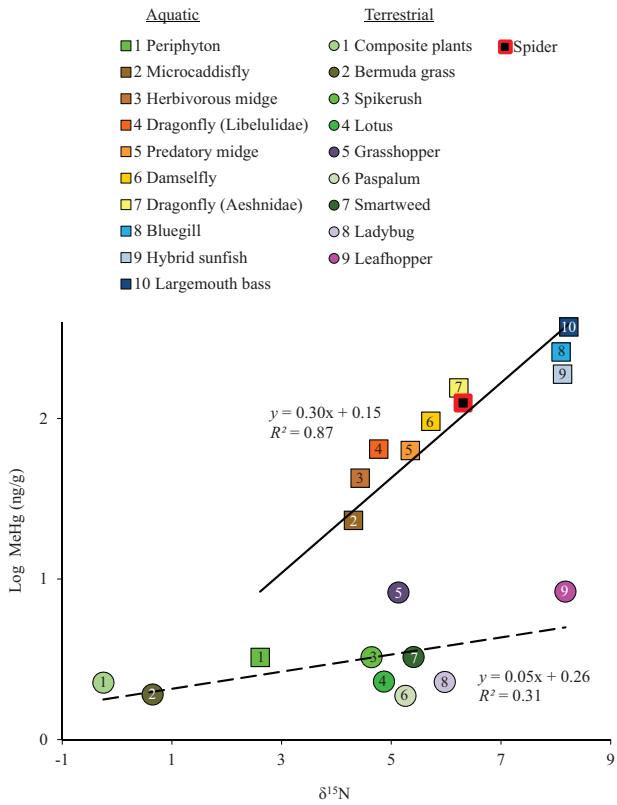

Figure 1. Relationship between log-transformed methyl mercury (MeHg) and nitrogen stable isotope values for aquatic and terrestrial taxa. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

webs along the shores of the ponds were observed to contain small emergent aquatic insects (Figure 2). Our findings are consistent with a previous study [11] that found a strong correlation between small emergent insect MeHg flux (calculated as biomass of emerging chironomids and microcaddisflies $\times$ their MeHg concentration) and MeHg concentrations in long-jawed orb weaver spiders. Given the low levels of MeHg in the terrestrial food chain, the terrestrial food chain does not appear to

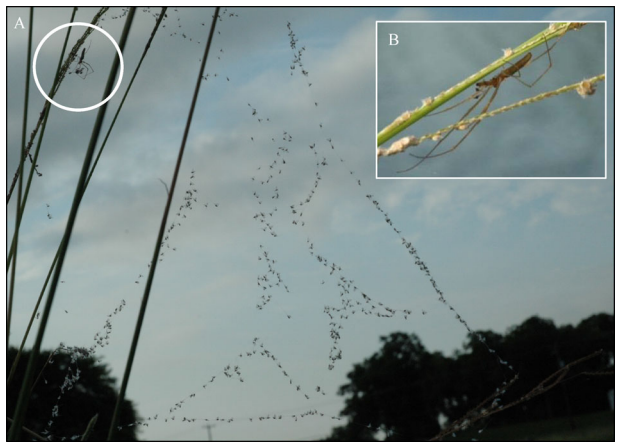

Figure 2. Pictures of long-jawed orb weaver spiders and webs taken during the present study. (**A**) Small emergent aquatic insects were frequently observed in the webs of long-jawed orb weavers along the shorelines of the study ponds. A long-jawed orb weaver is circled in the top left corner. (**B**) Close-up picture of long-jawed orb weaver. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

serve as a source of MeHg to long-jawed orb weaver spiders in these pond ecosystems. It is possible that other taxa of spiders, or long-jawed orb weaver spiders in other ecosystems, could become contaminated with MeHg from the floodplain as hypothesized by Cristol et al. [9], and this possibility should be explored in future studies.

The positive relationship between MeHg concentrations and $\delta^{15}N$ values in the aquatic taxa indicates that biomagnification of MeHg is occurring in the aquatic food chain [28]. The slope of the relationship between MeHg concentrations and $\delta^{15}N$ values, called the trophic magnification slope, is a measure of biomagnification, with larger values representing a larger increase in tissue Hg concentration with trophic position relative to lower values [28]. The trophic magnification slope observed in the present study (0.30) falls within the average range of trophic magnification slope values (0.24 ± 0.08) found in a review by Lavoie et al. [28]. In comparison, the weak positive relationship between MeHg concentrations and $\delta^{15}N$ values in the terrestrial taxa indicates that biomagnification of MeHg is not occurring in the terrestrial food chain. This is likely because terrestrial vegetation contains low concentrations of inorganic Hg, which typically does not biomagnify, and low concentrations of MeHg; therefore, organisms feeding in the terrestrial food chain would be expected to have low concentrations of both total Hg and MeHg [7].

How adjacent aquatic and terrestrial ecosystems are linked is a fundamental question in ecology [12,29]. In the past, $\delta^{15}N$ and $\delta^{13}C$ have been used as complementary tracers to explore the linkages between aquatic and terrestrial ecosystems. However, the utility of $\delta^{15}N$ and $\delta^{13}C$ is limited because in some systems carbon isotope ratios in terrestrial vegetation and periphyton are not sufficiently different to distinguish between these 2 sources [30]. In the present study, MeHg concentrations plotted as a function of $\delta^{15}N$ values produced different patterns in aquatic and terrestrial food webs. This finding suggests that MeHg and $\delta^{15}N$ isotope ratios can be used to distinguish between these 2 food chains, providing ecologists with another tool to examine and understand the fundamental interactions between aquatic and terrestrial ecosystems.

SUPPLEMENTAL DATA

Tables S1.
Figures S1–S2. (225 KB PDF).

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