Effects of Fish on Emergent Insect-Mediated Flux of Methyl Mercury across a Gradient of Contamination

Brent N. Tweedy,† Ray W. Drenner,‡ Matthew M. Chumchal,*† and James H. Kennedy‡

†Department of Biology, Texas Christian University, Winton Scott Room 401, 2800 South University Drive, Fort Worth, Texas 76129, United States
‡Department of Biology, University of North Texas, 1155 Union Circle No. 310559, Denton, Texas 76203, United States

ABSTRACT: We examined the effects of fish predation on emergent insect-mediated methyl mercury (MeHg) flux across a gradient of MeHg contamination in experimental ponds. Emergent insects were collected from ponds with (n = 5) and without fish (n = 5) over a six-week period using floating emergence traps. We found that the potential for MeHg flux increased with Hg contamination levels of the ponds but that the realized MeHg flux of individual insect taxa was determined by fish presence. Fish acted as size-selective predators and reduced MeHg flux by suppressing emergence of large insect taxa (dragonflies and damselflies) but not small insect taxa (chironomids and microcaddisflies). MeHg flux by small insect taxa was correlated with concentrations of MeHg in terrestrial spiders along the shorelines of the study ponds, demonstrating for the first time the cross-system transport of MeHg by emergent insects to a terrestrial spider.

INTRODUCTION

The fate and transfer of the methylated form of Hg (MeHg) in the environment is of particular concern to ecotoxicologists because MeHg readily bioaccumulates in the tissues of biota and is extremely toxic, negatively affecting the health of wildlife.1 Worldwide anthropogenic emissions of inorganic forms of Hg coupled with a dynamic, global atmospheric cycle have resulted in contamination of most ecosystems on Earth with levels of Hg that exceed preindustrial baselines.2 Because the conversion of inorganic forms of Hg to MeHg is a process primarily mediated by aquatic microbes,3 MeHg was previously thought to only threaten aquatic biota and consumers of aquatic organisms. However, recent studies have found elevated concentrations of MeHg in terrestrial consumers.3−6

Contaminants, such as MeHg, that enter aquatic food webs have the potential to be transferred to terrestrial food webs when aquatic insects that spend part of their life cycle in aquatic ecosystems emerge as adults.6−10 Although it is recognized that emergent aquatic insects provide critical energy subsidies to terrestrial food webs adjacent to aquatic systems,11−16 their role as biovectors of aquatic contaminants to terrestrial ecosystems is just beginning to be understood.16−19 Factors that control the magnitude of insect-mediated MeHg flux have rarely been studied,18 and the relationship between insect-mediated MeHg flux and MeHg concentration in terrestrial predators has not been examined.

In this study, we assess insect-mediated MeHg flux across a gradient of Hg contamination and determine how MeHg flux is affected by fish predation. We hypothesized that fish predation can reduce insect-mediated contaminant flux out of aquatic ecosystems by reducing aquatic insect biomass and altering insect community structure.18−23 Here, we use experimental pond ecosystems to test two hypotheses: (H1) the potential for insect-mediated MeHg flux from waterbodies is positively related to the overall level of food web contamination, but the realized MeHg flux is regulated by fish predation on emergent aquatic insects; and (H2) the MeHg flux of emerging insects is correlated with MeHg concentrations in terrestrial long-jawed orb weaver spiders (Tetragnathidae: Tetragnatha sp.). Spiders that live on the shorelines of aquatic ecosystems are predators of aquatic insects7−9,24−27 and play a key role in mediating contaminant flux from aquatic to terrestrial ecosystems because they are themselves consumed by terrestrial predators such as birds.3,8

METHODS

Study Site. We conducted the present study in 10 experimental ponds in Fort Worth, Texas, USA. The experimental ponds are whole ecosystems with earthen bottoms that contain complex communities of macrophytes, benthic invertebrates, and herptiles. Ponds are large and range in size from 0.23 to 0.54 ha with maximum and average depths of 1.2 and 0.6 m, respectively. Macrophyte communities were variable between ponds and were composed of several species of emergent and submerged taxa. An image from the
To examine the relationship between MeHg contamination in a terrestrial consumer to MeHg flux from the ponds, we collected long-jawed orb weaver spiders along the shorelines of the ponds. Long-jawed orb weavers are obligate shoreline species that specialize in consumption of aquatic insects.27 At our study site, we frequently observed webs of long-jawed orb weavers containing small emergent insects. We collected spiders on June 2 and June 16 from the emergent vegetation along the edge of the ponds using sweep nets. On each date, two samples were collected from two corners of each pond that yielded 99.5 ± 36.6 (average ± SE) long-jawed orb weavers per pond. Spiders were preserved in 85% denatured ethanol, pooled by pond, and dried at 60 °C for 72 h.

**MeHg Analysis.** Prior to MeHg analysis, emergent insects and spider composite samples were homogenized to a fine powder using a clean mortar and pestle or ball-mill grinder. A single subsample from each composite sample was analyzed for MeHg at the Dartmouth College Trace Element Analysis Core Lab using a MERX automated MeHg system (Brooks Rand, Seattle, WA) interfaced with an Agilent 7500c inductively coupled plasma—mass spectrometer.35,36 Samples of a certified reference material (NIST Musel 2976) were within certified ranges. A detailed description of MeHg analytical methods and QA/QC procedures is available in the Supporting Information. All MeHg concentration data is presented as nanograms per gram of dry weight. We estimated MeHg flux for each taxa over the course of the experiment by multiplying the total emergent biomass of each taxa by the concentration of MeHg in their tissues.

We used MeHg concentrations in herbivorous chironomids as a proxy for baseline MeHg contamination of the food web. MeHg concentrations in emergent diptera have been shown previously to be indicative of the level of ecosystem contamination.37 In this study, MeHg concentrations in herbivorous chironomids were positively correlated with MeHg in other insect taxa (Figure S3, Supporting Information), confirming that herbivorous chironomids are appropriate indicators of baseline food web contamination by MeHg.

**Statistical Analysis.** We used a series of analysis of covariance (ANCOVA; SPSS, Ver 20.0.0) models to determine the effect of MeHg concentrations in herbivorous chironomids (a proxy for overall level of MeHg contamination of the food web) (covariate) and fish presence (categorical variable) on total and taxa-specific MeHg flux (dependent variable). We first tested for an interaction effect between MeHg concentration in herbivorous chironomids and fish. If the interaction was not significant (an indication of homogeneous slopes and an assumption of ANCOVA), then the term was removed from the model to assess main effects of fish presence and MeHg concentration in herbivorous chironomids. If the interaction term was significant (indicating heterogenous slopes), then we tested for an effect of fish at the average of the covariate and used this statistic to assess the effect of fish on the dependent variable. We used ANCOVA (using the same procedure as above) and linear regression to examine the relationship between MeHg concentrations in long-jawed orb weavers, fish presence and absence, and emergent insect MeHg flux. Data met assumptions of normality and homogeneity of variance. Statistical significance for all analyses was inferred at P < 0.05.

**RESULTS**

Total MeHg flux, computed as the combined MeHg flux for the five insect taxa examined in this study, was positively and
significantly related to Hg concentrations in herbivorous chironomids with the total MeHg flux significantly suppressed in ponds with fish (Figure 1A). For each insect taxa except microcaddisflies, MeHg flux was positively and significantly related to MeHg concentrations in herbivorous chironomids (Figure 1B–E). MeHg flux of large taxa, dragonflies and damselflies, was suppressed in ponds with fish (Figure 1B,C). The MeHg flux of small taxa, herbivorous and predatory chironomids and microcaddisflies, was not significantly affected by fish presence (Figure 1D–F). These results indicate that the reduction of total MeHg flux in ponds with fish can be explained by the reduction of flux from large taxa, dragonflies and damselflies. The effect of fish on MeHg flux was achieved through the suppression of emergent biomass of dragonflies and damselflies and not alteration of MeHg concentrations (Figures S4 and S5, Supporting Information).

The difference in total MeHg flux between ponds with and without fish was greatest in ponds with high MeHg concentrations in herbivorous chironomids (Figure 1A). This pattern in total MeHg flux mirrors the pattern in dragonflies because dragonflies accounted for the largest component of MeHg flux in ponds with and without fish (73 ± 13% and 42 ± 18% (average ± SE), respectively; Figure 1B). Damselflies exhibited a similar but less pronounced pattern. However, damselflies only accounted for 12.6 ± 9.5% and 17.1 ± 10.2% of the total MeHg flux in ponds with and without fish, respectively (Figure 1C). Small insects, which were not impacted by fish, did not exhibit the pattern of diverging slopes across the MeHg contamination gradient observed in the larger taxa (Figure 1D–F). For total insect and dragonfly MeHg flux, but not damselfly MeHg flux, the MeHg concentration in herbivorous chironomids × fish interactions were almost significantly different between ponds with and without fish across the MeHg gradient (Figure 1B). These marginally significant interactions should be interpreted with caution but ignoring them risks a type II error because of the small number of ponds in the study and the limited statistical power to detect interaction effects.38

MeHg contamination of long-jawed orb weavers collected along the shorelines of the ponds was significantly and positively correlated with the MeHg flux of small insect taxa (the sum of predatory and herbivorous chironomids and microcaddisflies) but was not significantly affected by fish (ANCOVA; $F_{1,7} = 2.2; P = 0.18$; Figure 2). This relationship presumably results from spider predation on small emergent insect taxa and is consistent with our observation of small insects trapped in the webs of long-jawed orb weavers along the shorelines of the study ponds. MeHg concentrations of long-jawed orb weavers were not significantly correlated with the MeHg flux of large insect taxa (dragonflies and damselflies) (Figure S6, Supporting Information), and we never observed these large taxa trapped in spider webs.

Figure 1. Relationship between herbivorous chironomid MeHg concentration (a proxy for baseline MeHg concentration) and MeHg flux for (A) all taxa, (B) dragonflies, (C) damselflies, (D) herbivorous chironomids, (E) predatory chironomids, and (F) microcaddisflies in ponds with and without fish. Solid lines with blue squares represent ponds with fish (+ Fish) and dashed lines with yellow circles represent ponds without fish (− Fish). $P$ values from an ANCOVA testing for the main effects of chironomid MeHg concentration ([MeHg]) and fish presence (fish) and their interaction (F × Hg) on MeHg flux are included in each panel. The assumption of heterogeneous slopes was violated in the case of herbivorous chironomids; therefore, we report the treatment effect of fish at the average of the covariate. Complete ANCOVA statistics ($P$ values, $F$ statistics, and degrees of freedom) are reported in Table S3 in the Supporting Information. Note the scale of the $y$ axis differs for each taxa.
Our study indicates that the level of MeHg contamination of the food chain sets the potential for MeHg flux in emerging aquatic insects. In most aquatic ecosystems, the source of MeHg is inorganic Hg deposited from the atmosphere directly on the water body or its watershed and then converted to MeHg by microorganisms. Previous studies have shown a relationship between atmospheric deposition and Hg contamination of biota.  

Therefore, potential MeHg flux in aquatic insects would be expected to be correlated with atmospheric deposition of Hg. Mercury deposition and the conversion of inorganic Hg to MeHg vary widely across the landscape due to complex interactions between regional Hg emissions, atmospheric processes, and landcover that make some ecosystems more sensitive to Hg inputs. Therefore, we would expect considerable variation in MeHg flux in aquatic insects and the transfer of MeHg to terrestrial food chains across large geographic areas.

In this study, we also show that, for a given level of MeHg contamination, the quantitative and qualitative nature of the flux of MeHg in aquatic insects can be regulated by the presence and absence of fish. Compared to ponds without fish, ponds with fish had lower MeHg flux from large-bodied insects such as odonates, which are vulnerable to fish predation, while fish did not affect MeHg flux from smaller-bodied insects such as chironomids, which are less vulnerable to predation.

The net effect of fish was a reduction in total MeHg flux of up to a factor of 5 in the most Hg-contaminated ponds. Therefore, we hypothesize that a combination of fish predation and prey vulnerability determines the realized MeHg flux across a gradient of MeHg contamination (Figure 3). Aquatic insect taxa differ in their vulnerability to fish predation. The MeHg flux for invulnerable prey will be the same in ponds with and without fish. The MeHg flux for vulnerable prey will be suppressed by fish, and the magnitude of suppression will be greatest in ponds with high levels of MeHg contamination.

Our study is one of the first linking MeHg flux in emerging insects to a terrestrial spider, the long-jawed orb weaver. We found MeHg concentrations in long-jawed orb weavers were highly correlated with the MeHg flux of small insect taxa. Spider MeHg concentrations were not correlated with the MeHg flux in large insect taxa. Fish presence had no effect on the spider Hg contamination because fish primarily impact large insect taxa (such as dragonflies and damselflies) not consumed by long-jawed orb weavers. These results support the concept that contaminant monitoring programs could use shoreline spiders as biological monitors of aquatic pollution. Long-jawed orb weavers could be used as biosentinels of MeHg concentrations in small emergent insect taxa and thus an indicator of the overall level of food web contamination.

In conclusion, this study demonstrates the combined influence of aquatic pollution and community structure on the cross-system transport of contaminants out of ponds to terrestrial consumers. Small (<1 km²) ponds and lakes, where the processes examined in this study are likely to be important, represent the numerically dominant lentic ecosystem covering more surface area than large lakes worldwide. The species richness of aquatic birds, plants, amphibians, and invertebrates is often relatively high in small, shallow, isolated lakes. However, the benefits to biodiversity provided by small ponds and lakes may be partially offset by the production and transport of MeHg to surrounding terrestrial food chains and wildlife. Although some small ponds are semipermanent and are naturally colonized or stocked with fish, others periodically dry or experience winter oxygen stress and do not contain fish. Compared to ponds with fish, ponds without fish will...
have a greater total MeHg flux, and odonates will play a greater role as a biovector of Hg to terrestrial consumers. More work is needed to determine the magnitude of insect-mediated MeHg flux from systems with and without fish and how the effects of fish on MeHg flux vary with fish species. Given the prominence of small ponds on the landscape and the widespread nature of atmospheric Hg deposition, the impact of small ponds on terrestrial Hg cycling is probably quite significant.

ASSOCIATED CONTENT

Supporting Information
Additioanl information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

*E-mail: m.m.chumchal@tcu.edu.

Notes
The authors declare no competing financial interest.

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REFERENCES


