

Seasonality of Dipteran-Mediated Methylmercury Flux from Ponds

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Abstract: Methylmercury (MeHg) is an aquatic contaminant that can be transferred to terrestrial predators by emergent aquatic insects. We assessed the effects of month and pond permanence on dipteran-mediated MeHg flux (calculated as emergent dipteran biomass \times dipteran MeHg concentration) in 10 experimental ponds. Emergent dipterans were collected weekly from permanent ponds with bluegill (*Lepomis macrochirus*; $n = 5$) and semipermanent ponds without fish ($n = 5$) over a 7-mo period (February–August, 2015). We detected a significant effect of month on MeHg flux from 6 dipteran taxa and aggregate MeHg flux, with the highest MeHg flux from herbivorous/detritivorous chironomid midges and predatory midges in March; biting midges, phantom midges and herbivorous/detritivorous orthoclad midges in April; and mosquitoes in August. Aggregate dipteran-mediated MeHg flux peaked in April and then declined throughout the remainder of the summer. We did not detect a significant main effect of pond permanence or a significant month \times pond permanence interaction effect on MeHg flux for any of the taxa examined in the present study or for aggregate MeHg flux. Given their ubiquity in aquatic systems and their importance in food webs at the land–water interface, dipterans are important taxa that should not be overlooked as a part of the Hg cycle. *Environ Toxicol Chem* 2018;37:1846–1851. © 2018 SETAC

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INTRODUCTION

All aquatic ecosystems are contaminated with toxic methylmercury (MeHg) due to the global cycling of mercury (Hg). Inorganic and elemental forms of Hg released from natural and anthropogenic emission sources are deposited near their source or enter the global atmospheric pool and are eventually deposited on the landscape (Selin 2009). In aquatic systems, Hg is converted to MeHg by sulfate- and iron-reducing bacteria (Selin 2009). Methylmercury concentrates in the tissues of aquatic primary producers at the base of the food web (Miles et al. 2001; Pickhardt and Fisher 2007) and aquatic consumers are exposed to MeHg through their diet (Hall et al. 1997; Tsui and Wang 2004; Pickhardt et al. 2006). Methylmercury is transported to terrestrial ecosystems by MeHg-contaminated insects emerging from aquatic ecosystems (referred to as insect-mediated MeHg flux; Chumchal and Drenner 2015). Terrestrial consumers, like songbirds, bats, and spiders are exposed to

MeHg by consuming MeHg-contaminated emergent insects (Gerrard and St. Louis 2001; Tweedy et al. 2013; Gann et al. 2015; Schulwitz et al. 2015; Korstian et al. 2018; Williams et al. 2017; Becker et al. 2018). Previous research has suggested that insect-mediated MeHg flux can be high enough to pose a health risk to songbirds (Williams et al. 2017).

Human-made ponds can be important sources of insect-mediated MeHg flux to terrestrial food webs (Chumchal and Drenner 2015). Human-made ponds are the numerically dominant lentic ecosystem type in agricultural regions such as the Great Plains of the United States where ponds were constructed to provide water for livestock, erosion control, and recreation (Dendy 1963; Renwick et al. 2005). Over the past 100 yr, millions of ponds have been constructed in the Great Plains (Smith et al. 2002; Renwick et al. 2005, 2006; McDonald et al. 2012). In the southern Great Plains, ponds ≤ 1 ha are found at an average density of 2.5 ponds per km² (Chumchal et al. 2016). These ponds exist across a hydrology gradient from temporary ponds that hold water for weeks, to semipermanent ponds that hold water for months, to permanent ponds that continually hold water (Chumchal and Drenner 2015). Fish populations can only become established in permanent ponds (Chumchal and Drenner 2015). Like other water bodies,

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human-made ponds and their food webs are contaminated with Hg deposited from the atmosphere (Blackwell and Drenner 2009; Henderson et al. 2012). The construction of ponds in the Great Plains has unintentionally created millions of MeHg-producing aquatic ecosystems that contaminate not only aquatic consumers but also emergent aquatic insects and the terrestrial consumers that feed on them (Chumchal and Drenner 2015).

Insect-mediated MeHg flux from ponds can be regulated by season and pond permanence (Chumchal et al. 2017; Williams et al. 2017). Because insect development and emergence is affected primarily by temperature (Corbet 1999), strong seasonality in insect-mediated MeHg flux from ponds in the temperate zone would be expected, with low insect-mediated MeHg flux in winter and high MeHg flux in spring and summer (Williams et al. 2017). The taxonomic composition of insect emergence can be determined by pond permanence and the presence of fish (Tweedy et al. 2013; Chumchal et al. 2017; Williams et al. 2017). In semipermanent ponds without fish, both small midges (i.e., dipterans) and large dragonflies and damselflies (i.e., odonates) emerge, but the insect-mediated MeHg flux is dominated by odonates because of their large size and high MeHg concentration (Tweedy et al. 2013; Chumchal and Drenner 2015). In permanent ponds, the presence of fish results in suppression of odonates but not dipterans (Tweedy et al. 2013; Chumchal and Drenner 2015). Thus, in permanent ponds insect-mediated MeHg flux from small insects can be on the same scale as that from odonates. Although the effects of season and pond permanence on odonates has been recently studied (Williams et al. 2017), we have little information about the effects of season and pond permanence on small taxa such as dipterans. In the present study, we examined the monthly pattern of MeHg flux from dipterans in permanent ponds with fish and semipermanent ponds without fish.

METHODS

Experimental setup

We conducted the present study in 10 experimental ponds at the Eagle Mountain Fish Hatchery (32°52'32.95"N, 97°28'29.00"W) near Fort Worth, Texas, USA. Previous studies revealed that the ponds had food chains contaminated with Hg (Tweedy et al. 2013; Speir et al. 2014). The source of Hg to the ponds is atmospheric deposition to the pond surfaces and the watershed of a nearby drinking water supply reservoir, Eagle Mountain Lake, which is the source of water to the ponds. Ponds range in size from 0.23 to 0.54 ha and have an average depth of 0.8 m. The experimental ponds are whole ecosystems with earthen bottoms that contain complex communities of macrophytes, benthic invertebrates, reptiles, and amphibians. Macrophytes were dense in each pond but communities were variable among ponds and were composed of several species of emergent and submerged taxa including coontail (*Ceratophyllum demersum*), bushy pondweed (*Najas guadalupensis*), American lotus (*Nelumbo lutea*), paspalum (*Paspalum* spp.),

longleaf pondweed (*Potamogeton nodosus*), and cattail (*Typha* spp.).

In spring 2013, ponds were filled with water and stocked with bluegill (*Lepomis macrochirus*) purchased from a commercial fish hatchery (Table 1). Visual observation confirmed that bluegill had spawned in the ponds in the summers of 2013, 2014, and 2015. Bluegill are commonly present in warm-water fish communities throughout the United States (Lee et al. 1980) and feed on benthic insects as well as other prey (Mittelbach 1988).

On 1 April 2014, 5 of the 10 ponds were drained and fish removed to simulate drying disturbance (Table 1). Prior to refilling, we visually confirmed that the bottoms of the ponds were completely dry. The 5 dried ponds were refilled with water on 13 May 2014 to simulate semipermanent ponds without fish (Table 1). In this region, semipermanent ponds typically refill in May when precipitation is highest. The 5 ponds that were not drained simulated permanent ponds with insectivorous fish (Table 1).

The experiment of which the present study is a part involved 2 phases (Table 1). Phase 1 was conducted in 2014 and focused on recovery of insect-mediated MeHg flux immediately after the drying disturbance and refilling of semipermanent ponds (Chumchal et al. 2017). After the drying disturbance and refilling of semipermanent ponds, aquatic insect communities were reestablished by recruitment of larval insect populations from eggs deposited by adults that had migrated from other water bodies (Chumchal et al. 2017). Chumchal et al. (2017) found that within 1 mo after refilling semipermanent ponds, all 11 of the insect taxa emerging from the permanent ponds also began emerging from the semipermanent ponds, and total MeHg flux did not differ among the pond types. They concluded that insect-mediated MeHg flux can rapidly recover in ponds that have dried and refilled, especially in warm climates and in areas with nearby sources of adult insects to recolonize the ponds.

TABLE 1: Timeline showing dates of experimental setup and sampling

Date	Event
March 2013	All ponds filled with water and stocked with bluegill.
April 2014	5 of 10 ponds drained to simulate drying disturbance.
May 2014	5 drained ponds refilled to represent semipermanent ponds. 5 ponds that were not drained represent permanent ponds.
May–August 2014	Phase 1 of the experiment designed to assess the effect of drying disturbance on insect-mediated MeHg flux. Results published in Chumchal et al. (2017).
February 2015	Phase 2 of the experiment designed to assess seasonality of odonate- (Williams et al. 2017) and dipteran-mediated MeHg flux (the present study) begins.
February–August 2015	Collection of temperature and dipteran emergence data.

The present study is phase 2 of the experiment in which we examined the seasonality of insect-mediated MeHg flux from permanent fish ponds and semipermanent fishless ponds from winter through summer 2015 (Table 1). We have previously published a paper examining the seasonality of odonate-mediated MeHg flux from that experiment (Williams et al. 2017). The present study describes the seasonality of dipteran-mediated MeHg flux. We monitored temperature and dipteran emergence from each of the 10 ponds beginning 8 mo after semipermanent ponds were refilled (Table 1). Daily mean water temperatures were collected using temperature loggers (Onset Computer Corporation) staked near the maximum depth of each pond from 1 February to 31 August 2015. Dipteran emergence was monitored using pyramid-shaped floating emergence traps (0.53 m × 0.53 m [0.28 m²]; Supplemental Data, Figure S1) from 10 February to 24 August 2015. 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. Traps funneled emerging dipterans into a collecting bottle containing 95% ethanol. Collecting bottles were replaced every 3 to 10 d, resulting in 30 sampling periods over the 7-mo experiment. During 4 of the 30 sampling periods, 1 to 3 traps were damaged by weather or animals. Damaged traps were repaired or replaced and data from traps were excluded for the sampling period in which the damage occurred.

All individual dipterans collected from a given pond were identified and counted. Six taxonomic groups of dipterans were captured in adequate numbers for analyses: herbivorous/detritivorous chironomid midges (Diptera: Chironomidae: Chironominae), herbivorous/detritivorous orthoclad midges (Diptera: Chironomidae: Orthocladiinae) predatory midges (Diptera: Chironomidae: Tanytopodinae), phantom midges (Diptera: Chaoboridae), mosquitoes (Diptera: Culicidae), and biting midges (Diptera: Ceratopogonidae). We collected an average of 4233 ± 1009 (average \pm standard error) individual dipterans from each pond. All individual dipterans collected over the course of the 7-mo experiment were pooled by taxa within ponds, dried for at least 48 h at 60 °C, and weighed. We estimated emergent biomass on each sampling date by multiplying the weight of individual dipterans (Supplemental Data, Table S1) by the number of individuals emerging during the sampling period. The average weights of adult dipterans were determined from newly emerged adults captured in floating-emergence traps during phase 1 of the experiment (Chumchal et al. 2017).

In the present study, we define dipteran-mediated MeHg flux as the quantity of MeHg transported from aquatic to terrestrial ecosystems by adult emergent dipterans. Dipteran-mediated MeHg flux is the product of emerging dipteran biomass and MeHg concentrations of adult dipterans. We calculated MeHg flux by multiplying emergent biomass by MeHg concentration (Supplemental Data, Table S1). The average MeHg concentration of adult dipterans was determined from newly emerged adults captured in floating-emergence traps during phase 1 of the experiment (Chumchal et al. 2017). Methylmercury flux is expressed as ng MeHg/m²/d.

Statistical analysis

Statistical analysis was performed with SPSS (Ver 22). Data collected on a daily (temperature) or approximately weekly (MeHg flux) basis were averaged and expressed on a monthly basis. We used a repeated measures analysis of variance to examine both the main and interaction effects of month and pond permanence on response variables. Main effects are the independent impact of each treatment factor (month or pond permanence), and the interaction effect is the amount of measured variation in the response variables (dipteran-mediated MeHg flux) due to the interdependence between month and pond permanence. Because of low replication and statistical power, a probability level of $\alpha \leq 0.10$ was chosen to reduce the chance of making a type II error. Type II errors (accepting a false null hypothesis) can be controlled and power can be increased by increasing alpha (Keppel and Wickens 2004). In the present study, we reduced the chance of making a type II error and increased power by increasing alpha to $p \leq 0.10$ as we have done in previous pond experiments (Drenner et al. 1998; Chumchal et al. 2005; Tweedy et al. 2013; Williams et al. 2017).

RESULTS AND DISCUSSION

Temperature increased over the course of the experiment (Figure 1a). We detected a significant effect of month on temperature (Figure 1a; Supplemental Data, Table S2) but temperature was not significantly affected by pond permanence (Figure 1a; Supplemental Data, Table S2). We did not detect a significant month \times pond permanence interaction effect on temperature (Figure 1a; Supplemental Data, Table S2).

We found strong effects of month on dipteran-mediated MeHg flux but no effects of permanence (Figure 1b–h). When we began monitoring dipteran emergence in February, we detected low levels of MeHg flux from 5 of 6 taxa (herbivorous/detritivorous chironomid midges, predatory midges, biting midges, phantom midges, herbivorous/detritivorous orthoclad midges) and no MeHg flux from mosquitoes (Figure 1b–g). We detected a significant effect of month on MeHg flux from all dipteran taxa and aggregate MeHg flux (Figure 1b–h; Supplemental Data, Table S2) with the highest MeHg flux from herbivorous/detritivorous chironomid midges and predatory midges in March (Figure 1b and c); biting midges, phantom midges and herbivorous/detritivorous orthoclad midges in April (Figure 1d–f); and mosquitoes in August (Figure 1g). Aggregate dipteran-mediated MeHg flux peaked in April and then declined throughout the remainder of the summer (Figure 1h). Herbivorous/detritivorous chironomid midges and phantom midges comprised the largest proportion of aggregate flux. Herbivorous/detritivorous orthoclad midges and predatory midges comprised an intermediate proportion of aggregate flux. Biting midges and mosquitoes comprised the smallest proportion of aggregate flux. We did not detect a significant main effect of pond permanence or a significant month \times pond permanence interaction effect on MeHg flux for any of the dipteran taxa examined in the present study or for aggregate MeHg flux (Figure 1; Supplemental Data, Table S2).

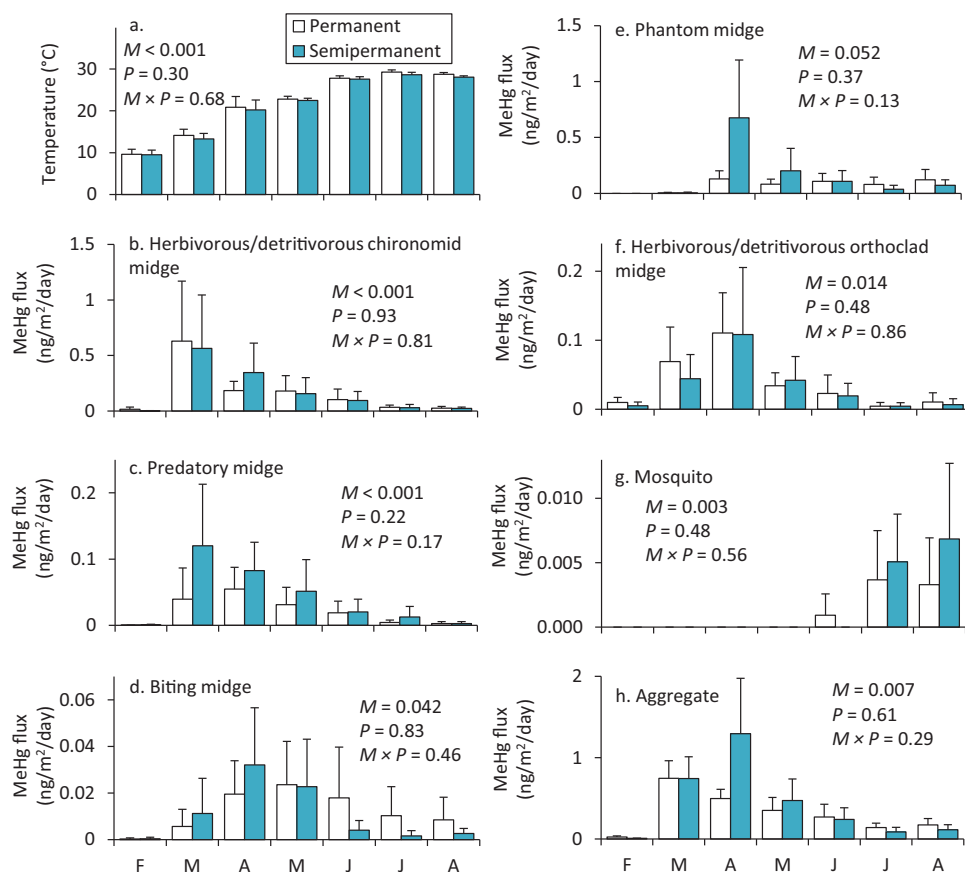


FIGURE 1: (a) Average (\pm standard error) monthly water temperature, and dipteran-mediated MeHg flux in permanent and semipermanent ponds for (b) herbivorous/detritivorous chironomid midge, (c) predatory midge, (d) biting midge, (e) phantom midge, (f) herbivorous/detritivorous orthoclad midge, (g) mosquito, and (h) aggregate. Shown on each panel are p values from repeated measure analyses of variance examining the main effects of month (M), pond permanence (P) and the month \times pond permanence interaction (M \times P). Complete statistical information is presented in Supplemental Data, Table S2. Note that the scale of the y-axis differs among panels.

Monthly patterns of dipteran-mediated MeHg flux corresponded to changes in emerging dipteran biomass over time (Supplemental Data, Figure S2). As was observed in a previous study focused on odonate-mediated MeHg flux (Williams et al. 2017), the pattern of dipteran emergence suggests that temperature is not the only factor controlling dipteran emergence and MeHg flux because temperature remains elevated in the late summer (Figure 1a) while emerging dipteran biomass (Supplemental Data, Figure S2) and MeHg flux decline (Figure 1b–f). Other environmental factors, such as oxygen levels, photoperiod, food availability, and food type, affect the rate of insect development and timing of dipteran emergence (Oliver 1971; Pinder 1986).

In the present study, MeHg flux from dipterans was not affected by pond permanence and the presence of fish. Several nonmutually exclusive hypotheses may explain this lack of effect. First, bluegill are size selective predators (O'Brien et al. 1976) that may have selected large-bodied prey over the small-bodied dipterans that we examined in the present study, resulting in no significant differences in dipteran-mediated MeHg flux between treatments. Second, the presence of dense vegetation in the ponds may have enhanced the secondary production of dipterans (Balci and Kennedy 2002) and thereby offset the

negative effects of fish predation, or the vegetation may have acted as a refuge, allowing dipterans to avoid fish predation (Diehl 1992; Batzer and Wissinger 1996). Indeed, Jones et al. (2013) found that in 500-L mesocosms with limited macrophyte growth, a congener of bluegill, green sunfish (*L. cyanellus*), was capable of suppressing dipteran-mediated MeHg flux. Third, dipteran emergence may have been suppressed by fish predation in the permanent ponds and by invertebrate predators (e.g., odonates) in fishless semipermanent ponds (Williams et al. 2017) resulting in a lack of difference in MeHg flux among treatments.

In conclusion, dipterans are present in all waterbodies and account for a substantial proportion of the MeHg flux from permanent ponds with fish (Tweedy et al. 2013). Schiesari et al. (2017) concluded that dipterans have a number of traits that make them keystone in the removal and export of contaminants from polluted freshwater ecosystems, including obligatory emergence of adults, high potential secondary productivity, high demographic responsiveness to eutrophication, tolerance to hypoxia, and tolerance to environmental contamination. Dipterans are consumed by a wide variety of terrestrial predators, such as spiders, bats, and songbirds (Busby and Sealy 1979; Gerrard and St. Louis 2001; Tweedy

et al. 2013; Gann et al. 2015; Schulwitz et al. 2015; Becker et al. 2018; Korstian et al. 2018). Songbirds can be exposed to Hg directly by consuming emergent dipterans or indirectly by consuming spiders that feed on dipterans. Because of their elevated trophic position, MeHg concentrations in shoreline spiders can be 2 to 5 times greater than MeHg concentrations in dipterans (Tweedy et al. 2013; Speir et al. 2014) and can pose a risk to nestling songbirds (Gann et al. 2015). In the present study, peak MeHg flux from most dipterans occurred in the spring during the nesting periods of common songbirds in our region (Arnold 2017). Future studies should determine the direct and indirect hazard that dipterans may pose to songbirds by performing a risk analysis (see Lazorchak et al. 2003). Given their ubiquity in aquatic systems and their importance in food webs at the land–water interface, dipterans are important taxa that should not be overlooked as a part of the Hg cycle.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4134.

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Data Availability—Data are available from the corresponding author (m.m.chumchal@tcu.edu).

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