Environmental Toxicology

SEASONALITY OF ODONATE-MEDIATED METHYLMERCURY FLUX FROM PERMANENT AND SEMIPERMANENT PONDS AND POTENTIAL RISK TO RED-WINGED BLACKBIRDS (AGELAIUS PHOENICEUS)

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Abstract: Methylmercury (MeHg) is an aquatic contaminant that can be transferred to terrestrial predators by emergent aquatic insects such as odonates (damselflies and dragonflies). We assessed the effects of month and pond permanence on odonate-mediated MeHg flux (calculated as emergent odonate biomass × MeHg concentration) in 10 experimental ponds and the potential risk to nesting red-winged blackbirds (Agelaius phoeniceus) posed by consuming MeHg-contaminated odonates. Emergent odonates were collected weekly from permanent ponds with bluegill (Lepomis macrochirus; n = 5) and semipermanent ponds without fish (n = 5) over an 8-mo period (January–August 2015). The MeHg flux from damselflies, aeshnid dragonflies, and libellulid dragonflies began in March and peaked in April, May, and June, respectively, and then declined throughout the rest of the summer. Odonate-mediated MeHg flux from semipermanent ponds without fish was greater than that from permanent ponds with fish. Nesting of red-winged blackbirds overlapped with peak odonate emergence and odonate-mediated MeHg flux. Because their diet can be dominated by damselflies and dragonflies, we tested the hypothesis that MeHg-contaminated odonates may pose a health risk to nesting red-winged blackbirds. Concentrations of MeHg in odonates exceeded wildlife values (the minimum odonate MeHg concentrations causing physiologically significant doses in consumers) for nestlings, suggesting that MeHg-contaminated odonates can pose a health risk to nesting red-winged blackbirds. Environ Toxicol Chem 2017;9999:1–5. © 2017 SETAC

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INTRODUCTION

Methylmercury (MeHg) is an environmental contaminant that is hazardous to the health of wildlife [1,2]. Inorganic and elemental forms of mercury (Hg) are emitted into the atmosphere from natural and anthropogenic sources [3]. When inorganic Hg is deposited into water bodies, aquatic bacteria can convert it to MeHg [3]. Methylmercury bioaccumulates in the tissues of aquatic consumers and can be transported from aquatic to terrestrial food chains via emergent aquatic insects [4–6], a process termed “insect-mediated MeHg flux.” Odonates (damselflies and dragonflies) are responsible for most of the insect-mediated MeHg flux [5]. Terrestrial predators, such as birds, can become contaminated with MeHg when they consume MeHg-contaminated emergent insects [7]. Methyl mercury has negative effects on the health, physiology, behavior, and reproduction of birds, with nestlings being more sensitive than adults [1,2].

The present study examines odonate-mediated MeHg flux from small ponds and the potential risk that MeHg flux poses to odonate-consuming birds. Chumchal and Drenner [8] hypothesized that pond permanence is an important environmental control of odonate-mediated MeHg flux. They defined permanent ponds as those ponds that hold water over long periods of time (years). Permanent ponds do not dry, allowing them to sustain fish populations [9,10]. Chumchal and Drenner [8] defined semipermanent ponds as ponds that contain water over long periods (months to years) but do not contain fish because of periodic drying. Compared with semipermanent ponds without fish, permanent ponds with fish have insect communities with smaller populations of taxa that are vulnerable to fish predation such as larval odonates [5,9–11]. In aquatic systems, larval odonates are top predators with high concentrations of MeHg [4], and when they emerge they transfer this MeHg to terrestrial ecosystems [5].

Because the rate of aquatic insect development and timing of emergence vary with temperature [12], we would predict large seasonal variation in odonate-mediated MeHg flux in the temperate zone. However, most of the research on insect-mediated MeHg flux has been conducted during a single season [4–6,13], and no studies have examined insect-mediated MeHg flux across seasons and its risk to insectivorous birds. We present the first experimental study of the seasonality of odonate-mediated MeHg flux from permanent and semipermanent ponds from winter through summer and the potential health risk odonate-mediated MeHg flux poses to nesting red-winged blackbirds (Agelaius phoeniceus). Because the diet of nesting red-winged blackbirds can be dominated by damselflies and dragonflies [14], we tested the hypothesis that MeHg-contaminated odonates may pose a health risk to nesting red-winged blackbirds.

METHODS

Experimental setup

We conducted the present experiment 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. 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 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. Macrophyte communities were variable between ponds and 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 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 [15] and feed on benthic insects as well as other prey [16].

On 1 April 2014, 5 of the 10 ponds were drained and the 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).

This experiment 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 [6]. 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 [6]. Chumchal et al. [6] 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 that total MeHg flux did not differ between 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.

The present study is phase 2 of the experiment in which we examined the seasonality of odonate-mediated MeHg flux from permanent fish ponds and semipermanent fishless ponds from winter through summer 2015 (Table 1). We monitored temperature and odonate 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) staked near the maximum depth of each pond from 1 January to 31 August 2015. Odonate emergence was monitored with 1.5 m (width × height) emergence platforms (Supplemental Data, Figure S1) from 12 January to 24 August 2015. Emergence platforms were constructed of fiberglass window screen material stretched across a rectangular polyvinyl chloride frame and anchored in ponds using plastic stakes and a fence post. Eight platforms were placed near the shore of each pond so that the bottom of the platform was in contact with pond sediment and the top of the platform extended 20 to 50 cm above the water depending on water depth (Supplemental Data, Figure S1). Larval odonates crawled up the platforms and underwent metamorphosis into adults, leaving behind their exuvia (i.e., cast-off outer skin). Exuviae were collected weekly by hand from emergence platforms and preserved in 95% ethanol.

Exuviae were identified to family in the laboratory and counted to estimate the relative number of emerging odonates from permanent and semipermanent ponds. We collected and identified 3792 odonate exuviae during the experiment belonging to 3 families: damselflies (Odonata:Zygoptera: Coenagrionidae), aeshnid dragonflies (Odonata:Anisoptera: Aeshnidae), and libellulid dragonflies (Odonata:Anisoptera: Libellulidae; Table 2). Each exuvia represented an adult odonate (damselfly, aeshnoid dragonfly, or libellulid dragonfly) that emerged from the pond.

In the present study, we define “odonate-mediated MeHg flux” as the quantity of MeHg transported from aquatic to terrestrial ecosystems by adult emergent odonates. Odonate-mediated MeHg flux is the product of emerging odonate biomass and MeHg concentrations of adult odonates. To calculate MeHg flux from adult odonates, we multiplied the number of exuviae collected from each pond during the present study by the average weight and the average MeHg concentration of adult odonates (Table 2). The average weight and MeHg concentration of adult odonates were determined from newly emerged adults captured in floating-emergence traps during phase 1 of the experiment [6]. Methylmercury flux is expressed as nanograms of MeHg per platform per day.

During the study, we observed red-winged blackbirds nesting in cattails in the ponds. We began weekly searches for active (i.e., parental activity, eggs, nestlings) red-winged blackbird nests on 25 April 2015 (Table 1). Searches were conducted for new nests by a single searcher for approximately 6 to 8 h/wk. Searches of cattails were conducted by wading through areas of ponds with cattails in a zigzag pattern until a nest was found. In addition to searching for new nests, nests identified as active during previous weeks were revisited. We did not find active nests on 15, 21, or 31 July; therefore, we stopped searching in subsequent weeks.

**Estimation of health risk to nestling red-winged blackbirds**

Odonate-based avian wildlife values were calculated to assess the exposure risk for nestling red-winged blackbirds at the study

### Table 1. Timeline showing dates of experimental setup and sampling

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 2013</td>
<td>All ponds filled with water and stocked with bluegill</td>
</tr>
<tr>
<td>April 2014</td>
<td>5 of 10 ponds drained to simulate drying disturbance</td>
</tr>
<tr>
<td>May 2014</td>
<td>5 drained ponds refilled to represent semipermanent ponds; 5 ponds that were not drained represent permanent ponds</td>
</tr>
<tr>
<td>May–August 2014</td>
<td>Phase 1 of the experiment designed to assess the effect of drying disturbance on insect-mediated MeHg flux; results published in Chumchal et al. [6]</td>
</tr>
<tr>
<td>January 2015</td>
<td>Phase 2 of the experiment designed to assess seasonality of odonate-mediated MeHg flux and risk to red-winged blackbirds (present study) begins</td>
</tr>
<tr>
<td>January–August 2015</td>
<td>Collection of temperature and odonate emergence data</td>
</tr>
<tr>
<td>April–July 2015</td>
<td>Monitoring of red-winged blackbird nesting</td>
</tr>
</tbody>
</table>

MeHg = methylmercury.
Seasonality of odonate-mediated MeHg flux

Table 2. Taxa, total number of exuviae collected on platforms, average individual weight (dry wt), and average methylmercury (MeHg) concentrations (dry and wet wt) of adult damselflies, aeshnid dragonflies, and libellulid dragonflies a

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Total number of exuviae collected on platform</th>
<th>Average weight per individual (mg dry wt)</th>
<th>Average MeHg concentration (ng/g dry wt)</th>
<th>Average MeHg concentration (ng/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damselflies</td>
<td>2749</td>
<td>3.2</td>
<td>112</td>
<td>30.1</td>
</tr>
<tr>
<td>Aeshnid dragonflies</td>
<td>211</td>
<td>109</td>
<td>120</td>
<td>32.3</td>
</tr>
<tr>
<td>Libellulid dragonflies</td>
<td>832</td>
<td>28</td>
<td>145</td>
<td>39.1</td>
</tr>
</tbody>
</table>

a Number of exuviae collected on platforms was determined during the present study. Average individual weight and average MeHg concentration of odonates were determined during phase 1 of a long-term experiment in the experimental ponds and have been published [6].


We detected a significant interaction effect of month and pond permanence on MeHg flux from all 3 taxa (repeated-measures ANOVA, damselfly $F_{1, 67, 13.4} = 6.92$, $p = 0.002$; aeshnid dragonflies $F_{2, 203, 16.6} = 5.79$, $p = 0.012$; libellulid dragonflies $F_{1, 308, 10.5} = 5.79$, $p = 0.029$; Figure 1b–d). We detected a significant main effect of month on MeHg flux of aeshnid dragonflies, with aeshnid dragonfly MeHg flux being higher in semipermanent ponds relative to permanent ponds (repeated-measures ANOVA, $F_{1, 8} = 4.72$, $p = 0.06$). We did not detect a significant main effect of pond permanence on MeHg flux of damselflies or libellulid dragonflies (repeated-measures ANOVA, damselfly $F_{1, 8} = 3.08$; $p = 0.12$; libellulid...
MeHg flux. Odonate-mediated MeHg flux occurred primarily in the spring and early summer, presumably when temperature, photoperiod, and food availability were optimal to stimulate emergence [26]. We observed a sequential taxon-specific timing of MeHg flux, with damselfly MeHg flux peaking first, followed by that of aeshnid and libellulid dragonflies. Methylmercury flux was affected by pond permanence such that semipermanent ponds without fish had higher MeHg flux than permanent ponds with fish.

Following emergence, the maximum life span of adult damselflies and dragonflies is 2 and 2.5 mo, respectively [26]. Visual surveys of adult dragonflies flying over the surface of the ponds indicated an increase in population size of adult dragonflies from spring to summer (Supplemental Data, Figure S3). This suggests that a pool of MeHg in adult odonates is available to terrestrial predators months after odonate-mediated MeHg flux has peaked.

**Red-winged blackbird nesting and MeHg risk**

We observed red-winged blackbird nests with eggs or nestlings at the present study site from 25 April to 10 July. We assessed risk to nesting red-winged blackbirds by calculating a risk quotient as MeHg concentrations of odonates divided by the MeHg concentration of the wildlife value (Supplemental Data) [19]. Methylmercury concentrations of odonates exceed wildlife values for red-winged blackbird nestlings when the risk quotient is >1. Red-winged blackbird risk quotients varied with the estimated percentage of odonates in their diet and the weight of nestlings. Risk quotients in nestling red-winged blackbirds were lower when we estimated that they consumed a low-odonate diet than when they consumed a high-odonate diet. Risk quotients declined with nestling weight and were 29% lower for 40-g nestlings compared to 4-g nestlings. The 4-g nestlings were the most sensitive to MeHg-contaminated odonates because they are small-bodied and have relatively low consumption rates (Supplemental Data, Table S1). Conversely, the 40-g nestlings were least sensitive to MeHg-contaminated odonates because they are large-bodied and have relatively low consumption rates (Supplemental Data, Table S1). All nestlings exceeded a risk quotient of 1, and the lowest and highest risk

![Figure 1.](image1.png)

*Figure 1. (a) Average (± standard error) monthly water temperature, (b) damselfly-mediated, (c) aeshnid dragonfly-mediated, and (d) libellulid dragonfly-mediated methylmercury flux in permanent and semipermanent ponds. The p values from repeated-measures analyses of variance examining the main effects of month, pond permanence, and the month × pond permanence interaction are shown in each panel (complete statistical results appear in the text). When a significant month × pond permanence interaction was detected, we tested for simple effects of pond permanence in a given month. Shaded region represents months when active nests were found at the study site. *p ≤ 0.10 and **p ≤ 0.05, significant simple effects between semipermanent and permanent ponds. MeHg = methylmercury; M = month; P = pond permanence.*

![Figure 2.](image2.png)

*Figure 2. Average risk quotients for 4-, 20-, and 40-g nestlings consuming low-odonate (52%) and high-odonate (87%) diets. A risk quotient of 1 (dashed black line) indicates the point at which methylmercury concentrations in odonates are equivalent to the avian wildlife value.*
quotients observed in the present study were 3.3 (40-g nestling, low-odonate diet) and 8 (4-g nestling, high-odonate diet), respectively. These risk quotients indicate that odonate consumption at the study site would result in MeHg exposures ranging from 3.3 to 8 times the wildlife values (Figure 2). The timing of breeding in birds is an important fitness-related life-history trait, and breeding is expected to be timed so that enough food is available during the nestling feeding phase [27]. In the present study, we observed a synchrony between red-winged blackbird nesting and the emergence of odonates, an important prey item of nestling red-winged blackbirds. Because larval odonates are predators, they have high concentrations of MeHg when they emerge as adults [4]. The present study is the first to demonstrate that MeHg flux from adult odonates may pose a health risk to nestling red-winged blackbirds. It is likely that this threat from MeHg-contaminated odonates to red-winged blackbirds is not unique to the present study site because red-winged blackbirds often nest in or near aquatic systems [28], all of which are contaminated with Hg from atmospheric sources.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3844.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (m.m.chumchal@tcu.edu).

REFERENCES