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Environmental Toxicology and Chemistry, Vol. 36, No. 10, pp. 2833–2837, 2017

Printed in the USA

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

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(Submitted 21 February 2017; Returned for Revision 5 April 2017; Accepted 1 May 2017)

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 nestling 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 nestling 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 nestling red-winged blackbirds. *Environ Toxicol Chem* 2017;36:2833–2837. © 2017 SETAC

Keywords: Odonate Insect-mediated methylmercury flux Pond permanence Seasonality Red-winged blackbird

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]. Methylmercury 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 nestling red-winged blackbirds (*Agelaius phoeniceus*). Because the diet of nestling 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 nestling 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.

This article includes online-only Supplemental Data.
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Published online 11 May 2017 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3844

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 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 [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 1×1.5 m (width $\times$ 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, aeshnid 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

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. [6]
January 2015	Phase 2 of the experiment designed to assess seasonality of odonate-mediated MeHg flux and risk to red-winged blackbirds (present study) begins
January–August 2015	Collection of temperature and odonate emergence data
April–July 2015	Monitoring of red-winged blackbird nesting

MeHg = methylmercury.

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]

Taxa[b]	Total number of exuviae collected on platform	Average weight per individual (mg dry wt)	Average MeHg concentration (ng/g dry wt)	Average MeHg concentration (ng/g wet wt)[c]
Damselflies	2749	3.2	112	30.1
Aeshnid dragonflies	211	109	120	32.3
Libellulid dragonflies	832	28	145	39.1

[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].
[b]Damselflies, Odonata:Zygoptera:Coenagrionidae; Aeshnid dragonflies, Odonata:Anisoptera:Aeshnidae; Libellulid dragonflies, Odonata:Anisoptera: Libellulidae.
[c]Methylmercury concentrations were initially determined using dried odonate tissues [6]. Water accounts for 73% of the body weight of dragonflies [29]. Therefore, we estimated wet weight MeHg concentrations by dividing dry weight MeHg concentrations by a conversion factor of 2.7 to account for weight change as a result of water loss.

site using methods modified from the US Environmental Protection Agency [17], Lazorchak et al. [18], Walters et al. [19], and Gann et al. [20] (Supplemental Data). Odonate-based avian wildlife values reflect the minimum MeHg concentrations in odonates, consumed by birds in normal proportions of the total diet, required to create physiologically significant doses [17,19]. A lower wildlife value is associated with a higher sensitivity of birds to consumption of MeHg-contaminated odonates [19].

Wildlife values were calculated for red-winged blackbird nestlings weighing 4, 20, and 40 g, which correspond to the weights of male nestlings 0, 4, and 10 d old [21]. The proportion of odonates in the diets of nestling red-winged blackbirds varies based on whether males participate in feeding nestlings, with nestlings fed by both males and females consuming a higher proportion of odonates (87% odonates) than those fed only by females (52% odonates) [14]. Therefore we also calculated wildlife values for nestlings with high-odonate diets (fed by males and females) and with low-odonate diets (fed by females only). We used life-history data from the literature to compute wildlife values [14,21,22] (Supplemental Data Table S2).

Following the approach of Walters et al. [19] and Gann et al. [20], we compared avian wildlife values with odonate MeHg concentrations to assess the relative MeHg exposure risk to nestling red-winged blackbirds from consuming odonates. Average MeHg concentrations of odonates were obtained from the study site during phase 1 of the experiment [6] (Table 2). We assessed risk to nestling red-winged blackbirds by calculating a risk quotient as MeHg concentrations of odonates divided by the MeHg concentration of the wildlife value. The risk quotient represents the proportional difference between dietary concentrations of MeHg and the concentration of MeHg expected to cause adverse effects in birds [19]. Methylmercury concentrations of odonates exceed wildlife values for red-winged blackbird nestlings when the risk quotient is >1.

Statistical analysis

Statistical analysis was performed with SPSS (Ver 22). Data collected on a daily (temperature) or weekly (MeHg flux) basis were averaged and expressed on a monthly basis. We used a repeated-measures analysis of variance (ANOVA) 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), whereas the interaction effect is the amount of measured variation in the response variables (odonate-mediated MeHg flux) attributable to the interdependence between month and pond permanence. When a month × pond permanence interaction effect was detected, we tested for simple effects of permanence during each month of the experiment using a one-way ANOVA. Because of low replication and statistical power, a probability level of alpha ≤ 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 [23]. 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 [5,24,25].

RESULTS AND DISCUSSION

Temperature and odonate-mediated MeHg flux

Temperature increased over the course of the experiment (Figure 1a). We detected a significant effect of month on temperature (repeated measures ANOVA, $F_{1.67, 13.4} = 1375$, $p < 0.001$), but temperature was not significantly affected by pond permanence (repeated measures ANOVA, $F_{1, 8} = 1.12$, $p = 0.32$). We did not detect a significant month × pond permanence interaction effect on temperature (repeated measures ANOVA, $F_{1.67, 13.4} = 0.50$, $p = 0.59$).

Odonate-mediated MeHg flux from damselfly emergence began in March and that from aeshnid and libellulid dragonfly emergence began in April (Figure 1b–d). Methylmercury flux from damselflies, aeshnid dragonflies, and libellulid dragonflies peaked in April, May, and June, respectively, and then declined throughout the rest of the summer. These temporal patterns in MeHg flux were driven by changes in odonate emergence over time (Supplemental Data, Figure S2). The pattern of emergence suggests that temperature is not the only factor controlling odonate emergence and MeHg flux because temperature remains elevated in the late summer (Figure 1a), while emergence (Supplemental Data, Figure S2) and MeHg flux (Figure 1b–d) decline. Other environmental factors, such as photoperiod and food availability, affect the rate of insect development and timing of insect emergence [26].

We detected a significant effect of month on MeHg flux from all 3 taxa (repeated-measures ANOVA, damselfly $F_{2.75, 22.0} = 6.92$, $p = 0.002$; aeshnid dragonflies $F_{2.03, 16.2} = 5.79$, $p = 0.012$; libellulid dragonflies $F_{1.308, 10.5} = 5.79$, $p = 0.029$; Figure 1b–d). We detected a significant main effect of pond permanence 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

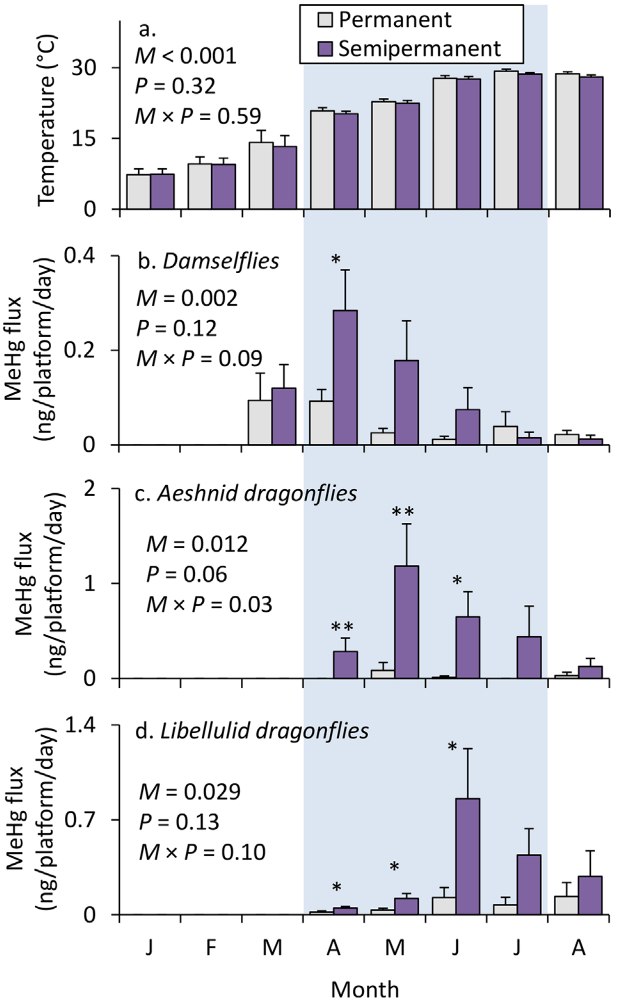

Figure 1. (**a**) Average (± standard error) monthly water temperature, (**b**) damselfly-mediated, (**c**) aeshnid dragonfly-mediated, and (**d**) libellulid dragonfly-mediated methylmercury flux in permanent and semipermanent ponds. The *p* values from repeated-measures analyses of variance examining 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. *$p \leq 0.10$ and **$p \leq 0.05$, significant simple effects between semipermanent and permanent ponds. MeHg = methylmercury; M = month; P = pond permanence.

dragonflies $F_{1,8} = 2.81$, $p = 0.13$). We detected a significant month × pond permanence interaction effect on MeHg flux for each of the 3 taxa, indicating that the effects of month and pond permanence were not independent of each other (repeated-measures ANOVA, damselfly $F_{2.75, 22.0} = 2.54$, $p = 0.09$; aeshnid dragonflies $F_{2.03, 16.2} = 4.61$, $p = 0.03$; libellulid dragonflies $F_{1.308, 10.5} = 3.13$, $p = 0.10$). Analysis of simple effects indicated that there was a significant effect of pond permanence on MeHg flux during some months of the experiment on damselflies (April), aeshnid dragonflies (April, May, and June), and libellulid dragonflies (April, May, and June). In all 3 taxa, MeHg flux was higher in semipermanent than in permanent ponds in the months that a significant simple effect was detected (Figure 1b–d).

The present study demonstrates that odonate-mediated MeHg flux from aquatic to terrestrial food webs is regulated by the interaction of month and pond permanence. Month of the year is an important factor regulating MeHg flux because for much of the year there is no or very little odonate-mediated 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 nestling 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 high 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

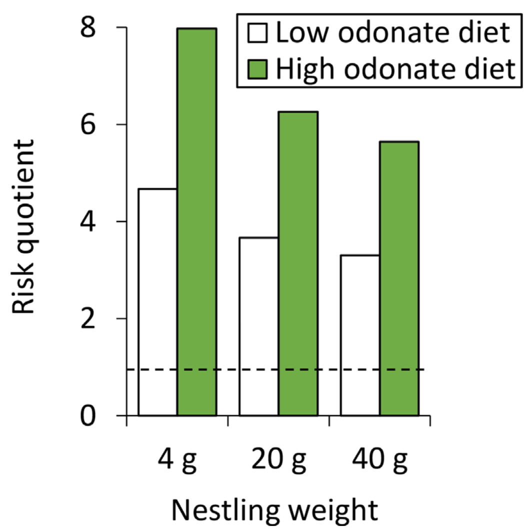

Figure 2. Average risk quotients for 4-, 20-, and 40-g nestlings consuming low-odonate (52%) and high-odonate (87%) diets. A risk quotient of 1 (dashed black line) indicates the point at which methylmercury concentrations in odonates are equivalent to the avian wildlife value.

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.

Acknowledgment—We thank D. Cross, M. Hall, M. Hannapel, K. Lauck, and K. Polk for assistance. The present study was supported by a Texas Christian University Research and Creative Activities Fund Grant, a Texas Christian University Invests in Scholarship Grant, the Texas Christian University Biology Department Adkin's Fund, and the Texas Christian University Biology Department. We are grateful to J. Luster and Luster Fish and Wildlife for stocking fish and the Tarrant Regional Water District for providing access to the ponds for this research.

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

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Corrigendum

Williams EB, Chumchal MM, Drenner RW, Kennedy JH. 2017. Seasonality of odonate-mediated methylmercury flux from permanent and semipermanent ponds and potential risk to red-winged blackbirds (*Agelaius phoeniceus*). *Environ Toxicol Chem* 36: 2833-2837. DOI:10.1002/etc.3844.

The authors of the above-noted paper hereby submit the following correction.

We made an error when calculating ingestion rates for nestling red-winged blackbirds. As a result, the ingestion rates as well as the wildlife values and risk quotients (which are calculated using ingestion rates and wildlife values, respectively) reported in our paper were incorrect. We have corrected the paper as described below.

1. On page 2836-2837, in the *Results and Discussion* section, *Red-winged blackbird nesting and MeHg risk* subsection, the text with corrected risk quotients should read: "All nestlings exceeded a risk quotient of 1, and the lowest and highest risk quotients observed in the present study were 1.2 (40-g nestling, low-odonate diet) and 2.8 (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 1.2 to 2.8 times the wildlife values (Figure 2)."
2. Risk quotients in Figure 2 have been corrected as follows:

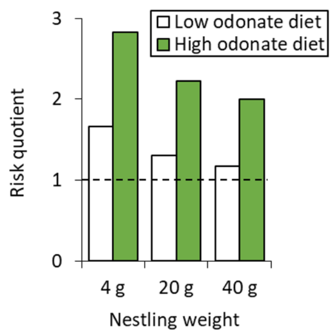

Figure 2. Average risk quotients for 4-, 20-, and 40-g nestlings consuming low-odonate (52%) and high-odonate (87%) diets. A risk quotient of 1 (dashed black line) indicates the point at which methylmercury concentrations in odonates are equivalent to the avian wildlife value.

3. On page S4, in the Supplemental Data, the text with corrected wildlife values should read: "Wildlife values for MeHg in nestling red-winged blackbirds, expressed as nanograms of MeHg per gram of odonate tissue per day (wet weight), consuming a low odonate diet (52% of diet) were 20.9, 26.6 and 29.5 ng/g/d for 4-, 20-, and 40-g nestlings, respectively. Wildlife values for MeHg in nestling red-winged blackbirds consuming a high odonate diet (87% of diet) were 12.5, 15.9 and 17.6 ng/g/d for 4-, 20- and 40-g nestlings, respectively."
4. Ingestion rates in Supplemental Data, Table S1, have been corrected as follows:

Table S1. Ingestions rate for nestling red-winged blackbirds used to calculate wildlife values.

Nestling age	Ingestion Rate ($kg_{food}/kg_{body\ weight}{}^{-d})^2$
0 d old	1.20
4 day old	0.94
10 d old	0.85

These corrections do not change the discussion or conclusions of the paper.

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DOI: 10.1002/etc.4357