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RECOVERY OF AQUATIC INSECT-MEDIATED METHYLMERCURY FLUX FROM PONDS FOLLOWING DRYING DISTURBANCE

MATTHEW M. CHUMCHAL,[a,*] RAY W. DRENNER,[a] FRANK M. GREENHILL,[a] JAMES H. KENNEDY,[b] ASHLYN E. COURVILLE,[a] CHARLIE A.A. GOBER,[a] and LUKE O. LOSSAU[a]
[a]Department of Biology, Texas Christian University, Fort Worth, Texas, USA
[b]Department of Biological Sciences, University of North Texas, Denton, Texas, USA

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Abstract: Small ponds exist across a permanence gradient, and pond permanence is hypothesized to be a primary determinant of insect community structure and insect-mediated methylmercury (MeHg) flux from ponds to the surrounding terrestrial landscape. The present study describes the first experiment examining the recovery of insect-mediated MeHg flux following a drying disturbance that converted permanent ponds with insectivorous fish to semipermanent ponds without fish. Floating emergence traps were used to collect emergent insects for 10 wk in the spring and summer from 5 ponds with fish (permanent) and 5 ponds that were drained to remove fish, dried, and refilled with water (semipermanent). During the 73-d period after semipermanent ponds were refilled, total MeHg flux from semipermanent ponds was not significantly different than total MeHg flux from permanent ponds, indicating that insect-mediated MeHg flux had rapidly recovered in semipermanent ponds following the drying disturbance. Methylmercury fluxes from dragonflies (Odonata: Anisoptera) and phantom midges (Diptera: Chaoboridae) were significantly greater from newly refilled semipermanent ponds than permanent ponds, but the MeHg fluxes from the other 8 emergent insect taxa did not differ between treatments. The present study demonstrates the impact of drying disturbance and the effect of community structure on the cross-system transport of contaminants from aquatic to terrestrial ecosystems. *Environ Toxicol Chem* 2017;9999:1–5. © 2017 SETAC

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INTRODUCTION

In small ponds, emergent aquatic insects are exporters of aquatic contaminants, such as highly toxic methylmercury (MeHg), to predators in terrestrial ecosystems [1,2]. Few studies have examined insect-mediated MeHg flux from small ponds [1,2], even though ponds are the numerically dominant lentic ecosystems in many regions [3] and often the only aquatic systems in dry upland areas that contribute MeHg to the terrestrial environment [4]. All ponds and their food webs are contaminated with MeHg produced by aquatic bacteria from inorganic Hg deposited from the atmosphere [5,6]. Methylmercury concentrates in bacteria and algae at the base of aquatic food webs and then biomagnifies, resulting in high concentrations of MeHg in the tissues of predators, such as fish and dragonflies [2,7,8]. Because of its toxicity and propensity to biomagnify and reach high concentrations, MeHg poses a health hazard to aquatic organisms and terrestrial wildlife with trophic linkages to aquatic food webs (e.g., those that consume emergent aquatic insects) [9–11].

Small ponds exist across a permanence gradient, and pond permanence is hypothesized to be a primary determinant of insect community structure [12] and insect-mediated MeHg flux from ponds [4]. Chumchal and Drenner [4] hypothesized that insect-mediated MeHg flux can be relatively low in permanent ponds because they usually contain fish that consume aquatic insects, but MeHg flux can be relatively high in semipermanent ponds because they periodically experience drying disturbances that eliminate insectivorous fish. Permanent ponds can shift to a semipermanent state if they dry and insectivorous fish are eliminated [4]. When a permanent pond dries, insect-mediated MeHg flux ceases; but after the pond refills, it is recolonized by insects, and insect-mediated MeHg flux recovers when adult insects begin to emerge and transport MeHg from aquatic to terrestrial ecosystems. The recovery of insect-mediated MeHg flux from semipermanent ponds after they refill with water has not been studied. In the present study, we present the first experiment examining the recovery of insect-mediated MeHg flux following a drying disturbance that converted permanent ponds with fish to semipermanent ponds without fish.

METHODS

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. 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 ha to 0.54 ha and have an average depth of 0.8 m. Water levels in the ponds often fluctuate weekly by approximately 0.3 m. The experimental ponds are whole ecosystems with earthen bottoms that contain complex communities of macrophytes, benthic invertebrates, and herptiles. Macrophyte communities were variable between 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). Daily mean water temperatures were collected from 5 June to 28 July 2014 using temperature loggers (Onset Computer) staked near the maximum depth of each pond. During the experiment, the average water temperature of the ponds was 27.8 ± 0.6 °C (average ± standard error [SE]).

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In spring 2013, ponds were filled with water and stocked with bluegill (*Lepomis macrochirus*) purchased from a commercial fish hatchery. Visual observation confirmed that bluegill had spawned in all of the ponds in the summers of 2013 and 2014. Bluegill are commonly present in warm-water fish communities throughout the United States [13] and feed on benthic insects as well as other prey [14].

On 1 April 2014, 5 of the 10 ponds were drained, and fish were removed to simulate drying disturbance (Supplemental Data, Figure S1A). 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 (Supplemental Data, Figure S1B). 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.

After drying disturbance and refilling of semipermanent ponds, aquatic insect communities can be reestablished by recruitment of larval insect populations from eggs deposited by adults that have migrated from other water bodies [15]. In the present study, we observed damselflies (Odonata: Zygoptera) and dragonflies (Odonata: Anisoptera) laying eggs in the ponds during refilling. To confirm that draining had eliminated all large-bodied macroinvertebrates from the semipermanent ponds, we used dip nets (mesh size = 3 mm) to collect larval damselflies and dragonflies from all ponds on 6 dates after semipermanent ponds were refilled (days 8, 15, 29, 43, 56, and 70 after refilling). Larval damselflies and dragonflies were captured in the permanent ponds but not in the semipermanent ponds 8 d after refilling (Supplemental Data, Figure S2). Fifteen days after refilling, larval damselflies and dragonflies were observed for the first time in semipermanent ponds (Supplemental Data, Figure S2). The density of larval damselflies and dragonflies in semipermanent ponds increased throughout the experiment, eventually becoming similar to permanent ponds (Supplemental Data, Figure S2).

Emergent insects were collected over a continuous 10-wk period from 16 May to 28 July 2014. We used pyramid-shaped floating emergence traps (0.53 m × 0.53 m area [0.28 m^2]) to sample emerging adult insects from each pond (Supplemental Data, Figure S1B). Four traps were deployed in each pond and were held in place with 2 plastic-coated stakes (1-cm diameter) pushed into the sediment by hand. Traps were staked at random locations near the corners of the ponds at a water depth of 52.1 ± 1.5 cm (average ± SE). Traps funneled most emerging insects into a collecting bottle containing 95% ethanol. Dragonflies and some damselflies and mayflies (Ephemeroptera: Baetidae) did not move into the collecting bottle and were captured by hand from the lower part of the trap and placed in the sample bottle. Data collected during previous studies indicated that MeHg concentrations in insects were not significantly affected by ethanol preservation (Supplemental Data). Collecting bottles were replaced, and traps were moved to new locations every 4 to 11 d. During one of the collection weeks, a trap was damaged and excluded from that week's analysis.

All individual insects collected from a given pond were identified and counted. Ten taxonomic groups of insects were captured in adequate numbers for analyses: herbivorous/detritivorous midges (Diptera: Chironomidae: Chironominae and Orthocladiinae), predatory midges (Diptera: Chironomidae: Tanypodinae), phantom midges (Diptera: Chaoboridae), mosquitoes (Diptera: Culicidae), biting midges (Diptera: Ceratopogonidae), micro-caddisflies (Trichoptera: Hydroptilidae), long-horned caddisflies (Trichoptera: Leptoceridae), mayflies, damselflies, and dragonflies. We collected an average of 4865 ± 1402 (average ± SE) individual insects from each pond. Total body length (from head to abdomen) was measured for 64 to 886 individuals of each taxa from all ponds using ImageJ® (US National Institutes of Health) and averaged across ponds (Supplemental Data, Table S1). All individual insects collected over the course of the 10-wk experiment were pooled by taxa within ponds, dried for at least 48 h at 60 °C, and weighed. We estimated the weight of individual insects from each taxa (Supplemental Data, Table S1) by dividing the composite weight of all insects collected from a pond by the number of insects captured from that pond. We then estimated emergent biomass on each sampling date by multiplying the weight of individual insects by the number of individuals emerging during the sampling period.

Prior to MeHg analysis, composite samples of each taxa of emergent insects from each pond were homogenized to a fine powder using a clean mortar and pestle or ball-mill grinder. Methylmercury analysis was conducted at the Dartmouth College Trace Element Analysis Core Lab using a MERX automated MeHg System (Brooks Rand) interfaced with an Agilent 7500c inductively coupled plasma–mass spectrometer [16,17]. Samples of 2 certified reference materials (National Institute of Standards and Technology [NIST] Mussel 2976 and National Research Council of Canada [NRCC] TORT-2) were analyzed for quality assurance. For NIST Mussel 2976, the average percentage of recovery of MeHg was 134% (range = 110–159%; $n = 2$). For NRCC TORT-2, the average percentage of recovery for MeHg was 101% (range = 96.2–106%; $n = 4$). For a 0.05-g sample (the typical sample weight analyzed in the present study), the method detection limit was 0.49 ng/g. The limit of quantification was 3 times the method detection limit. Concentrations of MeHg of all samples analyzed in the present study exceeded the limit of quantification. Methylmercury concentration data are presented as nanograms per gram dry weight (Supplemental Data, Table S1).

We calculated MeHg flux by multiplying emergent biomass by MeHg concentration. Because we found that for each insect taxa, MeHg concentration did not differ between treatments (Supplemental Data, Table S2), we averaged MeHg concentration data collected from both treatments over the course of the experiment to develop a robust estimate of MeHg concentration for each taxa (Supplemental Data, Table S1). We define total MeHg flux as the sum of MeHg flux of all taxa.

Statistical analysis

We used a repeated measures analysis of variance (ANOVA) to examine both the main and interaction effects of pond permanence and time on total MeHg flux on 10 dates after semipermanent ponds were refilled. Main effects are the independent impact of each factor (permanence or time), and the interaction effect is the amount of measured variation in the response variable (total MeHg flux) as a result of the interdependence between permanence and time. We considered insect-mediated MeHg flux in the newly refilled semipermanent ponds to have recovered when the total MeHg fluxes from permanent and semipermanent ponds were not significantly different. Because data did not meet the assumption of homogeneity of variance, we log-transformed (log + 1) all data prior to statistical analysis. Untransformed data are presented in Figure 2 to facilitate interpretation.

To determine whether the MeHg flux for each taxa differed by treatment during the experiment, we used a one-way

ANOVA to compare average MeHg flux from permanent and semipermanent ponds for each taxa for the period after that taxa began emerging from the semipermanent ponds. Data for dragonflies, damselflies, mayflies, mosquitoes, phantom midges, and micro-caddisflies did not meet the assumption of homogeneity of variance and were log-transformed (log + 1) prior to statistical analysis, but untransformed data are presented in Figure 3 to facilitate interpretation.

RESULTS AND DISCUSSION

The taxonomic composition of insect-mediated MeHg flux was relatively constant in permanent ponds throughout the experiment but changed in semipermanent ponds as the insect communities underwent succession after refilling (Figure 1A and B). In the permanent ponds, both small and large taxa (e.g., midges and dragonflies, respectively) contributed to the MeHg flux throughout the 10-wk experiment (Figure 1A). In the semipermanent ponds, we detected MeHg flux from the 8 smallest taxa 11 d after refilling (Figure 1B). The 3 largest taxa (mayflies, damselflies, and dragonflies) did not emerge and contribute to the MeHg flux from the semipermanent ponds until days 18, 25, and 32, respectively (Figure 1B). In combination, damselflies and dragonflies accounted for approximately half of the MeHg flux from both types of ponds (Figure 1C and D) because of their large body size and high concentrations of MeHg (Supplemental Data, Table S1).

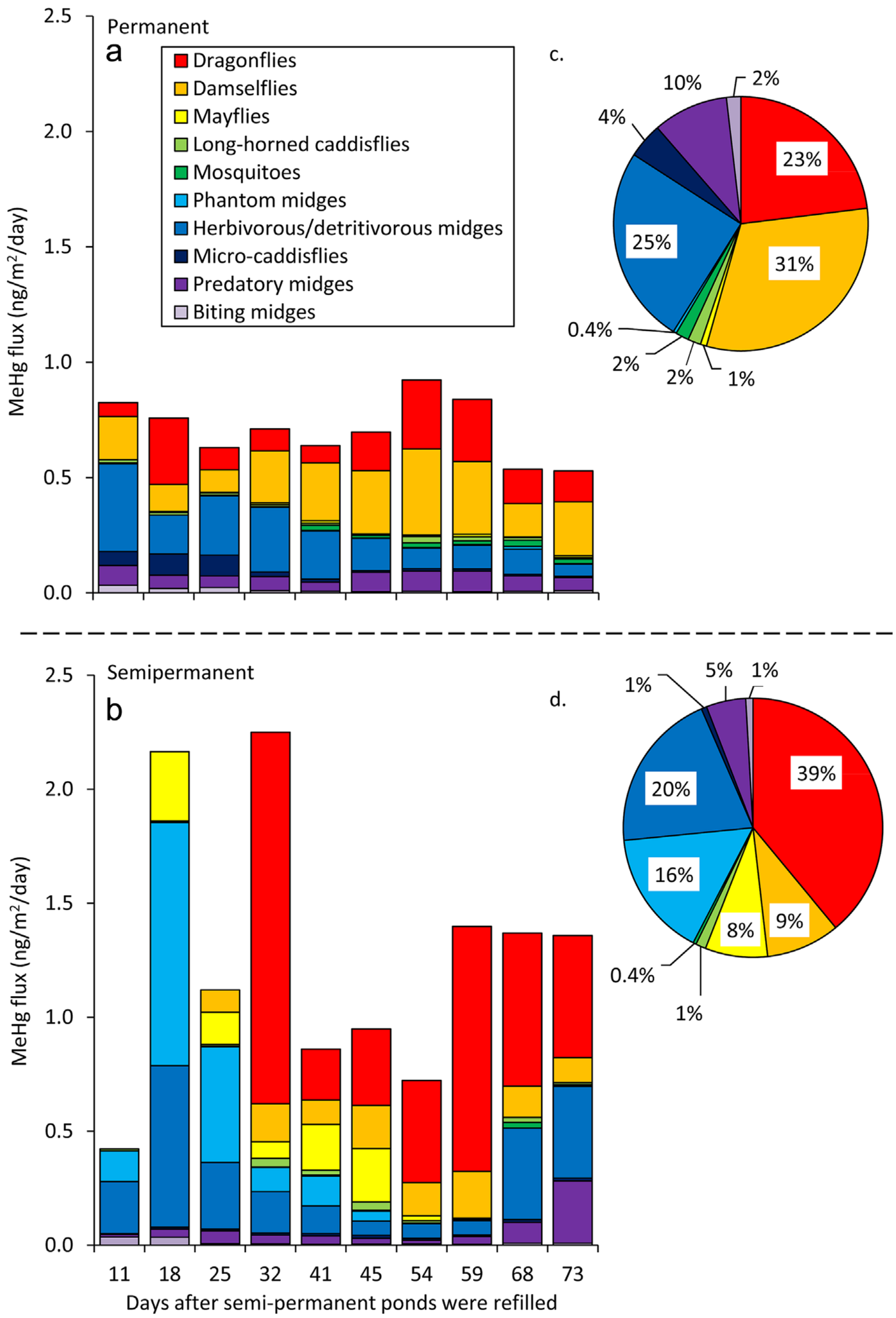

Figure 1. Average methylmercury (MeHg) flux (ng/m^2/d) for 10 emergent insect taxa collected on 10 sampling dates from permanent (**a**) and semipermanent (**b**) ponds, and percent contribution of emergent insect taxa to cumulative MeHg flux in permanent (**c**) and semipermanent (**d**) ponds. In the legend, taxa are listed in order of body length, from largest to smallest (Supplemental Data, Table S1).

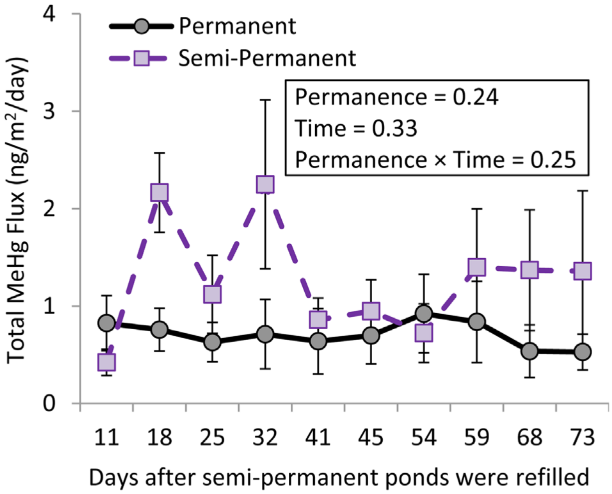

Figure 2. Average (± standard error) total methylmercury (MeHg) flux (ng/m^2/d) collected on 10 sampling dates from permanent and semipermanent ponds. The inset contains p values from a repeated-measures analysis of variance that examined both the main and interaction effects of pond permanence and time on total MeHg flux.

We did not detect statistically significant main effects of permanence (repeated measures ANOVA, $F_{1,8} = 1.64$, $p = 0.37$) or time (repeated measures ANOVA, $F_{6.02,48.1} = 1.18$, $p = 0.33$) on MeHg flux or a significant permanence × time interaction (repeated measures ANOVA, $F_{6.02,48.1} = 1.35$, $p = 0.25$) (Figure 2). These results indicate that insect-mediated MeHg flux had rapidly recovered in semipermanent ponds following the drying disturbance.

Over the course of the experiment, MeHg fluxes from dragonflies and phantom midges (Figure 3A and F) were significantly greater from semipermanent ponds than permanent ponds, but the other 8 taxa did not differ between treatments (Figure 3B–E, G–J). The effect of pond permanence on dragonfly and phantom midge MeHg fluxes was the result of a reduction in emerging insect biomass (Supplemental Data, Table S3) rather than a change in insect MeHg concentration (Supplemental Data, Table S2). These results are consistent with the prediction of Tweedy et al. [1] that fish can reduce insect-mediated MeHg flux for insect taxa vulnerable to fish predation. Bluegill are visually feeding predators that preferentially feed on large-bodied benthic insects such as larval dragonflies and large-bodied zooplankton such as larval phantom midges [14,18].

The present study is the first to demonstrate the rapid recovery of insect-mediated MeHg flux when a drying disturbance converts a permanent pond with fish to a semipermanent fishless pond. Rapid recovery of insect-mediated MeHg flux of semipermanent ponds in the present study may have been attributable in part to their close proximity (<1 km) to permanent ponds, a large reservoir, and a river, which likely served as sources of adult aerially colonizing insects [19]. Warm water temperatures in the spring and summer may have also contributed to the rapid recovery of MeHg flux in the present study because insect development times, from eggs to adults, are inversely related to temperature [20,21]. We would predict that the rate of recovery of insect-mediated MeHg flux from a drying disturbance may be slower during the winter, in ponds geographically isolated from permanent water bodies, or in ponds at higher latitudes and altitudes with cooler temperatures.

Drying and rewetting of sediments would be expected to create anoxic, carbon-rich conditions conducive to

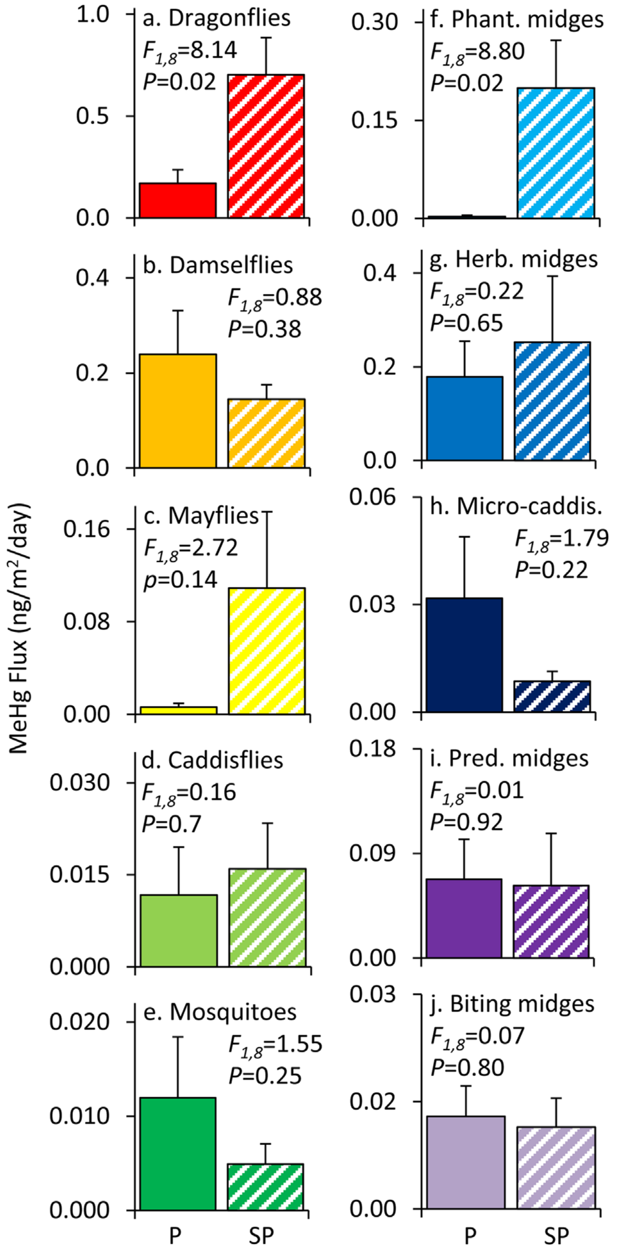

Figure 3. Average methylmercury (MeHg) flux (ng/m^2/d) for 10 insect taxa in permanent and semipermanent ponds. Panels (**a–j**) are arranged in order of insect taxa body length, from largest to smallest (Supplemental Data, Table S1). Phant. midges = phantom midges; Herb. midges = herbivorous/detritivorous midges; Micro-caddis. = micro-caddisflies; Pred. midges = predatory midges; Error bars represent standard error. F and p values are from a one-way analysis of variance comparing average MeHg flux from permanent and semipermanent ponds for the period after that taxa began emerging from the semipermanent ponds.

Hg-methylating bacteria [22,23], and several studies have reported a correlation between water-level fluctuation and MeHg concentrations in biota [4,23–27]. However, Henderson et al. [28] examined MeHg concentrations in invertebrates from permanent and semipermanent human-made ponds in the southern Great Plains and found no statistical difference between pond types. This lack of difference may have occurred because, even though permanent ponds do not dry completely, the water levels of permanent ponds fluctuate and cause sediments to experience wet and dry periods [4]. In the present study, we observed weekly fluctuations of water levels that exposed shoreline and basin sediments in both permanent and semipermanent ponds. As in Henderson et al. [28], we found no difference in MeHg concentrations in emergent insect taxa between permanence treatments (Supplemental Data, Table S2). Therefore, the difference in insect-mediated MeHg flux in semipermanent and permanent ponds in our study was not the result of changes in MeHg production associated with wetting and drying of sediments.

In conclusion, the present study demonstrates the impact of drying disturbance and community structure on the transport of contaminants from aquatic to terrestrial ecosystems. There are millions of small ponds worldwide, and they are the numerically dominant water body type in many regions [3,29,30]. Pond communities exist across a permanence gradient from permanent with fish to semipermanent without fish [12], and both types of ponds are common components of the landscape. For example, Chumchal et al. [31] estimated that 60% and 40% of the >500 000 ponds in the southeastern Great Plains (USA) were permanent and semipermanent, respectively. The present study indicates that insect-mediated MeHg flux from ponds that dry can rapidly recover after refilling and equal that of permanent ponds, but the taxonomic composition of MeHg flux can be different in permanent and semipermanent ponds. If semipermanent ponds retain water for a month or more, large populations of adult dragonflies can begin to emerge, eventually resulting in a higher MeHg flux from dragonflies in semipermanent ponds than in permanent ponds. Both permanent and semipermanent ponds play important but different roles in the Hg cycle and the contamination of terrestrial consumers by MeHg produced in these aquatic ecosystems.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3734.

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

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