EFFECTS OF FISH ON EMERGENT INSECTS AND THEIR TRANSPORT OF METHYL MERCURY FROM PONDS

by

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Introduction

Mercury (Hg) is a widespread environmental contaminant that poses a health hazard to wildlife and humans (Evers et al. 2007; Scheulhammer et al. 2007). Anthropogenic sources such as electric utilities, incinerators, industrial manufacturing and wastewater treatment plants release inorganic and elemental forms of Hg into the environment. (Driscoll et al. 2007). Inorganic Hg becomes methylated by bacteria into toxic methyl mercury (MeHg) in aquatic systems (Morel et al. 1998). Methyl mercury is concentrated in algae and biomagnifies as it moves through trophic levels in the aquatic food webs (Wiener et al. 2007). Because methylation occurs primarily in aquatic systems, Hg contamination has been thought to be exclusively a hazard for aquatic consumers (Wiener et al. 2007). However, MeHg may also pose a threat to terrestrial consumers because MeHg can be transported out of aquatic environments to terrestrial consumers via insect emergence (Tremblay et al. 1998; Gerrard and St Louis 2001; Cristol et al. 2008).

I hypothesized that aquatic insect emergence and the associated transport of MeHg out of ponds (MeHg flux) is regulated in part by fish predation. Visually-feeding fish are size-selective predators on large-bodied benthic macroinvertebrates and alter the biomass and species composition of the benthic communities through complex direct and indirect food web effects (Morin 1984; Maezono et al. 2005; Henderson et al. 2012). I would expect Hg flux would be lower in large-bodied taxa suppressed by fish and higher in small-bodied taxa indirectly enhanced by fish (Fig 1). Here I present a pond experiment examining how fish predation directly and indirectly affects insect emergence and the flux of MeHg from ponds across a range of MeHg contamination levels.

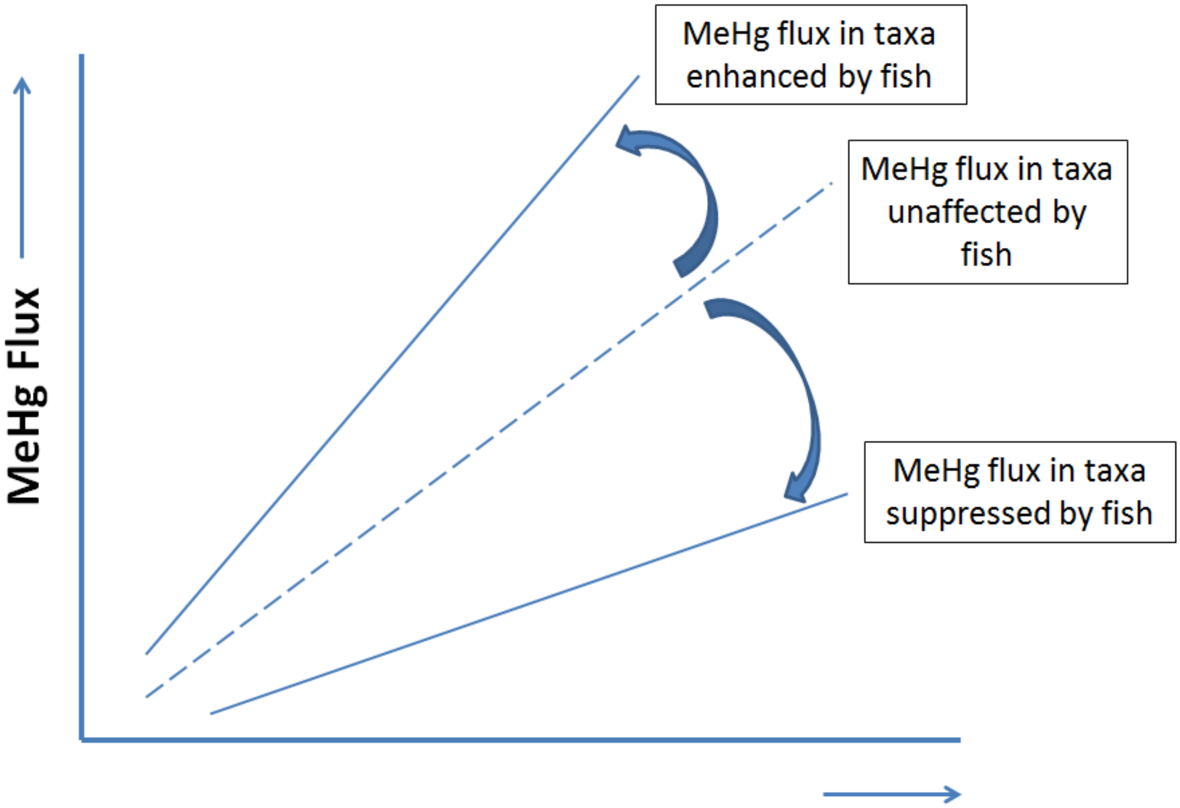

Figure 1. Predicted effects of fish on MeHg flux from ponds of varying contamination.

Materials and Methods

I conducted this study in 10 ponds at the Eagle Mountain Fish Hatchery, Fort Worth, Texas, USA. Ponds ranged in size from 0.23 to 0.54 ha and had maximum and average depths of 1.2 m and and 0.6 m, respectively. Ponds had earthen bottoms and persistent macrophyte communities. Macrophyte communities were highly variable between ponds and consisted largely of bushy pondweed (*Najas guadalupensis*), cattails (*Typha* spp.), coontail (*Ceratophyllum demersum*), hairyseed paspalum (*Paspalum pubiflorum*), horned pond weed (*Zannichellia palustris*), musk grass (*Chara* spp.), pondweeds (*Potamogeton nodosus*), sedge (*Carex cherokeensis* and *C. tetrastachya*), smartweed (*Polygonum hydropiperoides*), spikerush (*Eleocharis palustris* and *E. montevidensis*) and Vasey's grass (*Paspalum urvillei*). All ponds were filled with water from a eutrophic reservoir, Eagle

Mountain Lake. Ponds received continual water inputs from Eagle Mountain Lake to maintain water levels.

In June 2010, ponds were assigned one of two treatments: (1) ponds with no fish (n=5) and (2) ponds stocked with largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) (n=5). These fish species are commonly stocked into farm ponds and are known to feed on benthic invertebrates as well as other prey (Thomas et al. 2007). Visual observation and seining at the end of the study indicated that bluegill and largemouth bass had spawned in all ponds except one pond where only bluegill had survived.

To sample insects, I used 40 floating pyramid-shaped emergence traps, each with a 0.28 m^2 sampling area (Fig. 2). The traps funneled emerging insects into a collecting bottle containing 85% ethanol. I began six weeks of emergence trapping on May 10, 2011. Four traps were randomly deployed in each pond at approximately 45- and 55-cm depths. Traps were fixed in place using two 1.5-m stakes. The collecting bottles were replaced and traps moved to a new random location every seven days. Odonates in the lower part of the trap that had not moved into the sampling bottle were captured by hand, placed on ice, and frozen at -20C in the lab. Some traps were colonized by spiders and these traps were excluded from the study. Nine percent of the trapping effort was disrupted by spiders.

Figure 2. Photo of an emergence traps deployed on a pond. The net had a mesh size of 1.5mm.

I collected and identified five major taxonomic groups of emergent insects representing two functional feeding groups: predators, dragonflies (Odonata:Anisoptera), damselflies (Odonata:Zygoptera), chironomids (Chironominidae:Tanypodinae) and; herbivores, micro-caddisflies (Trichoptera:Hydroptilidae) and chironomids (Chironomidae:Chironominae and Chironominae:Orthocladinae) (Merritt and Cummins 1996). I composited the samples for each taxa in each pond for all six weeks. Samples were dried at 60˚C for 48 hours, weighed and then ground into powder. Ethanol-preserved and frozen damselflies were processed separately. Hg speciation was conducted at the Dartmouth Trace Elements Analysis laboratory using automated methylHg system MERX (Brooks Rand, Seattle, WA) interfaced with ICPMS instruments. I calculated MeHg flux for each taxa by multiplying the biomass of the composite samples multiplied by the Hg concentration of the taxa.

I analyzed data using SPSS. Because of high variability between the ponds and low sample size, I used $\alpha = 0.10$ to avoid Type II errors. Mean emergent insect biomass, MeHg concentration in insects, and MeHg flux were analyzed using a two-tailed *t*-test assuming equal variance. I used a series of analysis of covariance (ANCOVA; SPSS, Ver 20.0.0; SPSS) models to determine the effect of fish presence (categorical variable) and baseline MeHg contamination (covariate) on dragonfly and damselfly MeHg flux (dependent variable). I first tested for a significant interaction between fish presence and baseline MeHg contamination. If the interaction effect was not significant ($p > 0.10$) the interaction effect was removed from the model to determine significance of fish presence and baseline MeHg contamination. I used herbivorous chironomid MeHg as a proxy of baseline MeHg contamination in the ponds because the majority of members of these taxa are herbivorous (Merritt and Cummins 1996). The MeHg present in these organisms would be correlated with the MeHg available to the food chain.

Results and Discussion

Fish affected taxa as size-selective visual predators. Emergence biomass of large-bodied dragonflies and damselflies were significantly suppressed in the presence of fish (Fig. 3a and b). Smaller-bodied chironomids (predatory and herbivorous) were not significantly affected by fish presence (Fig. 3c and d) while caddisflies were significantly enhanced in the presence of fish and (Fig 3e). Other studies have shown that insectivorous fish have direct and indirect effects on aquatic invertebrates (Morin 1984; Maezono et al. 2005; Dorn 2008; Schilling et al. 2009; Wesner 2010; Henderson et al. 2012).

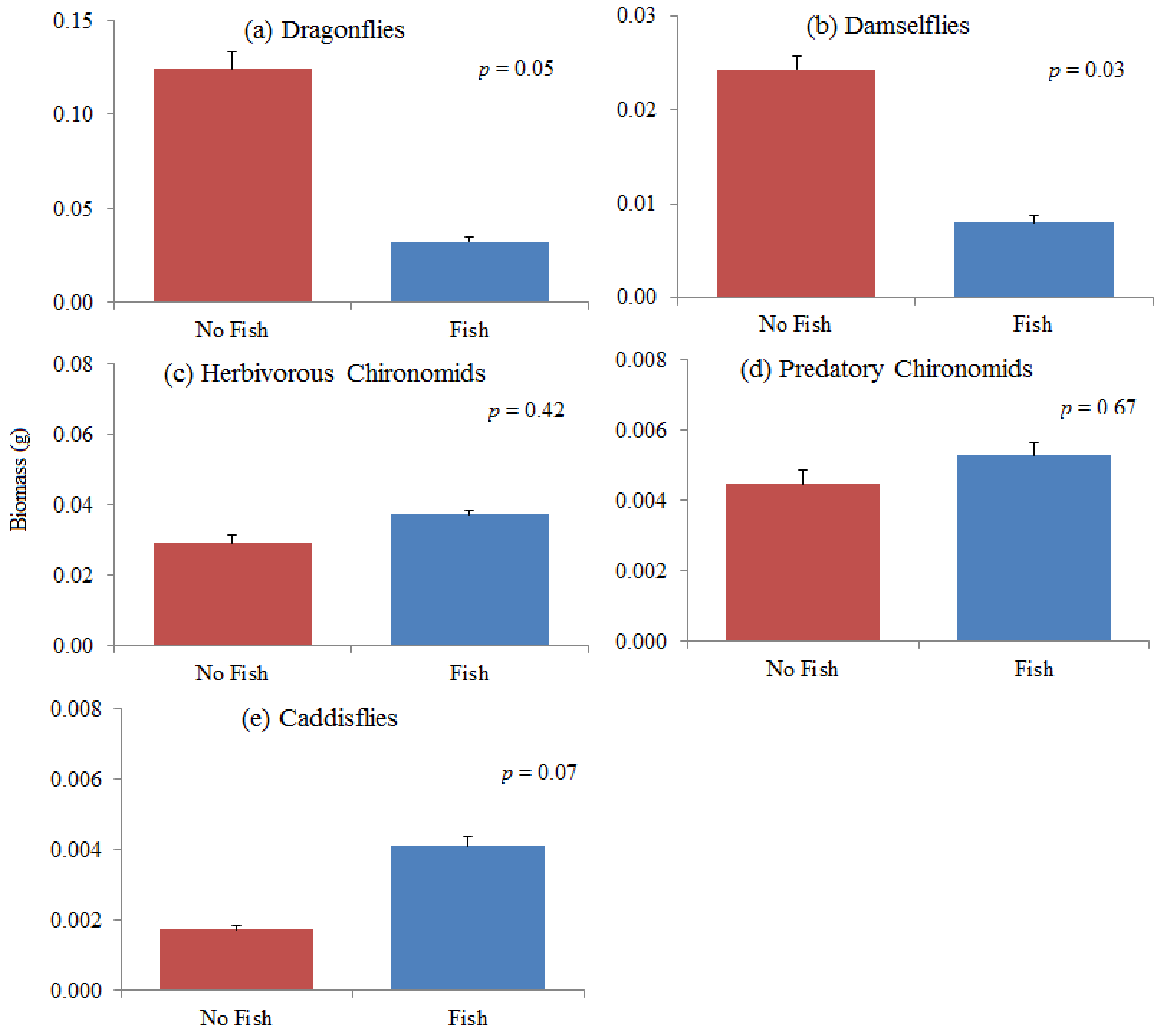

Figure 3. Average emergent insect biomass (g) per unit area for a) dragonflies, b) damselflies, c) herbivorous chironomids, d) predatory chironomids and e) caddisflies (mean ± 1 SE) for fish (n=5) and no fish (n=5) ponds. *p*-Values from a two tail *t*-test comparing mean biomass for each treatment are given on each panel. Note the *y*-axis scales differ for each taxa.

MeHg concentrations in emergent insect taxa were consistent with MeHg biomagnifying in the food web (Table 1). Highest concentrations of MeHg were found in predatory dragonflies and damselflies. Intermediate concentrations of MeHg were found in caddisflies and predatory chironomids. The lowest concentrations of MeHg were found in the herbivorous chironomids. No significant effect of fish on MeHg concentrations was observed for dragonflies and damselflies while MeHg concentrations of smaller taxa were marginally significantly higher in fish presence.

Table 1. MeHg Concentrations (ng\g) of Emergent Insect Taxa for Fish and Fishless Ponds

		Ponds With Fish						Ponds Without Fish						Treatment
	Taxa	**3**	**4**	**18**	**19**	**28**	**Mean**	**8**	**9**	**11**	**15**	**27**	**Mean**	***p*-value**
Predatory	Dragonflies	134.34	65.36	201.62	143.59	141.73	137.33	165.12	42.54	46.53	67.68	229.25	110.23	0.55
	Damselflies	138.44	52.48	191.03	279.02	144.55	161.10	178.25	43.13	84.37	76.83	122.76	101.07	0.21
	Predatory Chironomids	163.14	91.08	193.97	168.59	42.41	131.84	112.69	21.37	25.96	41.26	137.71	67.80	0.12
Herbivorous	Caddisflies	86.60	54.29	135.12	166.25	127.53	113.96	83.44	102.10	23.24	10.96	97.50	63.45	0.10
	Herbivorous Chironomids	74.94	42.02	105.72	78.90	65.87	73.49	61.23	14.17	16.86	35.55	90.76	43.71	0.13

MeHg contamination of ponds was variable as indicated by differing MeHg concentrations in emergent insect taxa (Table 1). For example, herbivorous chironomids feeding near the base of the food chain had MeHg concentrations varying from 14-105 ng\g. The cause of this MeHg variation is unknown but such variation affects the MeHg flux observed from each pond because MeHg flux in any given taxa is a function of the biomass specific MeHg concentration times the emergent biomass.

For each taxa in each pond, I computed the flux of MeHg by multiplying the biomass of the composite samples multiplied by the Hg concentration of the taxa. To examine fish effects, I plotted MeHg flux in ponds with and without fish as a function of MeHg concentration in herbivorous chironomids (Fig. 4). The concentration of MeHg in herbivorous chironomids was used as a proxy variable of mercury contamination in the ponds.

Fish affected the flux of MeHg from the ponds. The flux of MeHg in dragonflies and damselflies was significantly lower in the presence of fish (Fig. 4a and b). Fish had no effect on the flux of MeHg in predatory chironomids (Fig. 4d) but significantly enhanced the flux of MeHg in caddisflies (Fig 4c). Total MeHg flux, computed as the sum MeHg flux of all taxa, was greater in ponds without fish than those with fish.

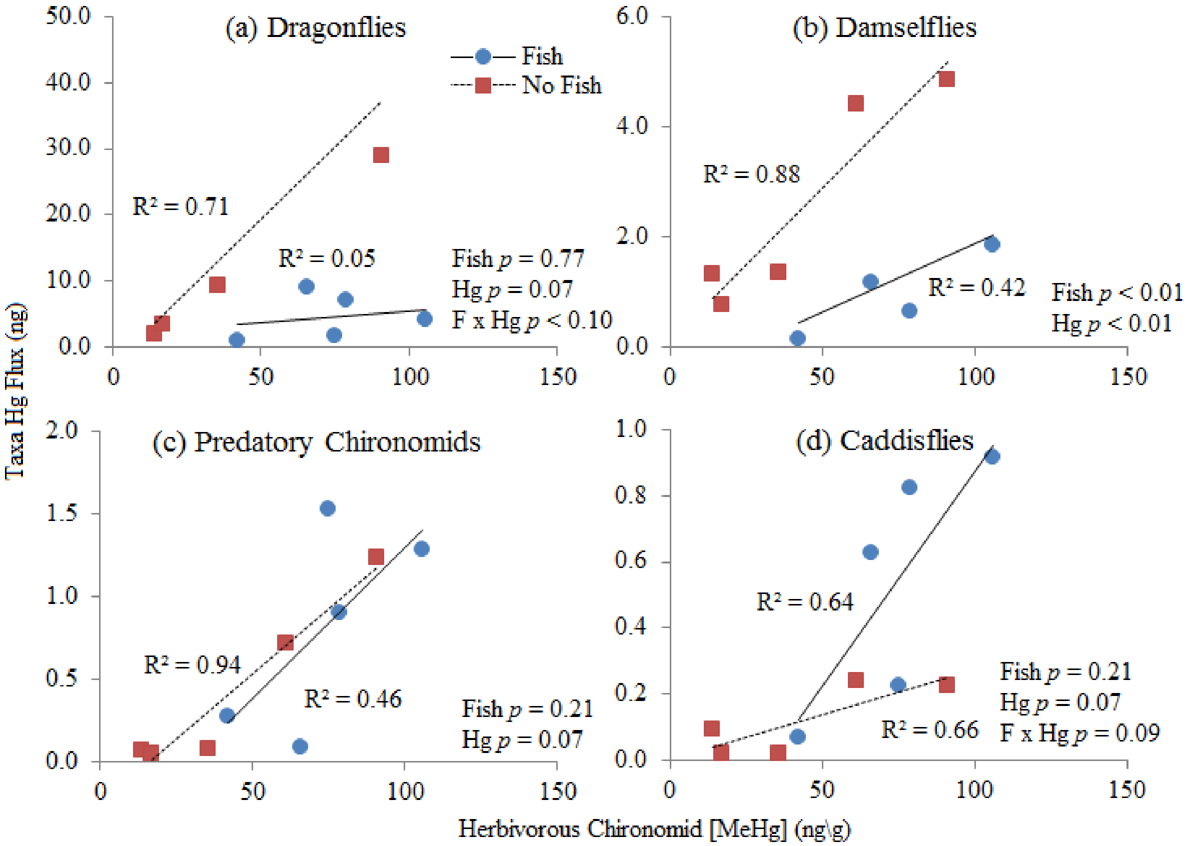

Figure 4. Relationship between baseline [Hg] (herbivorous chironomid [Hg]) and Hg flux for (a) dragonflies, (b) damselflies, (c) caddisflies, and (d) predatory chironomids. *p*-values for fish and Hg concentration are shown. If the interaction significance was *p* >0.10 it is not shown on the figure. Note the y scales differ for each taxa.

By altering the qualitative composition of emergence, fish could affect the risk Hg contamination of generalist terrestrial predators. For example, odonates can comprise 2-82% of a tree swallow's (*Tachycineta bicolor*) diet (Winkler et al. 2011). This suggests that tree swallows feed on odonates when available but rely on other food sources in their absence. The same is true of some lizards (Sabo and Power 2002), frogs (Werner et al. 1995), and bats (Rolseth et al. 1994). My results indicate that generalist predators of emergent insects will be at lower risk of Hg contamination near fish ponds because of the suppression of flux from those ponds by fish.

Taxa, such as chironomids, which are unaffected by fish presence may provide another, more consistent, pathway for Hg to enter terrestrial food webs. Riparian spiders prey heavily on emergent aquatic insects and a recent study found they consume mostly taxa unaffected by fish presence (Wesner 2010). Spiders have also been shown to be a major source of Hg in the diets of terrestrial feeding birds (Cristol et al. 2008). Therefore spiders may be a useful sentinel species for MeHg contamination (Walters et al. 2008).

In conclusion, small man made ponds are the dominant water body in the Great Plains. These ponds collect atmospheric Hg, which is converted by bacteria into MeHg that can enter food webs. How each pond contributes MeHg to the terrestrial environment is determined by the presence and absence of fish.

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ABSTRACT

EFFECTS OF FISH ON EMERGENT INSECTS AND THEIR TRANSPORT OF METHYL MERCURY FROM PONDS

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Methyl mercury (MeHg) is an environmental contaminant affecting the health of wildlife. It was once thought that only aquatic consumers were at risk of MeHg contamination, but we now know emergent insects transport MeHg from aquatic systems to terrestrial food webs (MeHg flux). Factors regulating MeHg flux in emergent insects are currently unknown. This study tests the hypothesis that fish predation regulates insect emergence and flux of MeHg. The experiment utilized five ponds stocked with fish and five ponds without fish. Floating emergence traps were used to capture emergent insects. Fish significantly suppressed Hg flux in dragonflies and damselflies, significantly enhanced MeHg flux in caddisflies, and did not affect Hg flux in midges. Total MeHg flux was significantly greater in ponds without fish. This is the first study to show that fish have complex direct and indirect effects on insect emergence and the flux of MeHg out of ponds.