

## EFFECTS OF FISH ON MERCURY CONTAMINATION OF MACROINVERTEBRATE COMMUNITIES OF GRASSLAND PONDS

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**Abstract**—Mercury is an environmental contaminant that negatively affects the health of vertebrate consumers such as fish, birds, and mammals. Although aquatic macroinvertebrates are a key link in the trophic transfer of Hg to vertebrate consumers, Hg contamination in macroinvertebrate communities has not been well studied. The purpose of the present study was to examine how Hg in macroinvertebrate communities is affected by the presence of fish. We sampled macroinvertebrates from five ponds with fish and five ponds without fish, at the Lyndon B. Johnson National Grassland in north Texas, USA. Ponds without fish contained a higher biomass of macroinvertebrates and taxa with higher concentrations of Hg, which led to a higher Hg pool in the macroinvertebrate community. A total of 73% of the macroinvertebrate biomass from ponds without fish was composed of taxa with the potential to emerge and transport Hg out of ponds into terrestrial food webs. The results of the present study suggest that small ponds, the numerically dominant aquatic ecosystems in the United States, may be more at risk for containing organisms with elevated Hg concentrations than has been appreciated. *Environ. Toxicol. Chem.* 2012;31:870–876. © 2012 SETAC

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## INTRODUCTION

Mercury is an environmental contaminant that has the potential to cause adverse behavioral, neurochemical, hormonal, and reproductive effects on fish, birds, and mammals [1]. Mercury has contaminated all landscapes because of widespread atmospheric deposition [2]. Sulfate and iron-reducing bacteria in aquatic ecosystems convert inorganic Hg to methylmercury (MeHg) [2], a highly toxic and bioaccumulative form of Hg [1]. Organisms at the base of the food web, such as phytoplankton and periphyton, concentrate MeHg directly from the water [3,4], whereas consumers are exposed to MeHg primarily through their diets [5–7]. Predators that feed at high trophic positions bioaccumulate higher concentrations of Hg in their tissues than consumers that feed at low trophic positions [8].

Most studies of Hg in aquatic consumers have focused on fish, because fish consumption is a major source of Hg to wildlife and humans [9]. Relatively few studies have examined Hg contamination of aquatic macroinvertebrates [10–15], even though macroinvertebrates are important in the trophic transfer of Hg to fish and wildlife [16–19]. Many macroinvertebrates have larval stages that are consumed by fish and waterfowl [20–22]. Macroinvertebrates also have adult stages that emerge from the water and can be consumed by terrestrial insectivores [17,18,21].

In ponds, macroinvertebrate biomass and species composition can be affected by the presence of fish [20,21,23–28], but few studies have examined how fish predation affects Hg

contamination of macroinvertebrate communities and thus the potential of macroinvertebrates to contaminate aquatic and terrestrial consumers. Ponds without fish are thought to contain macroinvertebrate communities with higher biomass and larger, more predatory species than ponds with fish [20,21,23–28], characteristics that could lead to a greater pool of Hg in the macroinvertebrate community. The purpose of the present study was to compare the taxonomic composition, biomass, Hg concentrations, and pool of Hg in macroinvertebrate communities of small manmade ponds with and without fish. Because macroinvertebrates undergo seasonal succession [29], we conducted the present study during two seasons, spring and summer. Small ponds are the numerically dominant water body type in the United States [30] and potentially play an important role in the Hg contamination of biota [31].

## METHODS

*Study site*

The present study was conducted at the Lyndon B. Johnson National Grassland in north Texas, USA (Fig. 1). The 8,000-ha property is composed of noncontiguous management units managed for livestock grazing and wildlife. As part of a plan to prevent soil erosion, the United States Department of Agriculture constructed numerous earthen dams, primarily in the mid-to-late 1970s. The dams created small ponds, most of which are smaller than 2,000 m<sup>2</sup> in surface area. Many of the ponds on the property go dry periodically and are devoid of fish [32]. The primary source of Hg in this region is atmospheric deposition (National Atmospheric Deposition Program; <http://nadp.sws.uiuc.edu>), and no known point-sources of Hg exist at the Lyndon B. Johnson Grassland. A previous study at the Lyndon B. Johnson Grassland found Hg in the tissues of macroinvertebrates from ponds without fish [31].

All Supplemental Data may be found in the online version of this article.

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Fig. 1. Lyndon B. Johnson National Grassland in north Texas, USA. The 8,000-ha property is composed of noncontiguous management units (shaded areas) managed for livestock grazing and wildlife. Black and white boxes indicate the location of ponds in the present study with and without fish, respectively, and contain each pond's identification code.

### Sampling

We sampled five ponds with fish and five ponds without fish. Ponds in the present study were chosen from a larger pool of ponds that were previously sampled to determine fish presence or absence [32]. To insure that ponds were geographically dispersed, we selected no more than one representative of each pond type (with/without fish) from a single management unit (Fig. 1). Pond area was calculated using National Agriculture Imagery Program orthoimagery from 2006 (<http://www.apfo.usda.gov/FSA/apfoapp?area=home&subject=prog&topic=nai;&topic=nai>; Table 1).

Each pond was seined during the present study to confirm the presence or absence of fish. Ponds with fish contained between one and 10 species of fish, including ribbon shiner (*Lythrurus fumeus*), golden shiner (*Notemigonus crysoleucas*), black bullhead (*Ameiurus melas*), yellow bullhead (*Ameiurus natalis*), channel catfish (*Ictalurus punctatus*), western mosquitofish (*Gambusia affinis*), green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), longear sunfish (*Lepomis megalotis*), redear sunfish (*Lepomis microlophus*), largemouth bass (*Micropterus salmoides*), white crappie (*Pomoxis annularis*), and black crappie (*Pomoxis nigromaculatus*) (Table 1). All of these

Table 1. Pond area and fish taxa present in ponds sampled at Lyndon B. National Grassland in north Texas, USA

Pond ID	Surface area (m <sup>2</sup> )	Fish taxa present
15B	383	Fishless
14A	485	Fishless
27C	620	Fishless
45C	958	Fishless
38A	2,205	Fishless
76A	320	Golden shiner
16B	376	Ribbon shiner, yellow bullhead
27D	668	Golden shiner
31G	853	Golden shiner black bullhead, western mosquitofish, green sunfish, bluegill, longear sunfish, redear sunfish, largemouth bass, white crappie
38D	1,205	Golden shiner, black bullhead, channel catfish, bluegill, black crappie

species generally function as carnivores in aquatic food webs and consume macroinvertebrates (Texas Freshwater Fishes; <http://www.bio.txstate.edu/~tbonner/txfishes/>). Mercury concentration and trophic position of fish were not examined in the present study.

Macroinvertebrate communities in ponds were sampled once during the spring (April–May) and once during the summer (July–August) in 2009. We used dip nets (mesh size = 250  $\mu$ m and 3 mm) to sample major habitat types, including the vegetation, top layers of sediment, and water column. We also sampled ponds with a 2.4-m seine (mesh size = 5 mm). These mesh sizes did not effectively sample small taxa, such as chironomids, and they are not included in the present study. More effort was required to collect enough organisms for Hg analysis in ponds with fish; thus, total time spent sampling varied from 1.6 to 5.8 person-hours per pond. We recorded total sampling time (combination of all methods) and determined catch-per-unit-effort biomass (mg dry wt/min) for each taxon within each pond. This sampling methodology allowed us to determine relative differences in biomass of macroinvertebrate communities in ponds with and without fish.

### Macroinvertebrate processing

Immediately after collection, macroinvertebrates were placed in plastic bags filled with spring water for 2 to 5 h before being transported to a laboratory and frozen. Macroinvertebrates were identified to lowest taxonomic resolution, usually to genus, using dichotomous keys of Merritt and Cummins [29] and Thorp and Covich [33] and other taxonomic guides [34–38]. In our analyses, we included the most abundant taxa that accounted for more than 1% of the total number of individuals in either fish or fishless ponds. Total body lengths of specimens were measured to the nearest millimeter. Specimens were then rinsed with deionized water and oven-dried at 60°C for at least 72 h. Macroinvertebrates from each pond were composited by taxa, and the dry weight of each taxonomic group was determined to the nearest milligram. Composite samples were ground into a fine powder using a ball-mill grinder before Hg and stable isotope analysis.

### Mercury analysis

In the present study, total Hg was used as a proxy for MeHg. Most of the Hg in higher-level consumers (secondary and above) is MeHg; however, the percentage that is MeHg, especially in lower trophic level invertebrates, can be variable [8,9]. We determined total Hg concentrations in composite samples of macroinvertebrates using a Milestone Direct Hg Analyzer (DMA 80, Milestone), which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy [39]. Quality assurance included reference (National Research Council of Canada Institute for National Measurement Standards) and duplicate samples. Reference samples (MESS-3, DORM-2, or DOLT-3) were analyzed every 10 samples, and the average percentage of recovery was 102% (range = 96–109%;  $n = 53$ ). Duplicate samples were analyzed every 20 samples, and the average relative percentage of difference was 2.48% (range = 0.004–10.9%;  $n = 27$ ). All Hg values are reported in ng/g dry weight unless otherwise noted.

### Trophic position

Stable isotopes of nitrogen were used to estimate trophic positions of macroinvertebrates. Stable nitrogen isotopes are used differentially in cellular processes [40], resulting in a predictable increase in the heavy isotope, <sup>15</sup>N, relative

to  $^{14}\text{N}$  with each increase in vertical trophic level [41]. Macroinvertebrate tissues were analyzed at the University of California-Davis stable isotope facility using a Europa Hydra 20/20 continuous-flow isotope ratio mass spectrometer. Tank nitrogen gases calibrated with known standards were used as working reference materials in daily laboratory operation. Nitrogen isotope results are given as

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000 \quad (1)$$

where  $R$  is  $^{15}\text{N}/^{14}\text{N}$ . Standard for  $\delta^{15}\text{N}$  was air  $\text{N}_2$ .

To calculate trophic position,  $\delta^{15}\text{N}$  values in macroinvertebrates were first corrected for differences in basal  $\delta^{15}\text{N}$  values between ponds using the method of Anderson and Cabana [42]. We selected *Heliosoma* spp., *Hexagenia* spp., Physidae, and Sphaeriidae as our baseline indicators because they were generally the organisms with the lowest  $\delta^{15}\text{N}$  values. We used multiple baseline indicators because no single taxa of primary consumer occurred in all ponds. We did not collect any baseline indicators from one fishless pond in the summer (27C), so we were unable to calculate trophic position for taxa from this pond during the summer. To reduce potential biases related to the use of several primary consumers with variable  $\delta^{15}\text{N}$  values, the  $\delta^{15}\text{N}$  values of *Heliosoma* spp., Physidae, and Sphaeriidae were corrected relative to the  $\delta^{15}\text{N}$  value of *Hexagenia* spp. as described in Anderson and Cabana [42]. Finally, the corrected  $\delta^{15}\text{N}$  values of all of the baseline indicators present were averaged to yield baseline  $\delta^{15}\text{N}$  values for each pond. Baseline corrected  $\delta^{15}\text{N}$  values were then calculated for each macroinvertebrate taxa by subtracting the baseline  $\delta^{15}\text{N}$  value for the pond from which they were collected. We determined trophic position for macroinvertebrates using the equation

$$\text{Trophic position}_{\text{macroinvertebrate}} = ([\delta^{15}\text{N}_{\text{macroinvertebrate}} - \delta^{15}\text{N}_{\text{baseline}}] / f) + 2 \quad (2)$$

where  $\delta^{15}\text{N}_{\text{macroinvertebrate}}$  is the  $\delta^{15}\text{N}$  value for each macroinvertebrate for which trophic position was estimated;  $\delta^{15}\text{N}_{\text{baseline}}$  is the  $\delta^{15}\text{N}$  value of the baseline organisms; 2 is the expected trophic position of the organisms used to estimate baseline  $\delta^{15}\text{N}$ ; and  $f$  is the fractionation factor expected between a predator and its prey [42]. We used a fractionation factor of 3.4‰ [42].

#### Data analysis

Statistical analyses were conducted with SPSS 15.0 (SPSS, Inc). A two-way analysis of variance was used to determine the effects of fish presence and season on average biomass, Hg concentration, Hg pool, trophic position, and average total body length of the macroinvertebrate community in ponds with and without fish. Our initial analysis of variance models included the fish presence  $\times$  season interaction term. The interaction term was not significant in any of the models ( $p \geq 0.24$  in all models); therefore, we removed it and tested for main effects of fish presence and season. A one-way analysis of variance was used to compare average Hg concentration and trophic position of predator and nonpredator macroinvertebrates. A paired  $t$  test was used to compare average Hg concentrations of those macroinvertebrate taxa found in both fish and fishless ponds during each season (spring/summer). Statistical significance was determined at  $p \leq 0.05$ , and all data met the assumptions of normality and equal variances, confirmed by Anderson-Darling's test of normality and Levene's test for equal variance, respectively.

## RESULTS

### Taxonomic differences in ponds with and without fish

The taxonomic compositions of the ponds were dependent on fish presence and season (Fig. 2A, B). In the spring, ponds without fish contained nine taxa of macroinvertebrates not found in ponds with fish. The biomass of fishless ponds was dominated by *Heliosoma* spp., *Anax junius*, *Tramea* spp., and *Streptocephalus seali*. The biomasses of these taxa were approximately four to eight times greater than any of the taxa found in ponds with fish. In the spring, ponds with fish contained five species of macroinvertebrates not found in ponds without fish, with *Gomphus* spp. and *Hexagenia* spp. having the highest biomasses. The biomasses of most taxa were greater in the summer, and many of the taxa found only in ponds without fish in the spring were found in both fish and fishless ponds in the summer. In the summer, *Heliosoma* spp., *Anax junius*, and *Tramea* spp., continued to have some of the highest biomasses in ponds without fish, but these taxa also reached high biomasses in ponds with fish. *Gomphus* spp. and *Hexagenia* spp. were still present in ponds with fish in the summer, but they no

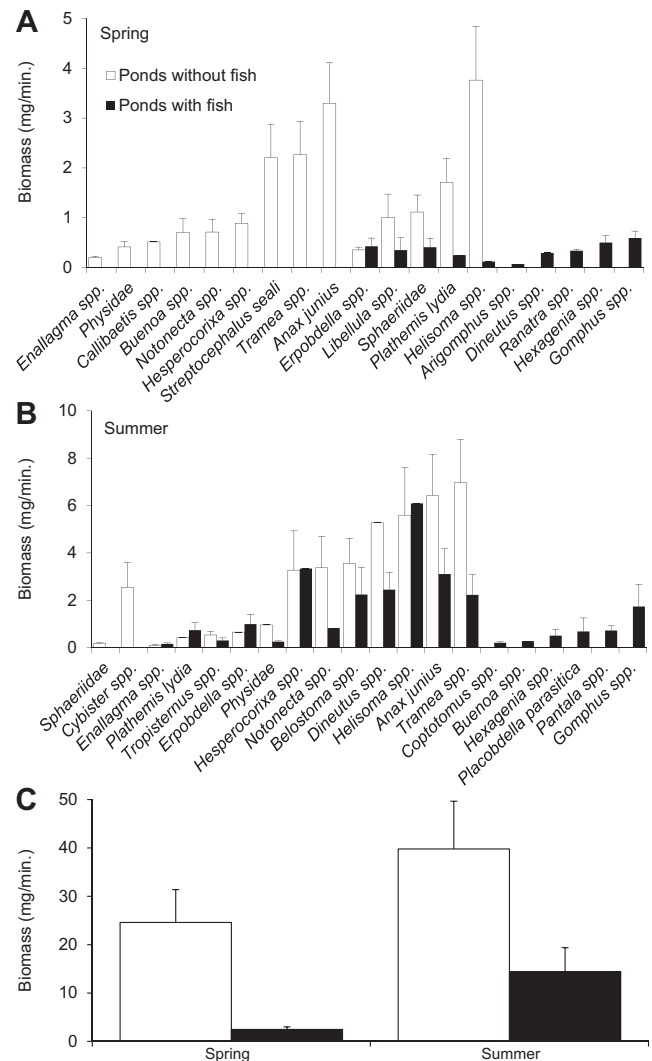


Fig. 2. Average catch-per-unit-effort biomass of macroinvertebrates from ponds with and without fish. Average biomass for each taxa collected in the (A) spring and (B) summer and for the (C) macroinvertebrate community during both seasons. Error bars represent standard error. Note that the scale of the y axis differs in each panel.

longer had the highest biomasses. The average biomass of the macroinvertebrate communities reflects the patterns observed in individual taxa (Fig. 2C). The average biomass of the macroinvertebrate community was higher in ponds without fish than in ponds with fish ( $F = 14.1$ ;  $df = 1,17$ ;  $p = 0.002$ ) and was higher in the summer than in the spring ( $F = 4.6$ ;  $df = 1,17$ ;  $p = 0.046$ ). Fish presence explained more than twice the variation in macroinvertebrate community biomass than season (partial  $\eta^2 = 0.45$  and  $0.21$ , respectively).

Mercury concentrations in macroinvertebrates ranged from 21.9 ng/g (*Callibaetis* spp. collected during the spring) to 865 ng/g (*Notonecta* spp. collected during the summer) (Fig. 3A, B). Predatory macroinvertebrates generally had higher Hg concentrations, although the difference was not significant ( $F = 1.72$ ;  $df = 1,22$ ;  $p = 0.2$ ), and had a significantly higher trophic position than nonpredators ( $F = 11.3$ ;  $df = 1,22$ ;  $p = 0.003$ ; Fig. 4; Supplemental Data, Table S1). During both seasons, *Notonecta* spp. and *Buenoa* spp. had higher Hg concentrations than most other taxa. The average Hg concentration

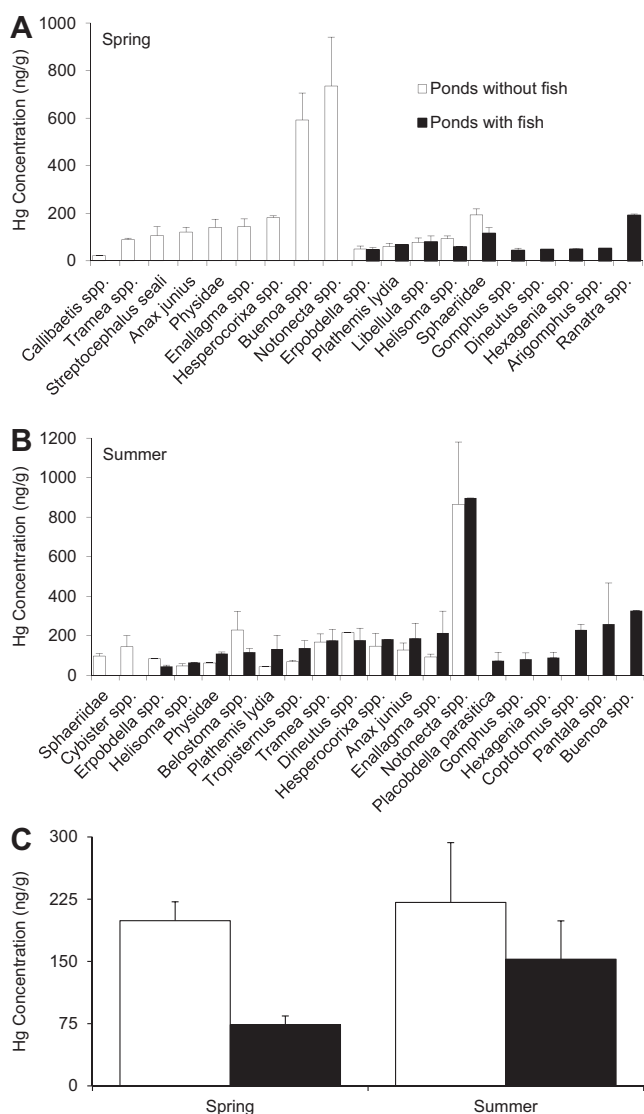


Fig. 3. Average total Hg concentration of macroinvertebrates from ponds with and without fish. Average Hg concentration for each taxa collected in the (A) spring and (B) summer and for the (C) macroinvertebrate community during both seasons. Error bars represent standard error. Note that the scale of the y axis differs in each panel.

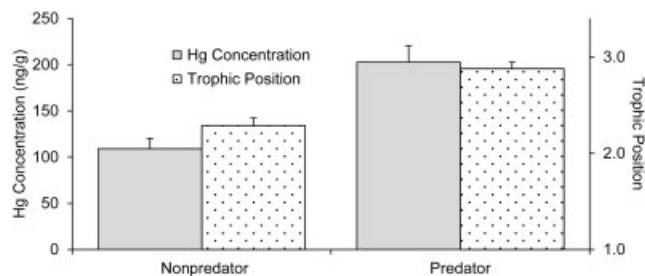


Fig. 4. Average total Hg concentrations and trophic position in nonpredator and predator invertebrates examined in the present study. Predator status was determined using Merritt and Cummins [29] and Thorp and Covich [33]. Refer to Supplemental Data, Table S1, for more information about taxa included in this figure. Error bars are standard error.

of the macroinvertebrate community was strongly affected by fish (Fig. 3C) and was approximately two times greater in fishless ponds than in ponds with fish ( $F = 4.9$ ;  $df = 1,17$ ;  $p = 0.041$ ). The average Hg concentration of the macroinvertebrate community did not differ between seasons ( $F = 1.3$ ;  $df = 1,17$ ;  $p = 0.27$ ) (Fig. 3C). Some macroinvertebrate taxa were found in both fish and fishless ponds, and the Hg concentrations of these taxa did not differ between pond types ( $t = 1.4$ ;  $df = 1,4$ ;  $p = 0.25$  for spring and  $t = -1.3$ ;  $df = 1,11$ ;  $p = 0.22$  for summer). The similarity in Hg concentrations of taxa that were found in ponds with and without fish suggests that the amount of Hg available for incorporation into the food chain did not differ between fish and fishless ponds.

We estimated the pool of Hg in the macroinvertebrate community within each pond by multiplying the catch-per-unit-effort biomass of each taxa by the concentration of Hg in their tissues and then summing the results for each taxa in the pond. We then used the Hg pool determined for each pond to calculate the average Hg pool for ponds with and without fish. The higher biomass of most macroinvertebrate taxa, combined with elevated Hg concentrations in some taxa, resulted in a larger pool of Hg in the macroinvertebrate community of fishless ponds (Fig. 5;  $F = 9.3$ ;  $df = 1,17$ ;  $p = 0.007$ ). A higher biomass of most macroinvertebrate taxa in ponds during the summer led to a larger pool of Hg in the macroinvertebrate community in the summer than in the spring ( $F = 5.8$ ;  $df = 1,17$ ;  $p = 0.03$ ). Fish presence explained more of the variation in Hg pool than season (partial  $\eta^2 = 0.36$  and  $0.25$ , respectively).

Although the taxonomic composition and biomass of the macroinvertebrate community in ponds with fish differed from that in ponds without fish, other expected effects of fish on macroinvertebrate community structure were not observed (Fig. 6). The average trophic position and total body length

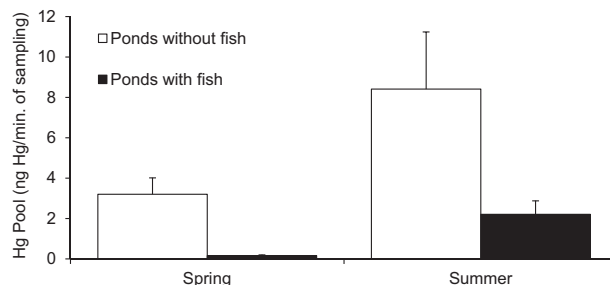


Fig. 5. Average pool of Hg in the macroinvertebrate community from ponds with and without fish during the spring and summer. Error bars represent standard error.

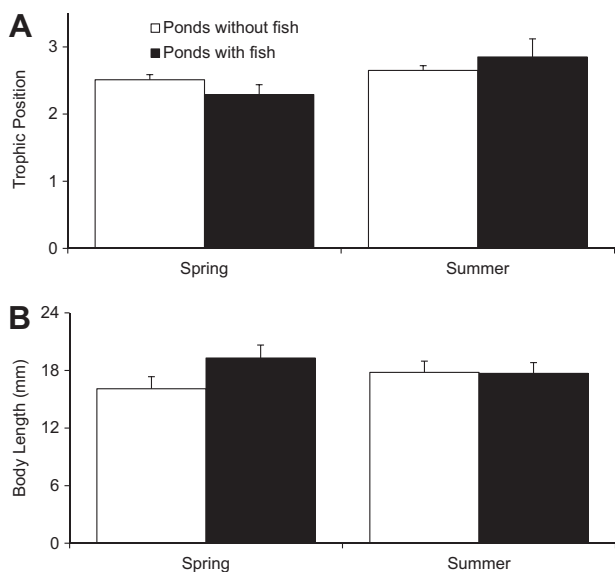


Fig. 6. Average (A) trophic position and (B) total body length of the macroinvertebrate community in ponds with and without fish from spring and summer. Error bars represent standard error.

of macroinvertebrates were not significantly different in the presence of fish (trophic position:  $F = 0.02$ ;  $df = 1,16$ ;  $p = 0.90$ ; body length:  $F = 1.7$ ;  $df = 1,17$ ;  $p = 0.21$ ) and did not differ between seasons (trophic position:  $F = 4.1$ ;  $df = 1,16$ ;  $p = 0.059$ ; body length:  $F = 0.03$ ;  $df = 1,17$ ;  $p = 0.87$ ).

## DISCUSSION

The presence of fish had major effects on macroinvertebrate communities that resulted in a substantial difference in Hg contamination of the macroinvertebrate community in ponds with and without fish. The pool of Hg in the macroinvertebrate community in ponds without fish was much greater than in ponds with fish. The difference in Hg pool between pond types could primarily be attributed to the suppression of macroinvertebrate biomass by fish.

Higher Hg concentrations in some macroinvertebrate taxa from ponds without fish also contributed to the elevated Hg pool in fishless ponds. Most of the difference in Hg concentration can be explained by the presence of Notonectidae (*Buenoa* spp. and *Notonecta* spp.) in ponds without fish in the spring. Previous studies have also found that Notonectidae are abundant in fishless environments relative to those containing fish [28]. When we removed Notonectidae from our analyses, we did not find a significant difference in Hg concentrations between ponds with and without fish ( $F = 0.17$ ;  $df = 1,17$ ;  $p = 0.69$ ), but there were still significant differences in biomass and Hg pool ( $F = 12.9$ ;  $df = 1,17$ ;  $p = 0.002$ , and  $F = 6.8$ ;  $df = 1,17$ ;  $p = 0.018$ , respectively). Notonectidae had the highest concentrations of Hg observed in the present study, and other studies have found high concentrations of Hg in this family [12,14,15,31]. Although Notonectidae had above average concentrations of Hg, their trophic positions were similar to those of other predatory macroinvertebrates (Supplemental Data, Table S1), suggesting that the high concentrations of Hg in the Notonectidae in the present study were not caused by elevated trophic position.

Fish have complex direct and indirect effects on prey communities [43]. Fish are thought to impact macroinvertebrate

communities by reducing the number of predatory species and the overall size of taxa [21,24]. Therefore, we hypothesized that fishless ponds would contain macroinvertebrate communities with higher Hg concentrations relative to ponds with fish, because larger, more predatory organisms tend to accumulate higher concentrations of Hg in their tissues [8,15]. We did not detect a significant difference in the average trophic position of the macroinvertebrate community or the average total body length of taxa between ponds with and without fish. Fish did cause changes in the Hg concentration of the macroinvertebrate community; however, as discussed, this appears to be through an effect on Notonectidae.

Although the average body length and trophic position of macroinvertebrates was similar in ponds with and without fish, macroinvertebrate taxa differed between pond types with regard to adaptations that allowed them to coexist with fish. For example, macroinvertebrate taxa found only in ponds with fish such as *Gomphus* spp., *Arigomphus* spp., and *Hexagenia* spp. are burrowing species [29], a characteristic that makes these organisms less vulnerable to fish predation [44]. Other invertebrate taxa such as *Coptotomus* spp. and *Dineutus* spp. found in ponds with fish belonged to families with chemical defenses that make them unpalatable to fish [45]. Conversely, some of the macroinvertebrate taxa found predominantly in ponds without fish had behaviors that would make them vulnerable to fish predation [24], such as high activity levels (e.g., *Anax junius*, *Callibaetis* spp., *Notonecta* spp.) or a planktonic lifestyle (*Streptocephalus seali*) [29,33].

Season also had a substantial influence on the pool of Hg in the macroinvertebrate community. Although the average Hg concentrations of taxa in the community were not different between seasons, the biomass of macroinvertebrates increased in the summer, and many taxa found only in ponds without fish during the spring were also found in both fish and fishless ponds during the summer (Fig. 2A, B). Although not quantitatively assessed, we observed that macrophyte abundance in the ponds increased from spring to summer. Macrophytes serve as a potential predation refuge for macroinvertebrates that are otherwise vulnerable to fish predation [44], and their increased abundance in summer may have allowed macroinvertebrate biomass to increase and the taxonomic composition of the macroinvertebrate communities of ponds with and without fish to become more similar.

The observed difference in the size of the pool of Hg in macroinvertebrate communities from ponds with and without fish has significant implications, because small ponds may represent an important source of Hg to aquatic and terrestrial consumers. Waterfowl such as ducks consume macroinvertebrates in aquatic habitats [21,22], whereas terrestrial consumers, such as spiders, birds, and bats, could be exposed to Hg by consuming adult emergent macroinvertebrates [17,18]. In ponds without fish, 13 macroinvertebrate species, representing 73% of the macroinvertebrate community biomass, were capable of emerging from aquatic habitats (emergence status determined using Merritt and Cummins [29]). Some of the Hg concentrations of macroinvertebrates in the present study exceeded the proposed concentration (100 ng Hg/g wet wt; ~400–500 ng Hg/g dry wt) to protect sensitive species of birds that regularly consume aquatic invertebrates [46]. Mercury concentrations in adult macroinvertebrates are up to 2.9 times higher than in immature stages [19], which suggests that Hg concentration of adult insects from the Lyndon B. Johnson grasslands may be higher than what we observed in immature stages.



The results of the present study should not be interpreted to mean that only ponds without fish pose a risk to aquatic and terrestrial consumers. We hypothesize that in ponds with fish, part of the Hg pool is transferred from macroinvertebrates into the fish community. Therefore, ponds with fish would pose risks to fish-eating wildlife and humans, as well as to wildlife that consume invertebrates. Thus, ponds with and without fish are potential sources of Hg to biota, but the presence or absence of fish determines which types of consumers are most affected.

Small manmade ponds (both fish and fishless) are found throughout the world, with at least 2.6 million in the conterminous United States [30]. The presence of large numbers of constructed water bodies in landscapes that historically had few or no lakes or wetlands constitutes a qualitative change to the landscape, with major potential ecological consequences [30]. The results of the present study and others [31] suggest that these ecosystems may be more at risk for containing organisms with elevated Hg concentrations than has been appreciated.

#### SUPPLEMENTAL DATA

**Table S1.** Average ( $\pm$  standard error) total mercury concentration and trophic position and predator status for macroinvertebrate taxa examined in this study. (8 KB DOC).

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