MERCURY CONTAMINANTION OF MACROINVERTEBRATES FROM PONDS WITH AND WITHOUT FISH AT THE LBJ NATIONAL GRASSLAND, TEXAS

BYRON LEE HENDERSON

Bachelor of Science, 2008
Texas Christian University
Fort Worth, Texas

Bachelor of Arts, 2008
Texas Christian University
Fort Worth, Texas

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INTRODUCTION

Mercury is an environmental contaminant that causes adverse behavioral, reproductive, and neurological effects in wildlife (Weiner et al., 2003). Anthropogenic emissions resulting from human activities, such as coal combustion, waste incineration, chlor-alkali facilities, and other industrial and mining processes, account for two-thirds of annual mercury emissions into the atmosphere (Harris et al., 2003). Mercury resides in atmospheric circulation, sometimes traveling long distances before being deposited onto the Earth's surface in an inorganic form (Jackson, 1997). Inorganic mercury is non-toxic but in aquatic ecosystems sulfate-reducing bacteria convert inorganic mercury to highly toxic methylmercury (Ullrich et al., 2001). Methylmercury is the most toxic and accumulative form of mercury (Morel et al., 1998). Organisms at the base of the food web, such as phytoplankton and periphyton, concentrate methylmercury directly from the water (Miles et al., 2001), whereas consumers are exposed to methylmercury primarily through diet (Hall et al., 1997; Tsui and Wang, 2004).

Most studies on mercury in aquatic consumers focus on fish because fish consumption is an important route of mercury exposure to wildlife and humans (Weiner et al., 2003). Few studies have examined mercury contamination of macroinvertebrates, which are important in the trophic transfer of mercury to fish and wildlife (Ackerman et al., 2010). Many aquatic macroinvertebrates occur as a larval stage in the water, where they can be consumed by fish and waterfowl (Marklund et al., 2002), before metamorphosing into aerial adults, where they can be consumed by terrestrial insectivores (Gray, 1989; Nakano et al., 2001).

Macroinvertebrate species composition and biomass in ponds is strongly influenced by the presence of fish (Wellborn et al., 1996). Ponds without fish typically have macroinvertebrate communities with high biomass (Nyström et al., 2001) that is composed of large macroinvertebrate taxa, many of which are predatory. Macroinvertebrate communities in ponds with fish have a smaller biomass (Nyström et al., 2001) and contain species with adaptations to avoid fish predation like small-body size, inactivity and burrowing (Wellborn et al., 1996). These differences in macroinvertebrate biomass and species composition in ponds with and without fish could impact mercury contamination of the macroinvertebrate community. The purpose of this study was to compare the biomass, mercury concentration, and mercury burden (an estimate of the quantity of mercury within the community determined using concentration and biomass) of macroinvertebrate communities in ponds with and without fish. We also assessed mercury concentrations of fish and compared them to mercury concentrations of macroinvertebrates.

METHODS

Study Site

The study was conducted at the Lyndon B. Johnson (LBJ) National Grassland, Wise County, in north-central Texas (Fig. 1). The 8,000-ha grassland contains 62 non-contiguous management units. Primary management priorities for the grassland are maintaining quality grass cover for livestock grazing, increasing abundance of wildlife, and preventing soil erosion (J. Crooks, pers. comm.). As part of the plan to prevent soil erosion, the United States Department of Agriculture constructed many small dams, mostly in the mid-to-late 1970s. The dams created hundreds of small ponds, most of which are less than 2,000 m^2 in surface area and go dry periodically. A previous study at the LBJ Grassland identified mercury in the tissues of macroinvertebrates from grassland ponds without fish (Blackwell and Drenner, 2009). There are no known point-sources of mercury at the LBJ Grassland. The region receives 8-10 $\mu g/m^2$ annually of wet mercury deposition (NADP, 2008).

Fig. 1 – Map of Lyndon B. Johnson (LBJ) National Grassland, Wise County, Texas. Shaded areas are LBJ Grassland management units and white areas are privately-owned land. Black and white stars indicate the location of ponds in this study with and without fish, respectively.

Sampling

I sampled ten grassland ponds, five ponds with fish (n= 5) and five ponds without fish (n= 5). Study ponds were chosen from a larger pool of ponds that had been previously sampled to determine fish presence/absence. The only selection criterion was that no more than one representative of each pond type (with/without fish) could be located within a single management unit. This approach insured the ponds were distributed across the LBJ Grassland. Because macroinvertebrate communities vary seasonally in grassland ponds (Williams, 1996; Verberk et al., 2008), ponds were sampled during two seasons, spring (April - May) and summer (July - August) 2009.

I used dip nets (mesh size = 250-µm and 3 mm) and a 2.4m seine (mesh size = 5mm) to collect macroinvertebrates and fish (when present). Each pond was seined during both sampling periods to confirm the presence or absence of fish. I sampled all habitat types, including the vegetation, top layers of sediment, and the water column with dip nets. In addition, I sampled the vegetation and water column with a seine. I recorded total sampling time, regardless of sampling method, and determined catch per unit effort (mg min^{-1}) for each taxa. All macroinvertebrates collected were placed in plastic bags filled with spring water for at least 2-5 hours. A representative subsample of each fish species (typically five per length class) were euthanized immediately after collection in buffered MS-222 and stored in labeled bags on ice. Macroinvertebrates and fish were transported to a lab where they were frozen prior to further processing.

Lab Processing

Macroinvertebrates were identified to lowest taxanomic resolution, usually genus, using dichotomous keys of Merritt et al. (1996). Macroinvertebrate taxa that did not account for at least 1% of the total number individuals in ponds with or without fish, for either spring or summer samples were not included in this study. Macroinvertebrates were rinsed with deionized water prior to oven-drying at 60°C for at least 72 hours. Macroinvertebrates from each pond were pooled by taxa and the dry weight of each taxa was determined to the nearest milligram. Macroinvertebrates were ground into a fine powder using a ball-mill grinder prior to analysis.

Fish Processing

Fish were indentified to species using Thomas et al. (2007) and recorded the length of individual fish to the nearest millimeter. A fillet of epaxial muscle was dissected from individual fish and samples of skinless tissue were collected from the center of each fillet using a scalpel and forceps. All dissection equipment was rinsed with 95% ethanol and deionized water between samples. Individual samples were oven-dried at 60°C for at least 72 hours. Dried fish tissue samples were ground into a fine powder using a ball-mill grinder prior to mercury analysis. Composite samples were created for fish taxa of each pond based on length so that the smallest fish in each composite was at least 75% the length of the largest fish.

Mercury Analysis

I determined total concentrations of mercury in composite samples of macroinvertebrates and fish using a Milestone Direct Mercury Analyzer (DMA 80, Milestone, Inc. Monroe, Connecticut), which uses thermal decomposition, gold amalgamation, and atomic-absorption spectroscopy (United States Environmental Protection Agency, 1998). A calibration curve was generated using two reference materials from the National Research Council of Canada Institute for National Measurement Standards: MESS-3 (marine sediment, certified value = 91 ± 9 Ng/g total mercury [dry weight]) and DORM-2 (dogfish muscle, certified value = 4,640 ± 260 Ng/g total mercury [dry weight]). Quality assurance included reference and duplicate samples. Reference samples (MESS-3, DORM-2 or DOLT-3) were analyzed every 10 samples and the mean percent recovery was 102% (range = 96–109%; n = 53). Duplicate samples were analyzed every 20 samples and the mean relative percent difference was 2.48% (range = 0.004–10.91%; n =27). Total mercury was used as a proxy for methylmercury because 95–99% of total mercury in fish tissue is methylmercury (Bloom, 1992) and 65-95% of total mercury in omnivorous and predatory macroinvertebrates is methylmercury (Tremblay et al., 1996).

Data Analysis

I categorized all macroinvertebrate taxa as emergent, semi-emergent, and non-emergent using Merritt et al. (1996). The emergent category represents macroinvertebrate taxa adapted to emerge from aquatic habitats, after metamorphosing, as aerial adults. The semi-emergent category represents macroinvertebrate taxa adapted to emerge from the water to fly or crawl to move between aquatic habitats. The non-emergent category represents macroinvertebrate taxa adapted to spend their entire lifecycle in aquatic habitats.

I recorded the time-spent sampling each pond as a measure of effort (E). For taxa (t) of each pond, I used the method described by Cremona et al. (2008) to calculate biomass:

(1) $BM_t = W_t / E_p$

where BM_t is the biomass of each taxon within a pond in mg/min; W_t is mg DW of each taxon of macroinvertebrates within a pond; and E_p is the sampling effort of a pond in min. Then, the biomass of each taxon ($BM_{t1}, BM_{t2}, \ldots, BM_{tn}$) within a given pond (p) were added as follows:

(2) $BM_{p1} = BM_{t1p1} + BM_{t2p1} + \ldots + BM_{tnp1}$

where BM_{p1} is the macroinvertebrate community biomass within a pond in mg/min.

For taxa (t) of each pond, I calculated Hg burden with the following equation:

(3) $\text{Hg Burden}_t = [\text{Hg}]_t \times W_t$

where Hg Burden_t is in ng/min; [Hg] is in ng/g DW; and W_t is in mg DW for each taxon of macroinvertebrates within a pond. Then, the Hg burdens of each taxon (Hg Burden_{t1}, Hg Burden_{t2}, …, Hg Burden_{tn}) within a given pond (p) were added as follows:

(4) $\text{Hg Burden}_{p1} = \text{Hg Burden}_{p1t1} + \text{Hg Burden}_{p1t2} + \ldots + \text{Hg Burden}_{p1tn}$

where Hg Burden_{p1} is the macroinvertebrate community Hg burden within a pond in ng/min.

All data analyses were calculated using SPSS 15.0 (Field, 2005; SPSS, Inc., 2006). A two-way ANOVA was used to determine significant effects of season and fish presence on mean mercury concentration, total macroinvertebrate biomass and total mercury burden of macroinvertebrate communities in ponds with and without fish. These data met the assumptions of normality, confirmed by Anderson-Darling's test of normality, and equal variances, confirmed by Levene's test for equal variance. Interactions between fish presence and season were tested for all models but the interaction terms were not significant in any model. Therefore I removed the interaction term and tested for main effects of fish presence and season. Statistical significance was determined at $p \leq 0.05$ unless otherwise noted.

RESULTS

I collected 25 macroinvertebrate taxa in the ponds, including *Anax junius*, *Arigomphus* spp., *Belastoma* spp., *Buenoa* spp., *Callibaetis* spp., *Coptotomus* spp., *Cybister* spp., *Dineutus* spp., *Enallagma* spp., *Erpobdella* spp., *Gomphus* spp., *Helisoma* spp., *Hesperocorixa* spp., *Hexagenia* spp., *Libellula* spp., *Notonecta* spp., *Pantala* spp., *Physidae*, *Placobdella parasitica*, *Plathemis Lydia*, *Ranatra* spp., *Sphaeriidae*, *Streptocephalus seali*, *Tramea* spp., and *Tropisternus* spp. Species composition differed between pond types. In the spring nine macroinvertebrate taxa were unique to ponds without fish, five taxa of macroinvertebrate were unique to ponds with fish, and five taxa were shared between both pond types (Fig. 2). Many of the taxa found only in fishless ponds during the spring were found in both pond types (with/without fish) during the summer (Fig 3).

Macroinvertebrate community biomass in ponds without fish was approximately four times greater than in ponds with fish (F = 14.1, P-value = 0.002, Fig. 4a) and was approximately two times greater in the summer than in the spring (F = 4.6, P-value = 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). The mean mercury concentration of macroinvertebrate communities in ponds without fish was approximately two times greater than ponds with fish (F = 4.89, P-value = 0.041, Fig. 4b) but did not differ between seasons. Macroinvertebrate community mercury burden in ponds without fish was approximately five times greater than ponds with fish (F = 9.34, P-value = 0.007, Fig. 4c) and was approximately three times greater in summer than spring (F = 5.76, P-value = 0.028). Fish presence explained more of the variation in pond macroinvertebrate community mercury burden than season (partial $\eta^2 = 0.36$ and 0.25, respectively).

Ponds contained between one and ten species of fish including *Ameiurus melas* (black bullhead), *A. natalis* (yellow bullhead), *Gambusia affinis* (western mosquitofish), *Ictalurus punctatus* (channel catfish), *Lepomis cyanellus* (green sunfsh), *L. macrochirus* (bluegill), *L. megalotis* (longear sunfish), *L. microlophus* (redear sunfish), *Lythrurus fumeus* (ribbon shiner), *Micropterus salmoides* (largemouth bass), *Notemigonus crysoleucas* (golden shiner), *Pomoxis annularis* (white crappie), and *P. nigromaculatus* (black crappie). I determined mercury concentrations of fish tissues to assess whether mercury was bioaccumulating in these organisms. The concentration of mercury in fish tissue ranged from 371 to 1528 ng/g DW and on average was nine times greater than the mercury concentration of the macroinvertebrate community found in ponds with fish (Fig. 5).

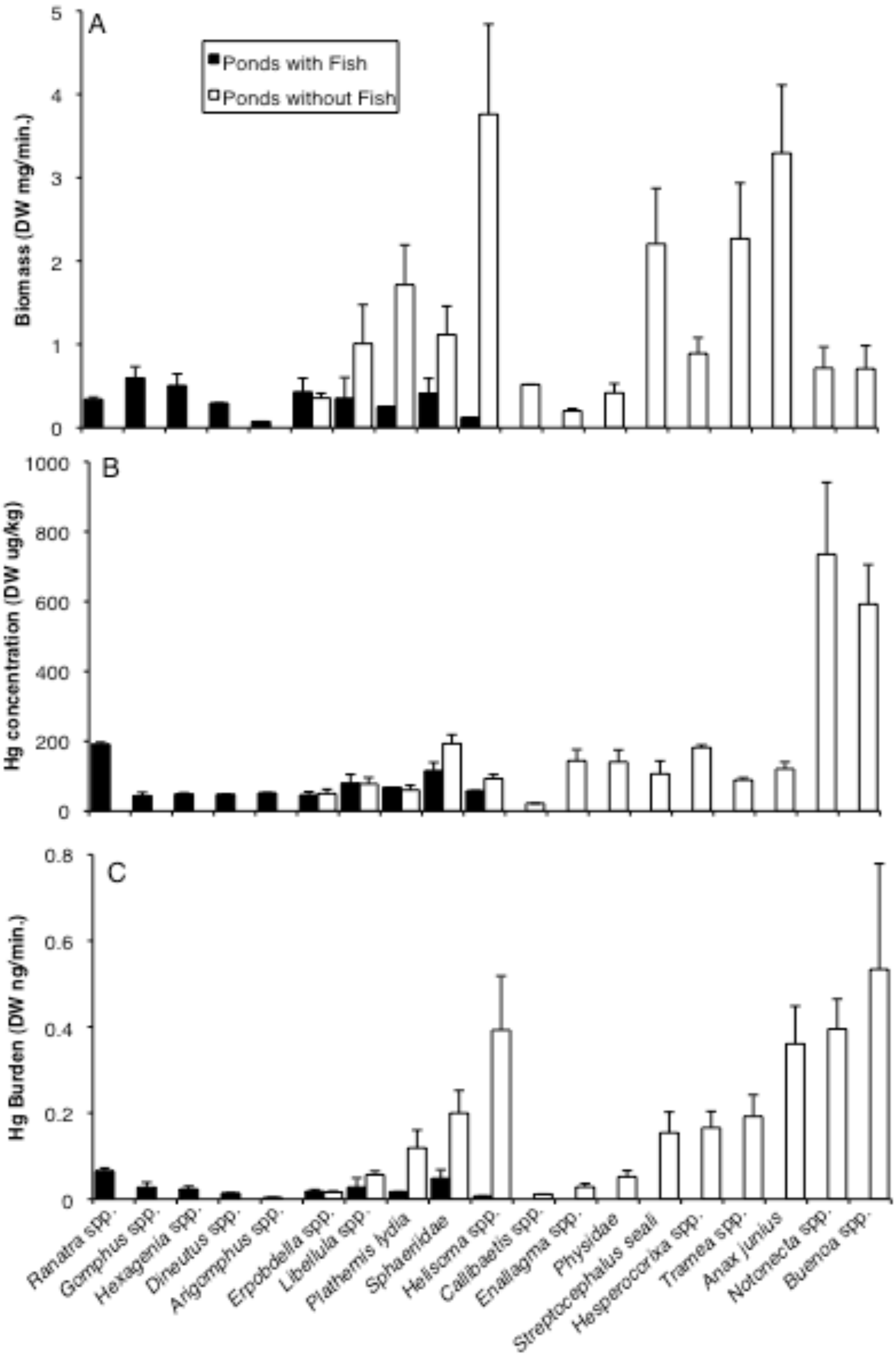

Fig. 2 – a) Mean biomass of macroinvertebrate taxa in ponds with fish and ponds without fish from spring. b) Mean mercury concentration of macroinvertebrate taxa in ponds with fish and ponds without fish from spring. c) Mean mercury burden of macroinvertebrate taxa in ponds with fish and ponds without fish from spring. Error bars represent standard error.

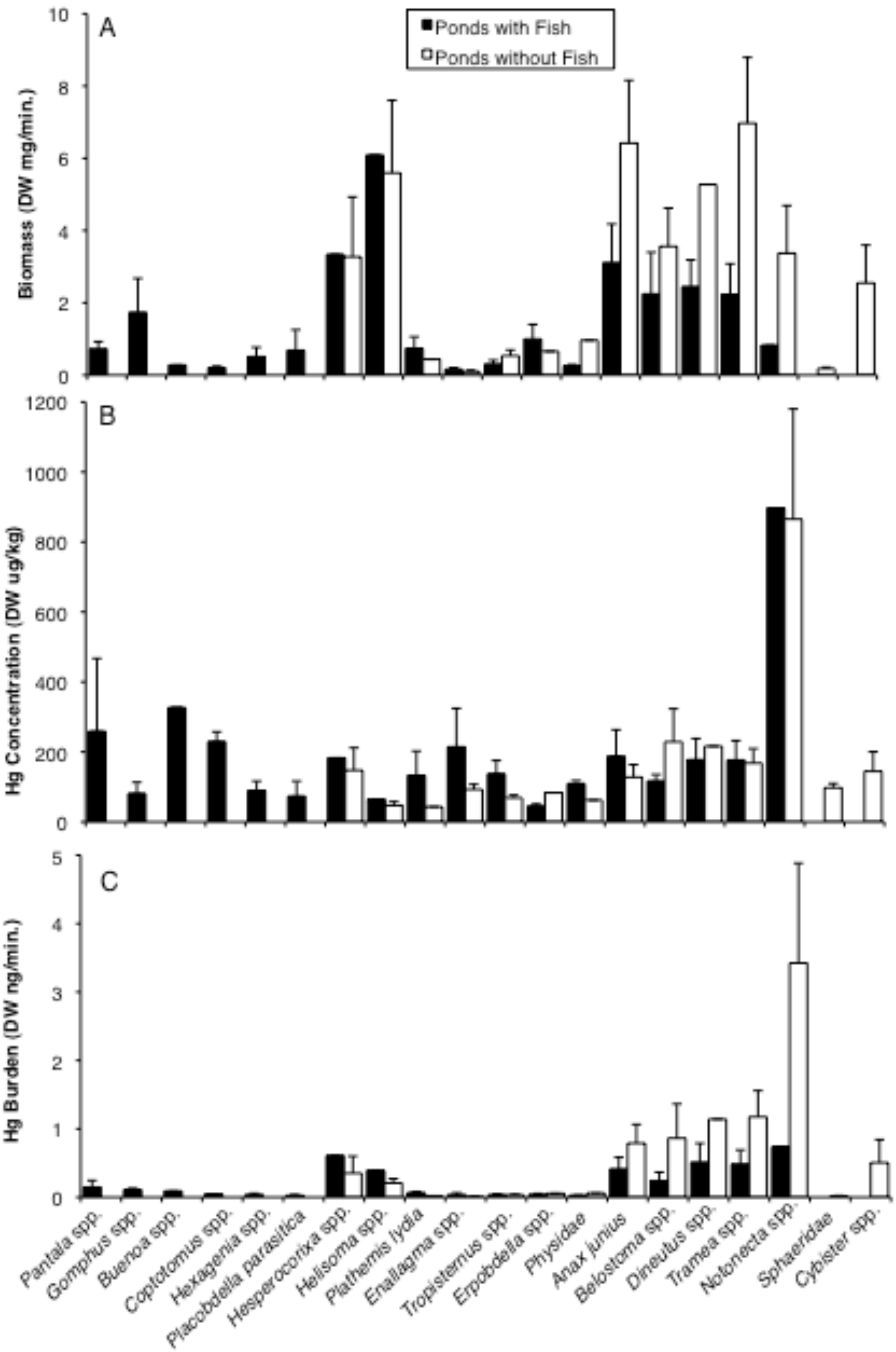

Fig. 3 – a) Mean biomass of macroinvertebrate taxa in ponds with fish and ponds without fish from summer. b) Mean mercury concentration of macroinvertebrate taxa in ponds with fish and ponds without fish from summer. c) Mean mercury burden of macroinvertebrate taxa in ponds with fish and ponds without fish from summer. Error bars represent standard error.

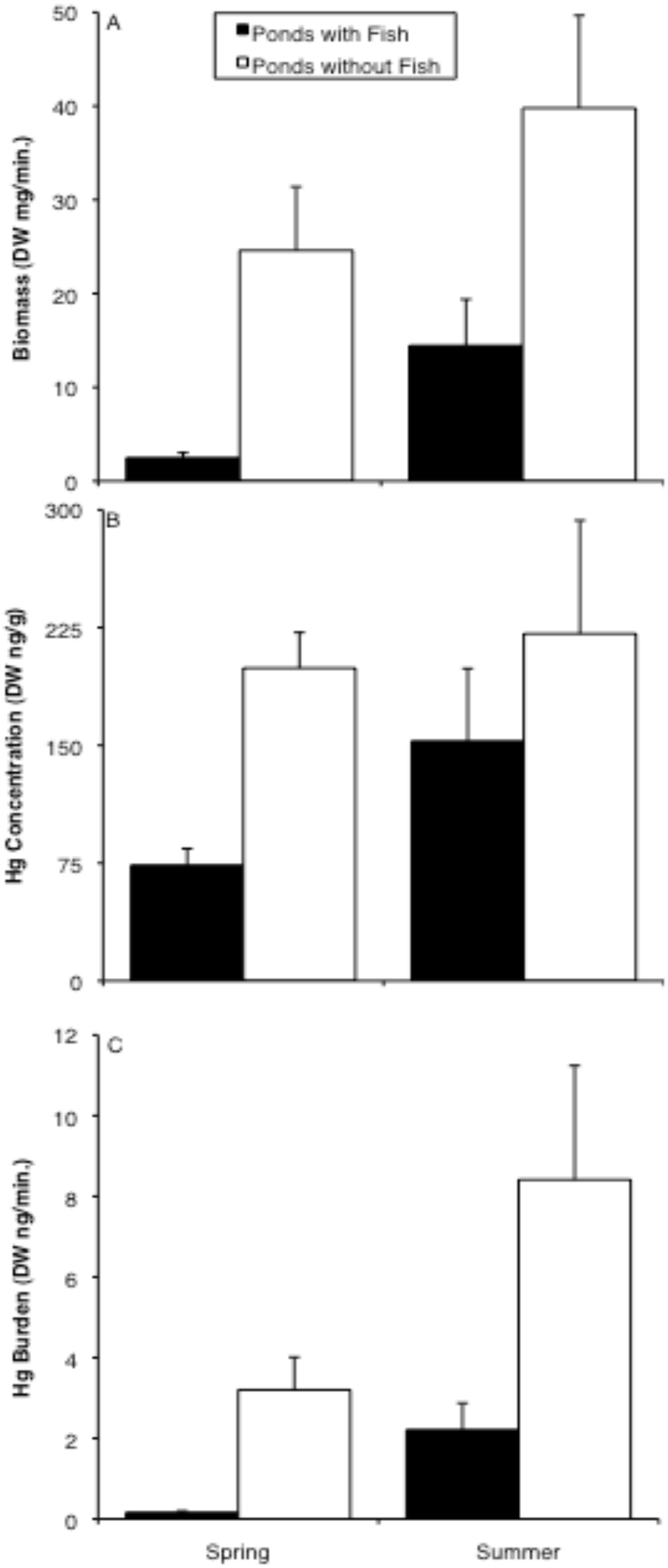

Fig. 4 – a) Mean biomass of macroinvertebrate community in ponds with fish and ponds without fish from spring and summer. b) Mean mercury concentration of macroinvertebrate community in ponds with fish and ponds without fish from spring and summer. c) Mean mercury burden of macroinvertebrate community in ponds with fish and ponds without fish from spring and summer. Error bars represent standard error.

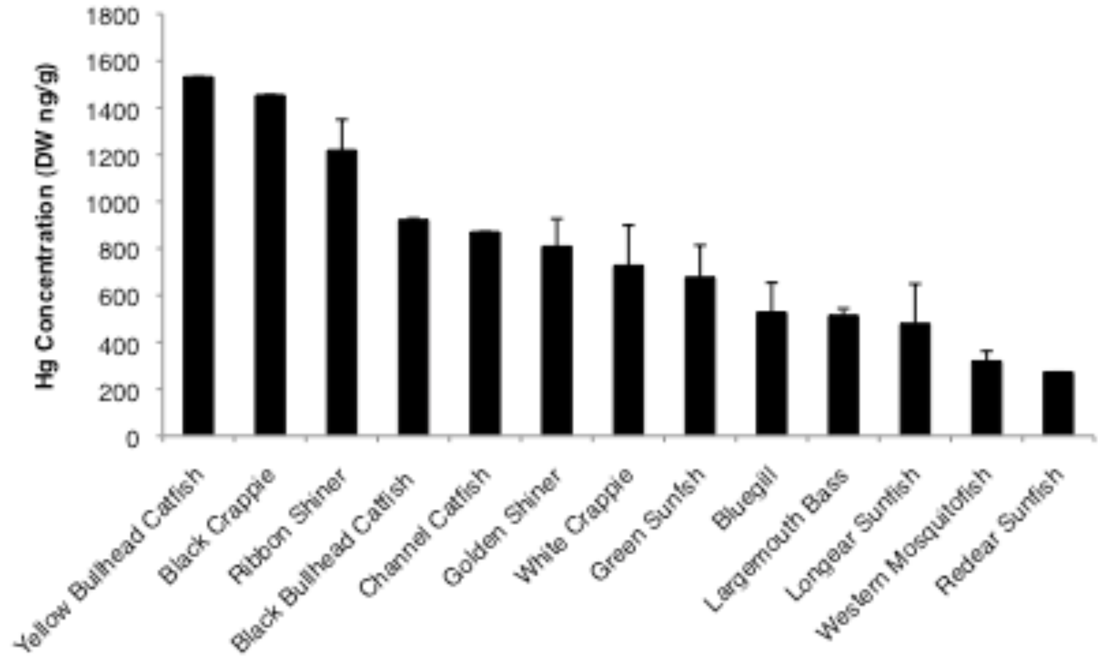

Fig. 5– Mean mercury concentration of fish taxa sampled in spring and summer. Error bars represent standard error.

DISCUSSION

The biomass of macroinvertebrates was greatest in ponds without fish. Many of the macroinvertebrate taxa unique to ponds without fish were large-bodied (*Anax junius*, *Tramea* spp., and *Enallagma* spp.), free-swimming (*Streptocephalus seali*, *Hesperocorixa* spp., *Notonecta* spp., *Buenoa* spp., and *Callibaetis* spp.), and climbing (*Physidae*) (Merritt et al., 1996), which are characteristics that would make them vulnerable to fish predation (Pope, 2009). In spring, many of the macroinvertebrate taxa unique to ponds with fish were burrowing macroinvertebrates (*Gomphus* spp., *Arigomphus* spp., and *Hexagenia* spp.), or diving macroinvertebrates (*Dineutus* spp.) (Merritt et al., 1996), characteristics which allow these organisms to avoid fish predation (Wellborn et al., 1996) (Fig. 2a). Many of the taxa found only in fishless ponds during the spring were found in both pond types (with/without fish) during the summer (Fig. 3a). Increasing macrophyte densities in the summer (personal observation) was hypothesized to provide a refuge for macroinvertebrates that are otherwise vulnerable to fish predation (Diehl and Kornijów, 1998; Hornung and Foote, 2006).

In general, macroinvertebrate taxa that were found in both ponds types had similar mercury concentrations in spring and summer (Fig 2b and Fig 3b, respectively). These findings suggest that the amounts of mercury available for uptake by the food chain in the two pond types were equivalent. However, mercury concentrations in macroinvertebrate taxa found only in ponds without fish were generally higher than taxa found only in ponds with fish. The reason for this pattern is not clear but I hypothesize differences in feeding ecology of macroinvertebrate taxa may have been a contributing factor. Although macroinvertebrates found only in ponds without fish in the spring were found in both pond types in the summer, mercury concentration was still greater in the macroinvertebrate community in ponds without fish.

The observed difference in mercury burden results from a higher biomass of most macroinvertebrate taxa in ponds without fish, combined with elevated mercury concentrations in macroinvertebrate taxa unique to ponds without fish in spring (Fig. 2c) and summer (Fig. 3c). Although I did not collect data on fish biomass and cannot determine mercury burden in the fish community, elevated concentrations of mercury in fish suggests that the majority of the mercury in ponds with fish was accumulating in fish tissue, rather than in macroinvertebrates.

Larval aquatic macroinvertebrates are important food source to aquatic and terrestrial consumers. Waterfowl, like ducks, consume macroinvertebrates in aquatic habitats (Ackerman et al., 2010). While terrestrial consumers, such as songbirds and spiders, could be exposed to mercury by macroinvertebrates that undergo metamorphosis and emerge as aerial adults or develop wings for aquatic habitat relocation (Cristol et al., 2008). Some of the macroinvertebrate mercury concentrations exceeded the threshold (100 ng/g wet weight; ca. 400-500ng/g dry weight) for items in diets of sensitive species of birds (Eisler, 1987). Because of the large difference in mercury burden between pond types, ponds without fish may represent a significant source of mercury to aquatic and terrestrial consumers. In this study thirteen species, representing 84% of the macroinvertebrate community mercury burden, in ponds without fish were adapted to emerge from aquatic habitats as aerial adults or are semi-emergent organisms.

Small ponds, like the ones examined in this study, may be more at risk for containing organisms with elevated mercury concentrations than has been appreciated. Small ponds (loosely defined to have surface areas smaller than approx. 10^4 m^2) are found in ecosystems around the globe and approximately 2.6 million small ponds are found in the conterminous United States (Smith et al., 2002). In regards to mercury contamination, small ponds are unstudied relative to their abundance. Because mercury-contaminated macroinvertebrates have negative health effects on aquatic and terrestrial consumers, future studies should focus on small ponds as sources of mercury contamination to terrestrial ecosystems, especially in the Great Plains region, where these habitats are extensive (Smith et al., 2002).

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VITA

Personal Background	Byron Lee Henderson	
	Benbrook, Texas	
	Son of Patrick and Amy Henderson	
Education	Master of Science, Environmental Science	
	Texas Christian University	
	May 2010	
	Bachelor of Science, Environmental Science	
	Texas Christian University	
	May 2008	
	Bachelor of Arts, Geography	
	Texas Christian University	
	May 2008	
	Diploma, Arlington Heights High School, Fort Worth, Texas	
	2004	
Experience	2008-2010	Teaching Assistant
		Texas Christian University
	2007-2009	GIS Analyst
		Freese and Nichols, Inc.
Professional Memberships	American Society of Limnology and Oceanography	

ABSTRACT

MERCURY CONTAMINATION OF MACROINVERTEBRATES FROM PONDS WITH AND WITHOUT FISH AT THE LBJ NATIONAL GRASSLAND, TEXAS

by Byron Lee Henderson, M.S., 2010
Department of Environmental Science
Texas Christian University

Thesis Advisor: Matthew M. Chumchal, Assistant Professor of Biology

The purpose of this study was to examine mercury in macroinvertebrate communities from grassland ponds with and without fish communities. We sampled macroinvertebrates from five ponds with fish and five ponds without fish, at the LBJ National Grassland in North Texas. In ponds without fish, the biomass of macroinvertebrates was significantly higher than in ponds with fish. The average mercury concentration of macroinvertebrates from ponds without fish was significantly higher than the average mercury concentration in ponds with fish. Because ponds without fish contained a higher biomass of macroinvertebrates and unique taxa with higher concentrations of mercury, the total amount of mercury in the macroinvertebrate community in ponds without fish was significantly higher than in the ponds with fish. In ponds with fish, the average mercury concentration of the fish community was 13 times greater than mercury concentration of the macroinvertebrates community. These data suggest that when fish are present, mercury accumulates in fish rather than in the macroinvertebrate community, which has implications for the movement of mercury into terrestrial ecosystems when macroinvertebrates emerge as aerial adults.