MERCURY CONTAMINATION OF MACROINVERTEBRATES IN EPHEMERAL GRASSLAND PONDS

by

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INTRODUCTION

Mercury is an environmental contaminant that is hazardous to the health of wildlife that consume mercury-contaminated prey (Wiener et al. 2003). Atmospheric deposition of inorganic mercury from anthropogenic atmospheric emissions is the primary source of mercury to most aquatic ecosystems (Driscoll et al. 2007). In aquatic ecosystems, inorganic mercury is converted into toxic methyl-mercury primarily by sulfur-reducing bacteria in anoxic sediments (Morel et al. 1998). Methyl-mercury biomagnifies in aquatic food webs and may reach harmful levels in top predators (Wiener et al. 2003).

In many areas of the United States, small ponds and wetlands contain water only in the rainy seasons, creating communities that are devoid of fish yet have high populations of macroinvertebrates. Because of the high populations of macroinvertebrates found in ephemeral aquatic environments, these areas offer a unique opportunity to study mercury contamination of macroinvertebrates. Relative to fish, few studies have focused on mercury contamination of aquatic macroinvertebrates, even though they are an intermediate link in the food chain and a possible pathway of contamination to wildlife and fish (Tremblay et al. 1998). The purpose of this study was to conduct a survey of mercury concentrations in macroinvertebrates of ephemeral ponds on a Texas grassland.

METHODS

STUDY AREA

The study was conducted at the Lyndon B. Johnson (LBJ) National Grassland in north-central Texas (Fig. 1). The 8000-ha grassland is made up of multiple non-contiguous units. The primary management priorities for the grassland are maintaining quality grass cover for livestock grazing, increasing wildlife abundance, and preventing soil erosion (Jim Crooks, USDA Forest Service, pers. comm.). As part of the soil erosion prevention plan, many small dams were constructed, mostly in the mid to late 1970s. These dams have created hundreds of small ponds, most of which are less than 2000 m² in surface area and go dry periodically. Although there are no known point sources of mercury on the LBJ Grassland, it is located in an area of Texas that is considered to have moderate amounts of atmospheric mercury deposition (3-10 µg/ m²/year, USEPA 1997).

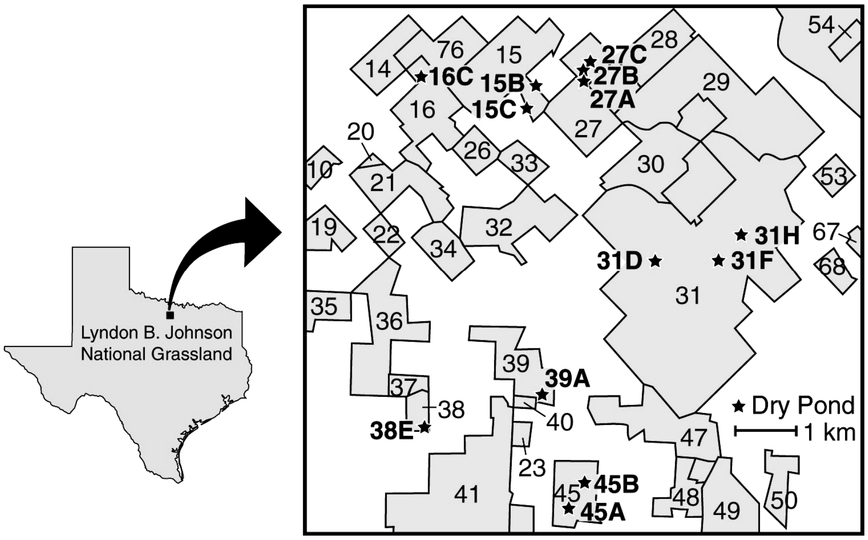

FIG. 1--Map of the LBJ National Grassland north of Decatur, TX. The gray-shaded, numbered areas are units of the grassland, and the white-shaded areas are privately-owned land. The stars indicate the locations of the ponds. Pond names are derived from the number of the unit on which they are located and an arbitrary letter assigned for identification.

FIELD METHODS

Following an extreme drought in 2005, many of the ponds on the grassland were dry. In January and February of 2006, the grassland was surveyed and dry ponds were located for potential study sites. Spring rains filled the dry ponds in late March and April of 2006, and the ponds were colonized by macroinvertebrates. Exploratory sampling of the ponds in April and May 2006 revealed immature invertebrates and few mature individuals.

In June 2006, 13 formerly dry ponds that contained high numbers of late-instar and mature macroinvertebrates were selected as sample sites. Between June 5 and 30 of 2006, a 250-µm mesh dip net was used to collect macroinvertebrates along the shorelines and in areas of aquatic vegetation. Only late-instar nymphs and adult macroinvertebrates were collected. Macroinvertebrates were sorted by taxa and placed into plastic bags filled with spring water. Macroinvertebrates were kept in the bags for 4-6 h to allow clearance of gut contents, after which the water was removed from the bags and the macroinvertebrates were frozen. Surface areas of the ponds were obtained using Google Earth or a measuring tape. Water samples were collected to analyze for total phosphorus (TP) and total nitrogen (TN) to determine trophic state of the ponds (Wetzel 2001).

LAB METHODS

Macroinvertebrates were identified using dichotomous keys of Merritt and Cummins (1996). Macroinvertebrates were sorted to family except for damselflies, which were grouped together by suborder (Zygoptera). Macroinvertebrates were rinsed with deionized water and dried at 60° C for 72 h. Macroinvertebrates from each pond were pooled together by family or suborder (five to ten individuals per taxa), ground into a fine powder using a ball-mill grinder, and refrigerated in acid-washed polypropylene vials.

Total mercury concentrations of 76 composited invertebrate samples were determined using a Milestone Direct Mercury Analyzer (DMA) 80 (Milestone, Inc. Monroe, CT), which uses thermal decomposition, gold amalgamation, and atomic absorption spectroscopy (USEPA 1998). The DMA-80 was calibrated using Canada Institute for National Measurements certified reference standards MESS-3 (certified value = 91 ± 9 ng Hg/g dry weight), PACS-2 (certified value = 3040 ± 200 ng Hg/g dry weight) and DORM-2 (certified value = 4,640 ± 260 ng Hg/g dry weight). During analysis, TORT-2 (certified value = 270 ± 60 ng Hg/g dry weight) was also analyzed as an external check standard. Previous studies have shown that a high percentage of total mercury is present as methyl-mercury in predatory macroinvertebrates (Mason et al. 2000), so total mercury was used as an indicator of methyl-mercury contamination.

Water samples were digested using a modified persulfate autoclave digestion method (Koroleff 1983). Digested samples were analyzed for TP using an Astoria segmented flow analyzer (Astoria Pacific Inc., Clackamas, OR) and TN using a Westco Smartchem (Westco Scientific Instrument, Inc., Brookfield, CT).

STATISTICS

For all statistical tests, mercury data were log-transformed to approximate normal distributions and homogeneity of variances (Quinn and Keough 2002). All analyses and data plots were generated using SPSS 15.0 (Chicago, IL). A two-way ANOVA was used to determine significant effects of taxon and pond on macroinvertebrate mercury concentrations. ANOVA post-hoc testing was performed using Tukey's test.

RESULTS

The ponds differed in surface area, nutrient concentrations, and macroinvertebrate taxa (Table 1). Surface area of the ponds ranged from 70-1925 m^2. Concentrations of TP and TN ranged from 31-307 and 670-2130 μg/L, respectively, which would classify the ponds as eutrophic (Wetzel 2001). Macroinvertebrates were collected from eight taxonomic groups: one suborder, Zygoptera, and seven families, including the beetles Gyrinidae and Hydrophilidae, dragonflies Aeshnidae and Libellulidae, and hemipterans Belostomatidae, Corixidae, and Notonectidae. Not all macroinvertebrate taxa were found in each pond, and the median value was six macroinvertebrate taxa per pond (range 4-8, SD 1.3). Only one pond contained all eight macroinvertebrate taxa.

Pond	Area (m^2)	TP (μg/L)	TN (μg/L)	A	B	C	G	H	L	N	Z
15B	70	307	1830	X	X	---	---	X	X	---	---
15C	590	50	1040	X	X	X	X	X	X	X	X
16C	995	55	1440	---	X	X	X	X	X	X	X
27A	1700	42	670	X	X	---	X	X	X	X	X
27B	255	85	1820	X	---	X	---	X	X	X	---
27C	1925	56	1210	X	---	X	---	---	X	X	X
31D	1485	52	820	---	X	X	X	X	X	X	---
31F	790	34	680	X	X	X	---	X	X	X	X
31H	480	31	870	---	X	---	X	X	X	---	---
38E	720	37	2130	X	X	---	---	X	X	X	---
39A	345	163	2080	X	X	X	---	X	---	X	---
45A	1630	69	1580	X	X	---	X	X	X	X	X
45B	395	82	2020	X	X	X	---	X	X	X	---

TABLE 1--Pond name, surface area, nutrient concentrations, and presence (X) or absence (---) of taxa (A, Aeshnidae; B, Belostomatidae; C, Corixidae; G, Gyrinidae; H, Hydrophilidae; L, Libellulidae; N, Notonectidae; Z, Zygoptera).

Mercury concentrations of macroinvertebrates varied among the taxa and the ponds (Fig. 2 and Fig. 3, respectively). Results of the two-way ANOVA showed that both taxa and pond had a significant effect on mercury concentrations ($F = 34.9$, $p < 0.001$; $F = 10.6$, $p < 0.001$, respectively). However, because not all taxa were collected from each pond, the ANOVA test could be confounded. For this reason, the ANOVA was also run on a subset of six ponds (15C, 27A, 31F, 38E, 45A, 45B) that contained five taxa common to each of the six ponds (Aeshnidae, Belostomatidae, Hydrophilidae, Libellulidae, Notonectidae). The ANOVA detected a significant effect of taxa and pond on mercury concentrations of macroinvertebrates in the subset of data ($F = 49.9$, $p < 0.001$; $F = 12.1$, $p < 0.001$, respectively).

Using Tukey's post-hoc testing with the entire data set, I determined that mercury concentrations of taxa were statistically different from each other. Notonectidae mercury concentrations were significantly higher ($p < 0.001$), and Hydrophilidae mercury concentrations were significantly lower ($p < 0.005$) than all other taxa. Aeshnidae mercury concentrations were significantly higher than Libellulidae ($p = 0.012$).

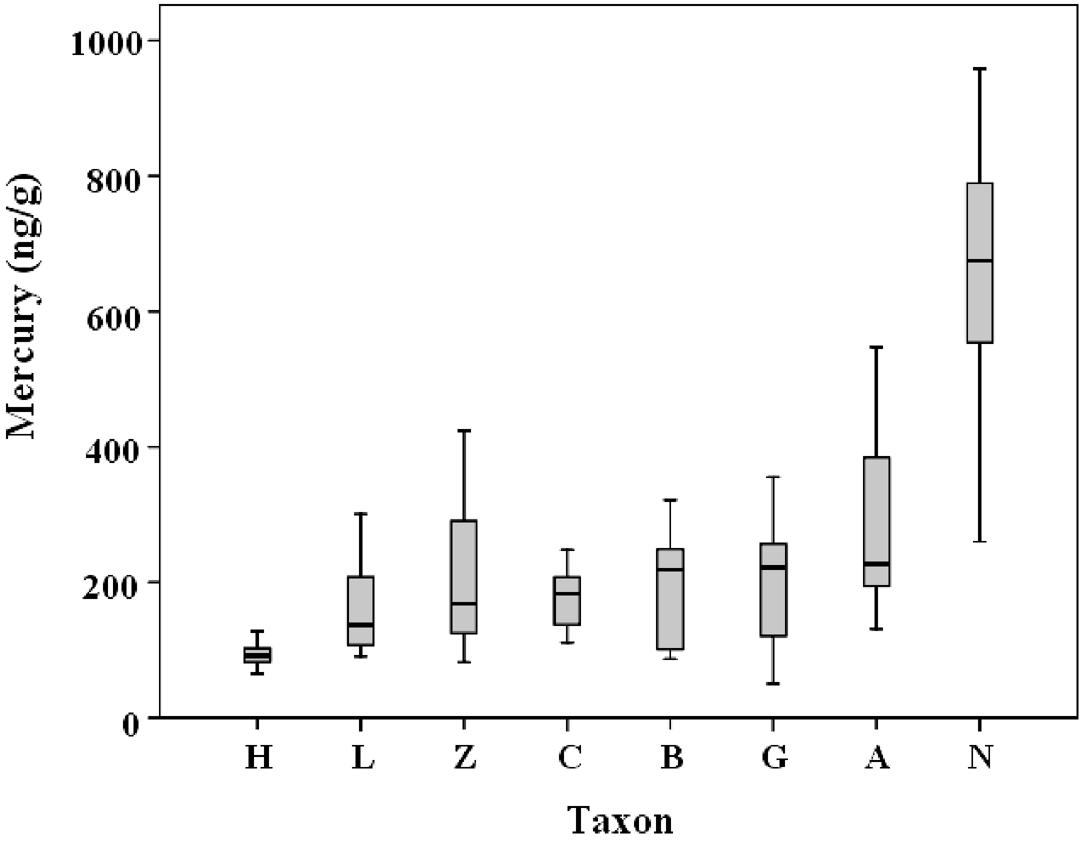

FIG.2--Box-and-whisker plot of mercury concentrations in macroinvertebrate taxa. The horizontal black line within each box represents the median, and the ends of the boxes represent the upper and lower quartiles. The ends of the whiskers mark the 5th and 95th percentiles. (A, Aeshnidae; B, Belostomatidae; C, Corixidae; G, Gyrinidae; H, Hydrophilidae; L, Libellulidae; N, Notonectidae; Z, Zygoptera).

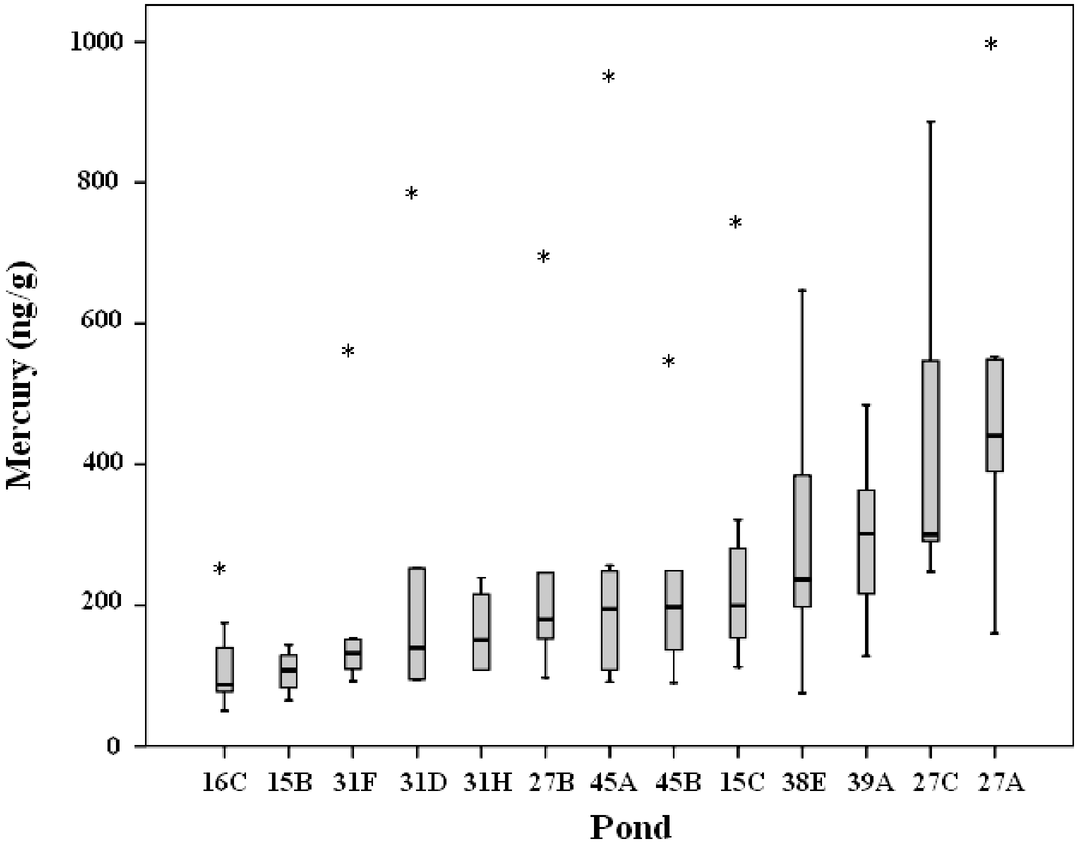

FIG. 2--Box-and-whisker plot of mercury concentrations of macroinvertebrates in ponds. See Figure 2 for an explanation of the plot. Stars represent statistical outliers, all of which are Notonectidae samples.

DISCUSSION

Mercury concentrations of macroinvertebrates differed significantly among the invertebrate taxa. Other studies of macroinvertebrates have also demonstrated wide variation in mercury concentrations both within and among taxa (Table 2). The highest mercury concentrations in this study were found in Notonectidae, a top predator in fishless ponds. Other studies also found high mercury concentrations in Notonectidae (Hall et al. 1998; Allen et al. 2005).

	Aeshnidae	Corixidae	Gyrinidae	Libellulidae	Notonectidae
Parkman and Meili 1993	97-593			93-487	
Tremblay et al. 1996[a]	34-276	66-793	22-405		
Hall et al. 1998[b]	19.4-645.2	36.9-462.8	13.1-172.7		25.9-845.8
Haines et al. 2003[a]	487-523			183-280	
Allen et al. 2005[ab]	30-247				118-219
This study	131-545	111-248	50-355	90-441	260-959

[a] Study reported average mercury concentrations from multiple samples at each study site

[b] Reported numbers are for methyl-mercury rather than total mercury

TABLE 2--Comparisons of invertebrate mercury concentrations in this study to previous studies of lentic systems. All concentrations are reported as ng Hg/g dry weight.

Trophic level is considered to be an important factor influencing mercury concentrations of macroinvertebrates because predatory macroinvertebrates generally have higher mercury concentrations than herbivorous or omnivorous macroinvertebrates (Tremblay et al. 1996). Difference in trophic level might explain why the omnivorous Hydrophilidae had lower mercury concentrations than the other taxa, which were mostly predators. Even among predatory taxa, significant differences in mercury were detected. Past studies have hypothesized that feeding habit accounts for differences in mercury among taxa (Parkman and Meili 1993; Tremblay et al. 1996), and this might explain some of the mercury differences among predators. For example, Notonectidae, Belostomatidae, and Aeshnidae are highly predatory species, but each has different feeding behaviors, including different mouthparts that feed on different tissues in prey items, which could result in differences in mercury accumulation.

Mercury concentrations of macroinvertebrates also differed among the ponds. Mercury in biota from different bodies of water is affected by numerous environmental factors, including atmospheric mercury loading (Orihel et al. 2007), plankton densities (Chen and Folt 2005), pH (Lange et al. 1993), food-web structure (Cabana et al. 1994), lake and watershed area (Chen et al. 2005), dissolved organic carbon (Driscoll et al. 1995), and hydroperiod (Snodgrass et al. 2000). The combined effects of the physical, chemical, and biological properties of each watershed ultimately determine the amount of mercury that is available to the macroinvertebrates of each pond. Differences in environmental variables between aquatic ecosystems can produce variation in mercury concentrations of biota, even in systems that are in close proximity to each other and have similar mercury inputs. In a study of nine southeastern depression wetlands, Snodgrass et al. (2000) found that variation in mercury concentrations among wetlands was greater than variation among fish species. In my study, mean mercury concentrations of macroinvertebrates in ponds varied by approximately a factor of five even though the ponds were within a few km of each other.

Aquatic ecosystems are sites of methyl-mercury production, potentially resulting in methyl-mercury contamination of surrounding areas (Rudd 1995). Dams prevent water from flowing downstream except in times of high rainfall when ponds can overflow and deliver methyl-mercury to downstream environments. The emergence of insects from ponds provides another transport pathway of methyl-mercury to surrounding environments. Many macroinvertebrates spend only a portion of their life cycle in aquatic environments and inhabit terrestrial environments as adults. A study of Canadian reservoirs and a natural lake estimated the flux of methyl-mercury from emerging insects to be between 55 and 224 ng Hg m^2 year $^{-1}$ (Tremblay et al. 1998). These systems contained fish and Tremblay et al. (1998) estimated that about half of immature insects were consumed by fish. They concluded that insects were an important mercury source for predators that consume them. At the LBJ Grassland, fish were not present in the ephemeral ponds, and populations of macroinvertebrates were very high in these ponds. Surveys of nine nearby grassland ponds with fish revealed few macroinvertebrates**.** Because of the lack of fish predation and high populations of macroinvertebrates, I would hypothesize that the mercury flux from the ephemeral ponds would be higher than ponds with fish. This hypothesis could be tested by examining mercury flux via macroinvertebrate emergence from ponds with and without fish.

Because some macroinvertebrates from my study had higher mercury concentrations than fish from other studies (Snodgrass et al. 2000; Swanson et al. 2006), wildlife feeding on these macroinvertebrates could be exposed to the same risks of mercury contamination as piscivorous wildlife. Studies of mercury concentrations in terrestrial and semi-aquatic wildlife provide evidence of mercury leaving aquatic environments and contaminating terrestrial consumers. Mercury body burdens in tree swallows (*Tachycineta bicolor*) living near a reservoir increased by nearly 1,000 ng after the reservoir was flooded, an increase that was attributed to the tree swallows feeding on insects emerging from the reservoir (Gerrard and St. Louis 2001). Insectivorous birds, including red-winged blackbirds (*Agelaius phoeniceus)* and song sparrows (*Melospiza melodia*) that I observed at the LBJ Grassland, have also been shown to contain moderate to high concentrations of mercury in their blood (Evers et al. 2005). Approximately 20% of invertebrate samples from my study were above the recommended threshold (100 ng/g live weight; ~ 400-500 ng/g dry weight) in diet items of sensitive bird species (Eisler 1987), which indicates that these ponds could pose a contamination threat to wildlife on the LBJ Grassland.

In conclusion, ephemeral ponds could serve as an important source of mercury to terrestrial ecosystems. The ponds in my study were originally designed to prevent soil erosion, and similar ponds are widely distributed throughout the prairie areas of the United States. Because of their high nutrient concentrations and lack of fish predation, these ponds are major sites of aquatic macroinvertebrate production. Their anoxic sediments and frequent drying and re-flooding promote methyl-mercury production. Therefore, the benefits of building dams to combat soil erosion may be partially offset by the production of methyl-mercury within the ponds and the transport of methyl-mercury to surrounding terrestrial food chains and wildlife. In my study, the mercury concentrations of some macroinvertebrates were above recommended thresholds for consumption by bird species. Future studies need to address transport of mercury by macroinvertebrates from ephemeral ponds to terrestrial food webs and its potential impact on wildlife.

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ABSTRACT

MERCURY CONTAMINATION OF MACROINVERTEBRATES IN EPHEMERAL GRASSLAND PONDS

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This study surveyed mercury concentrations of aquatic macroinvertebrates collected from ephemeral ponds on the Lyndon B. Johnson National Grassland, Texas. Macroinvertebrates representing eight taxonomic groups were collected from 13 ponds in June 2006. Significant differences in mercury concentrations were detected among the taxonomic groups, with the omnivore Hydrophilidae and the predator Notonectidae containing the lowest and highest concentrations of mercury, respectively. I also detected significant differences in mercury concentrations of macroinvertebrates in the different ponds. The mercury concentrations of some macroinvertebrates were above recommended thresholds for consumption by bird species. This study suggests that ephemeral ponds can produce large populations of mercury-contaminated macroinvertebrates that could be harmful to aquatic and terrestrial consumers.