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MERCURY CONTAMINATION OF MACROINVERTEBRATES IN FISHLESS GRASSLAND PONDS

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

RESUMEN—Se evaluó la concentración de mercurio en colecciones de macroinvertebrados acuáticos provenientes de estanques sin peces del Lyndon B. Johnson National Grassland, condado de Wise, Texas, USA. Macroinvertebrados representando ocho grupos taxonómicos fueron recolectados en junio del 2006 en 13 estanques. Se detectaron diferencias significativas en las concentraciones de mercurio entre los grupos taxonómicos, con el omnívoro Hydrophilidae y el depredador Notonectidae conteniendo la más baja y la más alta concentración de mercurio, respectivamente. También se detectaron diferencias significativas en las concentraciones de mercurio en los macroinvertebrados entre los estanques. La concentración de mercurio en algunos macroinvertebrados estuvo por encima de los límites para el consumo por aves. Nuestro estudio sugiere que los estanques sin peces pueden producir grandes poblaciones de macroinvertebrados contaminados con mercurio que podrían ser dañinos para consumidores terrestres y acuáticos.

Mercury is an environmental contaminant that can be 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 temporarily contain water only in rainy seasons, creating communities that are devoid of fish yet have high populations of macroinvertebrates. Because of the large populations of macroinvertebrates in temporary 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, although they are an intermediate link in the food chain and a possible pathway of contamination to wildlife (Tremblay et al., 1998). Frequent drying and rewetting cycles that are common in temporary ponds and wetlands stimulate production of methyl-mercury (Driscoll et al., 2007) and increase the likelihood that macroinvertebrates in these systems contain high concentrations of mercury. The purpose of this study was to quantify concentrations of mercury in macroinvertebrates of fishless ponds in a grassland in Texas and evaluate the potential of fishless systems to introduce mercury into aquatic and terrestrial food chains.

Materials and Methods—The study was conducted at the Lyndon B. Johnson National Grassland, Wise County, in north-central Texas (Fig. 1). The 8,000-ha grassland contains multiple non-contiguous 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, many small dams were constructed, mostly in the mid-to-late 1970s. These dams created hundreds of small ponds, most of which are <2,000 m^2 in surface area and go dry periodically. Although there is no known point source of mercury on the Lyndon B. Johnson Grassland, it is located in an area of Texas that is considered to have moderate amounts of deposition of atmospheric mercury (3–10 μg/m^2/year; United States Environmental Protection Agency, 1997).

Following an extreme drought in 2005, many ponds on the grassland were dry. In January and February 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 2006, and ponds were colonized by macroinvertebrates. Exploratory sampling of ponds in April and May 2006 revealed immature invertebrates and few late-instar individuals.

In June 2006, 13 formerly dry ponds that contained large numbers of late-instar and mature macroinvertebrates were selected as sample sites. Ponds selected for this study had maximum depths ≤1.5 m, and in 2006, ponds contained water mid-March through mid-August. Hydrologic patterns of the ponds in previous years are unknown, but inspection of ponds before they filled revealed no remains of fish, indicating that the ponds experience regular drying events. All nearby ponds that maintain water year-round contained populations of fish. A survey of grassland ponds revealed that fishless ponds had larger-bodied crustacean zooplankton than ponds with fish (Drenner, 2008).

During 5–30 June 2006, a 250-μm mesh dip net was used to collect macroinvertebrates along shorelines and in areas of aquatic vegetation. Only late-instar nymphs and adult macroinvertebrates were collected. Macroinvertebrates were sorted to taxa and placed into plastic bags filled with bottled spring water. Macroinvertebrates were kept in the bags for 4–6 h to allow clearance of intestinal contents, after which the water was removed from the bags and the macroinvertebrates were frozen. Surface areas of ponds were obtained using Google Earth or a measuring tape. Water samples were collected to analyze for total phosphorus and total nitrogen to determine trophic state of the ponds (Wetzel, 2001).

Macroinvertebrates were identified using dichotomous keys of Merritt and Cummins (1996). Macroinvertebrates were sorted to family except for members of the suborder Zygoptera, which were grouped together so that samples would be sufficient for analysis of mercury. Macroinvertebrates were rinsed with deionized water and oven-dried at 60°C for 72 h. Macroinvertebrates from each pond were pooled together by family or suborder (5–10 individuals/taxa), ground into a fine powder using a ball-mill grinder, and refrigerated in acid-washed polypropylene vials.

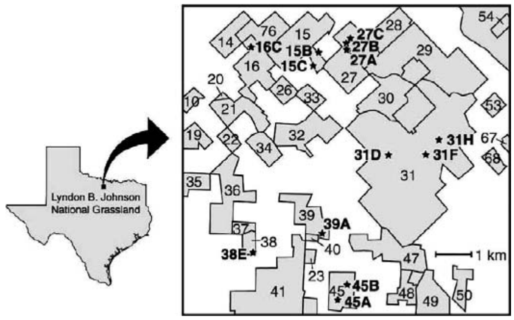

Fig. 1—Gray-shaded, numbered areas are units of the Lyndon B. Johnson National Grassland, Wise County, Texas, and white areas are privately-owned land. Stars with numbered letters indicate names and locations of ponds where concentrations of mercury in macroinvertebrates were assessed.

Total concentrations of mercury in 76 composited samples of invertebrates were determined 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). 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 = 3,040 ± 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) also was analyzed as an external check standard. Previous studies have shown that ca. 65–95% of total mercury is present as methyl-mercury in omnivorous and predatory macroinvertebrates (Tremblay et al., 1996), 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 total phosphorus using an Astoria segmented flow analyzer (Astoria Pacific Inc., Clackamas, Oregon) and total nitrogen using a Westco Smartchem (Westco Scientific Instrument, Inc., Brookfield, Connecticut).

For all statistical tests, data for mercury 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 (Field, 2005; SPSS, Inc., 2006). A two-way ANOVA was used to determine significant effects of taxon and pond on concentrations of mercury in macroinvertebrates. ANOVA post-hoc testing was performed using Tukey's test (Field, 2005; SPSS, Inc., 2006).

Results—The ponds differed in surface area, concentrations of nutrients, and taxa of macroinvertebrates (Table 1). Surface area of ponds were 70–1,925 m^2. Concentrations of total phosphorus and total nitrogen were 31–307 and 670–2130 μg/L, respectively, which would classify the

TABLE 1—Pond, surface area, concentrations of nutrients, and presence (X) or absence (—) of taxa on the Lyndon B. Johnson National Grassland, Wise County, Texas. Names of ponds correspond to those in Fig. 1.

Pond	Area (m^2)	Total phosphorus (µg/L)	Total nitrogen (µg/L)	Aeshnidae	Belostomatidae	Corixidae	Gyrinidae	Hydrophilidae	Libellulidae	Notonectidae	Zygoptera
15B	70	307	1,830	X	X	—	—	X	X	—	—
15C	590	50	1,040	X	X	X	X	X	X	X	X
16C	995	55	1,440	—	X	X	X	X	X	X	X
27A	1,700	42	670	X	X	—	X	X	X	X	X
27B	255	85	1,820	X	—	X	—	X	X	X	—
27C	1,925	56	1,210	X	—	X	—	—	X	X	X
31D	1,485	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	2,130	X	X	—	—	X	X	X	—
39A	345	163	2,080	X	X	X	—	X	—	X	—
45A	1,630	69	1,580	X	X	—	X	X	X	X	X
45B	395	82	2,020	X	X	X	—	X	X	X	—

ponds as eutrophic (Wetzel, 2001). Macroinvertebrates were collected from eight taxonomic groups: one suborder, Zygoptera, and seven families, including the coleopterans Gyrinidae and Hydrophilidae, odonates Aeshnidae and Libellulidae, and hemipterans Belostomatidae, Corixidae, and Notonectidae. Not all macroinvertebrates were in each pond, and the median value was 6 taxa/pond (range 4–8, *SD* 1.3). Only one pond contained all eight taxa of macroinvertebrates.

Concentrations of mercury in macroinvertebrates varied among taxa and ponds (Figs. 2 and 3, respectively). Results of the two-way ANOVA showed that both taxa and pond had a significant effect on concentrations of mercury ($F = 34.9$, $P < 0.001$; $F = 10.6$, $P < 0.001$, respectively). However, because not all taxa were collected from each pond, the ANOVA could be confounded. For this reason, ANOVA also was used to assess 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). ANOVA detected a significant effect of taxa and pond on concentrations of mercury in 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 dataset, we determined that concentrations of mercury in taxa were statistically different from each other. Concentrations of mercury in Notonectidae were significantly higher ($P < 0.001$) and concentrations of mercury in Hydrophilidae were significantly lower ($P < 0.005$) than all other taxa. Concentrations of mercury in Aeshnidae were significantly higher than Libellulidae ($P = 0.012$).

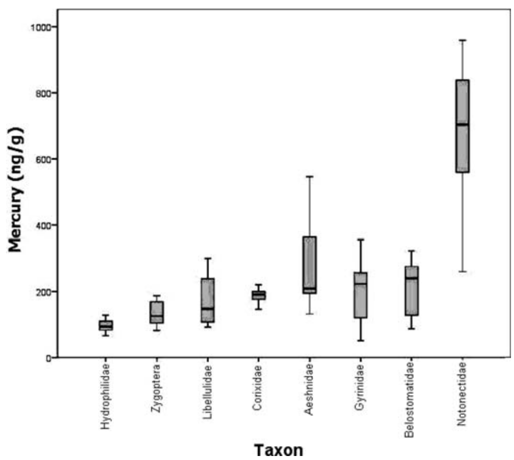

FIG. 2—Concentrations of mercury in macroinvertebrates on the Lyndon B. Johnson National Grassland, Wise County, Texas: the horizontal line within each box represents the median, ends of boxes represent upper and lower quartiles, and ends of vertical lines mark 5th and 95th percentiles.

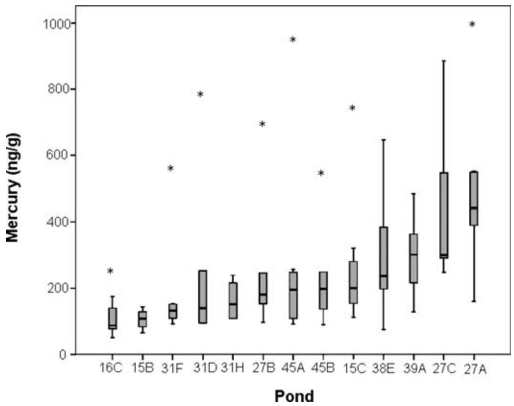

FIG. 3—Concentrations of mercury in ponds on the Lyndon B. Johnson National Grassland, Wise County, Texas: the horizontal line within each box represents the median, ends of boxes represent upper and lower quartiles, ends of vertical lines mark 5th and 95th percentiles, and asterisks represent statistical outliers, all of which were samples of Notonectidae. Names of ponds on the X-axis correspond to those in Fig. 1.

DISCUSSION—Concentrations of mercury in macroinvertebrates differed significantly among taxa. Other studies of macroinvertebrates also have revealed wide variation in concentrations of mercury within and among taxa (Table 2). The highest concentrations of mercury in this study were in Notonectidae, a top predator in fishless ponds. Other studies also reported high concentrations of mercury in Notonectidae (Hall et al., 1998; Allen et al., 2005). Trophic level is considered to be an important factor influencing concentrations of mercury in macroinvertebrates because predatory macroinvertebrates generally have higher concentrations of mercury than herbivorous or omnivorous macroinvertebrates (Tremblay et al., 1996). Difference in trophic level might explain why the omnivorous Hydrophilidae had lower concentrations of mercury than other taxa, which were mostly predators. Even among predatory taxa, significant differences in mercury were detected. Past studies have hypothesized that feeding habits account for differences in mercury among taxa (Parkman and Meili, 1993; Tremblay et al., 1996), and this might explain some of the 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 accumulation of mercury.

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

TABLE 2—Concentrations of mercury (ng Hg/g dry weight) in invertebrates in this study at Lyndon B. Johnson National Grassland, Wise County, Texas, and concentrations reported in previous studies of lentic systems.

Source	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] Reported average concentrations of mercury from multiple samples at each study site.
[b] Reported numbers are for methyl-mercury rather than total concentration of mercury.

our study, mean concentrations of mercury in macroinvertebrates in ponds varied by about a factor of five, although ponds were within a few kilometers of each other.

Aquatic ecosystems are sites of production of methyl-mercury, potentially resulting in contamination of surrounding areas by methyl-mercury (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. 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 55–224 ng Hg m^2 year $^{-1}$ (Tremblay et al., 1998). These systems contained fish and Tremblay et al. (1998) estimated that ca. 50% of immature insects were consumed by fish. They concluded that insects were an important source of mercury for predators that consume them. At the Lyndon B. Johnson Grassland, fish were not present in the temporary ponds, and populations of macroinvertebrates were large in these ponds. Nine nearby grassland ponds that contained fish were rigorously sampled using the same methods that were used for sampling fishless ponds, but these surveys yielded few macroinvertebrates. Because of the lack of predation by fish and large populations of macroinvertebrates, we hypothesize that the flux in mercury from temporary ponds would be higher than ponds with fish. This hypothesis could be tested by examining flux in mercury via emergence of macroinvertebrates from ponds with and without fish.

Because some macroinvertebrates from our study had higher concentrations of mercury 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 contamination with mercury as piscivorous wildlife. Studies of concentrations of mercury in terrestrial and semi-aquatic wildlife provide evidence of mercury leaving aquatic environments and contaminating terrestrial consumers. Body burdens of mercury 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 we observed at the Lyndon B. Johnson Grassland, also have been shown to contain moderate to high concentrations of mercury in their blood (Evers et al., 2005). Some samples of invertebrates from our study were above the threshold (100 ng/g live weight; ca. 400–500 ng/g dry weight) for items in diets of sensitive species of birds (Eisler, 1987), which indicates that these ponds could pose a contamination threat to wildlife on the Lyndon B. Johnson Grassland. Mercury in emerging insects may be further amplified in the food chain if it moves into intermediate terrestrial-invertebrate predators that are consumed by birds or bats. For example, spiders feeding near a mercury-contaminated river had higher total mercury than fish (Cristol et al., 2008). By feeding on terrestrial spiders, birds can functionally increase the length of their food chains and increase biomagnification of mercury from aquatic macroinvertebrates to terrestrial birds (Cristol et al., 2008). This would suggest that macroinvertebrates that are low in mercury could still be the source of harmful amounts of mercury to terrestrial predators depending on its biomagnification through the food chain.

In conclusion, temporary fishless ponds could serve as an important source of mercury to terrestrial ecosystems. Ponds in our study were originally designed to prevent soil erosion, and similar ponds are widely distributed throughout prairie areas of the United States. Because of their high concentrations of nutrients and lack of predation by fishes, these ponds are major sites of production of aquatic macroinvertebrates. Their anoxic sediments and frequent drying and re-flooding promote production of methyl-mercury. Therefore, benefits of building dams to combat soil erosion may be partially offset by production of methyl-mercury within ponds and transport of methyl-mercury to surrounding terrestrial food chains and wildlife. Some macroinvertebrates collected in this study contained concentrations of mercury above the mercury threshold for consumption by wildlife. Macroinvertebrates emerging from fishless aquatic systems represent a potentially significant pathway for introduction of methyl-mercury into terrestrial food chains, especially to invertebrateconsuming birds and bats that feed in the vicinity of aquatic and semi-aquatic environments. Future studies need to address transport of mercury by macroinvertebrates from temporary fishlesss ponds to terrestrial food webs and its potential impact on wildlife.

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