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Mercury Concentrations in Birds from Two Atmospherically Contaminated Sites in North Texas, USA

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Abstract Mercury (Hg) is a ubiquitous and highly toxic contaminant that can have negative effects on wildlife. Only a few studies have measured Hg concentrations in birds from the south central United States, and the potential threat of Hg contamination to birds in this region is largely unknown. In the present study, we assess Hg concentrations in blood and feathers from five bird species [eastern bluebird (*Sialis sialis*), Carolina wren (*Thryothorus ludovicianus*), wood duck (*Aix sponsa*), great egret (*Ardea alba*), and great blue heron (*Ardea herodias*)] that occupy different trophic levels at Caddo Lake and Lewisville Lake, located in northeast and north central Texas, respectively. Both sites are contaminated with Hg from the atmosphere. Adult passerines had higher Hg concentrations in their blood than conspecific nestlings. Mercury concentrations in feathers differed between species by more than an order of magnitude with large piscivorous species having higher concentrations than smaller insectivorous species. Mercury concentrations in eastern bluebirds were higher at Caddo Lake than Lewisville Lake. The present study represents one of the first studies of Hg concentrations in multiple bird species in north Texas and suggests that Hg concentrations in birds from atmospherically polluted sites in this region may be high enough to compromise fitness in those species.

Methyl mercury (MeHg) is a ubiquitous and highly toxic contaminant that negatively effects the nervous, reproductive, and immune systems of vertebrates, including birds (Scheuhammer et al. 2007; Franceschini et al. 2009). Although mercury (Hg) can be discharged into aquatic systems directly from point sources resulting in local contamination issues, the source of Hg in most ecosystems is atmospheric deposition (Selin 2009). Atmospherically deposited Hg originates from natural and anthropogenic emission sources, the largest of which include fossil fuel combustion and artisanal or small-scale gold mining (Krabbenhoft and Sunderland 2013). Once emitted, mercury can remain in the atmosphere for long periods of time (months to more than a year) and be deposited far from the original source, which has resulted in most areas of the planet becoming contaminated with levels of Hg that exceed pre-industrial baselines (Selin 2009). Mercury is deposited in relatively non-toxic inorganic forms but it can be converted to the toxic and accumulative methyl form by microbes in aquatic systems (Selin 2009). Primary producers concentrate MeHg directly from the water (Miles et al. 2001; Pickhardt and Fisher 2007), but consumers are exposed to MeHg through their diet (Hall et al. 1997; Tsui and Wang 2004; Pickhardt et al. 2006). MeHg biomagnifies in food webs reaching concentrations in higher order organisms, like birds, that are millions of times greater than that found near the base of the food web (Lavoie et al. 2013).

Concern exists that Hg contamination has contributed to population declines in birds (Braune et al. 2006; Edmonds et al. 2010; Seewagen 2010), but relatively few studies

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have examined Hg concentrations in birds compared to well-studied taxa like fish. Previous surveys of Hg concentrations in North American birds at sites contaminated by atmospheric deposition have focused on the northeastern U.S. (Evers et al. 1998, 2007), the Great Lakes (Evers et al. 1998), the Everglades (Frederick et al. 2004), and sites in western Canada (Hipfner et al. 2011; Guigueno et al. 2012). Little information is available regarding Hg concentrations in birds from other areas in North America not directly linked to a point source. In particular, relatively few studies have quantified concentrations of Hg in bird species from the south central U.S. (but see King et al. 1985; King and Cromartie 1986; Mora et al. 2008), though previous work has shown that the region experiences high levels of Hg deposition and that fish are highly contaminated with Hg, with concentrations similar to those observed in the northeastern U.S. (Drenner et al. 2011, 2013).

In the present study, we assessed Hg concentrations in birds sampled from two sites in north central and northeast Texas, Lewisville Lake and Caddo Lake, respectively and determined if differences in Hg concentrations exist based on age, trophic level, and location. The present study is the first to document Hg concentrations in multiple bird species in north Texas at sites polluted with atmospheric Hg.

Our study suggests that Hg concentrations in birds from atmospherically polluted sites in this region have concentrations of Hg as high as in other studies where negative impacts on behavior, reproductive success, or other aspects of life history have been observed (Heinz 1979; Hallinger et al. 2010; Jackson et al. 2011; Frederick and Jayasena 2011; Jayasena et al. 2011).

Methods

Study Areas

Lewisville Lake

Lewisville Lake, located in north central Texas within the northern portion of the Dallas-Fort Worth metro area, is an open water reservoir with an adjacent protected wetland habitat managed by the U.S. Army Corps of Engineers (Fig. 1). Mercury deposition is relatively low and the surrounding ecoregion does not have landscape characteristics associated with elevated deposition or methylation of Hg (Drenner et al. 2011, 2013). A previous study at Lewisville Lake found relatively low Hg concentrations in largemouth bass (*Micropterus salmoides*; Drenner et al. 2011).

Caddo Lake

Caddo Lake is a forested wetland ecosystem on the border between Texas and Louisiana (Fig. 1). The region is known to have high levels of Hg wet deposition (>11 μg/m^2/year, Drenner et al. 2013), landscape characteristics associated with increased Hg methylation (Drenner et al. 2011, 2013), and Hg levels in largemouth bass high enough to pose a significant hazard to human health (Drenner et al. 2013). Consequently, high Hg concentrations have been documented in multiple trophic levels of non-avian taxa, including invertebrates, fish, amphibians, reptiles, and mammals from Caddo Lake (Giggleman et al. 1998; Chumchal and Hambright 2009; Chumchal et al. 2011; Drenner et al. 2011). Mercury contamination has been found to negatively effect piscivorous spotted gar (*Lepisosteus occulatus*) from Caddo Lake (Barst et al. 2011). Fish and invertebrates from Caddo Lake have Hg concentrations (Chumchal et al. 2011; Gann et al. 2015) that are similar to concentrations shown to adversely affect birds when consumed (Heinz 1979; Frederick and Jayasena 2011; Jayasena et al. 2011; Varian-Ramos et al. 2014) and a risk analysis found that Hg concentrations in shoreline spiders were high enough to pose a potential risk to arachnivorous nestling songbirds (Gann et al. 2015). The only previous study to document Hg concentrations in birds at Caddo Lake examined great blue heron nestlings ($n = 7$) and found that individuals possessed liver Hg concentrations as high as 19,900 ng/g ww (Giggleman et al. 1998).

Nest Monitoring, Capture, and Sampling

All sampling at Lewisville Lake was conducted at the Lewisville Lake Environmental Learning Area (LLELA) in Denton County in north central Texas (Fig. 1). At Caddo Lake samples were collected from the Caddo Lake National Wildlife Refuge, Caddo Lake Wildlife Management Area, and a lakefront residential area in north Harrison and south Marion counties (Fig. 1).

We used bluebird and wood duck nest boxes at both sites to attract nesting birds during the 2010 breeding season. From March to July, we collected breast feathers (adults) and sampled blood (adults and nestlings) directly from Carolina wrens and eastern bluebirds that utilized the nest boxes (Table 1). Female wood ducks line their nests with feathers plucked from their breasts. We also collected wood duck nest-lining feathers from nest boxes (Table 1). Blood was collected in capillary tubes after puncturing the brachial vein on adults or the tibial vein on nestlings using a sterile 26-guage needle. We also opportunistically collected adult great egret feathers (i.e., flight feathers, coverts, and breeding plumes) and great blue heron feathers

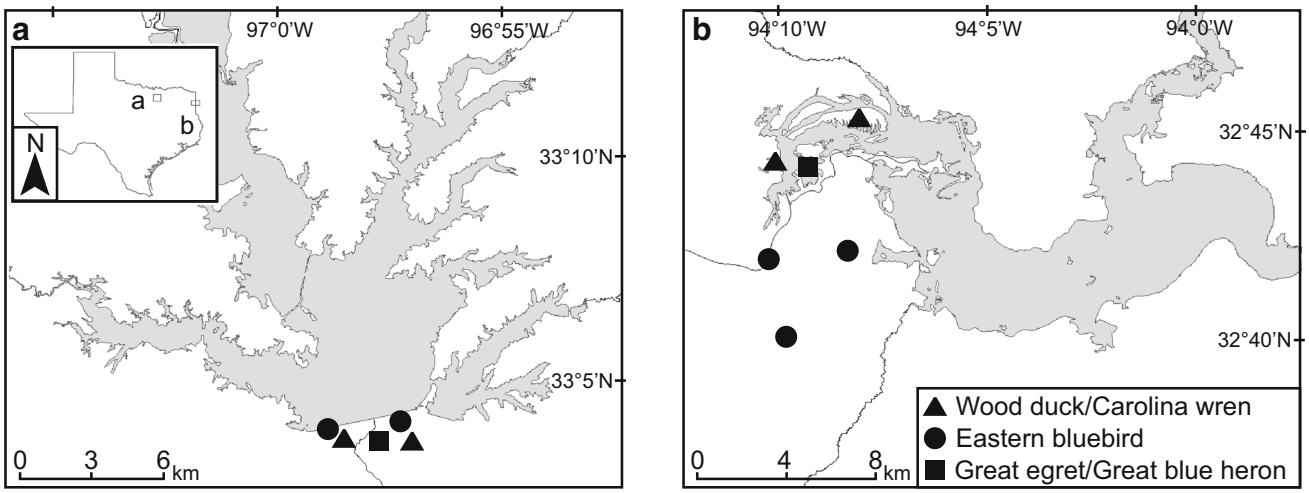

Fig. 1 Map of sampling locations of avian species at Lewisville Lake (**a**) and Caddo Lake (**b**) in Texas

Table 1 Sample sizes of bird tissues sampled at Caddo Lake and Lewisville Lake, TX

Species	Site	Age				Trophic guild	Major food items
		Nests (nestlings)		Adults			
		Feather	Blood	Feather	Blood		
Wood duck (*Aix sponsa*)	Lewisville Lake	0	0	6	0	Omnivore	Seeds, fruits, aquatic and terrestrial invertebrates (Hepp and Belrose 2013)
	Caddo Lake	1 (3)	0	28	0		
Eastern bluebird (*Sialis sialis*)	Lewisville Lake	0	5 (17)	7	6	Insectivore	Insects and small fruits (Gowaty and Plissner 2015)
	Caddo Lake	0	6 (23)	13	12		
Carolina wren (*Thryothorus ludovicianus*)	Lewisville Lake	0	0	0	0	Insectivore	Insects and spiders (Haggerty and Morton 2014)
	Caddo Lake	0	3 (9)	3	3		
Great egret (*Ardea alba*)	Lewisville Lake	0	0	14	0	Piscivore	Mainly fish; also invertebrates, amphibians, birds, reptiles, small mammals (McCrimmon et al. 2011)
	Caddo Lake	0	0	4	0		
Great blue heron (*A. herodias*)	Lewisville Lake	0	0	7	0	Piscivore	Mainly fish; also invertebrates, amphibians, birds, reptiles, small mammals (Vennesland and Butler 2011)
	Caddo Lake	0	0	0	0		

For nestling samples the number of nests sampled are given with the total number of individual chicks sampled given in parentheses

(i.e., flight and body feathers; Table 1). These feathers were assumed to be from different individuals but this was not verified. Sampled species represented different trophic guilds (Table 1; Hepp and Belrose 2013, Gowaty and Plissner 2015; Haggerty and Morton 2014, McCrimmon et al. 2011; Vennesland and Butler 2011) and occupy each study site year round, thus minimizing potential issues related to differences in Hg concentrations due to the timing of molt and Hg deposition at unknown locations. Wood ducks, great egrets, and great blue herons primarily feed directly from the aquatic environment (McCrimmon et al. 2011; Vennesland and Butler 2011; Hepp and Belrose 2013). Eastern bluebirds and Carolina wrens feed directly in terrestrial habitats, but may consume emergent aquatic prey items (e.g. Odonata, Ephemeroptera, some Diptera), especially for those birds nesting closer to the waterfront (Gowaty and Plissner 2015; Haggerty and Morton 2014). Our sampling methodology was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Texas.

Mercury Analysis

All samples were transported from the field and stored at −20 °C until analysis. All feather samples were rinsed 3× with ddH_2O to remove dirt and debris, blotted dry with a Kimwipe tissue and allowed to dry at room temperature prior to analysis. Blood samples were dried at 55 °C and removed from capillary tubes with a stainless steel plunger for dry weight (dw) analysis.

Samples were analyzed for total Hg concentration with a direct Hg analyzer (DMA-80; Milestone) that used thermal decomposition, gold amalgamation, and atomic absorption spectrometry (U.S. EPA 1998). Total Hg was used as a proxy for MeHg as previous studies have found that >95 % of total Hg in feathers and avian blood is in the form of MeHg (Thompson and Furness 1989; Spalding et al. 2000; Rimmer et al. 2005). Quality assurance included reference and duplicate samples. Samples of National Research Council Canada reference materials (MESS-3, marine sediment, certified value = 91 ± 9 ng Hg/g dw, or DOLT-3, dogfish liver, certified value = 3370 ± 140 ng Hg/g dw) were analyzed approximately every 10 samples. The mean percentage recovery of reference materials was 102.1 % ($n = 23$). Duplicate samples were analyzed approximately every 25 samples, and the mean relative percent difference was 12.8 % ($n = 6$). Concentrations are reported as ng of total Hg per g dw for both feathers and blood. The limit of detection (LOD) for the Hg analyzer was 0.2174 ng of Hg (i.e., ~40 and ~8 ng/g dw based on mean sample weight for blood and feather samples, respectively). Mercury concentrations in blood were below the LOD for 100 and 26 % of eastern bluebird nestlings at Lewisville Lake and Caddo Lake, respectively. Mercury concentrations were above the LOD for all other blood samples and all feather samples examined in the study. For samples with Hg concentrations below the LOD proxy Hg concentrations were calculated using one-half the LOD (i.e., 0.1087 ng) divided by the sample weight (g).

Statistical Analysis

At each site, we determined if Hg concentrations were significantly different between (1) age classes (adult vs. nestling) of a given species and (2) species of a given age-class. We also determined whether Hg concentrations in a given species and age-class differed between sites. The two tissue types (i.e., blood and feather) were analyzed separately in all comparisons.

Nonparametric statistics were used to compare Hg concentrations, because the datasets were not normally distributed or possessed small sample sizes. Mann–Whitney U two-sample test with Z approximation and continuity correction and Kruskal–Wallis tests were used to test for differences in Hg concentrations between two and more than two samples, respectively. Significant results for Kruskal–Wallis tests were followed with Tukey multiple comparison tests on ranked data. Because blood Hg concentrations from nestmates may be considered pseudoreplicates (Hurlbert 1984), comparisons that included nestlings were conducted with the median value from each nest. All statistical analyses were conducted using SAS® 9.3 (SAS Institute, Cary, NC). Findings were considered to be statistically significant when $p < 0.05$.

In the present study, total Hg data is presented on a dw and estimated wet weight (ww) basis to facilitate comparisons with other studies. Water accounts for approximately 75–80 % of the weight of blood in birds (Scanlon 1982; Rimmer et al. 2010). We estimated ww total Hg concentrations by multiplying dw total Hg concentrations by a conversion factor of 0.2 to account for weight change due to water loss after drying.

Results

Median Hg concentrations in blood ranged from 21.7 ng/g dw in nestling eastern bluebirds from Lewisville Lake to 3360 ng/g dw in adult Carolina Wrens from Caddo Lake

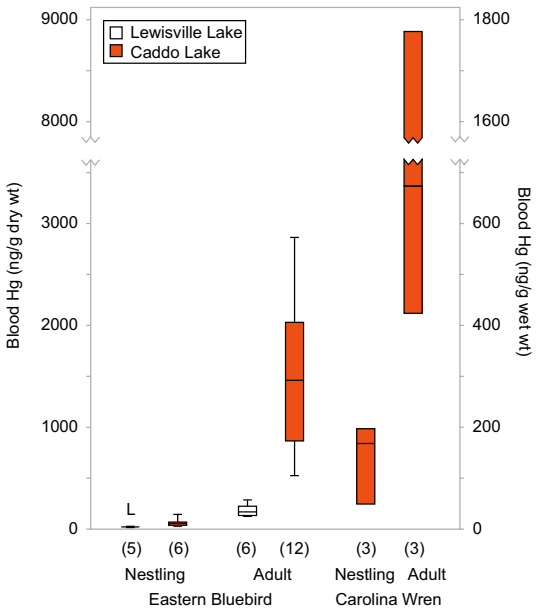

Fig. 2 Mercury concentrations in blood for eastern bluebird and Carolina wren. Actual measurements presented in dry weight (ng/g). Estimated wet weight (ng/g) presented on *right axis*; conversion based on Scanlon (1982). Sample size indicated in *parentheses*. *Boxes* represent first quartile, median, and third quartile and whiskers represent the range. The *letter* "*L*" on the figure indicates that nesting eastern bluebirds were collected from Lewisville Lake. Note break in *y*-axis

(Fig. 2). Median Hg concentration in feathers ranged from 279 ng/g dw in adult eastern bluebirds from Lewisville Lake to 13,923 ng/g dw in adult great blue herons at Caddo Lake (Fig. 3).

Age-Class Comparisons

At each site, adult passerines had higher concentrations of Hg in their blood than nestlings but the difference was not always significant (Fig. 2; Table S1). Mercury in blood from adult eastern bluebirds were ~1–3 orders of magnitude higher than in nestlings (df = 9, $Z = -2.78$, $p = 0.006$ and df = 16, $Z = -3.32$, $p = 0.0009$ at Lewisville Lake and Caddo Lake, respectively). Although the ranges of Hg concentration in blood for nestlings (240–983 ng/g dw) and adult (2100–8805 ng/g dw) Carolina wrens did not overlap (Fig. 2; Table S1), we did not detect a significant difference in Hg concentrations between age classes (df = 4, $Z = 1.75$, $p = 0.08$). The lack of significance is likely due to lack of power from low sample sizes ($n = 3$ from both age classes).

Within Site Comparisons

Lewisville Lake

Differences in the concentrations of Hg in feathers were detected between species from Lewisville Lake ($H = 6.37$, df = 2, $p = 0.04$; Fig. 3; Table S2). Great egrets had higher concentrations of Hg in feathers than eastern bluebirds (post hoc analysis with Tukey grouping on ranked data, $p < 0.05$), whereas wood duck Hg concentrations were intermediate and not statistically different from either of the other two species ($p > 0.05$).

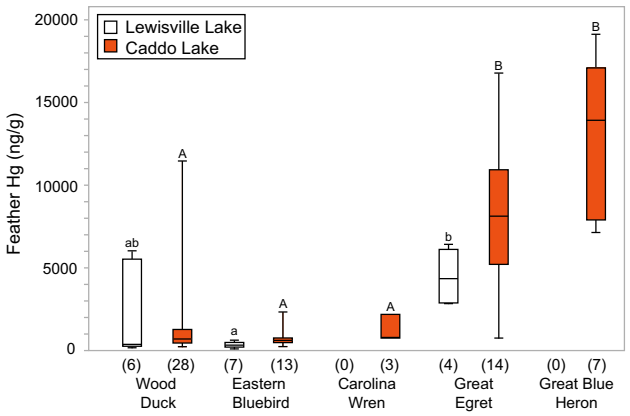

Fig. 3 Mercury concentrations in feathers from adult birds from Lewisville Lake and Caddo Lake. Sample sizes are given in *parentheses*. *Boxes* represent first quartile, median, and third quartile and whiskers represent the range. Within each site, species with Hg concentrations that were significantly different are indicated with different *lowercase* (Lewisville Lake) or *uppercase* (Caddo Lake) *letters*

Caddo Lake

At Caddo Lake, we observed differences in the concentrations of Hg in blood between species. Adult and nestling Carolina wrens had significantly higher concentrations of Hg in blood than adult and nesting eastern bluebirds, respectively (df = 13, $Z = 2.19$, $p = 0.03$ and df = 7, $Z = 2.09$, $p = 0.04$, respectively; Fig. 2; Table S1). Differences in concentrations of Hg in feathers were also detected between species (Kruskal–Wallis test, $H = 38.7$, df = 4, $p < 0.0001$). Herons and egrets had higher concentrations of Hg in feathers than passerines and wood ducks (post hoc analysis with Tukey grouping on ranked data, $p > 0.05$; Fig. 3; Table S2).

Among Site Comparison

For eastern bluebirds, Hg concentrations in blood were significantly higher at Caddo Lake than Lewisville Lake for both nestlings (df = 9, $Z = 2.78$, $p = 0.006$) and adults (df = 16, $Z = -3.32$, $p = 0.0009$; Fig. 2; Table S1). The same pattern was observed for eastern bluebird feather samples (df = 18, $Z = -2.89$, $p = 0.004$; Fig. 3; Table S2). Average Hg concentrations of feathers for adult wood ducks and great egrets were higher at Caddo Lake than Lewisville Lake but the difference was not significant (df = 32, $Z = -0.97$, $p = 0.33$ and df = 16, $Z = -1.75$, $p = 0.08$ for wood ducks and great egrets respectively; Fig. 3; Table S2). The power to test for statistical differences between the two locations was low, however, due to the small sample sizes from Lewisville Lake.

Discussion

Age-Class Comparisons

Blood Hg concentrations were consistently lower in eastern bluebird and Carolina wren nestlings compared with conspecific adults by nearly an order of magnitude, a finding that is consistent with previous studies that have compared Hg concentrations in nestling and adult birds (Scheuhammer et al. 1998; Evers et al. 2005; Brasso and Cristol 2008; Condon and Cristol 2009). This pattern is likely the result of rapid feather growth during the nestling period when continuous Hg sequestration from the blood occurs until feather growth has ceased (Fournier et al. 2002; Condon and Cristol 2009). In addition, differences in total Hg-exposure time between adults and nestlings also may

contribute to the observed differences. Blood Hg concentrations in several bird species have been shown to gradually increase over time during the intermolt period when feather growth is not occurring (Bearhop et al. 2000b; Ackerman et al. 2008; Varian-Ramos et al. 2014). As both eastern bluebird and Carolina wren undergo molt from July through October, we collected samples fairly late in adults' intermolt period when elevated concentrations of Hg in the blood are expected (Haggerty and Morton 2014; Gowaty and Plissner 2015).

Within Site Comparisons

Mercury concentrations differed among species within sites and mostly corresponded with trophic guild. Piscivores had the highest concentrations of Hg, followed by insectivores and omnivores. It is well established that MeHg concentrations increase with trophic level and is highest in fish-eating birds (Campbell et al. 2005; Evers et al. 2005; Rimmer et al. 2010; Lavoie et al. 2010; Keller et al. 2014).

Within trophic guilds, more fine-scale feeding specialization may have influenced Hg concentrations (Bearhop et al. 2000a; Keller et al. 2014). For example, Carolina wrens consume a greater percentage of animal-derived tissues as opposed to plant material in their diet compared to eastern bluebirds (94 vs. 59 % on annual basis, respectively, although insects may consist of up to ~85 % of the bluebird diet during spring and summer months; Beal et al. 1941). Carolina wrens are also known to consume more spiders than eastern bluebirds (Beal et al. 1941). Because animal-derived tissue would be expected to have a higher concentration of Hg than plant-derived tissue (Chumchal et al. 2011), and because spiders may contain higher Hg concentrations due to increased trophic position (Cristol et al. 2008; Rimmer et al. 2010), feeding specialization may have contributed to the differences observed between the two passerine species in the present study even though both species are commonly considered to be primarily insectivorous (Haggerty and Morton 2014; Gowaty and Plissner 2015).

Additionally, the differences in Hg concentrations between eastern bluebirds and Carolina wrens may have been impacted by foraging micro-habitat (Evers et al. 2005; Chumchal et al. 2008; Chumchal and Hambright 2009; Walters et al. 2009). Terrestrial organisms that forage closer to contaminated sites may be exposed to greater contaminant loads (Evers et al. 2005; Cristol et al. 2008; Walters et al. 2009; Barnes and Gerstenberger 2015). The three Carolina wren nests sampled in the present study were located immediately adjacent (e.g. 1–15 m) to aquatic habitat at Caddo Lake, whereas the eastern bluebird nests were located between 0 and 5 km from Caddo Lake. Because songbirds are known to forage in the vicinity of their nest, Carolina wrens may have fed on insects and spiders connected to the aquatic food web with high concentrations of Hg (Cristol et al. 2008; Walters et al. 2009; Keller et al. 2014; Gann et al. 2015).

Among Site Comparison

The majority of species sampled tended to have higher Hg concentrations at Caddo Lake than at Lewisville Lake. This pattern is in agreement with previous studies that identified a west to east gradient in Hg concentrations of largemouth bass in north Texas (Drenner et al. 2011, 2013). Largemouth bass sampled at Caddo Lake had 3–4× higher Hg concentrations as compared to those sampled at Lewisville Lake (Drenner et al. 2011). This gradient in Hg contamination of fish was correlated with Hg in wet deposition and conifer coverage, both of which increase from west to east across this region (Drenner et al. 2013). Additional studies with sampling sites in and between both regions should be conducted to assess definitively whether the west to east gradient in Hg contamination observed with fish also exists for birds.

Implications of Observed Hg Concentrations

Our results suggest that some birds sampled at both Caddo Lake and Lewisville Lake may be negatively impacted by high Hg exposure. Piscivorous wading birds at both sampling locations had feather Hg concentrations similar to those associated with endocrine disruption in white ibis (Frederick and Jayasena 2011; Jayasena et al. 2011). Likewise, Hg concentrations in wood duck feather samples from both Caddo Lake and Lewisville Lake were high, >5000 ng/g dw, with one sample >11,000 ng/g dw, similar to concentrations associated with negative effects in mallards (Heinz 1979). It should be noted that the adult wood duck feathers with the highest Hg concentration in the present study (11,426 ng/g dw) were collected from a nest box at Caddo Lake. The nest box contained three chick carcasses with feather Hg concentrations that ranged from 8605 to 18,310 ng/g dw (data on Hg concentration in nestlings was not included in Fig. 3 or Table S2).

Mercury concentrations may be high enough to pose risk and compromise fitness in populations of some songbird species at Caddo Lake. Two adult Carolina wrens had a blood Hg concentration that approached or exceeded 700 ng/g ww (i.e., 670 and 1760 ng/g ww) and a Carolina wren and an eastern bluebird had feather Hg concentrations that approached 2400 ng/g dw (i.e., 2117 and 2383 ng/g dw, respectively), which are all within the range at which adverse effects have been documented in previous studies focused on birds. For example, in free-living Carolina wrens in Virginia, Jackson et al. (2011) modeled the

response of nest survival to female Hg concentration among other competing risks and showed that a 10 % reduction in nest success was associated with 700 ng/g ww in blood and 2400 ng/g dw Hg in body feathers, whereas a 30 % reduction in nest success was associated with 1700 ng/g ww in blood. Additionally, tonal frequency, note diversity, and length were reduced in the songs of both Carolina wrens and house wrens (*Troglodytes aedon*) at the same Hg contaminated study site in Virginia (Hallinger et al. 2010).

Our results indicate that high Hg concentrations may be a cause for concern in some bird species at both Lewisville Lake and Caddo Lake. This finding is notable because both sites are polluted with Hg from the atmosphere, rather than a direct point source. Given that all aquatic ecosystems in the region are contaminated with atmospheric Hg (http://nadp.sws.uiuc.edu/mdn/), the present study suggests that Hg may pose a risk to birds residing in ecosystems throughout the South Central United States.

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