DETECTION OF MERCURY AMONG AVIAN TROPHIC LEVELS AT CADDO LAKE AND LAKE LEWISVILLE, TX

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Mercury (Hg) is a globally distributed toxicant that has been shown to have negative effects on birds. In the United States, avian taxa have been shown to possess high Hg concentrations in the northeast, Great Lakes and Everglades ecosystems; however, few studies have measured avian Hg concentrations in other geographic regions. Previous studies have documented high Hg concentrations in multiple organisms in east Texas, but birds were not included in these studies. The main objective of the present study was to quantify Hg concentrations in birds in differing trophic levels at Caddo Lake and Lake Lewisville, TX. Results suggest that Hg concentrations may be high enough to negatively impact some bird taxa, particularly those at high trophic levels, residing at both Caddo Lake and Lake Lewisville.

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INTRODUCTION

Mercury (Hg) is a globally distributed toxicant that has been shown to have neurotoxic, reproductive and immune response effects on vertebrates (Scheuhammer et al. 2007; Crump and Trudeau 2009; Franceschini et al. 2009). Mercury is available in the environment through natural sources such as volcanic eruptions and forest fires, but it also enters the environment in large quantities through anthropogenic processes, such as fossil fuel combustion and artisanal or small scale gold mining (Pacyna et al. 2010). Elemental Hg is transformed into organic methylmercury (MeHg) primarily by anaerobic, sulfate-reducing bacteria in anoxic waters and sediment (Compeau and Bartha 1985). Methylmercury is the most toxic form of Hg due to its ability to cross lipid membranes in living organisms and become incorporated within tissues of the organism as opposed to complete excretion (Morel et al. 1998; Scheuhammer et al. 2007). In addition, due to its high stability and lipophilicity, MeHg will bioaccumulate with increasing trophic levels (Fimreite 1974; Becker et al. 2002; Campbell et al. 2005; Ackerman et al. 2007).

Concern exists that Hg contamination has contributed to population declines in birds (Braune et al. 2006; Edmonds et al. 2010). Exposure to high concentrations of MeHg has negatively affected adult survival, while exposure to sublethal concentrations has negatively affected reproduction (Fimreite and Karstad 1971; Bennett et al. 2009; Frederick and Jayasena 2011). For example, captive red-tailed hawks (*Buteo jamaicensis*) were fed diets containing 6,000, 12,000 and 18,000 ng/g MeHg dicyandiamide (Fimereite and Karstad 1971). Three hawks (of six) fed the 18,000-ng/g diet and one hawk (of six) fed the 12,000-ng/g diet experienced significant weight loss

(20-36%) and died within 78 days. There were no overt signs of Hg intoxication in birds fed the 6,000-ng/g diet. Captive American kestrels (*Falco sparverius*) were fed diets containing 0, 3,000, 6,000, or 12,000 ng/g MeHg chloride (Bennett et al. 2009). All kestrels fed the 12,000-ng/g diet (*n* = 5) showed signs of Hg intoxication by 26 days and died between 39 and 49 days. Seven of nine kestrels fed the 6,000-ng/g diet exhibited Hg intoxication after 42 days and one died after 75 days. There were no obvious signs of Hg intoxication in birds fed the 3,000-ng/g diet. Prior to death, hawks and kestrels exhibited poor balance, weakness of extremities, and inabilities to control muscle movements (Fimereite and Karstad 1971; Bennett et al. 2009). Zero percent duckling survival was reported for female black ducks (*Anas rubripes*) fed a 3,000-ng/g diet (Finley and Stendall 1978).

At exposure to lower Hg concentrations (200-500 ng/g), Burgess and Meyer (2008) found that the number of nestlings produced per pair in free-living common loons (*Gavia immer*) decreased by more than 50% with increased exposure to Hg. Frederick and Jayasena (2011) reported an increased frequency of male-male pairings, altered courtship behavior, decreased egg productivity and decreased fledgling production in captive white ibises (*Eudocimus albus*) exposed to environmentally relevant concentrations of MeHg (50, 100 and 300 ng/g wet weight in diet). These changes were associated with altered estradiol and testosterone concentrations in adults of both sexes (Jayasena et al. 2011).

As the above studies show, the majority of studies quantifying the effects of Hg in birds have been primarily conducted with carnivorous or aquatic species that occupy high trophic levels, such as raptors, wading birds and common loons (Fimreite 1974;

Blus et al. 1985; Frederick et al. 1999; Bond and Diamond 2009; Seewagen 2009). A few recent studies, however, have focused on terrestrial, lower trophic level birds such as insectivorous passerines. Passerines comprise the largest order of birds (Passeriformes) and many species are in steep decline (Edmonds et al. 2010; Nebel et al. 2010). A growing number of studies have documented behavioral, physiological, and reproductive effects associated to Hg exposure in free living birds (Cristol et al. 2008; Hallinger et al. 2010; Winder and Emslie 2011). For example, mercury was the leading predictor of nest success of Carolina wrens (*Thryothorus ludovicianus*), more so than year, date in season, age of nest, or cavity type; nest success decreased incrementally in relation to female blood Hg concentration (Jackson et al. 2011). To my knowledge this was the first study to report a negative correlation in blood Hg concentrations and nest survival in a free-living population. Additionally, Carolina wrens and house wrens (*Troglodytes aedon*) at a Hg contaminated site had shorter songs with lower tonal frequencies than those at an uncontaminated site (Hallinger et al. 2010). Additionally, adrenocortical responses were suppressed in nestling tree swallows (*Tachycineta bicolor*) at a Hg-contaminated compared to an uncontaminated site (Wada et al. 2009). More work is required investigating the effects of Hg exposure with terrestrial passerine species because many are likely exposed to Hg through their diet (specifically insectivores, Nebel et al. 2010), and yet little is known regarding concentrations that cause observable effects in these birds (Seewagon 2009).

Studies quantifying Hg concentrations in birds in the U.S. have been generally concentrated in the northeast US, the Great Lakes and the Everglades ecosystems where avian taxa have had high Hg tissue concentrations (Evers et al. 1998; Frederick et al. 2004; Evers et al. 2007). Mercury contamination in these areas has not been linked to a single source, but rather has been thought to be a combination of local and global atmospheric Hg deposition (i.e. non-point source). An exception to this pattern, is studies that are conducted at sites where the source of contamination is known, such as historic dumping sites of nuclear productions facilities and abandoned industrial facilities (i.e. point-source; Wolfe and Norman 1998; Halbrook et al. 1999; Kennamer et al. 2005; Hawley et al. 2009; Wada et al. 2009).To my knowledge, little information is available regarding mercury concentrations in birds sampled from non-point sources outside of northeast U.S., Great Lakes and Florida Everglades regions.

Regions in east Texas, for example, may possess birds with increased Hg concentrations. Reasons for this include their close proximity to several high mercury emitting coal-fired power plants (CFPPs) and landscape characteristics, such as high percentages of forested and wetland areas, which have been associated with increased Hg deposition and Hg methylation (Evers et al. 2007; Chumchal and Hambright 2009; Drenner et al. 2011). In 2010, twenty of the approximately 350 CFPPs in the U.S. were located in east and southeast Texas, including six of the top ten Hg emitting power-plants in the U.S. (Fig. 1; Madsen and Randall 2011). Although, studies have documented elevated Hg concentrations in the environment in close proximity to CFPPs, it has not been unequivocally shown that Hg emissions from specific facilities are the primary source of Hg documented in organisms in their surrounding environment. With that said, however, total Hg concentration in a high trophic level fish such as the largemouth bass (*Micropterus salmoides*) generally increase from central to east Texas where the majority of coal-burning power plants exist (Drenner et al. 2011).

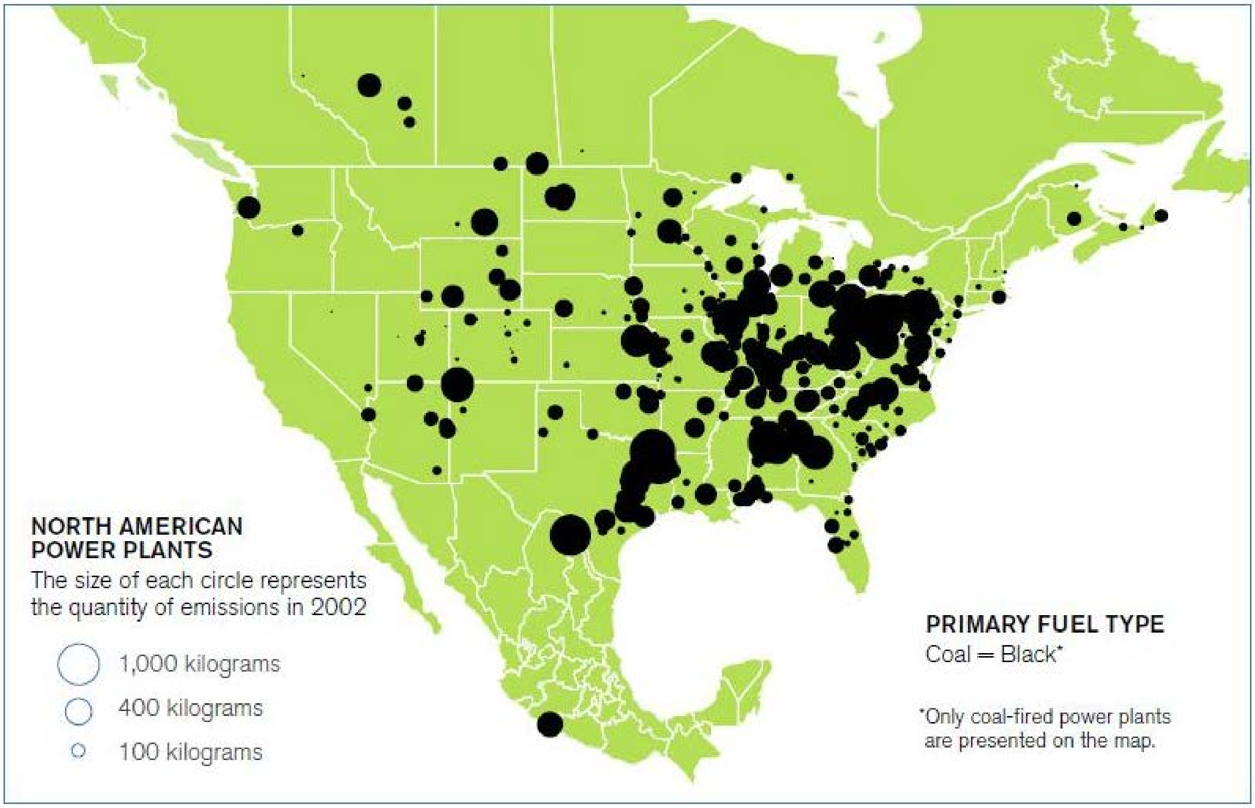


Fig. 1 Geographic distribution of power plant Hg emissions in North America in 2002 (Miller and Van Atten 2004)

In addition, studies at Caddo Lake, a Cyprus swamp ecosystem located on the border of Texas and Louisiana, have documented several groups of non-avian organisms with elevated Hg concentrations. Giggleman et al. (1998) found that sediment Hg concentrations at Caddo Lake exceeded non-enforceable state and federal government guideline values based on known chronic toxicological trends that were established to protect aquatic wildlife in inland waters. The study concluded that MeHg concentrations in spotted gar (*Lepisosteus oculatus*) and redear sunfish

(*Lepomis microlophus*) exceeded the avian predator protection concentration of 100 ng/g wet weight (Giggleman et al. 1998).

Further, a multi-trophic level study that included invertebrates, fish, amphibians, reptiles and mammals showed a positive correlation between Hg concentrations and trophic position at Caddo Lake (Chumchal et al. 2011). Some species sampled had Hg concentrations above the avian predator protection limit (~500-1,500 ng/g dry wt in whole bodies and ~800-2,400 ng/g dry wt in muscle tissue) (Chumchal et al. 2011). Table 1 provides length, Hg concentrations, and MeHg concentrations of organisms sampled in this study that may serve as potential prey items for avian species (Chumchal et al. 2011). The highest MeHg concentrations were found in the livers of the spotted gar, northern raccoon (*Procyon lotor*), and cottonmouth (*Agkistrodon piscivorus*), all of which occupied high trophic positions. Liver Hg concentrations in raccoons were below concentrations known to harm adult mammals and authors were unable to predict the risks associated Hg concentrations in reptiles sampled, as little is known regarding sub-lethal effects of Hg in reptiles (Chumchal et al. 2011). Alternatively, Hg concentrations reported in fish from Caddo lake were similar to those that have been associated with adverse physiological and reproductive effects. In fact, the authors concluded that fish Hg concentrations may be sufficiently high to impair fish health as well as pose a threat to exclusively piscivorous organisms (Chumchal et al. 2011).

Table 1 Number of samples, tissue type, mean (± 95% confidence interval) total length, and mean (± 95% confidence interval) total and methylmercury (MeHg) concentrations (ng/g dry weight) in muscle and whole body from Caddo Lake (TX/LA, USA). Some species sampled had Hg concentrations above the avian predator protection limit (~500-1,500 ng/g dry wt in whole bodies and ~800-2,400 ng/g dry wt in muscle tissue). Adapted from Chumchal et al. 2011.

Common Name	No. of samples (mean No. per composite)	Tissue Analyzed	Total length (cm)	Hg	MeHg (No. analyzed)
Invertebrates					
Grass shrimp (*Palaemonetes kadiakensis*)	6 (44.5)	Whole	3.29 ± 0.13	601 ± 114	435 ± 131 (3)
Dragonfly larvae (Libellulidae)	6 (36.8)	Whole	1.65 ± 0.06	174 ± 33.3	134 ± 52.7 (3)
Dragonfly larvae (Aeshnidae)	2 (6)	Whole	6.9 ±1.9	314 ± 104	265 ± 116 (2)
Giant water bug (*Belostoma* sp.)	6 (40.5)	Whole	1.91 ± 0.08	346 ± 70.8	294 ± 68.0 (3)
Crayfish (Cambaridae)	4 (3.3)	Muscle	7.47 ± 0.9	577 ± 260	405 ± 97.9 (3)
Vertebrates					
Fish					
Bluegill (*Lepomis machochirus*)	8 (4.6)	Muscle	9.97 ± 1.87	640 ± 110	632 ± 171 (4)
Largemouth bass (*Micropterus salmoides*)	8(4)	Muscle	24.9 ± 6.33	1,718 ± 386	1,442 ± 469 (6)
Golden topminnow (*Fundulus chrysotus*)	9 (5.9)	Whole [a]	4.76 ± 0.52	570 ± 161	578 ± 158 (3)
Pirate perch (*Aphredoderus sayanus*)	3 (11.7)	Whole [a]	3.89 ± 0.67	605 ± 80.5	512 ± 63.5 (3)
Red-ear sunfish (*Lepomis microlophus*)	12 (3.8)	Muscle	11.5 ± 1.86	445 ± 54.7	385 ± 39.4 (4)
Spotted Gar (*Lepisosteus oculatus*)	5 (4.6)	Muscle	50.6 ± 2.88	2,611 ± 478	2,224 ± 374 (4)
Amphibian					
Bullfrog (*Rana catesbeiana*)	5	Muscle	14.6 ± 1.92	620 ± 196	545 ± 288 (3)
Reptiles					
Cottonmouth (*Agkistrodon piscivorus*)	6	Muscle	67.6 ± 13.5	3,292 ± 2,186	3,075 ± 3,349 (3)

[a] Eviscerated and decapitated prior to analysis.

To my knowledge, the only study to investigate Hg in avian taxa at Caddo Lake was done with seven great blue heron chicks (*Ardea herodias*; Giggleman et al. 1998).

While range and geometric mea Hg concentrations were reported for liver (wet weight), feathers, and kidney (dry weight) tissues, the report did not specify age of chicks nor whether the chicks were from a single or multiple nests. From the seven great blue heron chicks, liver, feather, and kidney Hg concentrations ranged from 387 to 19,900 ng/g (mean = 896 ng/g), 1,230 to 14,500 ng/g (mean = 2,160 ng/g), and 513 to 2,370 ng/g (mean = 760 ng/g), respectively. Liver Hg concentration (19,900 ng/g) in one of the great blue heron chicks was similar to concentrations documented in a south Florida great white heron (*A. h. occidentalis*) population possessing elevated occurrences of chronic disease and parasites post-mortem compared to those that had died from physical collisions or dinoflagelate toxins (Spalding et al. 1994).

Birds are an important component of the Caddo Lake food web that could play an important role in transporting Hg to other organisms and geographic areas (e.g., Cristol et al., 2008). A more comprehensive study investigating Hg concentrations in bird species at Caddo Lake is therefore warranted. In this study, concentrations of Hg were measured in three avian species that occupy different trophic levels at Caddo Lake, TX. I predicted that Hg concentrations should increase with increasing trophic level, similar to other studies investigating Hg in vertebrate species (Chumchal et al. 2011).

METHODS

Description of Species Targeted for Study

Taxonomic representatives from different trophic levels were identified for sampling while considering the following factors: (1) year-round residency (i.e., non-migratory), (2) breeding season began no earlier than mid-March and continued through June or July, and (3) nests were accessible for sampling purposes (Baicich and Harrison 1997). From these criteria, the three species chosen to represent low, intermediate and high trophic levels were wood duck (*Aix sponsa*), eastern bluebird (*Sialia sialis*) and great egret (*Ardea alba*), respectively (Baicich and Harrison 1997).

Wood ducks are cavity nesters and frequently utilize artificial nest boxes located near or above water. Breeding begins in early March in Texas and usually ends by mid-July. Females raise a single brood of typically 8-10 eggs per breeding season (Baicich and Harrison 1997). Wood duck diet consists primarily of vegetation with some insects, but diet may vary between sexes and female breeding status (Drobney and Fredrickson 1979). For example, a study in south Missouri documented a similar percentage (34-36%) of insect remains in the stomach contents of males during the breeding season compared to the fall, whereas insect stomach content for females varied over time with 54% for pre-laying females, 79% for laying females, 43% for post-laying females, and 33% for females during the fall (Drobney and Fredrickson 1979). In this study, Diptera and Coleoptera were consistently ranked the top two insect Orders consumed by wood ducks (Drobney and Fredrickson 1979). To my knowledge, no studies have documented Hg concentrations in free-living wood ducks.

Eastern bluebirds nest in cavities and frequently utilize artificial nest boxes. Breeding begins between mid-March and early April and pairs usually raise two, sometimes three broods per breeding season, with 4-5 eggs per nest on average (Baicich and Harrison 1997). Major food items include ground arthropods during the breeding season and small fleshy fruits from late summer and into winter (Gowaty and Plissner 1998). Eastern bluebird nestlings are typically fed an arthropod diet prior to fledging. For example, Pinkowski (1978) documented a nestling diet consisting of 58% Lepidopterous larva and Orthopterans, with additional taxa (in order of decreasing percentage) Araneae, Coleoptera, Oligochaeta, Hymenoptera, and Diplopoda. A separate study found that eastern bluebird adults (*n* = 855) fed primarily on insects but that they shifted their diet to include fruits and other plant matter during the winter months when insect abundance was low. (Beal 1915; see also Pitts 1979, Gowaty and Plissner 1998). To my knowledge, the only study to quantify Hg in eastern bluebirds found that adults at a mercury-contaminated river in Virginia, USA had blood Hg concentrations of 1,210 $\pm$ 570 (wet weight, *n* = 86) and that nestlings had blood Hg concentrations an order of magnitude lower than their parents of 90 $\pm$ 60 ng/g (wet weight, *n* = 156) (Condon and Cristol 2009).

Great egrets nest in trees of woodland and Cyprus swamps. In Texas, they have also been observed nesting in scrub habitat of islands, singly or forming large colonies often with other heron species (*Ardea* spp.) and/or wood storks (*Mycteria americana*). Breeding begins in mid-April with 3 eggs on average (McCrimmon et al. 2011). Great egret diet has been shown to consist primarily of fish but also invertebrates, crustacean, amphibians, reptiles and small mammals (Hoffman and Curnow 1979; Frederick et al.

1999; Mccrimmon et al. 2011). They forage primarily in wetland habitats at a water depth of 20-40 cm (McCrimmon et al. 2011). Previous studies have investigated Hg concentrations in the diets of great egret nestlings in the Florida Everglades, where annual mean mercury concentration across a 4-year study was 410 ng/g (Frederick et al. 1999). They concluded that this concentration of Hg ingestion was similar to a dietary intake of 500 ng/g at which captive great egret nestlings displayed decreased appetite, strength, and fledgling mass, lower packed-cell volume, and increased lethargy (Frederick et al. 1999).

Sample Collection

Between March and July 2009, birds were sampled from Caddo Lake and Lake Lewisville, TX. Nest sites at Caddo Lake included those from the Caddo Lake National Wildlife Refuge, Caddo Lake Wildlife Management Area and a lakefront residential area in north Harrison and south Marion counties (32°42’ N, 94°08’ W). All sampling at Lake Lewisville was conducted at the Lake Lewisville Environmental Learning Area in Denton County in north central Texas (33°03’N, 96°58’W). The latter site was included to assess Hg concentrations in the same set of species, but at a different geographic location in Texas other than Caddo Lake.

Adult female wood duck feathers were sampled directly from nest boxes once during mid-breeding season in June (n = 28 Caddo Lake; n = 6, Lake Lewisville). Blood was collected from females captured on active nests (n = 3). Only one live nestling was sampled because they typically fledge from the nest within 24 hours after hatching.

However, one nest box contained dead nestlings (*n* = 3) at Caddo Lake, which were collected and their feathers were included in the analyses.

During the pre-breeding season in February, bluebird nest boxes were emptied of old nest material and checked regularly for nesting activity through the remainder of the nesting season. Adult and nestling eastern bluebirds were trapped in nest boxes. Adult female bluebirds (*n* = 13, Caddo Lake; *n* = 6, Lake Lewisville) were captured during incubation or prior to nestlings fledging and two adult males (*n* = 1, Caddo Lake; *n* = 1, Lake Lewisville) were captured prior to nestlings fledging. Breast feathers and blood were collected from adults. Blood was collected in capillary tubes by either puncturing the brachial vein for adults or a leg vein for nestlings using a 26-guage needle. Feathers were not collected from nestlings. Three active Carolina wren nests were found in wood duck nest boxes and sampled using similar methods.

Great egret feathers were collected below a single roosting site in a group of trees on Caddo Lake. Several great egrets (*n* > 20) were observed flying away from the roosting site as the boat approached during the early evening. An area approximately 40 m^2 was searched for feathers. More than twenty feathers were collected but only fourteen were positively identified as great egret feathers and subsequently analyzed. All feathers were assumed to be from different individuals, although this assumption has not been verified. Great egret feathers (*n* = 4) were opportunistically collected at Lake Lewisville, each from a different location presumably from four different individuals.

In addition, feathers and blood were sampled from two unrelated black vulture (*Coragyps atratus*) nestlings and four related eastern screech owl (*Megascops asio*) nestlings at Caddo Lake. Great blue heron feathers (*n* = 7) were also collected below a roosting site at Caddo Lake, and blue jay feathers from three different individuals were collected on three occasions from a property owner's yard in the lakefront residential study site at Caddo Lake. The blue jay feathers were most likely from predated individuals based on the number of feathers found at each location.

Museum specimens from the UNT's Elm Fork Natural Heritage Museum were used to identify feathers that were not collected from individuals in hand. Feather samples were stored in sterile plastic bags at -20°C until analysis. Samples were rinsed 3x with ddH_2O to remove dirt and debris, blotted dry with a Kimwipe and allowed to dry at room temperature prior to analysis. Surface binding of Hg to feathers is not considered to be a significant problem (Burger and Gochfeld 1997). Blood samples were dried at 55°C and removed from capillary tubes for analysis dry weight.

Mercury 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 (US EPA 1998). Total Hg was used as a proxy for MeHg as previous studies have found that >95% of total Hg in feathers is in the form of MeHg (Thompson and Furness 1989; Spalding et al. 2000). Quality assurance included reference and duplicate samples. Samples of National Research Council Canada reference materials (MESS-3, marine sediment, certified value = 91 $\pm$ 9 ng mercury/g dry weight, or DOLT-3, dogfish liver, certified value = 3370 $\pm$ 140 ng mercury/g dry weight) were analyzed approximately every 10 samples. The mean percentage recovery of reference materials was 102.1% (n = 23). Duplicate samples were analyzed approximately every 20 samples, and the mean relative percent difference was 17.9% (n = 9). Concentrations are reported as nanograms of total mercury per gram dry weight for both feathers and blood. The limit of detection for the Hg auto analyzer was 0.2174 ng.

Statistical Analysis

For samples below the limit of detection (0.2174 ng), the maximum likelihood estimation (MLE) method of Helsel and Cohn (1988) was used to estimate maximum possible concentrations for these samples (also see DeCaprio et al., 2005). In addition, means and SD were calculated using U.S. E.P.A. (1998) methods of substitution with zero and with one-half the limit of detection. The MLE method is the most conservative and thus MLE values were used for statistical comparison when necessary, while both the MLE and zero-substitution method are presented in Table 2 for reference. However, variation between samples using the substitution methods reflects variation in sample mass and is not a true estimator of variation in Hg concentration.

Not all data met parametric assumptions of normality and equality of variance, therefore non-parametric statistics were used to compare Hg concentrations between sites and species unless otherwise noted. Mann-Whitney U tests were used to test for differences in Hg concentrations between similar samples (i.e. same species, age, and tissue type) at Caddo Lake and Lake Lewisville. Kruskal-Wallis was subsequently used to test for differences in Hg concentrations among multiple species within each location when similar samples of tissue types were available. Significant results were followed with nonparametric comparisons for each pair using Wilcoxon method. All statistical analyses were conducted using JMP ® 9.0.2 2010 (SAS Institute, Cary, NC, USA). Although nonparametric statistics were used to analyze the data, Hg concentrations are presented as mean $\pm$ SD to allow better comparisons with results from other studies.

RESULTS

Mercury Concentrations in Birds at Caddo Lake and Lake Lewisville

Mercury was detected in feathers and adult blood of all species sampled in the present study from both Caddo Lake and Lake Lewisville, with the exception of one wood duck female at Lake Lewisville which had blood Hg below the limit of detection. Mercury was detected in all nestlings of Carolina wrens, black vultures, and eastern screech owls at Caddo Lake. Mercury was below detection limit for 26% (n = 23) and 100% (n = 17) of eastern bluebird nestlings at Caddo Lake and Lake Lewisville, respectively.

Mercury was highly variable among tissues and species (Table 2). Mean total Hg concentrations at Caddo Lake ranged from 748 (adult eastern bluebird) to 13,139 (great blue heron) ng/g dry wt in feathers and from below detection limit (nestling eastern bluebird) to 4,756 (Carolina wren) ng/g dry wt in blood. Mean total Hg concentrations at Lake Lewisville ranged from 110 (nestling wood duck) to 4,470 (great egret) ng/g dry wt in feathers and from below detection limit (nestling eastern bluebird) to 181 (adult eastern bluebird) ng/g dry wt in blood.

Table 2 Hg concentrations (ng/g) in birds from two lakes in northeast and north central Texas, Caddo Lake (C) and Lake Lewisville (L)

Species[a]	Site	Age	Tissue[b]	n	Hg ng/g dry weight Mean ± SD (min/max)[c]	Mean ± SD (min/max)[d]	# nests	% below MDL
WODU	C	Adult	F	28	1,345 ± 2,240 (230/11,426)		NA	0
			B	1	437		NA	0
		Nestling	F	3	12,380 ± 5,196 (8,605/18,310)		1	0
	L	Adult	F	6	2,112 ± 2,846 (193/6,024)		NA	0
			B	2	(22/120)	(0/120)	NA	50
		Nestling	F	1	110		1	0
EABL	C	Adult	F	13	748 ± 54 (208/2,383)		NA	0
			B	12	1,555 ± 834 (525/2,920)		NA	0
		Nestling	B	23	92 ± 71 (34/340)	78 ± 82 (0/340)	6	26
	L	Adult	F	7	310 ± 118 (175/499)		NA	0
			B	6	181 ± 66 (121/291)		NA	0
		Nestling	B	17	42 ± 14 (24/78)	0 ± 0 (0/0)	5	100
CAWR	C	Adult	F	3	1,686 + 1,284 (720/2,117)		NA	0
			B	3	4,756 ± 3,564 (2,102/8,805)		NA	0
		Nestling	B	9	925 ± 799 (199/2,942)		3	0
BLJA	C	Adult	F	3	3,070 ± 2,066 (1,036/5,167)		NA	0
BLVU	C	Adult	F	1	2,308		NA	0
		Nestling	F	2	(1,182/3,147)		2	0
			B	2	(205/1,032)		2	0
	L	Adult	F	2	(394/6,222)		NA	0
EASO	C	Nestling	F	4	2,605 ± 300 (2,269/2,294)		1	0
			B	4	1,004 ± 71 (910/1,078)		1	0
GREG	C	Adult	F	14	8,314 ± 4,286 (777/16,747)		NA	0
	L	Adult	F	4	4,470 ± 1,872 (2,790/6,369)		NA	0
GBHE	C		F	7	13,139 ± 4,520 (7,085/19,068)		NA	0

[a]WODU wood duck, EABL eastern bluebird, CAWR Carolina wren, BLJA blue jay, BLVU black vulture, EASO eastern screech owl, GREG great egret, GBHE great blue heron
[b] feather (F), blood (B)Mean
[c] (SD) of all samples with MLE substitution for samples below detection limit as described under Methods.
[d] Mean (SD) of all samples with zero substituted for samples below detection limit.

Site Comparison

Hg concentrations among adult wood duck feathers were not different between Caddo Lake and Lake Lewisville (Mann-Whitney test, $Z = 0.971$; $p = 0.3314$; Fig. 2). In contrast, significantly higher concentrations of Hg were observed with eastern bluebird adult feather (Mann-Whitney test, Z = 2.89345; $p = 0.0038$), adult blood (Mann-Whitney test, Z = 3.32488; $p = 0.0009$) and nestling blood (Mann-Whitney test, $Z = 3.37923$; $p = 0.0007$, MDL substituted values) samples from Caddo Lake compared to Lake Lewisville (Fig. 3). Mean great egret feather Hg concentrations were nearly significantly different (Mann-Whitney test, $Z = 1.75228$, $p = 0.0787$; Fig. 2) with higher values observed in egrets from Caddo Lake.

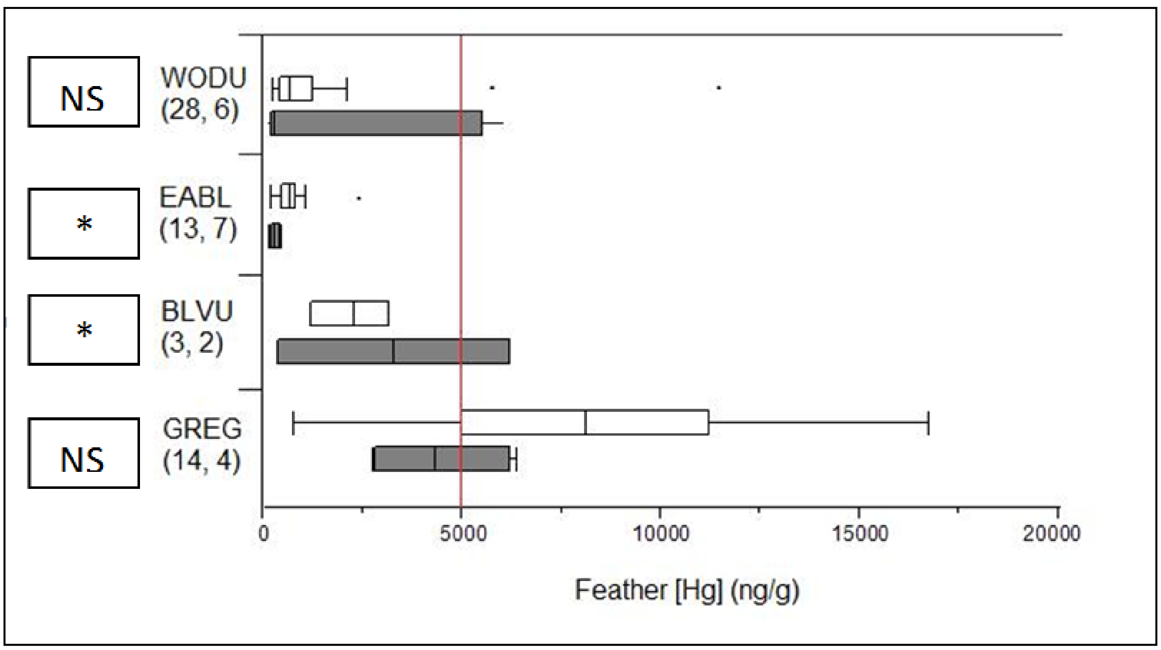


Fig. 2 Concentrations of total Hg (ng/g) in adult feathers of different species from Caddo Lake, TX (open box) and Lake Lewisville, TX (grey boxes). Shown is five-number summary. Sample sizes of Caddo Lake and Lake Lewisville, respectively, are indicated in parentheses below species abbreviation. Significant (*) comparisons are distinguished from non-significant (NS) comparisons to the left of species abbreviation. Vertical red line indicates concentration of adverse effects associated with feather Hg concentrations. WODU wood duck, EABL eastern bluebird, BLVU black vulture, GREG great egret.

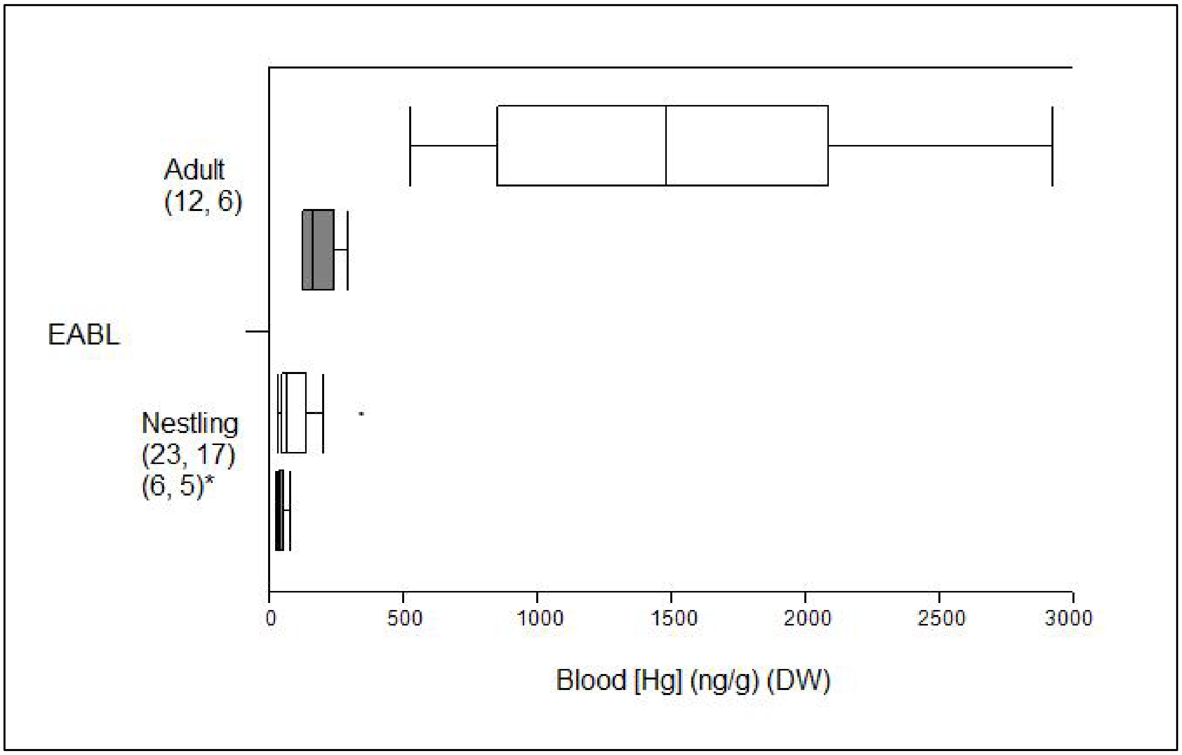
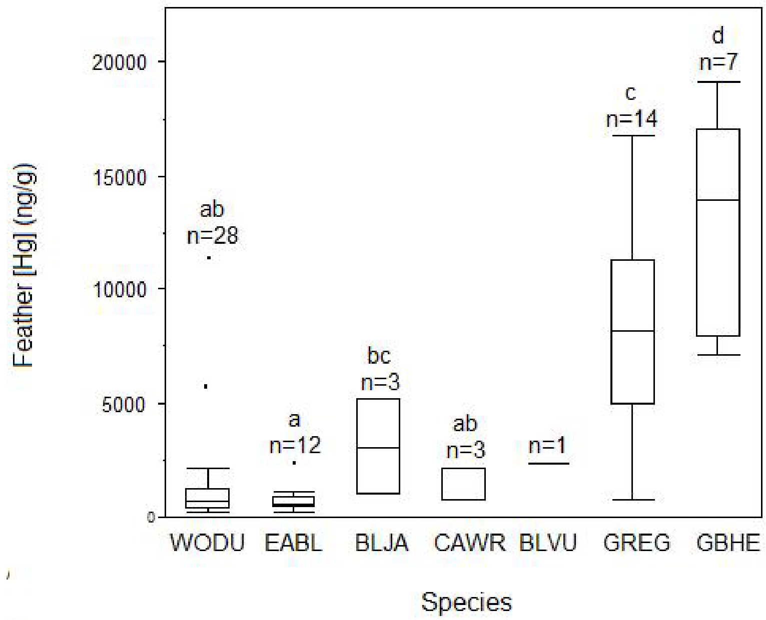


Fig. 3 Concentrations of total Hg (ng/g) in blood of adult (top) and nestling (below) eastern bluebirds from Caddo Lake, TX (open box) and Lake Lewisville, TX (grey boxes). Shown is five-number summary. Sample sizes of Caddo Lake and Lake Lewisville, respectively, are indicated in parentheses below species abbreviation. Significant (*) comparisons indicated to the left of species abbreviation.

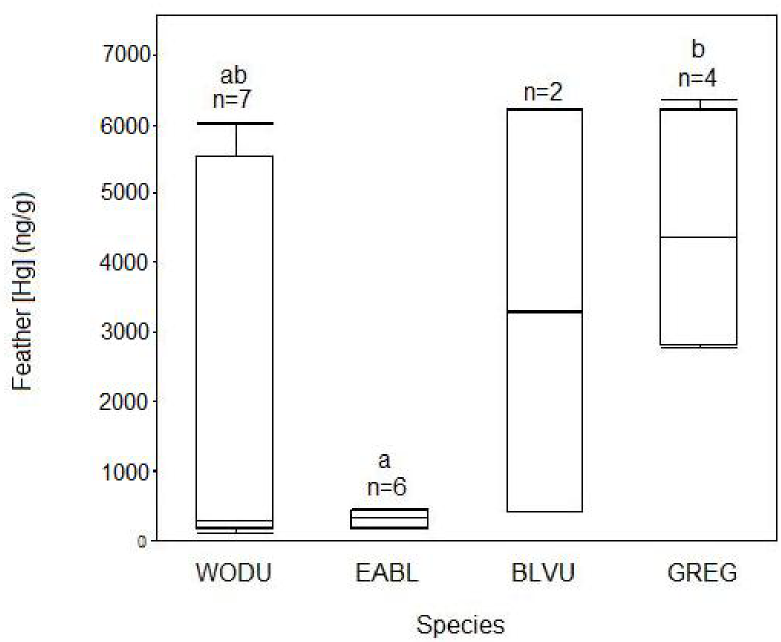
Species Comparisons

Differences in Hg concentrations were detected between species sampled at both Caddo Lake and Lake Lewisville (Fig. 4). In general, higher trophic levels species tended to have higher Hg concentrations. For example, at both sites, wading birds (great egret and great blue heron) had significantly higher feather Hg concentrations than eastern bluebirds. However, two adult wood ducks at both Caddo Lake and Lake Lewisville had Hg concentrations above 5,000 ng/g while the remaining adult wood ducks sampled at Lake Lewisville and Caddo Lake were below 300 ng/g and 2,500 ng/g, respectively. One adult wood duck sampled at Caddo Lake had a feather Hg concentration of 11,426 ng/g, and the three dead wood duck nestlings collected from the same nest box had a mean feather Hg concentration of 12,385 ± 5,196.

Nestling blood Hg concentrations were significantly different among four species at Caddo Lake (Kruskal-Wallis Test, $X^2 = 26.1767$, $p < 0.0001$). Nonparametric comparisons for each species pair (Bonferroni correction, $\alpha = 0.05/6 = 0.0083$) indicated that eastern bluebird nestlings had significantly different blood Hg concentrations than Carolina wren ($Z = 4.28945$, $p < 0.0001$) and eastern screech owl (Z = 3.10548, p = 0.0019) with lower value observed in eastern bluebirds (Fig. 5). All other pairwise comparisons yielded non-significant differences in blood Hg concentrations. Nestling blood Hg concentrations were significantly different among nests (Kruskal Wallis Test, $X^2 = 32.9278$, $p = 0.0005$). However, after a Bonferroni correction no nonparametric comparisons for each nest was significant.



A.



B.

Fig. 4 Box-plots for adult feather Hg concentration (ng/g) by species for A. Caddo Lake, B. Lake Lewisville. Sample sizes are above each box-plot. Species are arranged on x-axis in order of increasing trophic level based on literature review. Letters above box-plots indicate statistically distinct groups as designated by a nonparametric comparison for pairs using the Wilcoxon method.

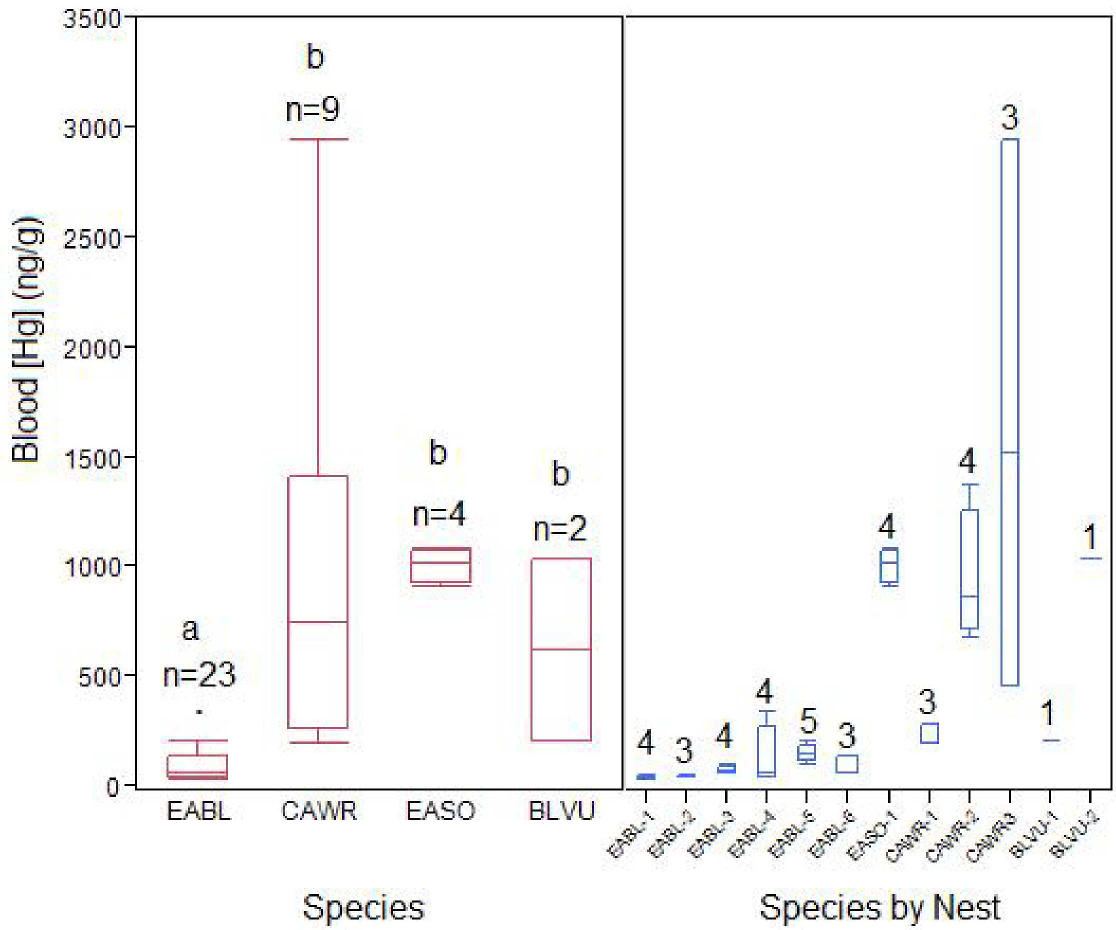


Fig. 5 Box-plots for nestling blood by species and by nest at Caddo Lake only. Sample sizes are above each box-plot. Species are arranged on x-axis in order of increasing trophic level based on literature review. Letters above box-plots indicate statistically distinct groups as designated by a nonparametric comparison for pairs using the Wilcoxon method.

DISCUSSION

Mercury Concentrations in Birds at Caddo Lake and Lake Lewisville

The main objective was to quantify Hg concentrations in birds in a region that has received little attention despite evidence that high Hg concentrations exist for other organisms in the region (Chumchal et al., 2011). To my knowledge, this is the first study to document Hg concentrations in birds from multiple trophic levels in northeast Texas.

Significant differences in Hg concentrations were observed between most pairwise comparisons based on trophic level criteria, with higher Hg concentrations generally observed with increasing trophic level. Further, significant differences in Hg concentrations of the same species were observed between Caddo Lake and Lake Lewisville in northeast and north central Texas, respectively. These latter results suggest that birds residing at Caddo Lake have much higher tissue Hg concentrations compared to birds at Lake Lewisville, with some samples possessing concentrations shown to cause adverse effects on reproductive success (Burger and Gochfeld 1997).

At both Caddo Lake and Lake Lewisville, the majority of the wood duck feather Hg concentrations were generally low (67% and 71% of samples were below 1000 ng/g, respectively). At least two individuals at both sites, however, had feather Hg concentrations above 5,000 ng/g. It is unknown why Hg was elevated in these samples.

At Caddo Lake, a sampled nest box contained adult wood duck feathers with high Hg concentrations (above 11,000 ng/g) and 3 dead nestlings possessing correspondingly high feather Hg concentrations (8,600, 10,200 and 18,300 ng/g). Other nest boxes located in close proximity had low feather Hg concentrations (<600 ng/g). It is unknown why Hg was elevated in these samples. Unfortunately, isotope data was not collected so it is unknown if C:N and feeding habits differences exist between boxes. As previously discussed, it has been shown that wood ducks increase the proportion of animal food items relative to vegetative materials before and during the breeding season (Drobney and Fredrickson 1979). Feeding on higher trophic level prey may increase individual and offspring exposure to potentially dangerous contaminants. Further research is warranted to determine how variability in wood duck feeding habits influence individual Hg concentrations.

Likewise, variability in feeding habits within trophic guild, such as insectivorous passerines, is also expected to influence Hg concentrations. In the present study, Carolina wren nestlings had significantly higher blood Hg concentrations than eastern bluebird nestlings at Caddo Lake. Differences between these two species may be due to a combination of prey choice and nest box proximity to Caddo Lake. Specifically, Carolina wren diet typically possess a greater percentage of animal matter in their diet during throughout the year than eastern bluebirds (94% vs. 68%; Beal 1915; Beal et al. 1927), which may help explain these results. However, Carolina wrens were sampled from artificial wood duck nest boxes located in an aquatic habitat on Caddo Lake, whereas eastern bluebird nest boxes were located within terrestrial habitat approximately 3 to 10 km from Caddo Lake. Thus, is it likely that habitat differences also influenced their exposure to Hg due to differences that may exist in Hg concentrations among their respective arthropod prey (Walters et al. 2009). For example, it has been shown that riparian spiders that consumed aquatic insects from contaminated water source had higher contaminant concentrations than upland spiders that consumed terrestrial insects (Walters et al. 2009). This pattern may also occur between birds that feed on aquatic versus terrestrial insects and spiders. These results suggest that several variables may contribute to Hg exposure risk. Further study is warranted to determine if the differences in Hg concentrations within trophic guilds are influenced more by nest location or prey type.

Hg concentrations in both species were similar to those previously associated with reduced fitness in wild birds (Frederick and Jayasena 2011; Jackson et al. 2011). Great egrets had higher feather Hg concentrations than all species other than great blue herons sampled at Caddo Lake. Eleven of fourteen (78%) and two of four (50%) great egret samples were above 5,000 ng/g at Caddo Lake and Lake Lewisville, respectively. Great blue herons feather Hg concentrations were all above 5,000 ng/g and higher than all other species sampled at Caddo Lake. Great blue heron feather concentrations were similar to the concentrations associated with endocrine disruption in white ibises that resulted in skewed sex hormone concentrations, same-sex mating, and reduced reproductive success (Jayasena et al. 2011).

Comparing species based on their trophic level within and among populations provides information useful for determining the range of Hg concentrations that may influence population viability differences among species. Most Hg studies to date have focused on high trophic level, often piscivorous species, yet recent studies have found that terrestrial insectivores may also be at risk and warrant further investigation (Rimmer et al. 2005; Cristol et al. 2008; Wada et al. 2009; Edmonds et al. 2010). The present study is unique in that it included bird species from differing trophic levels all from the same geographic area.

Implications for Avian Health at Caddo Lake and Lake Lewisville

A common challenge when conducting studies on Hg concentrations in free-living organisms is predicting the biologically significant risks to the organism's health (Eisler 1987; Burger and Gochfeld 1997). The dose-specific effects of dietary Hg in controlled studies have been investigated for decades in birds (Heinz 1979; Scheuhammer 1988; Bennett et al. 2009; Frederick and Jayasena 2011). However, few studies have reported subsequent Hg concentrations in tissues easily sampled from live birds (i.e. feather, blood; but see Heinz 1979; Burger and Gochfeld 1997; Frederick and Jayasena 2011). This information, particularly in relation to environmentally relevant Hg concentrations, would be useful for conservation purposes when trying to predict negative Hg-associated consequences in studies of free-living populations. A further challenge is that species-specific Hg sensitivity is highly variable. Therefore, predictive data based on a few species may be of limited value when monitoring multiple diverse taxa (Heinz et al. 2009). More work is needed identifying the negative effects associated with tissue Hg concentrations in the wild for a much larger number of species than currently studied. However, some studies have provided data on feather and blood Hg concentrations and associated effects in similar species to those sampled in this study (i.e. Anseriformes: Anatidae, Passeriformes, and Ciconiiformes). These may be compared to concentrations found in this study in order to predict potential risks of Hg concentrations found in birds at Caddo Lake and Lake Lewisville, TX.

While variation exists documenting adverse affects associated over a broad spectrum of Hg concentrations, the National Academy of Sciences (1978) has recommended Hg values should not exceed 5,000 ng/g in feathers and 3,000 ng/g in blood has been widely recognized as the lowest observed adverse effect level (LOAEL) in common loons, though a LOAEL has not been established for other species (Eisler 1987; Evers et al. 2008). Several studies have detected adverse physiological, reproductive or behavioral effects above these concentrations. For example, decreased reproductive success due to reduced number of intact eggs laid per day, increased eggs laid outside nest, and decreased number of ducklings that survived to one week was reported for mallard duck (*Anas platyrhynchos*) females with diets of 500 ng/g methylmercury which resulted in feather Hg concentrations of 9,000-11,000 ng/g (Heinz 1979). Of the females that produced offspring, their nestlings exhibited reduced response to maternal calls (Heinz 1979). Suppressed adrenocortical responses and thyroid hormone concentrations were reported in nestling tree swallows (*Tachycineta bicolor*) with blood Hg concentrations of 354 $\pm$ 22 ng/g (Wada et al. 2009). A ten percent reduction in nest success was associated with 2,400 ng/g Hg in body feathers and 700 ng/g (ww) in blood of free-living Carolina wrens (Jackson et al. 2011). In a dose-dependent manner, decreased egg production and altered courtship behavior including increased male-male pairing, decreased number of male head bobs, pair bows and approaches by female was reported in white ibises with diets of 0, 50, 100 and 300 ng/g MeHg which resulted in mean feather concentrations of 470- 740, 4,310-8,200, 14,130-17,960, and 23,860-51,320 ng/g, respectively (Frederick and Jayasena 2011).

In consideration of the previously discussed studies, tissue concentrations in the present study indicated that some, but not the majority of, birds at Caddo Lake and Lake Lewisville had Hg concentrations similar to those associated with adverse effects. One wood duck at Caddo Lake had feather Hg concentrations in the range associated with female mallards that exhibited reduced reproductive success and had less responsive nestlings. Interestingly, three dead nestlings were found in this nest box. All adult eastern bluebird and Carolina wren feather Hg concentrations in the present study were less than those associated with a 10% reduction in nest success in Carolina wrens, although some were very close (2,383 ng/g, eastern bluebird; 2,117 ng/g, Carolina wren). All eastern bluebird nestlings at Caddo Lake and Lake Lewisville had blood Hg concentrations below those associated with suppressed adrenocortical response in nestling tree swallows. Great egret feather Hg concentrations at Caddo Lake and Lake Lewisville were similar to feather concentrations associated with decreased egg production and altered courtship behavior in white ibises with diets of 50 ng/g Hg. Great blue heron feather Hg concentration at Caddo Lake was similar to feather concentrations associated with decreased egg production and altered courtship behavior in white ibises with diets of 50 and 100 ng/g Hg. Collectively, these studies suggest that Hg concentrations at Caddo Lake and Lake Lewisville may be high enough to negatively impact the health of some birds residing in these areas.

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