# MERCURY LEVELS IN THE FEATHERS OF TERRESTRIAL SONGBIRDS OF SOUTHEAST MICHIGAN

**A THESIS**

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## Abstract

Sublethal levels of methylmercury can have consequences for the reproductive, neurological and physical health of birds. Despite their lower trophic position, songbirds may be at risk for health effects from mercury exposure depending on their proximity to local pollution sources and high levels of bioaccumulation from contaminated diets. This study investigates mercury concentrations in terrestrial songbirds of Southeast Michigan, where historical and present-day emissions of heavy metals are elevated. We collected tail feather samples from 223 songbirds across four different species during summer and fall of 2018 and summer of 2019. The average mercury concentration across all samples is 222 ± 24.7 ng Hg/g of dry feather weight. Mercury levels vary significantly among foraging guilds and by age, with its concentrations nearly nine times higher in omnivores than in granivores, and approximately two times higher in juveniles than in adults. We find no significant difference between sexes. In comparing different study sites, American goldfinches in urban areas of Flint have significantly higher Hg levels than those at rural areas, and American robins in watered lawns have approximately three times the Hg levels than those in unwatered lawns, suggesting that local Hg emissions in addition to site-specific dietary intake and subsequent bioaccumulation could increase Hg exposure in songbirds. While our samples do not exceed sublethal levels, our findings provide an insight into the spatiotemporal and demographic patterns of mercury concentrations which may aid in understanding the threshold levels for various health effects and identifying vulnerable species.

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## Introduction

Mercury (Hg) is a widespread and persistent environmental pollutant that is harmful to humans and wildlife (Jackson et al. 2015). Once introduced to terrestrial and aquatic ecosystems, primarily through atmospheric deposition (Landis & Keeler 2002; Hammerschmidt & Fitzgerald 2006), it may be converted to methylmercury (MeHg) that is capable of bioaccumulating and biomagnifying within food chains (Cristol et al. 2008; Scoville & Lane 2013). Many species of bird have a long lifespan and occupy higher trophic positions, making them particularly vulnerable to the effects of Hg exposure (Evers et al. 2005). Sublethal levels of MeHg have consequences for their reproductive health (Brasso & Cristol 2007; Evers et al. 2008; Jackson et al. 2011b; Varian-Ramos et al. 2014), neurological functioning (Wolf et al. 2017; Scoville & Lane 2013) and immune systems (Lewis et al. 2013; Whitney & Cristol 2017) which could reduce overall fitness and impact their populations.

Much research surrounding the effects of Hg on avian physiology to date focuses on large aquatic and piscivorous birds (Evers et al. 2005; Scheuhammer et al. 2007; Lavoie et al. 2014; Frederick et al. 2004) due to the known biomagnification of Hg within aquatic food chains. However, less is known about the health effects of Hg exposure among songbirds (avian Order Passeriformes), especially those associated with terrestrial habitats. These species may also be at risk due to their proximity to local pollution sources (Jackson et al. 2011a), high level of bioaccumulation from foraging behavior (Keller et al. 2014) and contaminated diets (Cristol et al. 2008), and varying surface characteristics that increase the dry deposition of Hg (Miller et al. 2005; VanArsdale et al. 2005). Studies show that the total Hg level in terrestrial forest songbirds from a

historically contaminated site (Cristol et al. 2008) and blood Hg level in wetland insectivores like red-winged blackbirds (Evers et al. 2005) are higher than those of aquatic-feeding and piscivorous birds respectively. Although the threshold for detrimental effects on songbird health is not well-established (Seewagen 2010), there are reports of Hg-related decline in the proportion of eggs hatching (Hallinger & Cristol 2011) and fledgling success (Brasso & Cristol 2007; Hallinger & Cristol 2011) in free-living songbirds like tree swallows near contaminated sites. Similarly, European starlings dosed with 1.5 ppm MeHg show reduced energy expenditure during escape takeoff and increased molt rate than the controlled starlings, which could negatively affect their feather quality, thermoregulation ability and flight performance (Carlson et al. 2014).

Mercury is a metal of concern in the state of Michigan as well as the broader Great Lakes region where anthropogenic Hg emissions largely contribute to levels of MeHg in food webs that could harm humans and wildlife (Risch et al. 2014). Michigan and its surrounding states, Ohio and Indiana, are among the top 10 airborne emitters of Hg in the United States (Madsen & Randall, 2011). Atmospheric deposition of Hg is also especially elevated in urban areas of Michigan (Keeler et al. 2006; Liu et al. 2010) mainly due to combustion from coal-fired power plants. In southeast Michigan, Hg wet deposition represents a primary loading to lakes and watersheds (Risch et al. 2012) and its rate is two-fold higher as compared to the Upper Peninsula (Keeler & Dvonch, 2005). Due to the high concentrations of Hg deposited in the Great Lakes aquatic ecosystems, there are statewide consumption advisories on Hg in fish (Evers et al. 2011b) as well as a growing number of studies focusing on Hg toxicity among predatory fish and piscivorous wildlife (Evers et al. 2011a; Weseloh et al. 2011). Primary avian indicators, including the

common loons, show that the spatial distribution of Hg is heterogeneous in the region but proximity to local emission sources and landscape sensitivity likely increase biological hotspots (Evers et al. 2011a). However, to our knowledge, there are no published studies on Hg exposure among terrestrial birds in this area.

In our study, we measure feather Hg concentrations among four year-round terrestrial songbirds from Southeast Michigan. Past studies have commonly used feathers as one of the sampling substrates to assess Hg levels among avian species (Keller et al. 2014; Lane et al. 2020; Stenhouse 2019; Warner et al. 2012). Feather Hg concentrations are highly correlated with blood levels during the time of feather growth (Bearhop et al. 2000) and reflect short-term, site-specific dietary intake of MeHg in both juvenile and adult birds (Condon & Cristol 2009; Evers et al. 2005). In adults, especially those exposed to high levels of MeHg, feather Hg may also reflect long-term chronic body burden as MeHg is depurated into feathers from contaminated muscle tissues (Evers et al. 2005). Feathers are also a simple and non-invasive alternative to other tissues, and Hg in feathers are highly stable for an extended period of time (Teraoka et al. 2012). Despite feathers being a convenient proxy for dietary Hg exposure, Hg levels could vary across different feather tracts within the same individual (Low et al. 2019; Peterson et al. 2019). They could further be complicated by the timing of molt, migration and breeding, and the associated shift in diets with the sampling periods (Eagle-Smith et al. 2008; Ackerman et al. 2008; Low et al. 2019; Peterson et al. 2019), which may limit the ability to accurately compare and quantify exposure levels across different studies. However, feathers provide qualitative insight into the factors that affect and determine Hg levels among songbirds and are also suitable for sampling Hg levels in species that have limited annual

movement or other ecological attributes that provide information on the location of feather molt (as cited in Ackerman et al. 2019).

The primary objective of this study is to examine the effects of foraging guilds (omnivore and granivore), age (adult and juvenile), sex (male and female) and location (urban and rural areas of Flint and Ypsilanti, Michigan) in understanding the demographic and spatial variations of Hg bioaccumulation within and across different species of terrestrial songbirds in southeast Michigan. We assess tail feather Hg concentrations in two native species: American Goldfinch (*Spinus tristis*) and American Robin (*Turdus migratorius*), and two invasive species: European Starling (*Sturnus vulgaris*) and House Sparrow (*Passer domesticus*). In American robins, we also investigate the effects of watered lawns on their exposure to MeHg, and the relationship between sample Hg concentrations and body conditions.

## Methods

### Experimental Design

***Sample Collection.*** We collected feathers from 223 individual songbirds across four species: American goldfinch (n=109), American robin (n=66), European starling (n=18) and house sparrow (n=30) from May to October 2018 and April to July 2019. After we caught the birds with feeder traps, ground traps and mist-nets using acoustic lures, we immediately extracted them from the mist net or trap for age and sex identification via plumage and breeding indicators (brood patch and cloacal protuberance). We plucked two symmetrical tail feathers from each bird: one for dry weight measurement and the other for total mercury analysis. The feathers were labeled

and sealed in plastic bags, and shipped to Colorado College for analysis. Birds were equipped with a unique U.S. Fish and Wildlife Service band and were released unharmed.

***Study Species.*** We group American robins and European starlings as omnivores and American goldfinches and house sparrows as granivores following the Birds of the World in the Cornell Lab of Ornithology database. American robins forage largely on lawns, switching their diet between worms in spring and summer and berries in the winter (Vanderhoff et al. 2020). European starlings also tend to feed on varieties of worms, insects and berries and forage around rural farms (Cabe 2020). American goldfinches and house sparrows on the other hand are largely seed and grain eaters (McGraw & Middleton 2020; Lowther & Cink, 2020). In terms of habitat, we considered all species as mostly year-round residents in North America, although American robins and American goldfinches are partial migrators during the winter, and European starlings and house sparrows are invasive species. All of the species are local breeders in southeast Michigan and we caught the adults during their breeding seasons. We also expect that juveniles grew their feathers locally assuming that they did not disperse far before the capture. Only feathers collected before the molting period for each species were included, so we assume that the measured Hg levels reflect dietary MeHg from local pollution sources that accumulated throughout the period of feather development from the prior pre-alternate molt. Of the total samples, 105 were males, 100 were females, and 18 were of unknown sex; all of the individuals with unknown sex were juveniles.

***Study Sites.*** We captured birds from urban and rural sites of Flint and Ypsilanti in southeast Michigan (Fig. 1). These sites were categorized based on their proximity to

infrastructure such as buildings, parking lots and industrial sites as well as populated areas. Urban sites in Flint included the University of Michigan-Flint (UMF) campus, Charles Stewart Mott Library in Mott Community College (MCC), and Bassett and Mott community parks. The urban site in Ypsilanti was located at the Eastern Michigan University (EMU) campus. Rural sites included Fish Lake in Lapeer and a residential backyard on Clark Road in Ypsilanti. All five urban and two rural sites have local rivers and lakes nearby including a human-made pond on the EMU campus. The Huron River runs through Ypsilanti (0.23-1.1km from sampling sites) and the Flint River is in proximity to the urban sites in Flint (0.25-1.3 km). Urban sites including UMF, MCC and EMU also have lawns that are extensively watered (hereby referred to as watered sites) while the remaining four sites are unwatered.

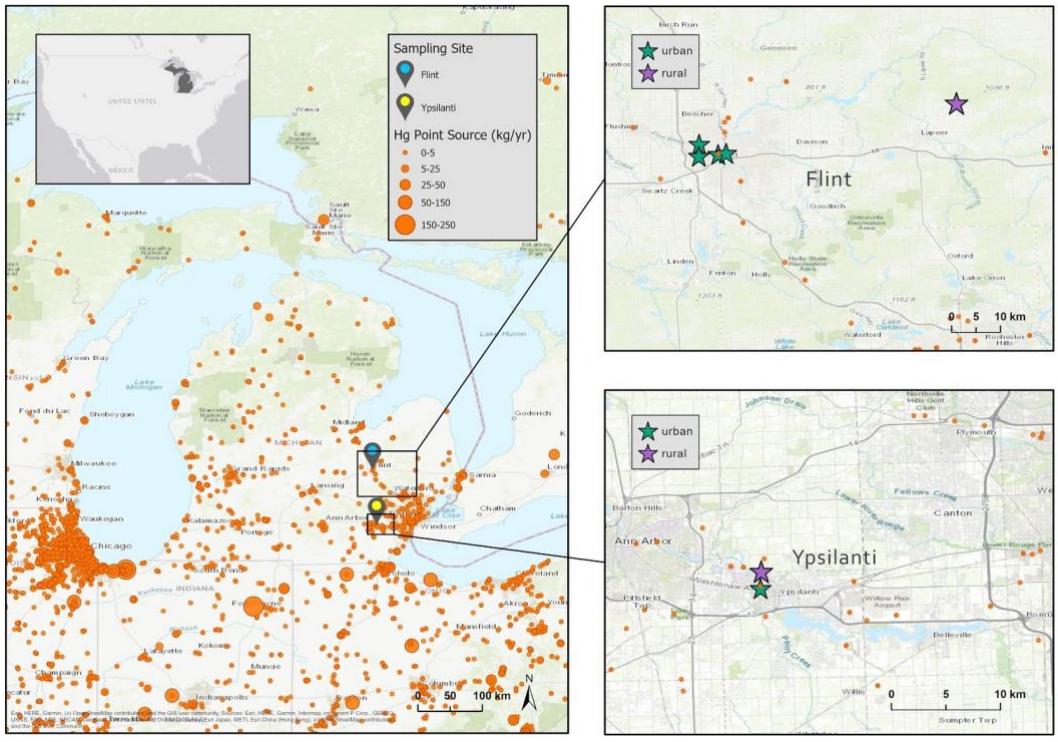

**Fig. 1**: Total annual atmospheric mercury emissions (kg/yr) in Michigan and its neighboring states (left). Urban and rural sampling sites at Flint (top right) and Ypsilanti (bottom right) in southeast Michigan. Map point source data: NEI and NPRI

**Laboratory Analyses**

***Sample Pre-treatment and Dry Weight Measurement.*** We washed each feather with Formula 409 and thoroughly rinsed them with Milli-Q water to remove any external contamination. After the cleaned samples were air-dried for 1.5 hours, the initial mass of the feather was measured using a precision analytical balance. One feather from each individual was then oven-dried (Lab-Line Imperial V Laboratory Oven) for 3 hours at a constant temperature of 105°C. Feather masses were measured again once they cooled to room temperature. We then applied percent weight change in each feather to its corresponding feather pair that was used for the total mercury analysis.

For samples collected before September 2018 (n=84), we applied an average dry weight correction due to our use of a lower precision balance (0.0001g) for determining feather masses during that period; all samples collected after September 2018 were weighed using a higher precision balance (0.000001g) which was more suitable for detecting our range of weight changes after oven-drying. To calculate the new dry weights for samples collected prior to September 2018, we took an average percent weight change for each species from samples collected after September 2018 (Table 1), and applied accordingly to the corresponding species. We then compared those values to the true dry weight corrected masses in that subset of samples (Fig. 2). The strong positive correlation (slope = 0.9974, $R^2$ = 0.9998) indicates a minimal difference between the actual and the average percent weight change, offering confidence in our application

of these average dry weight corrections to samples collected before September 2018. Dry weights for feathers collected after September 2018 are based on the true weight changes.

| Species | Count (n) | Average wet feather weight ± 1σ (g) | Average percent weight change ± 1σ (%) |
|---|---|---|---|
| American goldfinch | 73 | 0.00526 ± 0.00113 | 4.75 ± 0.77 |
| American robin | 49 | 0.0357 ± 0.00711 | 6.04 ± 1.64 |
| European starling | 15 | 0.0197 ± 0.00189 | 6.76 ± 0.97 |
| House sparrow | 25 | 0.0106 ± 0.00164 | 5.50 ± 0.53 |

**Table 1** Average wet weights and percent weight changes in feathers collected after September 2018 for each species

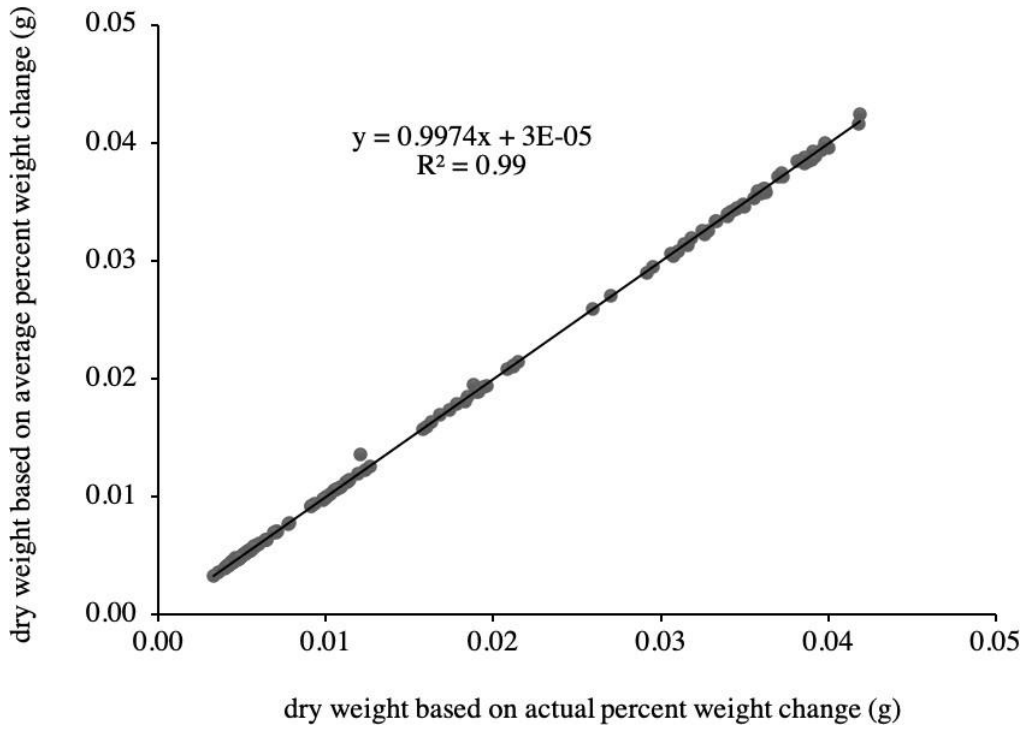


**Fig. 2** Linear regression and square of the correlation coefficient between dry weights of feather based on actual versus average percent weight change for samples collected after September 2018

***Feather Digestion.*** We adopted our digestion procedure for feathers from the *Tekran Series 2600 Total Mercury Analysis in Human Hair* described in Application Series: AN2600-08. Each digestion took place in a 40ml VOA vial with a Teflon-lined cap, both of which were previously washed with Citranox ®, soaked in 2% Bromine chloride overnight, and dried in a trace metal-clean laminar flow hood. All of the samples collected between May to October 2018 (n=125) were digested in 10ml of Reagent-Grade Nitric Acid (69.0 to 70.0%, Baker Instra-Analyzed™). For samples collected between April and July 2019 (n=98), samples were digested in either 5ml or 7ml of nitric acid (depending on the feather size) in order to increase sample concentrations and also lower

the acid content of digestions for optimal analytical conditions; smaller feathers including that of American goldfinches and house sparrows were digested in a lower volume of nitric acid while larger feathers of American robins and European starlings were digested in a higher volume of the acid. Following an overnight digestion, the samples were gradually heated at increments of 10°C for 1.5 hours using an SC 154 Environmental Express Hot block ®. The samples were then allowed to reflux at a constant temperature of 106°C for the following 3 hours and subsequently cooled to room temperature.

After the samples cooled, we removed a 1 ml aliquot of each digested sample into a separate vial for the eventual analysis of other heavy metals. The remaining aliquot was diluted with bromine chloride to preserve mercury within the sample and to achieve the detection limits (< 200 ppt) of the *Tekran 2600* mercury analyzer. Feather samples collected between May and October 2018 (n=125) were diluted in 15ml of 5% bromine chloride while samples collected between April and July 2019 (n=98) were diluted in 10ml of 2% bromine chloride. As with the modification to the digestion procedure, this change was similarly made to lower the acid concentration and increase sample concentration in an effort to optimize sample conditions for analysis on the *Tekran 2600*. Following the dilution, we sealed the samples tightly with Teflon tape and refrigerated until total mercury analysis was performed.

***Quality-control.*** We digested a human hair certified reference material (ERM-DBO-010198) in every batch of 20-30 feather samples to verify the accuracy of our methodology. The digestion and analysis procedure for reference material, including the digestion and dilution volumes, was consistent with procedures used in the corresponding batch of feather samples. The overall average percent recovery for mercury was 90.7 % ±

6.2% (n=29). In accordance to the aforementioned changes in the volume of acid used for digestion as well as the volume and concentration of bromine chloride for sample dilution (Section 2.2.2), we also digested additional reference materials (n=8) to ensure that the varying concentrations and volumes of bromine chloride did not alter the consistency of our total mercury detection. The average percent difference between these reference material samples was 1.96% ± 5.8%, indicating minimal variability in the Hg recovery regardless of the acid or dilution volume used.

Before the total mercury analysis and during the second dilution process, we also prepared a replicate every 10 samples to test for instrumental accuracy. The average percent difference for the replicate agreement was 4.85 % ± 6.8% (n= 33). We also prepared 5-10 ml of nitric acid lab blanks in a 40ml VOA vial consistent with the preparation and dilution procedure for feather samples, with the exception that they were not heated. The average lab blank was 0.36 ppt ± 0.13 ppt (n=6) and all of our samples were greater than the blank with the lowest concentration detected at 0.51 ppt.

***Total Mercury Analysis***. Depending on the variability of the feather sizes and anticipated Hg concentrations for each species, 1ml to 3ml of sample aliquot was pipetted and diluted up to a total volume of 25 ml using 0.5% bromine chloride in Milli-Q water, as per EPA Method 1631. We then performed total mercury analysis, following reduction by stannous chloride and hydroxylamine hydrochloride, using cold vapor atomic fluorescence spectrophotometry (CV-AFS) on a Tekran ® 2600 Automated In-Vial Sparging Mercury Analysis System. Samples were analyzed for total mercury, which approximates to the amount of methylmercury, given that 90 to 100% of mercury in avian blood and feathers is methylmercury (Condon & Cristol, 2009).

***Statistical Analysis and Mapping*.** We conducted all statistical analysis in Microsoft ® Excel 2016 and IBM ® SPSS Statistics 26. After Levene's Test of Equality of Variances for the assumption of homogeneity of variance, we used independent sample t-tests to find significant differences between two groups of variables. Mercury concentrations are reported in ng/g of dry weight feather ± standard error of the mean (SE).

We calculated body condition for each sex (adult male, adult female, and unknown (all juveniles)) by first running a principal component analysis (PCA) of keel, tarsus and wing chord, and using the first principal component (PC1) as an estimate of size. We then regressed PC1 against body mass and saved the residuals as an estimate of body condition, where positive values represent an above average body condition and negative values represent a below average body condition.

Emission point sources were mapped using ArcGIS ® Pro 2.4.2. We used the 2017 National Emission Inventory (NEI) data from the United States Environmental Protection Agency (EPA) and the 2017 National Pollution Release Inventory (NPRI) data from Environment and Climate Change Canada to plot the annual atmospheric mercury emission for the states in EPA Region 5 and Ontario respectively.

## Results and Discussion

### Overall species-level difference

The average tail feather Hg concentration of four different species is 222 ± 24.7 ng Hg/g (range: 18.5 ng/g to 2510 ng/g, N= 223). Mercury concentrations are above detection limits in all samples, and means significantly differ between the four species

(Fig. 3). The highest mean Hg concentration is in the feathers of American robins (562 ± 65.2 ng/g), followed by European starlings (252 ± 27.9 ng/g), house sparrows (84.0 ± 15.4 ng/g) and the lowest in American goldfinches (49.8 ± 2.18 ng/g). The mean level observed in our study is below the threshold of negative reproductive consequences studied among a free-living terrestrial songbird (Carolina wrens), where a 10% reduction in nest success is observed at tail feather Hg level above 3000 ng/g (Jackson et al. 2011 b). However, there is very little research upon which to base such benchmarks in songbirds more generally, and it remains unknown if Hg may have consequences at lower levels for physiology or behavior in other species (Seewagen 2010). We find that 4% of our total samples have levels greater than 1000 ng/g and three American robins have levels above 2000 ng/g.

Comparison of Hg levels among different studies is difficult due to variations in the species sampled and their corresponding habitats and diets, as well as the type of body tissues or specific feathers examined. Among the recent research that has specifically focused on terrestrial songbirds, which is relatively limited, the reported mean feather Hg values are a similar order of magnitude as our species averages (Warner et al. 2012; Stenhouse et al. 2019; Cooper et al. 2017), but there are geometric means as high as 110 ng/g in breast feathers of American goldfinches and 3220 ng/g in American robins (Ackerman et al. 2019). However, the levels reported by Ackerman et al. (2019) for other finches like the house finch (40 ng/g) and lesser goldfinch (70 ng/g) are similar to that of the American goldfinches we sampled. In tidal marshes of the northeastern USA, saltmarsh sparrows exposed to elevated levels of Hg via wetland ecosystems have average concentrations between 1800-2400 ng/g in their tail feathers (Lane et al. 2020),

which is comparable to some of the highest concentrations observed among American robins at our urban sites in Flint and Ypsilanti. Though we did not report any insectivores, one of the highest levels reported in the literature is among tree swallows at contaminated river sites in Virginia, USA with 13550 ng/g of Hg in their P1 primary feathers (Brasso & Cristol 2007), which is well above any levels observed in our data set.

**Foraging Guild**

Foraging guilds seem to be a key indicator of differences in Hg concentrations between species in this study (Fig. 3), and this result agrees with other studies that have confirmed contaminated diets as the primary exposure pathway for MeHg among terrestrial vertebrates (Cooper et al. 2017; Keller et al. 2014). Mercury concentrations are on average 8.7 times higher in omnivores (495 ± 53.4 ng/g) than in granivores (57.2 ± 3.89 ng/g) (Fig. 4). This difference may be explained by the fact that mercury biomagnifies in food chains and with higher trophic levels (Jackson et al. 2015; Keller et al. 2014). Although we classified house sparrows as granivores, we note that they often vary their diets with insects and other arthropods during their breeding season (Lowther & Cink, 2020) which may be why they have higher Hg concentrations than American goldfinches, which are more strict to their granivore diet.

Studies vary in their ability to assess how Hg level differs with foraging guild, in part due to sampling in different seasons and habitats. Mercury levels in breast feathers collected in contaminated riparian floodplains in Central Valley, California ranged from 30 ± 10 ng/g in granivores to 440 ± 100 ng/g in plant-eating omnivores (Ackerman et al. 2019). However, direct comparison with our study is difficult as species sampled in

California include both year-round as well as migratory songbirds that have different exposure periods for Hg accumulation (Ackerman et al. 2019; Knutsen & Varian-Ramos 2019). Our observed mean feather Hg in omnivores compares similarly to that of omnivorous and insectivorous songbirds studied in the Southern Appalachians, whose average Hg level in S1 secondary feathers is 460 ± 20.0 ng/g of Hg (range: 10.0 to 3740 ng/g, n = 458) (Keller et al. 2014). The average is also similar to the level observed in an insectivorous songbird (460 ng/g Hg tail feather) sampled at tidal marshes of Delaware, USA (Warner et al. 2012).

Methylmercury production mainly occurs in aquatic-associated habitats (Driscoll et al. 2013). A recent study indicates that some terrestrial songbirds could receive substantial aquatic subsidies of MeHg through emergent aquatic insects and the associated food web (Cristol et al. 2008). Birds do not necessarily have to be exposed to emerging aquatic insects directly, as predatory invertebrates like spiders can serve as a link in transferring and biomagnifying MeHg from aquatic to terrestrial food webs (Jackson et al. 2015). Although all of our sampled species are terrestrial songbirds, our sampling sites are close to nearby rivers and lakes (Fig. 1) which may favor MeHg production and make the species more susceptible to higher Hg consumption. Thus, despite their terrestrial foraging habitats, some terrestrial species could be exposed to higher levels of MeHg than expected.

**Age**

Understanding how Hg concentrations in songbirds vary during different life stages is important because their diets may change with physiological growth and also because early-life MeHg dynamics could influence Hg concentration into the fledgling

period and adult life (Jackson et al. 2015; Warner et al. 2012) if it impacts developmental processes. Studies suggest that the nestling stage, before internal mercury is depurated into growth feathers, is the developmental period with the greatest toxicological exposure (Ackerman et al. 2011) and also likely the most sensitive stage for producing life-long phenotypic changes from mercury exposure as their neural networks grow (Warner et al. 2012). Elevated Hg levels in juvenile feathers could indicate high Hg exposure during critical stages of early development. Juveniles in our study (431 ± 100 ng/g) have approximately 2.2 times higher Hg levels than adults (192 ± 23.8 ng/g), although when we compare ages within species, only juvenile robins and house sparrows have significantly higher Hg levels than their adult counterparts. Concentrations in juvenile house sparrows (245 ± 38.8 ng/g) are approximately four times higher than in adults (59.3 ± 10.3 ng/g) whereas in American robins, juvenile levels (897 ± 245 ng/g) are almost twice as high as compared to adults (509 ± 63.2 ng/g). Levels in both adult and juvenile American goldfinches are low and show no significant difference (adult: 49.9 ± 2.30 ng/g, juveniles: 46.8 ± 4.44 ng/g) (Fig. 5). We note that the small sampling sizes for juveniles among each species ($n < 10$) limit our ability to draw strong conclusions from these relationships. Nevertheless, we consider some possible explanations.

Firstly, the timing of feather growth and change in diet could be important as juveniles tend to grow their feathers in spring or early summer when they fledge from the nest. Adults, on the other hand, replace their tail feathers later in the summer - when their diet may vary. American robins, for example, consume more berries than insects when they are molting in the late summer, and thus may have a seasonal reduction in overall MeHg intake. Adults are also likely to feed their nestlings the highest-quality food or the

most protein-rich source to optimize nestling growth (as cited in Warner et al. 2012). Higher Hg levels among juveniles could therefore come from parents that selectively feed them more invertebrates such as worms. In addition to the potential for higher intake from their nestling diet composition, juveniles also have higher metabolic and nutritional demand to support their accelerated growth rates, which results in higher food and subsequent MeHg intake per body size (Warner et al. 2012).

Past studies have observed higher feather levels of Hg in juveniles and the authors have partly attributed this to the ability of juveniles to rapidly depurate substantial amounts of Hg from blood into their growing feathers (Ackerman et al. 2011; Condon & Cristol 2009; Eagle-Smith et al. 2008; Evers et al. 2005; Fournier et al. 2002). Fournier et al. (2002) found that the rate of Hg elimination from blood into feathers was faster in younger loons who were actively growing feathers as compared to older loons who were not, suggesting a possibility for similar age-related decline in the rate of Hg depuration into feathers among our adults. When new feathers develop, MeHg from the blood and muscle tissue binds to the keratin structure of growing feathers, which is then permanently sequestered into the feather tissue (Condon & Cristol 2009). This mechanism therefore explains why studies that examine blood Hg levels find lower concentrations in juveniles than in adults (Jackson et al. 2015; Fournier et al. 2002; Knutsen & Varian-Ramos 2019), but the opposite pattern in feathers.

**Sex**

We did not find significant differences in overall Hg levels among males (178 ± 31.0 ng/g) and females (199 ± 33.0 ng/g). Our results are consistent with other studies that report no sex-specific difference in feather Hg levels among passerines like northern

cardinals, grey crested flycatchers (Cooper et al. 2017) and tree swallows (Hallinger et al. 2011) and indicate that both sexes in most species are probably feeding on a similar diet at the same trophic level, and have consistent bioaccumulation rates (Cooper et al. 2017). However, this similarity may not be applicable to house sparrows that show a significant sex-difference (female: 101 ± 21.6 ng/g, n=20; male: 48.2 ± 10.6 ng/g, n=10). In addition to MeHg exposure and intake, detoxification may also be an important factor for the observed difference as the liver is able to demethylate MeHg into organic Hg. Different liver sizes among male and female therefore could be impacting their detoxifying capacity (Robinson et al. 2012); however, we note that our sample size for house sparrows is relatively small. In contrast, some studies have found lower Hg concentrations in females and have ascribed this to their smaller body mass and their tendency to get rid of contaminants through eggs and eggshells during their breeding season (Robinson et al. 2012; Burgess et al. 2005). Our data do not support this hypothesis as female robins are considerably smaller than males but did not have lower Hg levels. There is limited research on sex-specific differences in Hg exposure and elimination among songbirds and further study is needed to understand this relationship. In addition to studying sex-specific physiological differences in mercury metabolism (Robinson et al. 2012), examining blood Hg levels in females during the breeding and non-breeding seasons, as well as in eggs, could expand our understanding of the female-specific processes that may or may not reduce mercury body burden.

**Location**

The Hg levels among birds caught in Flint (235 ± 36.9 ng/g, n=97) and Ypsilanti (211 ± 33.3 ng/g, n=126) do not significantly differ. Although these two sites are

approximately 105 km apart, both cities have small-scale, local Hg emission sources including electric power generators, automobile and truck plants and compressor stations that emit Hg and may contribute to its deposition into local ecosystems (Fig. 1). Regional mercury emissions from industrial facilities in nearby cities could also be transported and deposited at our sampling sites depending on wind patterns and seasons (Liu et al. 2010).

In Ypsilanti, there are no significant differences in feather Hg between urban and rural sites in any species. Although the rural site is a forested area with no traffic and has fewer buildings compared to the urban site, the lack of any differences may be explained by the closeness of the two sites to one another, and perhaps their close proximity to the Huron River. The two sites are only about one kilometer apart and therefore do not have major differences in their nearby atmospheric emission sources (Fig. 1). In contrast, the urban and rural sites in Flint are 45 km apart, yet there is still no significant difference between them except in American goldfinches (urban: 55.7 ± 3.42 ng/g, rural: 45.8 ± 2.74 ng/g) (Fig. 6) for which the concentrations are already relatively low in general. American robins caught at urban sites in both Flint and Ypsilanti are higher than the ones in rural areas but the differences are not significant. We also did not catch house sparrows and caught only two European starlings in rural areas, which limited our ability to make comparisons in those species.

Mercury levels may have been significantly higher in urban American goldfinches but not in robins because of their contrasting foraging guilds and diet composition. As discussed earlier, robins feed on invertebrates that are indirectly associated with aquatic ecosystems and the lakes surrounding the rural site in Flint could accumulate similar levels of Hg as some of the urban sites - thus exposing robins to similar amounts.

American goldfinches, on the other hand, are strict granivores whose diet is less directly associated with aquatic habitats, thus making urban and rural differences more evident. Despite goldfinches having the lowest concentrations among all of our sampled species, this significant difference may suggest that urban areas could be more impacted by local Hg emissions (Fig. 1) compared to rural sites. A study based on an urban site in Detroit, MI and a rural site in Dexter, MI concluded that despite both sites being affected by regional Hg sources and/or meteorological conditions, Hg levels in Detroit are subject to substantial local source influences (Liu et al. 2010), supporting our reasoning for why urban levels might be higher in Flint goldfinches. We note that the link between atmospheric Hg emission and the bioavailability of MeHg for exposure in songbirds is affected by factors including landscape sensitivity (elevation, pH), watershed features and the associated biogeochemical cycling (Evers et al. 2011a). This may complicate the direct association between atmospheric emission and wildlife exposure, but some studies have observed a temporal decrease in fish and bird egg Hg concentrations as a result of the reduction in regional Hg emissions around the Great Lakes area (Evers et al. 2011b; Weseloh et al. 2011). Similar long-term studies focusing on the effects of local and regional Hg emissions as well as wet deposition on different habitats of terrestrial songbirds are necessary to better understand the relationships.

Aquatic environments are prone to MeHg formation and subsequent biomagnification in the food chain due to direct Hg atmospheric deposition, and input from river runoff and industrial discharge (Driscoll et al. 2007; Ahmadpour et al. 2016). In American robins, the average Hg concentrations in watered sites of both Flint (900.88 ± 239.03 ng/g, n=10) and Ypsilanti (634 ± 116.5 ng/g, n=28) are significantly higher than

the unwatered site in Flint (333.61 ± 56.09 ng/g, n =11) (Fig. 7); we do not have an unwatered site in Ypsilanti. Mercury concentrations for other species that occasionally feed on insects and worms, including European starlings and house sparrows, are also higher for watered sites, though not significantly so. Watered lawns could induce worm hatching as well as increase their dispersal movements, creating more opportunities for robins to feed on these prey and increase their MeHg exposure.

**Body condition**

We found a negative correlation ($R^2$ = 0.25, p<0.0001, F= 18.277) between Hg concentrations and sex-specific body condition in American robins (Fig. 8), implying an effect of Hg exposure on lowering body condition. However, we advise caution in the interpretation given the relatively small sample size (n=66) across the range of measured Hg concentrations in this subset of data. For example, the body conditions are more variable at lower to moderate levels of Hg, while just four robins have Hg concentrations greater than 1900 ng/g and have below-average body conditions; a Cook's D test suggests that three of these four values may be overly-influential and a larger dataset is needed to verify the observed relationship. Nevertheless, other studies including a model-predicted assessment among 52 different species of resident and migratory songbirds also found a negative correlation between Hg levels and body condition - where blood Hg levels decreased by 44% over a range of standardized body masses (Ackerman & Herzog 2019). Similarly, blood, as well as breast and head feather Hg levels in California clapper rails, were negatively correlated with body condition (Ackerman et al. 2012). In aquatic birds, including sea and diving ducks, masses of various internal organs also decreased with increasing Hg concentrations (Takekawa et al. 2002; Wayland et al. 2003). In

combination, these studies support our observation that Hg exposure may lead to reduced body condition, which could indicate negative impacts on fitness.

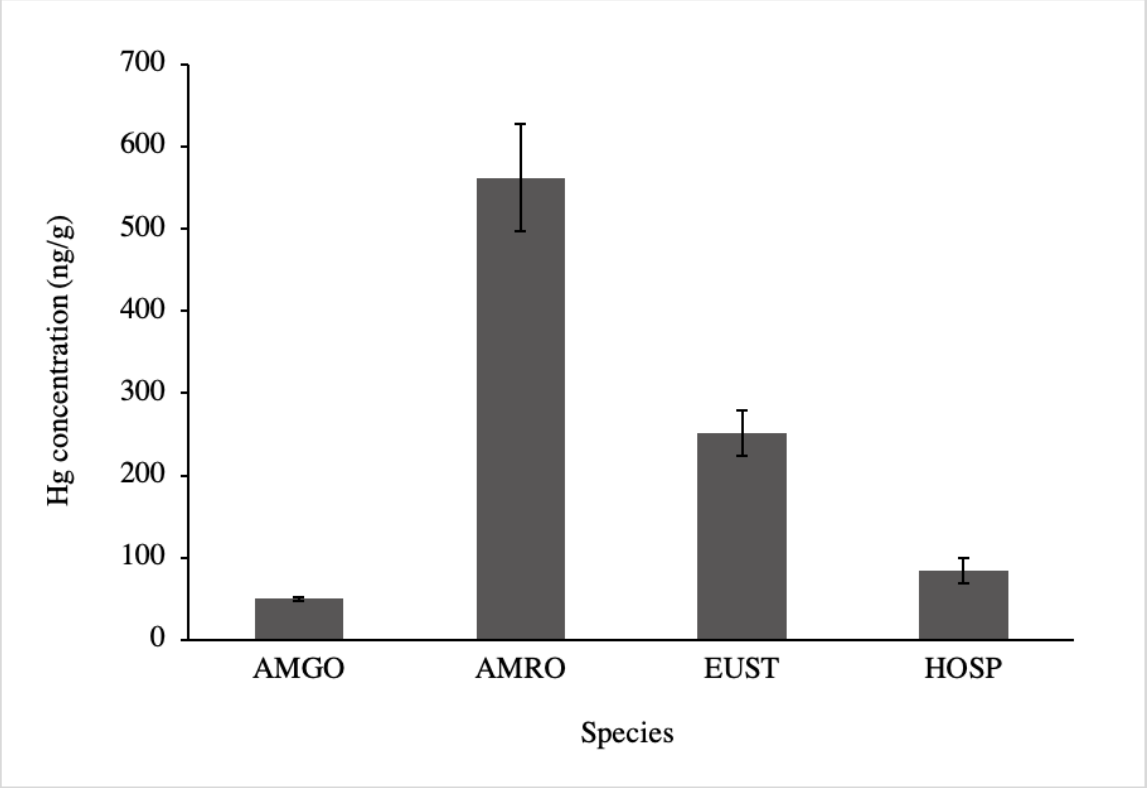


**Fig. 3** Average Hg concentrations across four species of songbirds from Southeast Michigan: American goldfinch AMGO (n=109), American robin AMRO (n=66), European starling EUST (n=18) and house sparrow HOSP (n=30). Mean values are significantly different ($p<0.05$) between each species. Error bars represent one SE of the mean

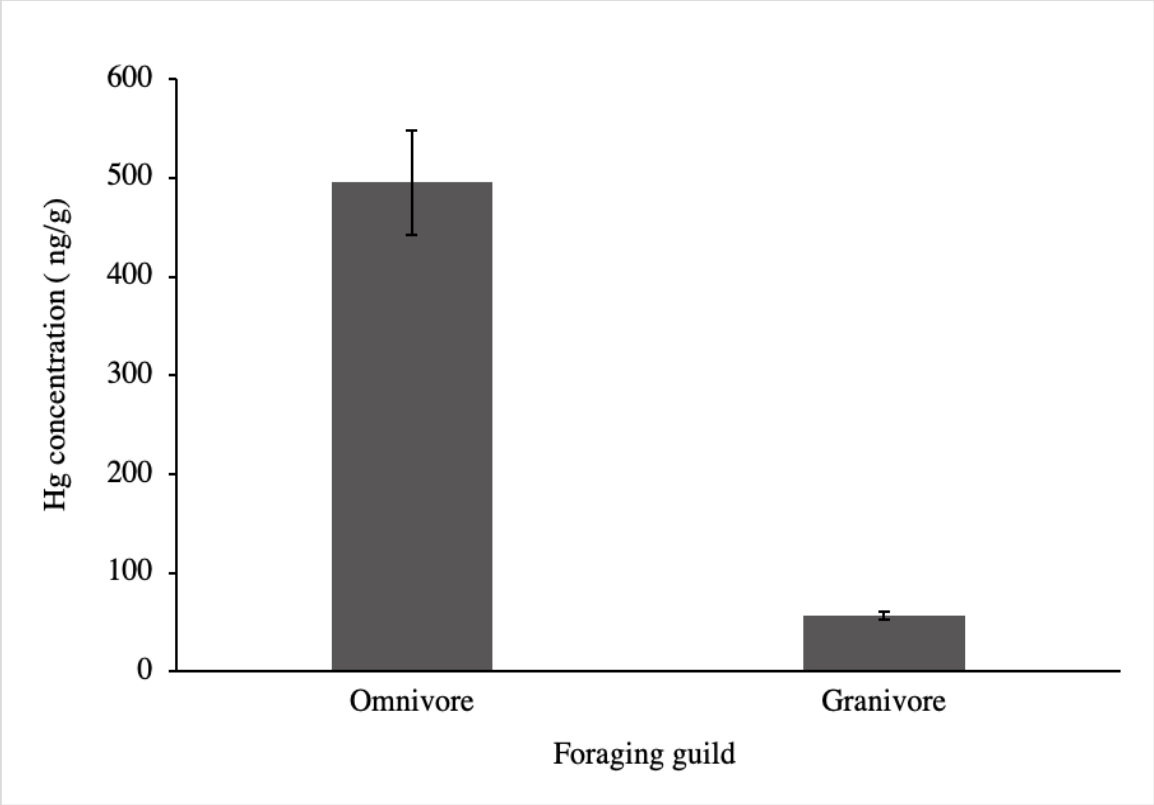


**Fig. 4** Average Hg concentration among omnivores (n=84) and granivores (n=139). Mean values are significantly different ($p<0.05$). Error bars represent one SE of the mean

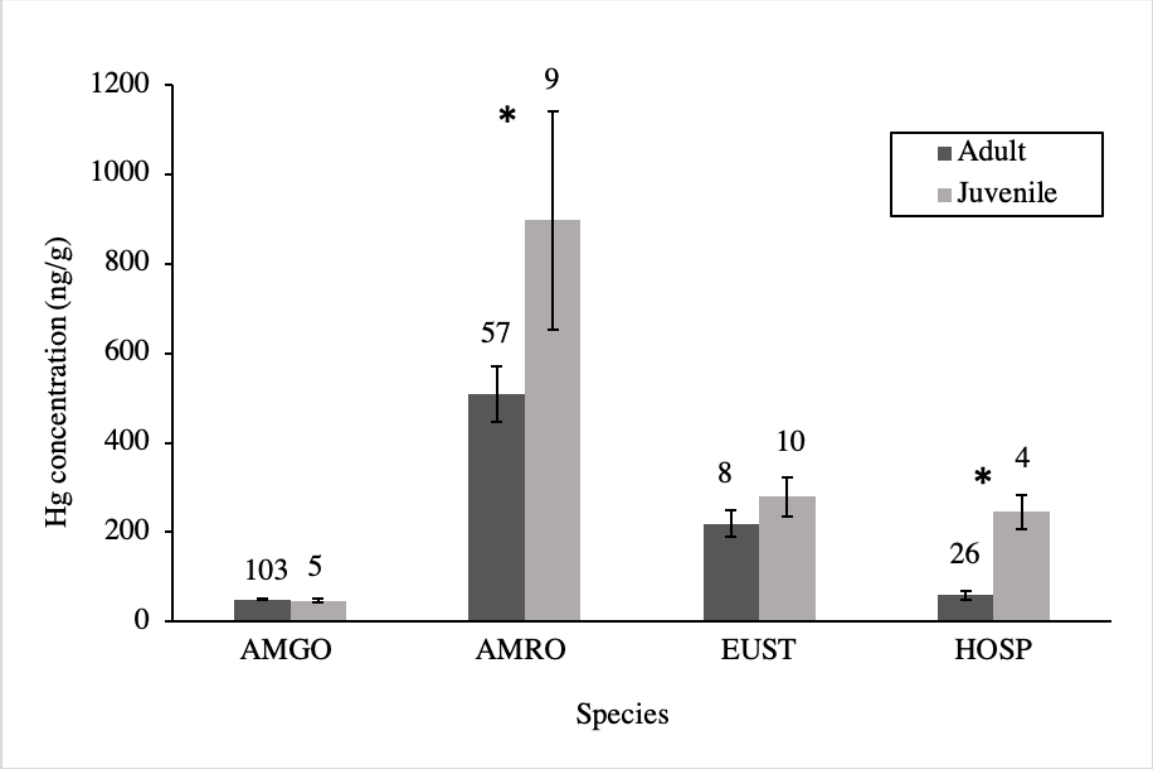


**Fig. 5** Average Hg concentration among adults and juveniles in each species: American robin (AMRO), American goldfinch (AMGO), European starling (EUST) and house sparrow (HOSP). Error bars represent one SE of the mean. Sample size is indicated above the error bars. Asterisk (*) above bars indicate statistically significant differences ($p<0.05$) in means within each pair group

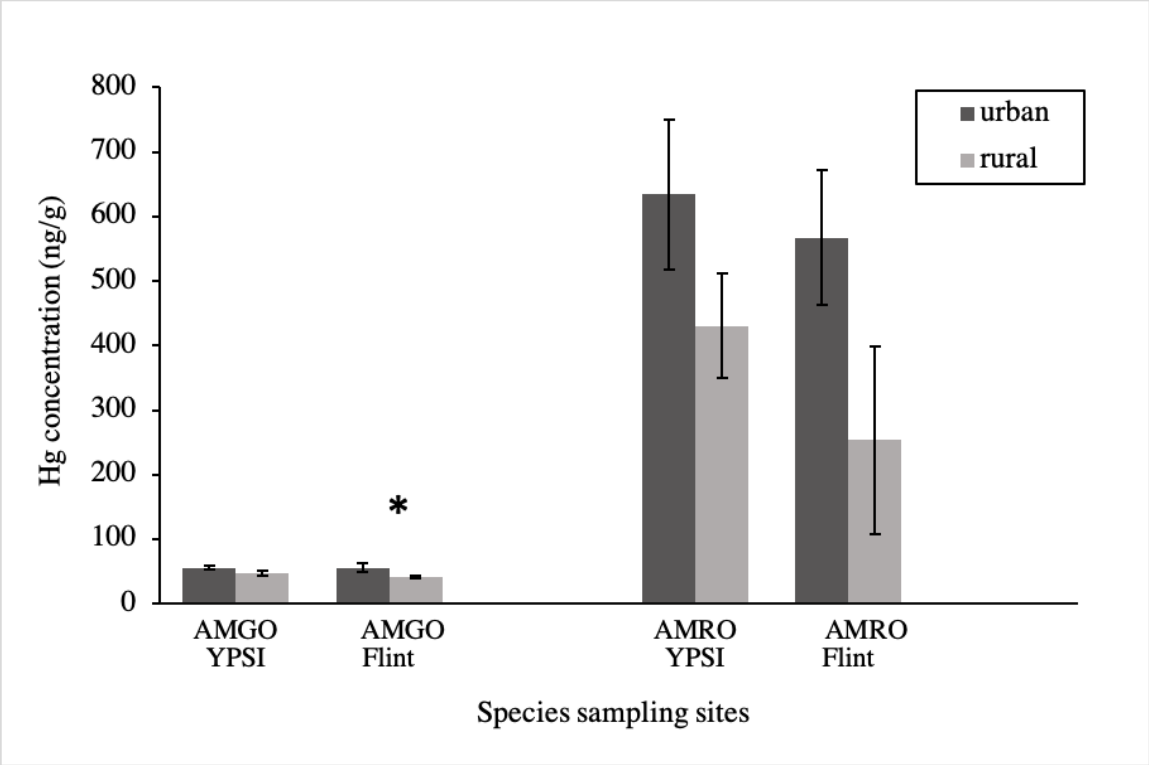


**Fig. 6** Average Hg concentration among American goldfinches (AMGO) and American robins (AMRO) in rural and urban areas of Flint and Ypsilanti. Error bars represent one SE of the mean. Asterisk (*) above bars indicate statistically significant differences ($p<0.05$) in means within each paired group

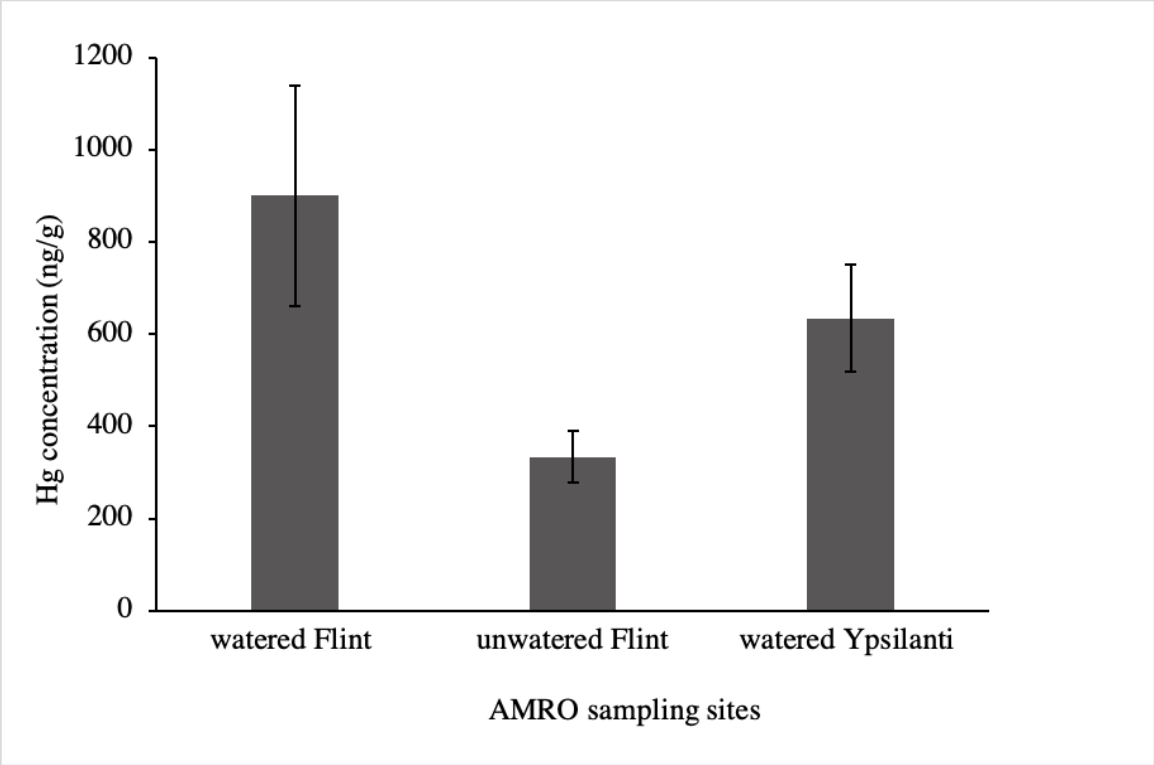


**Fig. 7** Average Hg concentration of American robins (AMRO) in watered sites of Flint (n =10) and Ypsilanti (n=27) and unwatered sites of Flint (n=11). Error bars represent one SE of the mean. Mean for unwatered Flint is significantly different ($p<0.05$) from watered Flint and watered Ypsilanti

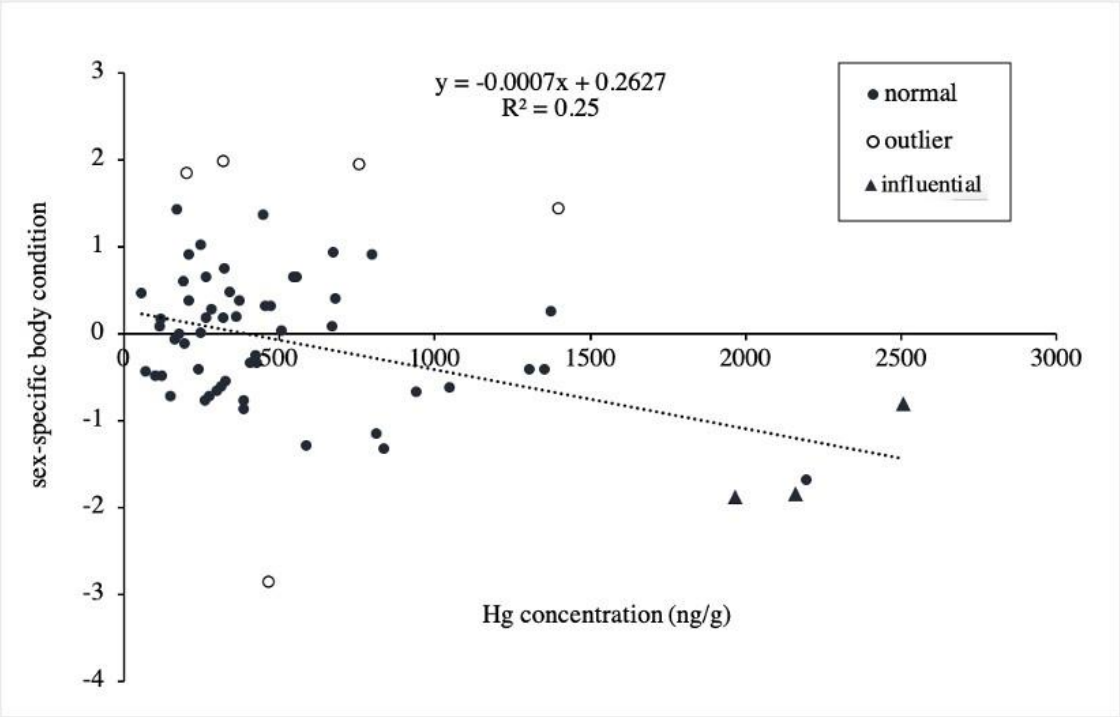


**Fig. 8** Linear regression analysis for Hg concentrations and sex-specific body condition in American robins (n= 57). Outliers for body condition (+/- 2$\sigma$) are displayed as open circles but these points are excluded in the regression analysis. Triangles represent potential influential points (Cook's D = 4/n) and are included in the regression analysis

## Conclusion

Feather analysis from our study reveals that foraging guild, age and location are key factors contributing to the variation in Hg levels among terrestrial songbirds in southeast Michigan. Omnivores ingest substantial amounts of MeHg through biomagnification within the terrestrial food chain whereas juveniles tend to receive more MeHg through selective feeding, and MeHg may be more bioavailable near urban sites that are in proximity to local emission sources than rural areas. Mercury levels between sexes are not different, suggesting a similar bioaccumulation rate between male and

female, and a lack of depuration into eggs among females. Our research adds to the growing body of literature that has documented Hg exposure among different species of terrestrial songbirds, but further research is needed to establish exposure levels for their health consequences. Future research may be strengthened by cross-comparing Hg levels within various feather tracts, as well as with other tissues like blood and internal organs in each sampled individual for a comprehensive understanding of Hg exposure levels and subsequent body burdens. Furthermore, studies focusing on songbirds that feed at higher trophic levels (including vermivores and omnivores), that forage in different habitats with varying Hg deposition rates, and that are associated with aquatic habitats could be conducted to broaden the knowledge on species and location vulnerability to Hg contamination around Michigan and its surrounding areas.

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