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The city blues of an iridescent canary: physiological, behavioral, and developmental impacts of lead (Pb) on songbirds along an urban-to-rural gradient

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MS Thesis

Submitted by

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Abstract

Urbanization is rapidly changing the environment and creating new challenges in the lives of animals across the globe. Anthropogenic contaminants found commonly in old construction materials—including heavy metals like lead— persist within the environment for prolonged periods of time and present a widespread problem for all who live near contaminated areas. Despite the phase-out of lead usage, it continues to threaten the health of all organisms but especially those from urban areas where historical lead use was more common. Currently, the sub-lethal effects of lead on wildlife are not well understood, though lead is known to affect physiology and behavior in humans. In response to higher concentrations of lead, animals may have to make trade-offs in order to survive within urbanized habitats. In this study, we used a common urban adapter, the European starling (*Sturnus vulgaris*), to explore how lead exposure is correlated with behavior, physiology, and feather development along a gradient of urbanization. We captured 125 free-living starlings to measure feather lead concentrations, along with aggression, corticosterone, and testosterone. We also measured soil lead concentrations across an urbanization gradient to test whether environmental availability was correlated to lead in starling feathers. Using linear mixed models, we found that urban starling nestlings had elevated feather lead concentrations compared to rural nestlings. However, we found no correlations between nestling feather Pb and endocrine, behavioral, or developmental traits. We also found no correlation between lead and urbanization in adults, as well as no significant differences in soil Pb concentrations between our study sites. Our findings suggest that nestling starlings may be a biomonitoring tool to detect lead in the local environment, and that non-invasive feather sampling is a promising tool beyond sampling soil alone. Further work needs to be conducted to understand the intricate relationship between heavy metals, behavior, morphological development, and physiology in free-living organisms.

Introduction

As urbanization increases globally, urban habitats are encroaching on wildlife habitats and impacting ecosystems. Human construction associated with urbanization has led to a marked increase in pollution and the impacts of pollution on wildlife are complicated and not fully understood (Seress & Liker, 2015). One class of environmental contaminants that are very toxic at low doses are heavy metals (Miranda et al., 2013). Heavy metal contamination influences the integrity of the ecosystem by accumulating in soil, water and eventually in animal tissues and can lead to sub-lethal poisoning or death, which ultimately alters animal populations and fitness (Millaku et al., 2015). Heavy metals in water or soil are non-degradable and can remain undetected for prolonged periods of time (Briffa et al., 2020). Lead (Pb) is one heavy metal contaminant that was historically commonly used in a variety of products. Despite Pb emissions decreasing due to the phase-out of lead products in the 1990s, historical lead emissions from as early as 1970 may still be present in the environment today while remaining undetected (Datko-Williams et al., 2014). Due to the fine-particle, aerosol nature of Pb, it can wash away from Pb-laden materials or structures into the environment (Mielke et al., 1984). Since Pb was commonly used in several construction materials, it is hypothesized that Pb contamination increases in areas that are more urban. In support of this idea, studies have discovered that soil Pb is found in higher concentrations in more urbanized areas compared to rural ones and soil Pb is positively correlated to human density (Datko-Williams et al., 2014; Grue et al., 1986; Mielke et al., 2011; Pouyat et al., 2015). As a result, organisms living in urban areas may be at greater risk of Pb exposure and may even accumulate heavy metals in their tissues more rapidly or at higher concentrations.

From laboratory studies, the negative effects of Pb exposure on physiology, behaviors, and morphological development have been well documented in multiple vertebrate species (Assi et al., 2016; Dumitrescu et al., 2014; He et al., 2020; Kumar et al., 2020). In birds, it has been shown previously that Pb affects hormone levels, morphological development—especially in reproductive organs—and critical early learning and survival behaviors (Burger & Gochfeld, 1988, 1995; Eeva et al., 2014; Leidens et al., 2018). However, these studies do not wholly reflect the real world and how organisms interact with their environment because they experimentally manipulated Pb dosages. The lethal and sub-lethal effects of Pb poisoning in free-living organisms remains less clear—where animals are exposed to environmental Pb naturally rather than treated with experimental doses—despite this being a critical question in the fields of urban ecology and conservation. For example, because sub-lethal effects could take time to accumulate and for negative impacts on fitness to manifest, some negative effects of Pb on wildlife behavior, physiology, and development are difficult to monitor (Buekers et al., 2009). In a recent study, McClelland et al. (2019) observed a sub-lethal effect of Pb exposure in northern mockingbirds (*Mimus polyglottos*): they compared birds living in neighborhoods with low Pb soil concentrations to ones in high Pb soil concentrations and found that birds exposed to higher Pb concentrations had elevated concentrations in their tissues and responded more aggressively to perceived threats. In another study that compared common blackbirds *(Turdus merula*) living across different levels of urbanization, trace element contamination burden increased with urbanization which resulted in endocrine disruption (Meillère et al., 2016). In both of these studies, the researchers collected feathers to measure Pb concentrations and in general, it is a popular, non-invasive sampling method in heavy metal research.

One concern for pollutants that are endocrine disruptors—such as Pb—is that they can negatively impact the way an organism behaves in the face of different stressors in their environment and thus impair their ability to cope with non-pollutant challenges. Two major vertebrate endocrine systems that influence an animal's behavior and fitness—the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis—may be disrupted by heavy metals. The HPA axis regulates vertebrate responses to environmental changes and acute stressors (Majer et al., 2019), including urbanization. The HPA axis, through a series of intermediaries, controls circulating glucocorticoid (CORT) concentrations. Glucocorticoids are a hormone that restores homeostasis and regulates energy, immune reactions, and behavioral responses for animals exposed to environmental stressors. When animals face a sudden stressor, CORT concentrations rise rapidly—called the stress response—which allows the individual to alter their behavior in order to overcome the stressor. Currently, it remains unclear the extent to which Pb exposure may disrupt the HPA axis and CORT due to differences in sampling methods (Meillère et al., 2016), though prior research found that nestling birds with higher Pb exposure showed increased CORT (Eeva et al., 2014). In addition, prior studies have suggested the effect that Pb has on CORT may vary between species or phase of annual cycle (Provencher et al., 2016), making it imperative to investigate each species individually. Similarly, the HPG axis is primarily responsible for regulating reproductive activity through releasing reproductive hormones (Couse et al., 2003; Vadakkadath Meethal & Atwood, 2005), and mediating trade-offs between mating and parental efforts (Chatelain et al., 2018). Testosterone (T) is a reproductive hormone used to initiate reproduction, courtship behavior, and territorial aggression, and thus often shapes reproductive success (Chatelain et al., 2018). Previous research has shown that Pb exposure may increase T levels which can result in increased aggression in birds (Wingfield, 1984, 1985) and humans (Wright et al., 2008). Although some studies have shown reproductive impairment from Pb exposure (Ding et al., 2019), the underlying physiological mechanism linking Pb and hormones remain unclear and one possibility is that these effects are mediated through disruption of the HPG axis.

It is important to understand the impacts of Pb on these endocrine systems, because they shape a number of important behaviors when animals face a sudden challenge or during breeding, which could in turn affect reproductive success and survival. For example, both CORT and T may shape important behaviors that are linked with fitness outcomes. For animals living across an urbanization gradient, adjusting behavior correctly based on the conditions that they face may be particularly important. We expect higher CORT and T to correspond with increased aggressive behaviors due to coping with the stressors related to sub-lethal Pb poisoning. In humans, the sub-lethal effects of Pb exposure can lead to antisocial behaviors such as aggression as well as increased physiological stress (Ahamed & Siddiqui, 2007; Wright et al., 2008). Similarly, researchers observed increased aggression post sub-lethal Pb exposure in laboratory animals (Cervantes et al., 2005; Delville, 1999; Li et al., 2003). However, the relationship between CORT and T levels and aggression are not well understood in free-living birds. Although aggression in both sexes is thought to be shaped in part by testosterone, there is some disagreement amongst the literature exploring these relationships in free-living birds (Fokidis et al., 2011; Ketterson et al., 2005; Lipshutz & Rosvall, 2021; Rosvall, 2013). Similarly, there is disagreement about the relationship between aggression and CORT levels (Davies et al., 2018; Fokidis et al., 2011). While understanding this relationship is critical for urban conservation, the exact mechanism between hormones or behavior and heavy metal exposure remains unclear.

We investigated the effects of Pb exposure on a common songbird —the European starling (*Sturnus vulgaris*) —who resides naturally across the urbanization gradient and has distinct rural and urban populations. Our study incorporates both adults and nestlings to explore the impacts of environmental Pb contamination across two cohorts of starlings. We use feathers as a less invasive method of monitoring lead in birds compared to collecting blood samples or organs for heavy metal analysis. Heavy metals accumulate in feathers at the time of feather growth, when compounds circulating in the blood of the animal at the time get passively deposited in the feather. To date, there has been one study showing a positive correlation between increased exposure to Pb and feather growth rates. In another urban-adapted species, the great tit (*Parus major*), researchers found that experimentally elevated concentrations of Pb in great tits decreased feather growth rate (Talloen et al., 2008). We would thus expect to see altered growth rates in feathers of birds that are exposed to higher concentrations of Pb.

We predicted that our urban study sites would have higher concentrations of soil Pb compared to our rural sites (Fig. 1). Following this pattern, we also predicted that urban starlings would have higher Pb concentrations, which would correlate with higher circulating concentrations of CORT and T, more instances of aggressive behaviors, and slower feather growth rates. In addition to improving our understanding of the sub-lethal ways in which Pb exposure may shape the lives of urban animals, motivation for this work includes understanding whether animals may serve as bioindicators of heavy metals to inform risk to all organisms within the environment, including humans (Cai & Calisi, 2016; Dip et al., 2001; Swaileh & Sansur, 2006). Although humans and animals differ in cognition and behavior, the effects of Pb on behavior and physiology may be conserved across vertebrates. Studies in animals can thus be informative to identify the effects of Pb poisoning in real-world settings in order to inform future research in humans (Davis et al., 1990).

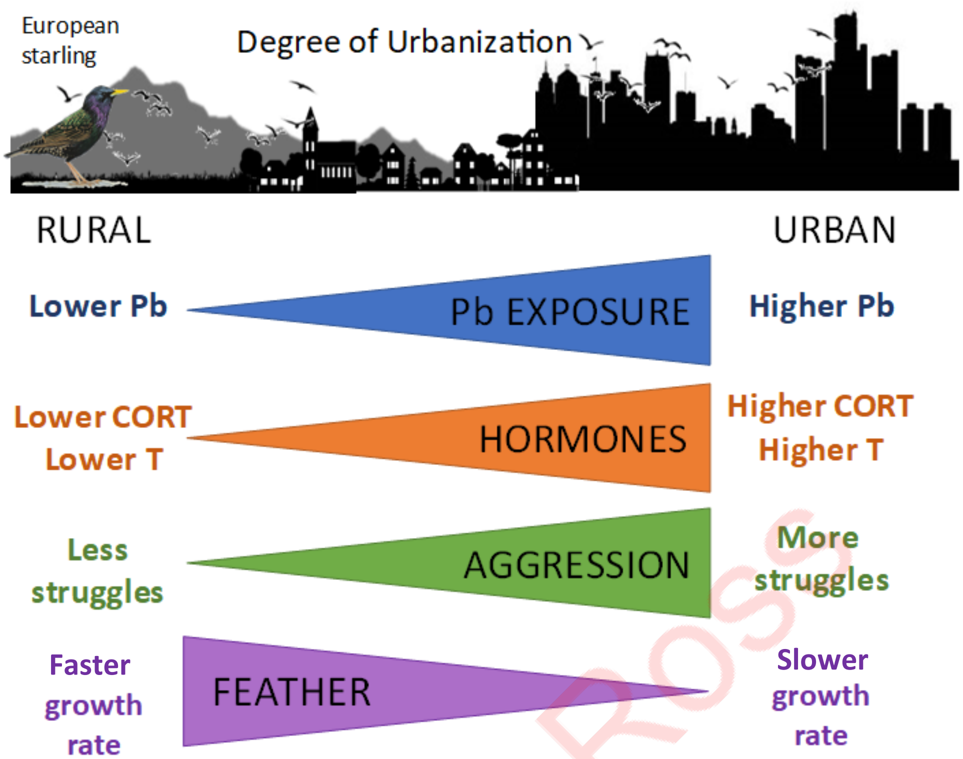

Fig. 1: This graphic shows the correlations we predict to see in our experiment. We predict that urban habitats and urban starlings will have higher concentrations of Pb which will correlate with higher CORT and T levels, intensifying aggressive behaviors, and smaller feather growth rate.

Statement of Integration

This research integrates methodologies from chemistry, environmental science and multiple subdisciplines of biology—including ecology, physiology, endocrinology, and behavior—with the ultimate goal of informing conservation and public health policy. My thesis blends techniques from different scientific disciplines in order to explore whether wildlife exposed to Pb in their environments have altered endocrine systems, behavior, or morphological traits. I used methods from ecology and ornithology to collect field-based data on free-living bird species across a variety of habitat types. I applied tools from environmental science and chemistry to determine the chemical makeup of soil and feather samples. These allowed me to explore the differences in Pb observed in soils and avian tissues across an urbanization gradient. In addition, I applied tools from physiology to assay for corticosterone and testosterone from small field-collected plasma samples. Both hormones are measured in the lab using commercially available enzyme immunoassays (see *Methods*). To explore the additional impacts altered endocrine systems may have on organisms, I applied tools from animal behavior to assess aggression in free-living animals. Finally, I tested previous literature claims in laboratory birds by assessing if feather growth rates were altered by sub-lethal Pb poisoning in free-living birds. The research questions in my thesis stem from an ecological framework and they have important conservation implications. While previous research has addressed some of the effects of Pb in biomedical literature, much less is understood about the impacts of Pb in the context of urbanization and in a natural setting. Results from my work can be applied to different study systems because the impacts of Pb on wildlife are shown to be similar across most vertebrates, including humans. In addition, I hope my findings will be used to inform public health policy by determining where soil remediation efforts are necessary or by using data from my study species as a bioindicator in metro-Atlanta.

Materials and Methods

The metro-Atlanta region is a sprawling urbanized area where the population between 2020-2022 has grown by 124,130 people and is predicted to add 2.9 million people by 2050 (Atlanta Regional Commission, 2022a, 2022b). The study area includes a cattle farm in Taylorsville, GA (34°05'50.7"N 84°54'10.8"W; average population density = 65.22/km^2), a rural park in Cartersville, GA (34°07'47.5"N, 84°49'41.0"W; average population density = 289.71/km^2), an old concrete plant that was converted to an urban farm in Acworth, GA (34°03'43.1"N 84°36'13.5"W; average population density = 732.4/ km^2), and an urban sports complex in Kennesaw, GA (34°00’12.6”N 84°37’07.7”W; average population density = 1,312.88/km2). Our study was conducted during the breeding season (March to June) in 2020-2022 across these four study sites. While both urban sites were monitored in all three years of the study, the rural cattle farm in Taylorsville was only monitored in 2020 and 2021, while the rural park in Cartersville was added in 2022 only. To compare the degree of urbanization between our field sites, we used ‘UrbanizationScore’ software (Lipovits et al., 2015) which scores the abundance of vegetation, buildings, and paved roads, ranking sites with a higher score as being more urbanized. We confirmed that our more rural sites differed from the more urban sites in their degree of urbanization (rural farm = -2.29, rural park = -2.01, urban farm = 2.13, urban park =2.17).

We monitored nest boxes with active nests (n = 115) and captured 125 starling individuals (n = 88 nestlings, n = 37 adults). Adults were captured with a Van Ert spring trap at the nest box when nestlings were 3-10 days old, while nestlings were captured and sampled at the nest box at 16-17 days of age. During the 2020 breeding season, we did not sample any adult starlings. From our urban park field site, we only successfully sampled adult birds as all nests found at this site were ultimately abandoned before nestlings reached 16-17 days of age.

At each site, we also collected soil samples to assess soil lead concentrations. We collected a core sample that was 25.4 mm deep and 101.6 mm in diameter. This soil depth reflects the depth at which starlings forage at. Samples were then stored at -20°C until they were thawed and shipped to University of Georgia’s Agricultural and Environmental Services Laboratory (UGA AESL) for analysis.

Biological sampling

When nestlings or adults were sampled, we began by collecting a baseline blood sample from the brachial vein within 3 mins of capture to measure baseline corticosterone (CORT) and testosterone (T) concentrations. Next, we measured handling aggression: holding the bird loosely, the observer placed the bird with its back on their palm and held the bird’s head between their index and middle finger with the legs unrestrained. The observer then counted the number of struggles (e.g. kicking, scratching, biting) for 30 s. Following the handling test, birds were immediately placed inside of a cloth bag and were suspended in the air by the observer. The observer then counted how many times the bird struggled for 30 s to assess isolated bag aggression. By placing the bird in a cloth bag, this eliminates visual stimuli and helps calm the bird, thus it gives us additional information about the bird's personality.

Following the isolated bag aggression, birds remained in the cloth bag and after 15 mins for nestlings and 30 mins for adults, we collected an additional blood sample to measure stress-induced CORT. We selected different time points for nestlings and adults, because nestlings have been shown to attenuate their CORT stress response more rapidly than adults (Bebus et al., 2020; Guindre-Parker et al., 2022). Both blood samples for each individual were stored on ice until centrifuged (5 min at 10,000 rpm) within 4 h. We then removed the plasma and stored the samples at -80°C until further analysis.

We then plucked the outermost right tail feather to assess feather growth rates and for Pb analysis. Starlings molt their feather during the pre-basic molt period after breeding once per year, between June and September, and thus the feathers represent one year of Pb exposure in adult birds during the prior breeding season. In contrast, feather Pb represents primary exposure in nestlings who molt their tail feathers in the nest box of the current breeding season (Carlson et al., 2014). Feather growth rate was determined by measuring the total feather length (not including the pin) and dividing by the number of growth bars on the tail feather sample. Each growth bar, a dark and light bar visible on the feather, represents feather growth for a 24-hour cycle (Brodin, 1993; Grubb, 2006). Feathers were stored at room temperature until they were sent off for Pb analyses.

Laboratory Analyses

Glucocorticoid Assay

CORT concentrations were quantified using a commercially available enzyme immunoassay kit from Arbor Assays (DetectX Corticosterone Enzyme Immunoassay Kit, K014-H5) following the manufacturer's protocol for small volumes and as previously validated for this species (Kilgour et al., 2022; Guindre-Parker et al., 2022). Each plate included a standard curve, ranging from 78 pg/ml to 10,000 pg/ml. We combined 10 µl of dissociation reagent and 10 µl of plasma sample together and waited for 5 min. Then, 230 µl of assay buffer was added to each sample and 50 µl of the diluted sample was loaded to a 96-well plate. Next, 25 µl of conjugate and 25 µl of antibody were added to all wells before incubating at room temperature for 1 h while shaking at 500 rpm. Plates were washed 4 times with 200 µl of wash buffer before 100 µl of TMB substrate was added to each well. The plate was left to incubate at room temperature in the dark for 30 mins (no shaking). After 50 µl of stop solution was added, the plate absorbance was read using a BioTek plate reader (ELX808) at 450 nm and CORT concentrations were calculated based on the standard curve. We ran both baseline and stress-induced samples for the same individual on the same plate, but the individuals selected on each plate and the position of the samples on the plate were randomized. In addition, samples were assayed in duplicate. We calculated the intra-assay coefficient of variation (CV) by comparing duplicates of the same sample which was 5.14%. In addition, we also assessed the inter-assay CV from a pooled plasma sample which was run on each assay plate. The mean inter-assay CV was 11.9%. Baseline CORT and stress-induced CORT concentrations are expressed in ng/ml.

Testosterone Assay

T concentrations were measured using a commercially available enzyme immunoassay kit from Arbor Assays (DetectX Enzyme Immunoassay Kit T Kit, K032-H5) according to the manufacturer's protocol. Prior to running the assay, we performed a diethyl ether extraction as recommended by the kit. We combined 40 µl of each plasma sample with 200 µl of DI water at room temperature. In addition, we assessed extraction recovery using a T standard (10 ng/ml) where we added 10 µl of standard to 200 µl of DI water. Next, 1 ml of diethyl ether was added to each sample or standard and tubes were vortexed for 2 min. The samples were flash frozen in a dry ice bath where the bottom layer (containing plasma and water) froze whereas the top layer (ether and T) was poured into a clean vial. T vials were left uncovered in a 30°C water bath for 40 mins to allow the ether to evaporate. We then capped and stored the vials at -20°C until we could perform the assays. We found an extraction efficiency of 134 ± 8.29% (note that the kit protocol also reports mean recoveries that exceed 100%).

We ran the T assay by creating a standard curve which ranged from 10,000 pg/ml to 40 pg/ml. We brought the extracted sample vials up to room temperature and reconstituted them by adding 150 µl of assay buffer and vortexing each sample. 50 µl of standard and samples were added to wells in duplicate. We added 25 µl of conjugate followed by 25 µl of antibody to each well and incubated the plate for 2 hr at room temperature while shaking at 500 rpm. Plates were washed out 4 times with 200 µl of wash buffer and 100 µl of TMB substrate was added to each well. Next, samples were incubated at room temperature in the dark for 30 mins (no shaking) before 50 µl of stop solution was added. Plate absorbance was read using a BioTek plate reader (ELX808) at 450 nm and T concentrations were calculated based on the standard curve. Similar to our CORT samples, we assessed the intra- and inter-assay CVs and found the mean intra-assay CV to be 2.99% and the mean inter-assay CV to be 32.6% (note that this includes variability caused by repeating the extraction as well as from running the same sample across plates). T concentrations are expressed in ng/ml.

Lead Assay

Individual tail feather samples and soil samples were analyzed for Pb at UGA AESL. At their facilities, both sample types were arranged in a tray and dried in an oven for ~24hr at 65°C. Extraneous material like plants, rocks, and roots were removed from the soil samples. Both sample types were passed through a 20-mesh screen Wiley mill and ground. UGA AESL uses the EPA Method 3052 (USEPA, 1995) to digest samples. Solutions of each digested sample were analyzed for Pb using EPA Method 200.8 (Creed, J.T. *et al.*, 1994). All results are reported in percent or parts per million (mg kg-1). Calibration standards are from a certified source. Independent laboratory performance checks are also run with acceptable deviations for recoveries set at 100 ± 5%.

Statistical analyses

We used linear mixed effect models (LMM) to assess how (1) soil Pb concentrations differed across sites or habitat types (urban vs rural), as well as how (2) feather Pb concentrations were correlated with habitat type, feather growth rate, and behavioral and endocrine coping styles. All Pb concentration values were log-transformed to meet the assumptions of LMM. First, we examined whether soil Pb concentrations differed across our urbanization gradient: we treated the soil Pb (log) as the dependent variable and included site name, habitat type (classified as urban vs rural) and date as predictor variables.

Next, we explored whether feather Pb concentrations varied with urbanization, feather growth rates, or behavioral and endocrine coping styles. We built one LMM each for nestlings and for adults, but with identical model structures. The dependent variable was feather Pb (log), and we included the following predictor variables: year of sampling, Julian date of sampling, mean soil Pb at site of sampling, habitat type (urban vs rural), feather growth rate, handling aggression, isolated bag aggression, baseline CORT concentration, stress-induced CORT concentration, and T concentration. In addition, we used nest ID as a random effect to account for sampling both parents or multiple nestlings from the same nest. All analyses were performed in R (version 4.2.2). We used package nlme (version 3.1-160) to perform all LMMs.

Results

First, we examined whether soil Pb concentrations differed across our study sites, expecting higher Pb in the soil of more urban compared to rural sites. We found that none of our field sites differed from one another (Fig. 2). However, the year the soil samples were collected was a significant predictor of soil Pb concentrations, with 2022 soil samples showing elevated Pb compared to 2021 (Table 1).

Next, we examined whether feather Pb concentrations differed between rural and urban starlings for both adults and nestlings separately. In the adult model, we found the feather Pb was uncorrelated to any of our predictor variables, including year, Julian date, site type (urban vs rural), or soil Pb at the breeding site (Table 2). Adult feather Pb concentrations were also unrelated to circulating hormone concentrations—including baseline CORT, stress-induced CORT or T—as well as feather growth rates, handling aggression or isolated bag aggression (Table 2). In contrast, we found the urban starling nestlings had elevated feather Pb concentrations compared to rural nestlings (Fig. 3). We also found an effect of year on nestling feather Pb concentrations, where feather lead appears to increase over the period of our study from 2020 to 2022 (Table 2). However, similarly to the adult starling results, nestling feather Pb concentrations were not correlated with Julian date or soil Pb at the breeding site. We similarly found that there were no significant correlations between nestling feather Pb concentrations and baseline CORT, stress-induced CORT, T, feather growth rates, handling aggression or isolated bag aggression (Table 2).

Table 1
We sampled 26 soil samples across four different field sites during the breeding seasons of 2021-2022. Our LMM results show no significant differences between field sites. However, our results do show a difference between the year the samples were collected, with 2022 showing elevated Pb. Estimates for each site below are compared relative to the rural farm site.

Fixed effects	estimate ± se	t-value	*p*-value
Intercept	-1581.1 ± 523.55	-3.02	**0.008***
Site (Rural Park)	0.15 ± 0.36	0.41	0.69
Site (Urban Farm)	0.09 ± 0.32	0.28	0.78
Site (Urban Park)	0.08 ± 0.32	0.26	0.80
Year	0.78 ± 0.26	3.02	**0.008***
Random effect	sdev		
Nest ID	$1.82*10^{-5}$		
Residuals	0.52		

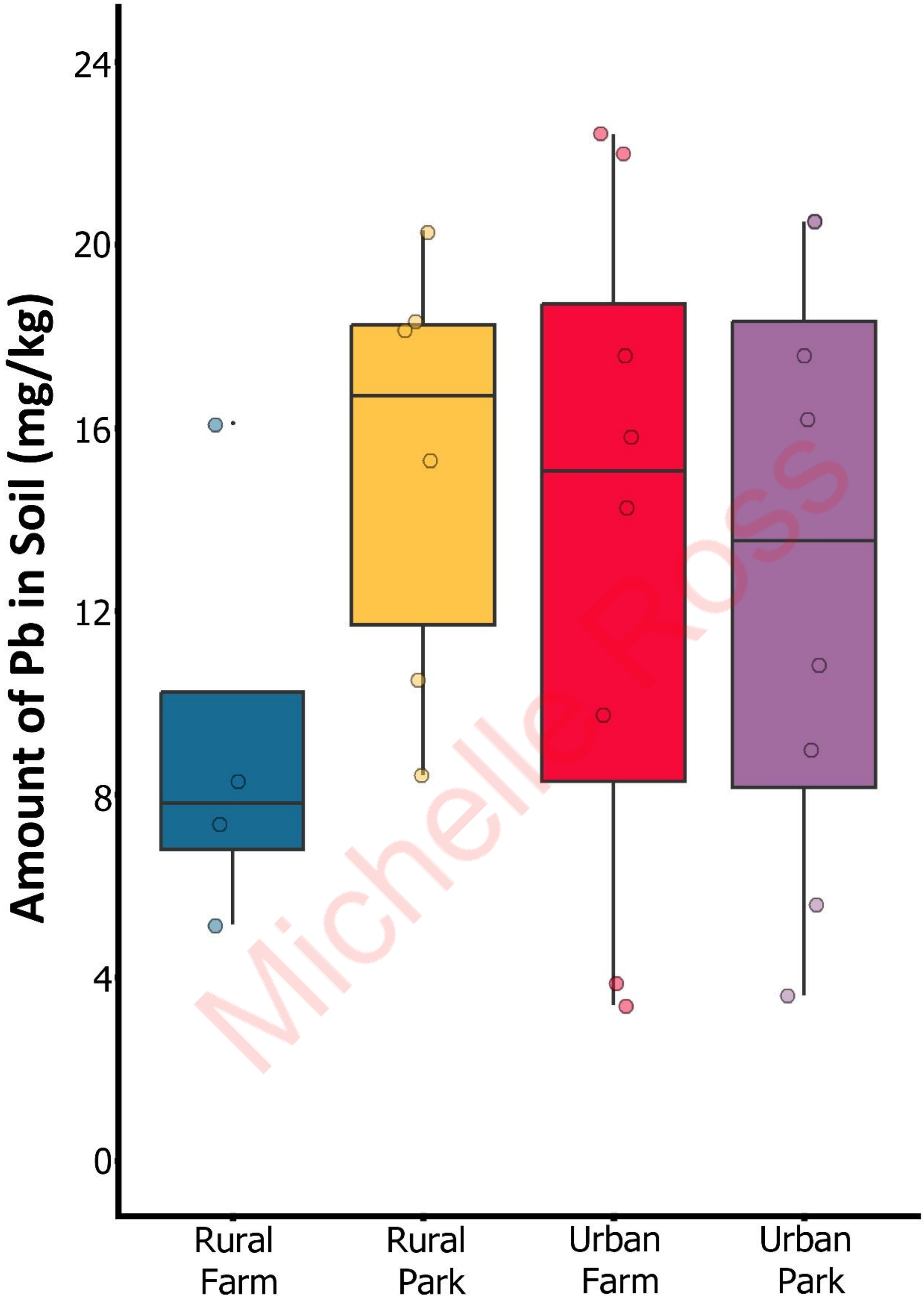

Fig. 2: While our Rural Farm site showed lower mean soil Pb compared to the other sites in our study, this difference was not statistically significant in our LMM (Table 1). Soil Pb was quite variable within each study site across locations and years of sampling.

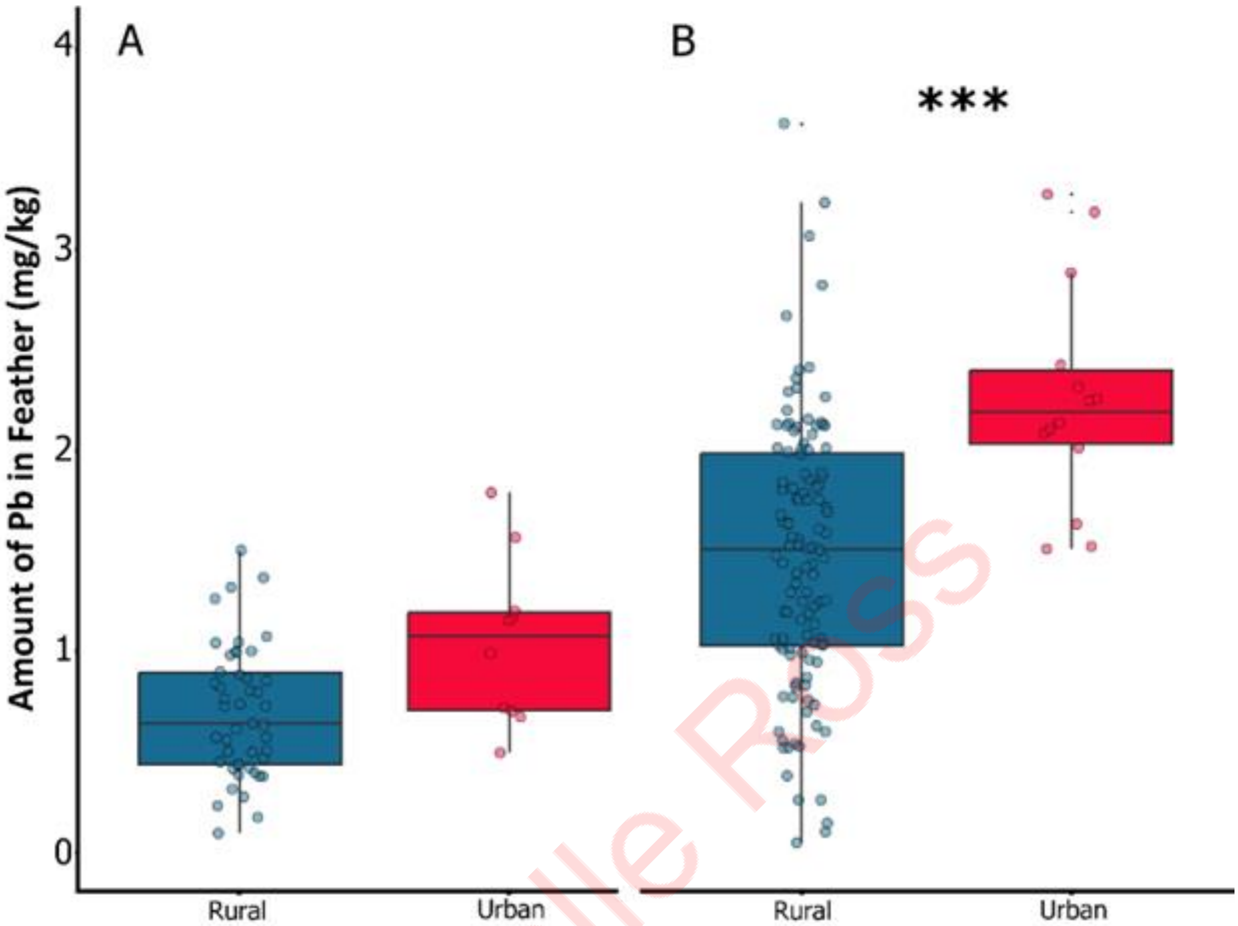

Fig. 3: We compared feather lead for (A) adult and (B) nestling starlings from urban versus rural sites. We found that urban starling adults did not differ in their feather Pb concentrations whereas nestlings did. Nestlings from urban sites had elevated feather lead compared to ones raised in rural sites. Asterisks indicate statistically significant differences among urban versus rural groups (p-value ≤ 0.05).

Table 2

We sampled 125 individual starlings (n = 37 adults and n = 88 nestlings) during the breeding seasons of 2020-2022. Here we present the results of our LMM for (A) adults and (B) nestlings separately. Asterisks and bolding denote significant predictor variables where *p*-value ≤ 0.05.

	A) Adults			B) Nestlings		
Fixed effects	estimate ± se	t-value	*p*-value	estimate ± se	t-value	*p*-value
Intercept	342.47 ± 934.97	0.37	0.72	936.94 ± 349.08	2.68	**0.008***
Year	-0.17 ± 0.46	-0.37	0.72	-0.46 ± 0.17	-2.68	**0.01***
Julian date	0.005 ± 0.006	0.70	0.50	0.009 ± 0.005	1.83	0.08
Site (urban)	0.16 ± 0.27	0.58	0.56	0.71 ± 0.33	2.11	**0.04***
Soil Pb	0.06 ± 0.08	0.68	0.50	-0.10 ± 0.05	-1.93	0.06
Baseline CORT	-0.01 ± 0.01	-1.16	0.28	-0.007 ± 0.004	-1.72	0.08
SI CORT	0.003 ± 0.004	0.60	0.56	0.002 ± 0.002	1.18	0.24
Testosterone	0.03 ± 0.09	0.36	0.73	-0.28 ± 0.25	-1.09	0.28
Feather growth rate	0.11 ± 0.24	0.47	0.65	0.09 ± 0.06	1.47	0.15
Handling aggression	-0.02 ± 0.03	-0.69	0.51	0.008 ± 0.01	0.71	0.48
Isolated bag aggression	0.01 ± 0.01	1.12	0.29	-0.006 ± 0.01	-0.51	0.61
Random effects	sdev			sdev		
Nest ID	0.11			0.38		
Residuals	0.44			0.47		

Discussion

Our study explored variation in soil and feather Pb across a gradient of urbanization to determine if urban starling populations are exposed to higher concentrations of Pb and as a result, have higher amounts of circulating Pb within their tissues. We found that although there were no consistent differences in soil Pb associated with increasing urbanization, soil Pb concentrations did vary significantly from year-to-year. Due to the non-homogenous nature of soil or Pb deposits, and the differences between sampling and analysis methods, Pb concentrations can vary, even when sampling the same area in different years (IAEA-TECDOC-1415, 2004). Therefore, soil Pb concentrations may not give precise estimations of the potential effects on the environment and its occupants. In contrast, our results did show that urban starling nestlings have higher concentrations of Pb compared to their rural counterparts which matches results from previous studies (Dauwe et al., 2000; Janssens et al., 2001; Janssens et al., 2003a; Roux & Marra, 2007). Additionally, although the result was statistically insignificant, we found a weak pattern showing that nestling feather Pb concentrations increased with soil Pb concentrations at the site of their nestbox ($p = 0.06$). This suggests that with a larger sample size, we may be able to detect a correlation between soil Pb and feather Pb concentrations and that nestling feathers may be a useful bio-indicator of environmental lead.

In contrast, there were no differences in feather Pb concentrations between the rural and urban adult starlings. Prior studies do show that heavy metal concentration differences between adult and juvenile bird samples is common (Lodenius & Solonen, 2013). Although adult birds might be exposed more directly to heavy metals than nestlings (i.e. by interacting with contaminated soil or water more directly), feathers of nestling birds appear to more accurately reflect the local pollution within the area where they are raised and show similar Pb levels in some internal tissues due to being confined to the nest during development (Burger & Gochfeld, 1993; Franson & Pain, 2011; Janssens et al., 2003b; Janssens et al., 2001). Since adults can experience a larger home range during the annual cycle, it may be less likely that feather Pb would correlate with environmental Pb at their breeding site (Espín et al., 2016; Franson & Pain, 2011). Similarly, feather Pb in adults reflects their exposure during the prior summer when they last molted their tail feather, and they may have been using a slightly different habitat at that time than the one they are currently breeding in nearly a year later. Another possible explanation is that there can be differences in Pb detected across different tissues and adult feathers are a poor indicator of current Pb exposure. For example, a study by Ek et al. (2004) showed heavy metals do vary in their distribution in different organs, feces, feathers, and eggs. They found that the concentration of Pb was significantly higher in kidney and blood samples compared to egg and feather samples. Different methodology may give additional information about our adult populations that we failed to detect (Lodenius & Solonen, 2013).

Our results show no correlation between elevated Pb levels and hormones in either adults or nestling birds. The nestling baseline CORT results suggest that there may be a positive relationship between the two that we failed to detect due to small sample size ($p = 0.08$). Although, there is some discrepancy amongst prior studies on whether increased Pb concentrations lead to increased hormone levels. In similar urban-adapted songbird species, studies showed a direct correlation between degree of urbanization, Pb and increased CORT concentrations (Bichet et al., 2013; Meillère et al., 2016). However, other studies on similar species showed no link between Pb and CORT levels nor T levels (Chatelain et al., 2018; Eeva et al., 2014; Provencher et al., 2016). To our knowledge, no study has shown a positive correlation between urbanization, heavy metal exposure, and T levels in birds; it remains an understudied topic. Overall, our results did not support that Pb exposure as detected in tail feathers altered endocrine traits in starlings.

Similarly, we found no correlation between elevated Pb concentrations and behavior. For nestlings, aggressive behavior may be limited in scope and occur primarily to compete with their siblings for food. Our results showed very low levels of T were present within nestlings which could drive low incidence of aggressive behaviors. However, another study in starlings did not detect a correlation between T levels and sibling competition (Gil et al., 2008). We sampled adult birds during the breeding season when T concentrations and territorial aggression would be at their highest. However, we found that adult feather Pb was unrelated to aggressive behavior in starlings. In other urban-adapted adult birds, studies showed no correlation between aggression and T or heavy metal exposure during the breeding season (Davies et al., 2018; Grunst et al., 2018). Although McClelland et al. found a positive correlation between neighborhoods with high Pb concentrations and increased aggression in adult birds, this study did not measure T levels (2019). In a different study, they castrated male European starlings and found that although control males had higher levels of circulating T, the castrated males were significantly more aggressive which suggests that T levels and aggressive behaviors may occur independently of one another (Pinxten et al., 2003). These results suggest that, contrary to the effects seen in humans, Pb as measured in feathers does not have a strong correlation with aggressive behavior in birds at the doses observed in our study.

Finally, we found no correlation between elevated Pb levels and feather growth rate in either adults or nestlings. Previous research suggests that heavy metals, especially Pb, may impact the development of chicks who are exposed to high concentrations of heavy metals (Burger & Gochfeld, 1988; Spahn & Sherry, 1999). However, our findings are in agreement with prior research that failed to find any significant effects of heavy metal pollution on feather growth in free-living, urban-adapted species (Dauwe et al., 2006). Although birds deposit heavy metals into their feathers, our results suggest that exposure to environmental Pb at the time of feather growth does not affect the development of the feather itself.

Conclusion

Here, our results show urban starling nestlings have higher concentrations of feather Pb despite failing to detect soil Pb concentration differences between our field sites. Additionally, these feather Pb differences do not influence nestling physiology, behavior, nor feather development. In contrast, we found no relationship between urban and rural adult feather Pb, nor did we find any correlation between adult feather Pb concentrations and hormones, behavior, or feather growth. These results suggest that non-invasively collected feathers from nestlings, but not adults, may be a useful biomarker of urbanization and Pb. Because parents provision their young from a relatively small provisioning range during breeding and the nestlings grow their tail feathers over a few short weeks in the nest, the feather Pb of nestlings appears to be a better representation of the local environment than feather Pb in adults. With the Pb doses in our study, we found no impacts of Pb on endocrine, behavioral, and developmental traits: future experimental studies using similar doses of Pb would be needed in order to better understand the sublethal impacts of Pb across an urbanization gradient. This work suggests that incorporating feathers of urban-adapted birds as bioindicators of heavy metals may be a valuable addition to assessing environments than soil sampling alone.

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