



Lead exposure is correlated with reduced nesting success of an urban songbird

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ABSTRACT

Lead exposure is a concern in urban ecosystems, with physiological and behavioral effects well documented in humans. Wildlife inhabiting urban ecosystems are also exposed to lead, yet little work has documented the sublethal effects of lead exposure in urban wildlife. We studied northern mockingbirds (*Mimus polyglottos*) in three neighborhoods of New Orleans, Louisiana, two with high soil lead and one with low soil lead, to better understand how lead exposure may influence mockingbirds' reproductive biology. We monitored nesting attempts, measured lead concentrations in blood and feathers of nestling mockingbirds, documented egg hatching and nesting success, and assessed rates of sexual promiscuity in relation to neighborhood soil lead levels. We found that nestling mockingbirds' blood and feather lead levels reflected the soil lead levels of their neighborhoods and nestling blood lead levels were similar to those of adult mockingbirds in the same neighborhoods. Nest success, as evaluated by daily nest survival rates, was higher in the lower lead neighborhood. Clutch sizes varied substantially across neighborhoods, but rates of unhatched eggs did not covary with neighborhood lead levels, suggesting that other drivers are influencing variation in clutch sizes and hatching success in urban habitats. At least one-third of nestling mockingbirds were sired by an extra-pair male, and there was no relationship between extra-pair paternity rates and neighborhood lead levels. This study provides insight on how lead contamination may influence reproduction in urban-dwelling wildlife and suggests that nestling birds could serve as useful bioindicators of lead levels in urban neighborhoods.

1. Introduction

Humans modify natural ecosystems in profound ways, and the development and expansion of urban areas is one of the most pervasive anthropogenic alterations to ecosystems worldwide (Aronson et al., 2014). Billions of wild animals currently live in urban environments, and these urban animals face different stressors and survival pressures than animals living in native, unaltered habitats (Chamberlain et al., 2008; Sepp et al., 2017). These differences potentially include changes in the availability of food (Bailly et al., 2016; Seress et al., 2020), suitable habitat (Han et al., 2019), higher population densities (Moller et al., 2012; Pipoly et al., 2019), noise pollution (Fuller et al., 2007), light (Dominoni et al., 2013), and contaminants (Janssens et al., 2003; McClelland et al., 2019). Studying the consequences of urban ecosystem

alterations on the survival and reproduction of urban-dwelling animals is increasingly important as urbanization continues expanding worldwide (Chance and Walsh, 2006; Chamberlain et al., 2008; Bailly et al., 2016; Han et al., 2019).

Lead is a toxic element that is common in urban environments and presents a threat to both humans and wildlife (Callendar and Rice, 2000; Mielke et al., 2016). Numerous studies have demonstrated the toxicity of lead, including behavioral and reproductive effects in humans (Schwartz, 1994; Sallmen, 2001; Sanders et al., 2009; Mielke and Zahrán, 2012; Smith et al., 2012) and in wildlife (Janssens et al., 2003; Kendall and Scanlon, 1981; Wadi and Ahmad, 1999; Chen et al., 2012). Recently, there has been increasing interest in examining the effects of exposure to lead in wildlife (Glucs et al., 2020; Levin et al., 2021). Although lead is known to be highly dangerous for childhood

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development (Smith et al., 2012), the effects of lead exposure during juvenile development in free-living wildlife are not well-known (Eeva et al., 2014; Ruuskanen et al., 2014). Of concern, lead exposure might disrupt reproduction and juvenile recruitment into populations, affecting the viability of wildlife populations (Finkelstein et al., 2010; Herring et al., 2017).

Lead exposure may influence reproductive success in wildlife via a number of mechanisms. Laboratory studies in mammalian and avian systems have linked lead exposure to male infertility (Kendall and Scanlon, 1981) or poor sperm quality (Wadi and Ahmad, 1999), and these physiological effects may directly reduce an individual's capacity to produce viable offspring. Additionally, in birds, high levels of lead exposure during the nesting stage can cause physiological damage to offspring that prevents fledging (Finkelstein et al., 2003). Since developing individuals such as nestling birds are more sensitive to lead exposure compared to adults (Burger, 1990; Burger and Gochfeld, 2005), the effects of exposure on nestlings are especially concerning (Herring et al., 2017). In addition, nestlings of altricial bird species are likely to be more sensitive to lead exposure as more of their development occurs after hatching, resulting in outcomes including poorer growth rates and higher mortality rates (Hoffman et al., 1985).

Lead might also influence reproduction indirectly via its impact on behavior. McClelland et al. (2019) found that increased lead exposure was associated with heightened aggression in free-living adult northern mockingbirds (*Mimus polyglottos*; hereafter "mockingbirds"), which has the potential to influence reproductive success. On the one hand, more aggressive individuals may be more competitive in the defense of high-quality mates, territories, and resources (Smith and Blumstein, 2008; Wiebe, 2016), and thereby increase reproductive output. Alternatively, excessive levels of aggression could trade off with offspring survival through decreased care and provisioning (Duckworth, 2006), or unintentionally increase the likelihood of offspring depredation through reduced vigilance at the nest site (Dunn et al., 2004; Hess et al., 2016). Increased aggression due to lead exposure could also indirectly influence cuckoldry rates. Many bird species engage in some degree of extra-pair copulations (Griffith et al., 2002), with females selecting extra-pair partners as insurance against reduced sperm quality or sterility of their social mates (Double et al., 2000). If increased aggression leads to increased social mate guarding by males it could reduce cuckoldry; alternatively, if increased aggression is directed primarily toward neighboring birds it could reduce mate guarding and lead to greater rates of cuckoldry (Westneat and Stewart, 2003; Moreno et al., 2010). The complex ways that behavioral changes related to lead exposure could affect reproduction remain unexplored, and filling this knowledge gap becomes more pressing as an increasing number of wild species become reliant on lead-contaminated urban habitats.

To investigate how subtle lead exposure affects reproduction in wildlife, we assessed lead exposure of nestling mockingbirds and mockingbird reproductive success in urban New Orleans, LA, USA in three neighborhoods with different soil lead levels (Mielke et al., 2016; McClelland et al., 2019). Soil lead levels can vary substantially throughout cities: older areas of cities bear a greater lead burden because they sustained more anthropogenic activity during periods in which leaded paint, gasoline, and other industrial sources of lead were in ubiquitous use (Laidlaw et al., 2016). In addition, highly territorial adult mockingbirds, with breeding territories of around 1 ha (Derrickson and Breitwisch, 1992), nesting in neighborhoods with differing levels of soil lead reflect the soil lead of their local environment (McClelland et al., 2019). Further, blood and feather lead concentrations of adult northern mockingbirds in New Orleans have been shown to reflect their neighborhood's soil lead levels (McClelland et al., 2019).

We tested the hypothesis that nestling mockingbird lead levels in blood and feathers differ with respect to neighborhood soil lead levels and that birds in high lead neighborhoods will have lower reproductive success. We predicted that mockingbird nests in high-lead neighborhoods would produce fewer eggs per clutch, have a higher rate of

unhatched eggs, and have reduced rates of daily nest survival. We also tested the hypothesis that aggressive, high-lead adults would produce fewer extra-pair offspring, putatively because they would mate-guard more effectively. Together, our study helps inform the effects of lead exposure on urban wildlife and the prospects for songbird nestlings as bioindicators of environmental health.

2. Materials and methods

2.1. Field methods

We collected data from March 2016–August 2018 in New Orleans, LA, USA in three primarily residential neighborhoods with differing background lead levels and vegetation cover (hereafter, "greenness"): Lakeshore, characterized by relatively low soil lead and high habitat greenness; Marigny-Bywater, characterized by high soil lead and low greenness; and Uptown, characterized by high soil lead and high greenness (Mielke et al., 2016; McClelland et al., 2019). Previous studies of New Orleans and other US cities have found that older areas of cities have higher levels of soil lead than newer areas that were developed following the ban of many leaded consumer products in the 1970s (Mielke et al., 2016; Laidlaw et al., 2016). High and low 'greenness' categories (McClelland et al., 2019) for study neighborhoods were scored by the approximated habitat cover of mockingbird territories via satellite image classification of the landscape of New Orleans sourced from April 2015 National Agricultural Imagery Program (NAIP). McClelland et al. (2019) identified the three study neighborhoods listed above, selecting two high-lead neighborhoods (Uptown and Marigny-Bywater) that had similar average soil lead levels, which were an order of magnitude greater than the average soil lead level of the selected low-lead neighborhood (Lakeshore). If Uptown birds more closely resemble birds from Lakeshore (low lead/high greenness), then greenness would be implicated as a probable causal factor, which would suggest that other habitat- or vegetation-related variables may be driving observed patterns. In contrast, if Uptown birds more closely resemble birds from Marigny-Bywater (high lead/low greenness), then lead would be implicated as a probable causal factor. During the mockingbirds' breeding season (March–August) in each year of the study, we searched the three focal neighborhoods for mockingbird nests. We located nests by observing parenting behavior of adult birds and systematically examining shrubs, trees, and sturdy vines for nests <4 m from the ground (Derrickson and Breitwisch, 1992).

We checked nests every 7 days during the nest construction, laying, and incubation stages and every 3–4 days during the nestling stage to monitor the development, survival, and approximate fledging date of offspring (Laskey, 1962; Derrickson and Breitwisch, 1992). At the conclusion of each nesting effort, we recorded the outcome as one of the following: nest failed due to infertile eggs, nest failed due to depredation in either egg or nestling stages, or nest succeeded in fledging at least one offspring.

We banded each nestling 6–9 days post-hatching with a uniquely numbered aluminum leg band on the right tarsus, and collected blood and feather samples before returning all individuals to the nest. We collected a small blood sample (<100 μ L) from nestlings for genetic analysis and lead concentration analysis using brachial venipuncture. Immediately following collection, blood samples were transferred from capillary tubes into trace metal clean micro-centrifuge tubes (for lead analysis) or sample tubes containing Longmire's lysis buffer solution (for genetic analysis). All blood samples were stored at -20 °C. For nestling birds, the third secondary feather of each wing was clipped and stored with desiccants at room temperature until processing for analysis of lead concentrations.

A one-way ANOVA was used to assess differences in clutch size between the neighborhoods. A chi-squared test was used to test for differences in the frequency of infertile eggs produced by neighborhood and by year. The Mayfield (1975) method was used to calculate nest

daily survival rates (dsr) for each nest and generate 95% confidence intervals for these daily survival rates across the three study neighborhoods during the 2018 breeding season. The Mayfield method was appropriate for this study system because the outcomes, approximate hatching and fledging dates, and approximate failure dates of all nests were known (Mayfield, 1975; Jehle et al., 2004).

2.2. Lead concentration analysis (2018 breeding season only)

Whole blood and feather samples were processed for analysis of lead concentrations following the established trace metal clean techniques and using ultra-pure reagents outlined in McClelland et al. (2019) with the exception that blood samples were transferred immediately to cleaned micro-centrifuge tubes after collection.

2.2.1. Whole blood

Blood (~5–70 μL) was transferred from heparinized capillary tubes into trace metal clean micro-centrifuge tubes within 60 s of collection, then stored at $-20\text{ }^{\circ}\text{C}$ until processing. Processing was performed at the University of California, Santa Cruz. Samples were weighed then dried overnight at $60\text{ }^{\circ}\text{C}$ to obtain dry weight values, and digested as follows: 100 μL (for samples with blood volume $<50\text{ }\mu\text{L}$) or 150 μL (for samples with blood volume $>50\text{ }\mu\text{L}$) of concentrated HNO_3 (optima, Fisher Scientific) were added to each sample and samples were digested cold for 10 h. 30% H_2O_2 (ultrax, JT Baker) and ultrapure water was added to each sample for a HNO_3 to H_2O_2 ratio of 2:1 and an approximate final concentration of HNO_3 of ~6%. Samples were vortexed and left to sit overnight before analysis.

2.2.2. Feathers

The entire feather was washed sequentially with acetone, ultrapure water, 1% HNO_3 , and ultrapure water to remove surface contamination. Feathers were then dried overnight at $60\text{ }^{\circ}\text{C}$, weighed, digested overnight in 2 mL sub-boiling concentrated HNO_3 (optima, Fisher Scientific) in closed Teflon vials, evaporated to dryness, and reconstituted in 5% HNO_3 for analysis.

2.2.3. Lead analysis

Lead concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) at the UC Santa Cruz Plasma Analytical Laboratory, RRID:SCR_021925, measuring masses of ^{208}Pb and ^{205}Tl isotopes (used as an internal standard). Approximately 20 μL of NIST SRM 955c (lead in blood, level 2) was digested using the methods described above for blood with an average recovery of $97\% \pm 1.8\%$ RSD ($n=5$).

2.2.4. Statistical analysis

All analyses were performed in R v.3.6.3 (R Core Team, 2020). All blood and feather lead concentration values were log-transformed for analyses in order to meet the assumptions of normality. Differences in nestling blood lead concentrations between the three study neighborhoods were tested using linear mixed effect models, with Bonferroni-adjusted post hoc pairwise comparisons using packages 'lme4' and 'nlme'. Correlations between nestling blood and feather lead concentrations were investigated using a linear correlation using package 'dplyr'. A two-sample *t*-test was used to test for differences in feather lead concentrations between the low lead neighborhood and one of the high lead neighborhoods; the other high-lead neighborhood was excluded from this analysis due to a low sample size of analyzed feathers ($n = 2$). A single mixed effects model was used to test for differences in blood lead concentrations among nestlings, with neighborhood as the fixed effect and nest as the random effect. Further, a repeatability analysis was performed to assess differences in nestling blood lead concentrations at the nest level and at the neighborhood level.

2.3. Molecular methods and library preparation

We used double-digest restriction site-associated DNA sequencing (ddRAD) to characterize rates of extra-pair paternity (EPP) in a subset of nests that we monitored. Because we were unable to capture and collect blood samples from a representative sample of adults to perform relatedness analyses, we evaluated whether siblings in the same nest were all full siblings, or whether at least one pair of nest-mates were half-siblings, which would provide evidence of extra-pair paternity in a given nest. We note that this measure of EPP rates is conservative because it is possible that nestmates could all be full siblings sired by an extra-pair male. Genomic DNA was extracted from blood with a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. We followed standard ddRAD protocols of Peterson et al., (2012) with modifications as described in Thrasher et al., (2018) (details in supplementary materials). We constructed ddRAD libraries for 160 individuals for single-nucleotide polymorphism (SNP) discovery to be used for individual genotyping.

Sequence read quality was assessed, and reads were trimmed, filtered, and demultiplexed before sequences were assembled de novo using STACKS v 2.3 (see supplemental materials), with a minimum stack depth of 10 ($m = 10$). We used the program Populations to only retain one SNP per stack using the `-write_single_snp` flag, and also only retained loci with a minimum allele frequency greater than 20%, and present in at least 95% of individuals. We then removed loci that were not in Hardy-Weinberg equilibrium using VCFTOOLS (1000 Genomes Project Analysis Group, 2011). This resulted in retaining 284 SNPs. We obtained a variant calling format (vcf) file, and converted that into a genepop format with PGDSpider version 2.1.1.5 (Lischer and Excoffier, 2012). We then used CERVUS version 3.0.7 (Kalinowski et al., 2007) to convert the genepop file into a .csv genotype file that can be used in relatedness analysis.

Relatedness testing was used to assign pairs of nestlings from the same nest as either full-siblings or half-siblings with the program COLONY (v 2.0.6.5) for both SNP datasets separately. For all runs, the parameters included: female polygamy, male monogamy, set error rates for 0.0001 for all loci, and a "medium" run using the "full likelihood method." Though both sexes could be polygamous, since we were only interested in assigning sib-ships within a nest, and an individual's paternity in multiple nests is irrelevant, we chose male monogamy for computational simplicity as described in (Turjeman et al., 2016). In addition, we did not provide genotypes for mothers, so the analysis was run blind of any information that individuals belonged in the same nest. The program produced family clusters ("BestCluster" output) with putative parent "identifications" – the output reported whether two individuals had the same or different mothers and fathers, and we used that information to extract nestling relationships. Individuals in the nest were assigned as either being full-siblings (FS) or half-siblings (HS).

Most individuals in the same nests were correctly assigned as having the same mother, confirming COLONY was correctly assigning individuals to the same family groups. However, three individuals were incorrectly assigned as having different mothers as their nestmates, possibly due to genotyping or coding error. Those 3 nests were removed from further analysis. Of those remaining nests ($n = 48$), nests were classified as FS if all individuals within the nest were full siblings, and nests were classified as HS if at least one pair of individuals within the nest were half siblings. A Fisher's exact test was used to determine whether there were differences in the number of FS nests relative to HS nests at both the neighborhood (Uptown, Marigny-Bywater, and Lakeshore) and neighborhood lead (high-lead v. low-lead) levels. We were not able to link nest-level lead to cuckoldry rates because only one study year had lead data.

3. Results

We identified and monitored a total of 212 mockingbird nests during

our three-year study period (2016: $n = 95$; 2017: $n = 58$; 2018: $n = 59$). During this time period, we banded and sampled 281 nestling birds from 94 nests (2016: $n = 102$ nestlings from 32 nests; 2017: $n = 76$, 27; 2018: $n = 103$, 35).

3.1. Lead concentrations in blood and feathers

Blood lead concentrations were analyzed for 92 nestlings from 36 nests sampled during the 2018 breeding season (Uptown: $n = 37$ nestlings from 16 nests; Marigny-Bywater: $n = 25$, 10; Lakeshore: $n = 30$, 10; Table 1). Lead concentrations from whole blood samples of nestlings differed significantly by neighborhood ($F = 22.10$, $p < 0.0001$; Fig. 1), with nestlings in both high lead neighborhoods having higher blood lead concentrations than nestlings in the low lead neighborhood (Bonferroni-adjusted pairwise comparisons: Lakeshore v. Marigny-Bywater $p = 0.0002$; Lakeshore v. Uptown $p < 0.0001$). There was no difference in blood lead concentrations between the two high lead neighborhoods (Uptown v. Marigny Bywater $p = 0.55$). Compared with adult mockingbird lead data previously published (McClelland et al., 2019), nestling blood lead levels were similar between the high lead and low lead neighborhoods, while nestling feather lead levels appeared lower than adults Table 1).

Feather lead concentrations were analyzed for a total of 22 nestlings from 8 nests sampled during the 2018 breeding season in one high lead neighborhood and one low lead neighborhood (Uptown: $n = 12$ nestlings from 5 nests; Lakeshore: $n = 10$ nestlings from 3 nests; Table 1). Lead concentrations in feather samples from nestling mockingbirds differed significantly between the two neighborhoods: concentrations were higher in the high lead neighborhood than the low lead neighborhood ($t = -4.9556$, $df = 20$, $p < 0.0001$; Fig. 1). The other high lead neighborhood (Marigny-Bywater) was excluded from this analysis due to a low sample size ($n = 2$), though the feather lead values from this neighborhood are informative for qualitative comparison against the other study neighborhoods (Fig. 1).

Single mixed effect models found that neighborhood lead level significantly predicted nestling blood lead levels ($t_{56} = 3.64$, $p = 0.0006$). Moreover, blood lead concentrations of nestlings from the same nest had a repeatability of 72.4%, while blood lead concentrations of nestlings from the same neighborhood had a repeatability of 50.0%, suggesting more consistent indices of lead exposure at the finer-scale

Table 1

Mean blood and feather lead concentrations (\pm SE) for nestling mockingbirds across study neighborhoods. Mean blood and feather lead concentrations (\pm SE) included for adult mockingbirds as published in McClelland et al. (2019). Marigny-Bywater feather lead concentrations were excluded from analyses due to low sample size.

Blood					
Neighborhood	Lead Category	Mean nestling blood lead ($\mu\text{g}/\text{dL}$) \pm SE	n	Mean adult blood lead ($\mu\text{g}/\text{dL}$) \pm SE (from McClelland et al., 2019)	n
Lakeshore	Low	2.11 \pm 0.34	30	3 \pm 0.6	7
Marigny-Bywater	High	8.61 \pm 1.49	25	10 \pm 2	7
Uptown	High	12.6 \pm 2.19	37	10 \pm 2	12
Feather					
Neighborhood	Lead Category	Mean nestling feather lead ($\mu\text{g}/\text{g}$) \pm SE	n	Mean adult feather lead ($\mu\text{g}/\text{g}$) \pm SE (from McClelland et al. 2019)	n
Lakeshore	Low	0.110 \pm 0.036	10	2.76 \pm 1.28	8
Marigny-Bywater	High	0.838, 1.1	2	13.19 \pm 1.97	12
Uptown	High	6.09 \pm 4.6	12	14.2 \pm 1.74	10

nest level than at the neighborhood level.

3.2. Difference in reproductive output between high and low lead neighborhoods

For 2018 nests, 95% confidence intervals of Mayfield analyses of nest daily survival rates (dsr) differed significantly between neighborhoods (Table 2). More specifically, nests in the low-lead Lakeshore neighborhood had higher dsr (dsr = 0.5662; 95% CI: 0.5459, 0.5865) than did nests in the two high-lead neighborhoods, Marigny-Bywater (dsr = 0.3583; 95% CI: 0.3316, 0.3851) and Uptown (dsr = 0.3050; 95% CI: 0.2803, 0.3296). There was a less pronounced, but still significant, difference between the daily survival rates of nests in the two high-lead neighborhoods.

For nests across all three study years, average clutch size differed between the three neighborhoods ($F_{2, 211} = 3.335$, $p = 0.038$). Clutch size in the Marigny-Bywater neighborhood (3.22 ± 0.065 SE) was significantly greater than the clutch size of nests in the Uptown neighborhood (3.02 ± 0.042 SE; $p = 0.038$). There was no difference in average clutch size between nests in the Lakeshore neighborhood (3.164 ± 0.077) compared with either the Marigny-Bywater ($p = 0.818$) or Uptown ($p = 0.216$) neighborhoods.

The proportion of unhatched eggs to overall clutch size varied significantly between the three study years ($\chi^2 = 30.328$, $df = 2$, $p < 0.0001$; 2016 = 4.5% infertile; 2017 = 1.2%, 2018 = 15.6%), but there was no significant variation in the frequency of unhatched eggs between the three study neighborhoods ($\chi^2 = 1.7717$, $df = 2$, $p = 0.4124$; Table 3).

3.3. Genetic relatedness analysis and sibling classification

Of the 48 total nests for which relatedness data were analyzed, 32 (66.7%) nests compromised full siblings, and 16 (33.3%) nests had at least one pair of half siblings in the nest (Table 4). We found no significant difference in the proportion of nests that were classified as full-siblings-only vs. half-sibling-containing by neighborhood (Fisher's exact test, $p = 0.54$) or by lead level category (Fisher's exact test, $p = 0.31$).

4. Discussion

The goal of this study was to investigate how sublethal lead exposure in an urban songbird may affect reproduction directly (i.e. clutch size, nest survival rates, frequency of infertile eggs), or indirectly (i.e., by mediating behaviors such as aggression that may, in turn, influence reproduction). We examined nests of northern mockingbirds breeding in three urban neighborhoods characterized by different lead levels and greenness to examine the relationship between nestling lead exposure, nest success, and extra-pair paternity of offspring. This study suggests potential consequences of lead exposure on urban wildlife and highlights the need to better understand behaviorally-mediated and other potential indirect effects of lead exposure on wildlife reproduction. We also suggest that nestlings of bird species whose nests are relatively easy to access may serve as useful bioindicators of local lead levels.

4.1. Lead exposure in nestlings and nestlings as an indicator of lead in urban systems

Nestling birds from neighborhoods with high lead levels had higher concentrations of lead in their blood and feathers relative to those in the low lead level neighborhood, consistent with lead exposure patterns documented for adult mockingbirds in the same study system (McClelland et al., 2019). Further, when comparing blood lead levels for nestling mockingbirds with those for adult mockingbirds reported in McClelland et al. (2019), blood lead levels were similar, suggesting that nestlings could be a good indicator of adult lead exposure. As such,

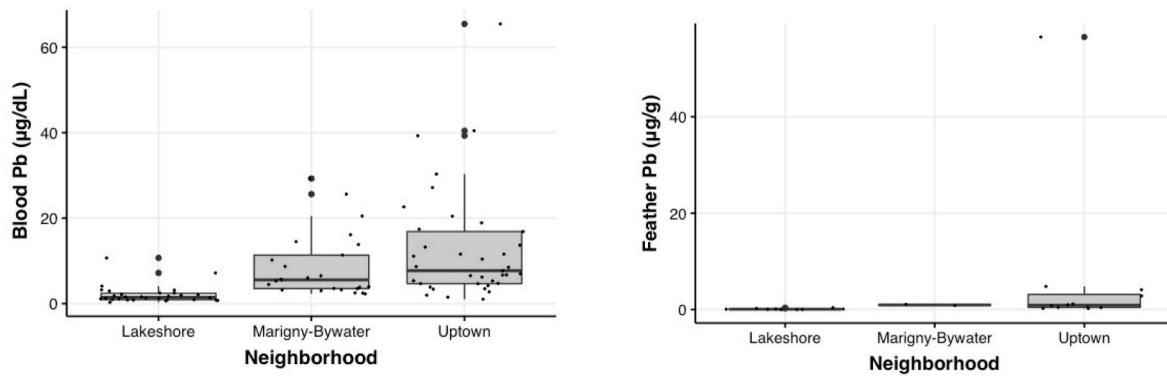


Fig. 1. Blood (left) and feather (right) lead concentrations for nestling mockingbirds across study neighborhoods. Marigny-Bywater feather lead concentrations were excluded from analysis due to low sample size (n = 2).

Table 2

Nest daily survival rate and associated 95% confidence intervals by neighborhood.

Neighborhood	Lead Category	Nest Daily Survival Rate (dsr)	95% CI Lower Bound	95% CI Upper Bound
Lakeshore (n = 16)	Low	.5662	.5459	.5865
Marigny-Bywater (n = 18)	High	.3583	.3316	.3851
Uptown (High) (n = 27)	High	.3050	.2803	.3296

nestling mockingbirds and potentially nestlings of common songbird species with easy-to-access nests could play a valuable role as bio-indicators of elevated lead levels in urban ecosystems, as has been proposed with urban-dwelling adult birds (Cai and Calisi, 2016). Since nestlings' lead exposures may reflect lead contamination at a fine spatial scale on account of the small home range size of most breeding songbirds, including mockingbirds (Biedenweg, 1983), assessing lead exposures at songbird nests in urban settings could be used to identify localized areas with high lead levels. Nestling birds can also be sampled with relative ease compared to flighted adult birds, making sampling more accessible. Our finding that birds within the same nest were more likely to have similar blood lead levels than birds from the same neighborhood also suggests that sampling at the nest-level will allow for a finer-scale assessment of local lead levels. Further, the nestling diet of invertebrates (O'Conner, 1984; Dickinson, 1999) could allow studies on an often-overlooked lead exposure source (e.g., insects; Custer et al., 2009).

4.2. Neighborhood lead and reproductive parameters

Nest success, as estimated by daily survival rate, covaried with neighborhood lead level, with lower nest success in high-lead neighborhoods. Although the blood lead levels observed in this study system for both nestling and adult mockingbirds in the high lead neighborhoods (~10 µg/dL) were less than threshold values associated with subclinical poisoning in multiple avian species (i.e., >20 µg/dL; Franson and Pain, 2011), we note that mockingbird adults in high lead neighborhoods were shown to have increased aggression compared to low lead neighborhoods (McClelland et al., 2019). In addition, studies have documented physiological effects (e.g., reduced blood δ-aminolevulinic acid dehydratase enzyme activity) in avian species at blood lead levels observed in mockingbird nestlings and adults in this study (Finkelstein et al., 2012; Work and Smith, 1996) as well as altered movement behavior at blood lead levels as low as 2.5 µg/dL (Ecke et al., 2017).

Table 3

Number of infertile eggs relative to the total number of eggs produced by neighborhood and by year. Values represent number of infertile eggs/total number of eggs (% infertile).

Neighborhood	Lead Category	2016	2017	2018	Total by Neighborhood
Lakeshore	Low	3/100 (3.0%)		8/50 (16.0%)	11/150 (7.3%)
Marigny-Bywater	High	8/155 (5.2%)		9/45 (20.0%)	17/200 (8.5%)
Uptown	High	1/9 (11.1%)	2/170 (1.2%)	10/78 (12.8%)	13/257 (5.1%)
Total by Year		12/264 (4.5%)	2/170 (1.2%)	27/173 (15.6%)	

Thus, the observed reduced daily survival rate in high lead neighborhoods, while most likely not a result of direct lead toxicity, might be related to some other sublethal effect of lead. For example, the increased aggression in adult mockingbirds living in high lead neighborhoods (McClelland et al., 2019) could result in more time invested in mate and territory defense at the expense of nestling care and provisioning (Duckworth, 2006), which could adversely affect nest success. Similarly, greater aggression aimed towards conspecific territory defense could trade off with reduced guarding of the nest site (Dunn et al., 2004; Hess et al., 2016), inadvertently resulting in higher nest predation.

There was no observed difference in clutch sizes between nests in high-versus low-lead neighborhoods, yet among the high-lead neighborhoods, nests in the neighborhood with low greenness produced more eggs per clutch than nests in the neighborhood with high greenness. This observation diverges from trends in the established urban ecology literature: nests in more urbanized areas tend to produce smaller clutches (Chamberlain et al., 2008; Sepp et al., 2017; Seress et al., 2020). Other factors such as foraging ecology and resource input differences could be contributing to this pattern (Anderies et al., 2007; Dominoni et al., 2013; Galbraith et al., 2015; Preiszner et al., 2017).

One might expect to observe lower hatching success in high-lead neighborhoods, since male infertility has been documented in birds

Table 4

Total number and proportion of nests comprised of full siblings vs. nests that had at least one pair of half siblings across three New Orleans neighborhoods. There were no significant differences across neighborhoods.

Neighborhood	Lead category	Full sibling nests (%)	Nests with at least one half-sibling pair (%)
Uptown	High	16 (72.7%)	6 (27.3%)
Marigny-Bywater	High	7 (53.8%)	6 (46.2%)
Lakeshore	Low	9 (69.2%)	4 (30.8%)

with chronic lead exposure (Kendall and Scanlon, 1981). Though the frequency of hatching success varied by year, there was not a clear connection between neighborhood lead level, greenness, or hatching success, with no significant difference observed between high- and low-lead neighborhoods. More work is needed to understand the drivers of variation in clutch size and hatching success among nests in different urban habitats. For example, although hatching success is an index of infertility, other factors may influence hatch rates, including egg viability (Aldredge et al., 2012), incubation onset timing (Wang and Beissinger, 2009), and egg quality (Eeva and Lehikoinen, 1995).

4.3. Extra-pair paternity & relatedness

Approximately one-third of nests analyzed in this study exhibited offspring sired by an extra-pair male. We note that this was a conservative measure, in that nests that were entirely sired by the social male parent are indistinguishable from nests that were wholly sired by an extra-pair male. To our knowledge, this study is the first to estimate rates of extra-pair paternity in northern mockingbirds and genetically demonstrate extra-pair paternity of offspring in the northern mockingbird. Despite the conservative index we used, the rate of extra-pair paternity presented here was higher than average for socially monogamous bird species (Brouwer and Griffith, 2019).

We had hypothesized that behavioral and physiological impacts of lead exposure indirectly influence extra-pair paternity rates in mockingbirds. However, using our approach, no relationship was found between neighborhood lead level and the presence of offspring sired by extra-pair males even though lead exposure was documented to be associated with increased aggression in adult mockingbirds (McClelland et al., 2019). Higher rates of extra-pair paternity have been reported in urban populations of other bird species due to stressors unique to the urban environment (Moore et al., 2012; Pipoly et al., 2019). There are many additional urban stressors, including changes in breeding synchrony (Stutchbury and Morton, 1995), higher breeding densities (Mayer and Pasinelli, 2013), altered circadian patterns (Dominoni et al., 2013), pollution (Pipoly et al., 2019), or food availability (Garcia-Navas et al., 2015) that may interact to shape patterns of promiscuity in urban birds. Further study, as well as a more refined metric of identifying paternity to estimate promiscuity rates, could provide insight into the potential role of lead exposure in the frequency of extra-pair mating behaviors in northern mockingbirds and other urban-dwelling birds.

4.4. Conclusion

Understanding the effects of lead exposure on reproduction in urban wildlife, including mockingbirds, is important for promoting the well-being and persistence of urban wildlife populations. This study provides insight into some of the questions that remain unanswered regarding the effects of lead exposure on the behavior and reproductive success of urban-dwelling wild birds. Recent scholarship does suggest that chronic lead exposure in birds may result in tradeoffs between lifespan and later reproductive success (Fritsch et al., 2019), though it is not yet known whether early-life lead exposure in wild birds influences altered adult behaviors, such as heightened aggression (McClelland et al., 2019). Consequently, future directions for this work should follow individuals exposed to lead as nestlings through later life stages to evaluate how early, chronic lead exposure influences interactions

between behavioral and reproductive outcomes in urban birds. Further, little is known about mockingbird dispersal patterns, but natal dispersal distances and outcomes in mockingbirds could be influenced by the behavioral and physiological impacts of lead (Dawideit et al., 2009; Serrano et al., 2021). Overall, we show that nests in high lead areas have poorer nest survival, that extra-pair offspring are present in at least one-third of mockingbird nests, and that nestling mockingbirds reflect local soil lead levels in their blood and feathers, and that their blood lead concentrations are similar to adults in the same neighborhood. Our study helps inform how urban lead contamination may influence reproduction in wildlife and suggests that nestling songbirds may serve as useful bioindicators for urban lead exposures in humans and wildlife.

Credit author statement

Conceptualization, Methodology, Investigation, & Writing – review & editing: LGH, SK, AB, MEF, ENKI, SCM, RDR, and JK. Formal analysis: LGH, SK, AB, MEF, and ENKI. Resources: LGH, SK, MEF, and JK. Data curation: LGH, SK, AB, MEF, ENKI, and SCM. Writing – original draft: LGH. Funding acquisition: LGH and JK.

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Use of animal subjects

The use of animal subjects for this observational study was conducted under supervision of the Tulane University Institutional Animal Care and Use Committee, approved protocol #395R2.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data presented here are available in a Dryad repository associated with this manuscript: <https://doi.org/10.5061/dryad.tht76hf3v>.

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Supplementary Information:

ddRAD library preparation and sequencing:

We quantified DNA concentrations for each individual using the Qubit fluorometer and dsDNA broad range assay kit (ThermoFisher Scientific, Q32853, Life Technologies, Carlsbad, CA, USA). For each library preparation, we used ~200–500 ng DNA, with concentrations ranging from 3 to 93

ng/uL. DNA was digested with SbfI and MspI restriction enzymes (New England Biolabs, MA, USA) and ligated to one of 20 P1 adaptors and a P2 adaptor using T4 DNA ligase (New England Biolabs). Samples were pooled into one of nine index groups by similar DNA concentrations. Samples were purified with 1.5X Agencourt AMPure XP beads (Beckman Coulter, Pasadena, California, USA) to remove enzymes and small DNA fragments. Fragments were size selected between 400 and 700 bp using the Blue Pippin (Sage Science, MA, USA) to ensure the same loci are recovered in all index groups. We performed low-cycle PCR with Phusion High-Fidelity DNA Polymerase (New England Biolabs) for each index group, purified the samples with 0.7X AMPure XP beads, and visualized the product on 1% agarose gel and fragment Bioanalyzer (Agilent Technologies, CA, USA). All index groups were combined and sequenced on one lane of the Illumina HiSeq 2500 Rapid Run at the Cornell University Biotechnology Resource Center, with a read length of 100 bp.

Bioinformatics:

After quality of reads were assessed using FASTQC version 0.11.8. (www.bioinformatics.babraham.ac.uk/projects/fastqc), all reads were trimmed to 97 bp and then filtered for quality using the FAST-X Toolkit (http://hannonlab.cshl.edu/fastx_toolkit). We removed sequences with Phred quality scores below 10 and sequences with more than 5% of bases with Phred quality scores below 20. We used the process_radtags commands in STACKS v 2.3 (Catchen et al., 2011) to demultiplex the remaining sequences. We applied additional filtering steps, retaining reads only if the following conditions were met: they passed the Illumina chastity filter, they contained an intact SbfI RAD site, they contained one of the unique barcodes, and they did not contain Illumina indexing adaptors. Following the recommended STACKS workflow (Richette and Catchen, 2017), we chose twelve individuals with the best read coverage to choose the best parameter combination for M and n. We ran the STACKS de novo pipeline eight times, with M and n values ranging from 1 to 8, where $M = n$ in each run and $m = 3$. Based on these tests, we found that the number of widely shared polymorphic loci (present in 80% of individuals) plateaus when M and n = 3 (Figure S1). After optimizing these parameters, all sequences were assembled de novo using STACKS v 2.3.

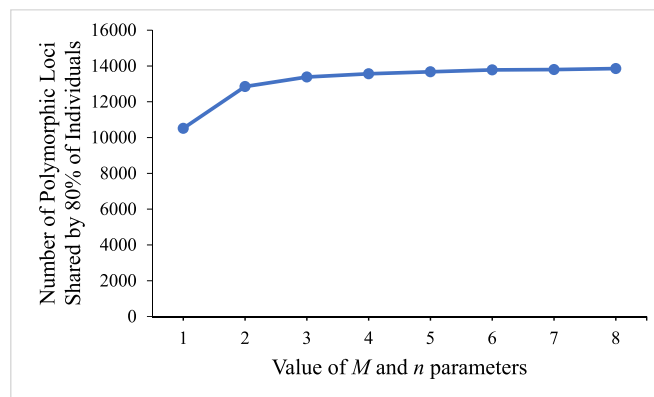


Fig. S1. Number of polymorphic loci shared by 80% of individuals as the M and n parameters changed in the STACKS de novo pipeline (n = 3 in all runs).

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