American Robin (Turdus Migratorius) Blood Lead Levels May Reflect Elevated Soil Lead Levels: Further Consequences of the Flint Water Crisis

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Abstract

High levels of pollutants often occur in urban environments and can pose a threat to human residents as well as local wildlife. The Flint, Michigan water crisis was caused by the corrosion of pipe infrastructure, resulting in high levels of lead (Pb) leaching into the drinking water. Irrigation with contaminated water may have introduced lead into the soil causing another source of exposure to humans as well as wildlife. A widespread songbird species, the American robin (*Turdus migratorius),* feeds heavily on earthworms and ingests large amounts of soil during foraging. This study investigated the impact of the Flint water crisis on American robin blood lead levels (BLL) during the breeding season in southeast MI by comparing BLL of birds captured at irrigated sites of Flint to those captured at unirrigated sites in Flint, irrigated sites in a nearby city (Ypsilanti) and rural sites. Robins captured at irrigated Flint sites had nearly double BLL compared to unirrigated Flint sites and all other control sites. Body condition declined with increasing BLL at these irrigated sites of Flint, suggesting a measurable fitness impact of lead at these levels. Because BLL in American robins is known to reflect soil lead levels and soil lead is a known driver of BLL in children, robins may act as a bioindicator for urban communities. Further research should determine the efficacy of using robin BLL as a bioindicator of soil lead and how lead might be impacting body condition and other long-term fitness metrics in urban wildlife.

Introduction

Expanding urbanization limits natural habitat and has resulted in a rising number of wild animals residing in or near human-altered landscapes. Urban-associated wildlife, such as songbirds, may have an increased exposure to contaminants compared to their counterparts in more rural or natural habitats (Roux and Marra 2007). Lead (Pb) is an environmentally persistent anthropogenic pollutant that has the potential to negatively impact the physiology and fitness of vertebrates if accumulated in the body (Burger and Gochfeld 2010; Grunst et al. 2019; McClelland et al. 2019). Elevated blood lead levels can reduce body condition through muscle atrophy and fat loss in birds, which is likely to reduce survivability (Newth et al. 2016). Understanding how birds vary in exposure to lead contaminants across landscapes, as well as the fitness consequences of different lead-burdens, is necessary to understand how urban growth increasingly impacts wild bird populations.

Past use of leaded-gasoline and paint, as well as current pollution emissions and the use of leaded pipes in drinking water infrastructure, increases lead exposure in humans and wildlife alike (Sordo and Casas 2006; Hanna-Attisha et al. 2016; Laidlaw et al. 2016). Lead from drinking water pipelines, for example, can be corroded if proper corrosion-control is not implemented (Edwards and Triantafyllidou 2007; Hanna-Atisha et al. 2016) and can result in direct consumption of contaminated tap water by humans and their pets. Further, irrigation with contaminated water can disburse lead more broadly into the environment where it may persist in soils for years (Ahmed and Goni 2009; Liu et al. 2005; Malan et al. 2015; Islam et al. 2018). Soil lead is the main pathway in child lead exposure and is linked to blood lead levels (BLL) in children, largely due to the top layer of soil being aerosolized in the dry season, resulting in inhalation of lead dust (Mielke et al. 1997; Zahran et al. 2013; Laidlaw et al. 2016). Additionally, outdoor play can result in physical contact with, and ingestion of, contaminated soil due to children's frequent hand-mouth activity (Mielke et al. 1997; Zahran et al. 2013; Laidlaw et al. 2016). Lead exposure in humans and other animals can therefore be driven by inhalation of lead dust or direct contact and ingestion of lead-contaminated substances (e.g., soil, food, water).

Lead is a well-established neurotoxin and can result in developmental delays and irreversible cognitive dysfunction in human children (Sordo and Casas 2006). It also mimics calcium, an ion essential for physiological function and can be absorbed through the diet via calcium receptors in the gut (Goyer and Mahaffey 1972; Sordo and Casas 2006). Residency time of lead in the body varies by lead dose and duration, and can be influenced by sex, age, and diet (Goyer and Mahaffey 1972). While most of a body's lead burden is stored in bone (Goyer and Mahaffey 1972), BLL reflects the amount of circulating lead following absorption and indicates current exposure of tissues (e.g., neurons) to lead. BLL may also provide the most comparable value of current lead burden across sites or exposures since lead may be allocated differentially to various tissues (Beyer et al. 2013). Blood lead analysis is a convenient, cost-effective, non-lethal method for estimating recent exposure and has been proposed to be the most useful measure of lead absorption in humans (Goyer and Mahaffey 1972), and is therefore likely appropriate for wildlife as well (Scheifler et al. 2006; Roux and Marra 2007; Cai and Calisi 2016; McClelland et al. 2019; Grunst et al. 2019).

This study investigates environmental lead exposure in American robins (*Turdus migratorius)*, a songbird species that ranges across North America and commonly breeds in both rural and urban habitats (Vanderhoff et al. 2020). We studied circulating BLL during spring and summer months in urban and rural sites in southeastern Michigan, including the city of Flint, following the heavily reported Flint Drinking Water Crisis (Pieper et al. 2017; Pieper et al. 2018). Briefly, in 2014, the drinking water of Flint was contaminated by lead when the city switched their water source from Lake Huron to the Flint River without implementing corrosion inhibitors, thereby causing corrosion of leaded pipelines and contamination of the drinking water (Pieper et al. 2017; Pieper et al. 2018). In the most impacted areas of Flint, 10% of children had BLL > 5 ug/dL in comparison to 1.2% of children residing outside of Flint in 2015 (Hanna-Attisha et al. 2016). By 2017, the lead levels in Flint's drinking water were below the assigned 'action level' of 15 ug/L (Pieper et al. 2018). While residents of Flint were advised not to consume their tap water during the three-year crisis, there was less focus or guidance concerning lawn irrigation using lead-contaminated water. Watering lawns with contaminated water can serve as a source of environmental exposure for the area's human and wildlife populations (Ahmed and Goni 2009; Liu et al. 2005; Malan et al. 2015; Islam et al. 2018). We investigated the BLL of American robins, a widespread songbird that specializes on soil-dwelling invertebrates (Gochfeld and Burger 1984), in lead-impacted areas of Flint at sites with and without irrigation, as well as control sites. We also assessed how body condition varied with BLL to better understand the dynamics of lead contamination and how it may impact fitness of this free-living bird.

Methods

Study Sites

Sites within Flint, MI (43°N 83°W) were chosen based on a lead pipe map provided by the City of Flint (created by the GIS center, University of Michigan-Flint; Moore 2016). Within affected areas of Flint, we chose sites where lawns were regularly irrigated, including: the University of Michigan (43°01'04.3"N 83°41'16.6"W) and Mott Community College (43°01'12.2"N 83°40'23.0"W) campuses, and sites that did not irrigate their lawns: Bassett (43°02'11.1"N 83°43'22.4"W) and Mott (43°00'52.1"N 83°43'24.7"W) community parks. Reference sites were chosen outside of the city of Flint and consisted of irrigated sites in Ypsilanti on the Eastern Michigan University campus (42°14'49.0"N 83°37'42.9"W), as well as two rural and unirrigated sites, one near Flint in Lapeer (43°06'42.5"N 83°14'57.7"W), and one in Ypsilanti (42°15'39.2"N 83°37'40.7"W). We chose University or College campuses as our irrigated site comparisons because we expected them to use more similar irrigation and landscaping practices than private residences. This allowed us to directly compare robin BLL at irrigated and unirrigated sites in Flint, as well as to an irrigated site in a different city and rural sites.

Animal and Hematological Procedures

American robins were mist-netted passively or using acoustic lures on their breeding territory from April-August of 2018 and 2019. Birds were immediately extracted from the mist net and age and sex were determined via plumage or breeding indicators (i.e., presence of brood patch and length of cloacal protuberance) (Pyle 1997). Morphometrics were measured (unflattened wing chord, length of metatarsus and keel) with analog calipers to the nearest 0.1 mm. Mass was measured using a Pesola spring scale to the nearest 0.5 g, and fat deposits were scored on a scale from 0-4 in the furcular and abdominal cavities and summed for a total fat score (following Helms and Drury 1960; Cornelius et al. 2021). Birds were equipped with a unique U.S.G.S. band and were immediately released following blood sampling.

Approximately 200 ul of whole blood was extracted via venipuncture of the alar vein and was collected into 75-ul microcapillary heparinized tubes. To measure BLL, a portion of whole blood was immediately transferred from one capillary tube into a standardized measuring tube provided by the LeadCareII blood lead measuring system (Magellan Diagnostics) and was immediately expelled into a proprietary solution containing hydrochloric acid, and inverted. This solution was then kept at room temperature until measurement in the LeadCareII blood lead monitor later in the day, as per manufacturer guidelines. Remaining blood samples were kept on ice and transferred to the lab within 7 hours of sampling for measurement of hematocrit and storage of plasma for other studies. Hematocrit was measured as the percent packed red blood cells after 8 minutes of centrifugation at 10,000 RPM in a clinical centrifuge. A small amount of the HCl-blood solution was placed onto a LeadCareII sensor strip and was inserted into the LeadCareII monitor to measure blood lead. A blood lead value (ug/dL) was reported within three minutes with a minimum detectability of 3.3ug/dL. Samples with undetectable lead as measured by the LeadCareII (i.e., < 3.3 ug/dL) were set to a value of 3.2 ug/dL. Note that setting these undetectable values instead to half-detectability (i.e., 1.6 ug/dL) did not change the statistical results or patterns.

The LeadCareII machine determines BLL via anodic stripping voltammetry (ASV) and is used frequently in wildlife studies given its ease of use, portability and low cost (Herring et al. 2018). The system, however, was designed and calibrated for use with human blood. Accuracy of LeadCareII ASV-derived BLL in birds has been tested by comparison to inductively coupled plasma mass spectrometry (ICP-MS); a trusted method that has shown to be accurate, precise, and highly correlated to other blood lead analysis methods such as graphite-furnace atomic absorption spectrometry (GFAAS) (Herring et al., 2018). Bird BLL derived from ASV methodology (LeadCareII) consistently underestimated blood lead by 30-38%, thus, for proper interpretation, it has been suggested that absolute ASV-derived values be increased by 30% to reflect accurate estimates of blood lead values in birds (Herring et al. 2018). We therefore adjusted our absolute values by +30%. Note that this does not alter the relative differences between sites, but rather provides more physiologically accurate blood lead values by which to estimate the impact of contamination and to compare absolute levels to other studies.

Statistical Analyses

Body condition was calculated by first using a principal component analysis (PCA) to reduce dimensionality of the morphometrics. PC1 explained 52.2% of the variance in wing, tarsus and keel lengths. To control body mass variation related to size, PC1 was then regressed against body mass and the residuals of each point to the best-fit line were saved as a metric of size-corrected body mass to reflect body condition. Body condition was calculated separately for males, females and juveniles due to egg-production impacts on body mass during the breeding season and differences in mass due to developmental stage.

Blood lead levels were analyzed with non-parametric assessments as the data were not normally distributed, did not meet model assumptions and transformations were determined ineffective. We used a Kruskal-Wallis comparison of means to determine if BLL varied by treatment (i.e., irrigated Flint, unirrigated Flint, irrigated Ypsilanti (urban control), and rural sites), followed by a Wilcoxon nonparametric multiple comparison test to determine which groups differed from each other. This test is appropriate given apriori predictions that BLL will be highest in irrigated Flint sites, followed by unirrigated Flint, irrigated Ypsilanti, and lowest levels at rural sites. Site specific relationships between BLL and body condition were analyzed with linear regression. All statistical analyses were performed with JMP Pro 15, using a significance level of $\alpha = 0.05$.

Results

Location

BLL in this study ranged from undetectable (< 4.2 ug/dL) to 32.0 ug/dL (Table 1). Kruskal-Wallis found that site significantly impacted BLL ($\chi^2 = 13.0$, $P = 0.005$). American robins had higher blood lead levels at irrigated Flint sites compared to all other sites (unirrigated Flint sites, irrigated Ypsilanti sites, and rural sites; Table 2). Birds captured at unirrigated Flint sites had higher BLL than those captured at rural sites, although BLL of robins captured at irrigated Ypsilanti sites did not differ significantly from those sampled at rural sites (Table 2; Figure 1).

Table 1
Range of blood lead values (ug/dL) at each location

Location	n	Minimum	Mean	Maximum
Irrigated Flint	18	4.2	14.0	32.0
Unirrigated Flint	13	4.2	8.8	13.0
Irrigated Ypsilanti	27	4.2	7.9	21.0
Rural	13	4.2	6.9	10.4

Table 2
Wilcoxon comparison of BLL across all sites. (*) indicates statistical significance

Location	Location	Z value	P value	Lower CI	Upper CI
Irrigated Flint	Unirrigated Flint	2.24	0.025*	1.04	8.32
Irrigated Flint	Irrigated Ypsilanti	2.85	0.0043*	2.34	9.10
Irrigated Flint	Rural	2.54	0.011*	1.69	10.8
Unirrigated Flint	Rural	1.94	0.057	-0.130	4.42
Unirrigated Flint	Irrigated Ypsilanti	1.56	0.12	-0.520	3.51
Irrigated Ypsilanti	Rural	0.56	0.57	-0.910	2.86

Body condition

There was a significant decline in body condition with increasing BLL in the irrigated sites of Flint (r^2 = 0.3, p = 0.02; Figure 2) but not in other sites (Table 3).

Table 3
Correlation between blood lead and body condition. (*) indicates statistical significance

Location	r^2	p-value
Irrigated Flint	0.30	0.02*
Unirrigated Flint	0.07	0.4
Irrigated Ypsilanti	0.001	0.9
Rural	0.09	0.4

Discussion

Following the well-publicized Flint Drinking Water Crisis, we sought to document the impact that this event had on circulating BLL in a wild bird species, the American robin, and to better understand the role of irrigation as a source of environmental exposure in this species. Robins had higher blood lead levels when captured in irrigated sites of Flint compared to unirrigated sites in Flint, and to surrounding sites. This result indicates that irrigation practices during the Flint Drinking Water Crisis likely served as a source of environmental contamination that continues to impact human and wildlife residents of this community to this day. Birds captured at irrigated sites of Flint with high BLL also tended to have lower body condition (Table 1). It should be noted that while BLL in birds at the Flint sites were higher, 81.2% of individual robins across all sites had lead levels that were higher than levels considered dangerous in children by the CDC (5 ug/dL).

Lead is a neurotoxin that can impact memory, processing speed, and comprehension in humans (Sordo and Casas 2006). In 2012 the CDC reduced the threshold BLL for identifying dangerous exposure levels in children to 5 ug/dL from the previous level of concern of 10 ug/dL, and stated that no BLL has been identified as safe (CDC 2020). Links between abnormal child development and relatively low lead exposures are well established in human children (Sordo and Casas 2006), and the risk of lead exposure to Flint residents may be reflected in the BLL of the local robins. In birds, lead has been documented to correlate with reduced brain weight in nestling starlings (*Sturnus vulgaris*) (Grue et al. 1986). Nestling growth, locomotion, balance, begging, behavioral thermoregulation, depth perception, and individual recognition were all negatively impacted in lead-dosed gulls (Burger and Gochfeld 2010), suggesting environmental pollutants may similarly impact avian cognition. In one study, BLL of rock-pigeons (*Columba livia*) in New York City (New York, USA) were correlated with child BLL when analyzed across neighborhoods (Cai and Calisi 2016), reflecting common local exposure. American robins and other widespread *Turdus* species with similar foraging habits and prey preferences (Scheifler et al. 2006) could be used as bioindicators of environmental lead contamination in neighborhoods across North America given their widespread distribution. American robins forage near their nests during the breeding season (Knupp 1977), interact directly and intensively with soil, and BLL in this species has been correlated with soil lead levels in previous studies (Blus et al. 1995; Johnson et al. 1999; Beyer et al. 2004; Roux and Marra 2007; Hansen et al. 2011; Sample et al. 2011; Beyer et al. 2013; Beyer and Sample 2017). Robins are thus likely to reflect local soil lead conditions and should be investigated further as a potential biomonitor for public health and human exposure to soil lead in urban environments.

In one study, urban robins had the highest blood lead levels (26.4 ug/dL) in comparison to other robins living in rural areas (7.9 ug/dL) and six other songbird species caught in both urban (5.3 - 18.7 ug/dL) and rural (1.1-5.3 ug/dL) habitats in Washington D.C. (Roux and Marra 2007). High lead exposure in robins has been attributed to the high percentage of soil in their diet (20%), which is greater than other urban wildlife, including opossum (5%), Canada geese (8%), racoons (9%) or American woodcock (10%), and other ground-foraging songbirds such as the song sparrow (17%) and the Swainson's thrush (1%), which is thought to be due to different behavioral modes of foraging (Beyer et al. 1994; Hansen et al.

2011). Modeling of lead exposure in American robins based on diet and average U.S. soil lead levels projected that 89% of a robin's lead burden is derived from earthworm tissues (42%) and soil (47%) that is either in or on their earthworm prey (Beyer and Sample 2017). Thus, American robins may accumulate soil lead more quickly and directly than other species, including humans, which are exposed largely through respiration of aerosolized soil lead (Mielke et al. 1997; Zahran et al. 2013; Laidlaw et al. 2016). These results suggest robins could act as an early indicator of soil lead contamination.

Relatively little is known about the health impacts of different levels of blood lead on songbirds (Hansen et al. 2011, Sample et al. 2011, Beyer et al. 2013), however we detected a negative relationship between BLL and body condition in irrigated sites of Flint. Body condition significantly declined with increasing BLL only in our irrigated sites of Flint, where BLL was the highest, implying that high lead burdens in some robins may be hindering health. Impacts on body condition may not be detectable until a lead burden threshold is reached, as seen in whooper swans (*Cygnus cygnus*) that only show a negative correlation between blood lead and body condition in individuals exceeding 40 ug/dL BLL (Newth et al. 2016). A decline in body condition in swans was hypothesized to be due to lead-induced muscle atrophy, particularly of the breast muscle, and the loss of both visceral and subcutaneous fat (Beyer et al. 1988, Newth et al. 2016). The authors suggest that reduction of muscle and fat levels may be detrimental as fuel stores are critical for overwinter survival (Haramis et al. 1986), breeding performance (Ankney and MacInnes 1978), migration (Ramenofksy 1990) and other important behaviors (Newth et al. 2016).

The cognitive impacts of elevated BLL in songbirds are also not well established, but there are several studies documenting changes in behavior with increased lead exposure. For example, increased nest aggression of urban northern mockingbirds (*Mimus polyglottos*) was found in a population with BLL averaging 10 ug/dL (McCleland et al. 2019). Another songbird (*Parus major*) showed reduced exploratory behavior with increasing BLL (range of 0 – 46 ug/dL) near a smelter (Grunst et al. 2019). Birds in our study had BLL similar to both of these songbird studies, suggesting cognitive impacts of lead exposure may contribute to the negative relationship found with body condition. More work is needed to determine the linkages between lead exposure and behavioral, cognitive or physiological effects in our study populations. Lead exposure has also been found to negatively impact metrics of reproductive success, including: thinning eggshells (Eeva and Lehikoinen 1994), interrupted laying (Janssens et al. 2003), reduced clutch size (Berglund et al. 2010), increased nestling mortality (Berglund et al. 2010; Jannssens et al. 2003) and dietary changes of invertebrate variety fed to nestlings (Eeva et al. 2005). These studies suggest that reproductive success may also be hindered by environmental lead contamination and warrant future research.

Future research should be aimed at identifying the fitness consequences of lead burdens with careful attention to potential thresholds for negative impacts. Such efforts should include investigation of the physiological mechanisms by which lead impacts health metrics, such as the link between reduced body condition and high lead exposure found in this study. Additional research regarding species-level variation in exposure by dietary niche and the degree or nature of association with human habitats will also help to inform conservation and management decisions following acute and chronic environmental contamination events. Lead contamination in human drinking water systems is a widespread issue - with more than 2,000 water systems across the country finding unsafe lead levels in their drinking water following the Flint crisis (USAToday, Young and Nichols 2017). It is therefore imperative to better understand the impacts of lead exposure on urban wildlife fitness, particularly given the ever-increasing prevalence of human-wildlife overlap.

Declarations

Statements and Declarations:

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Animal Research (Ethics): This work was approved by Eastern Michigan University Institutional Animal Care and Use Committee (IACUC) protocol #2015-072.

Consent to Participate (Ethics): N/A

Consent to Publish (Ethics): All authors read and approved the final manuscript.

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Figures

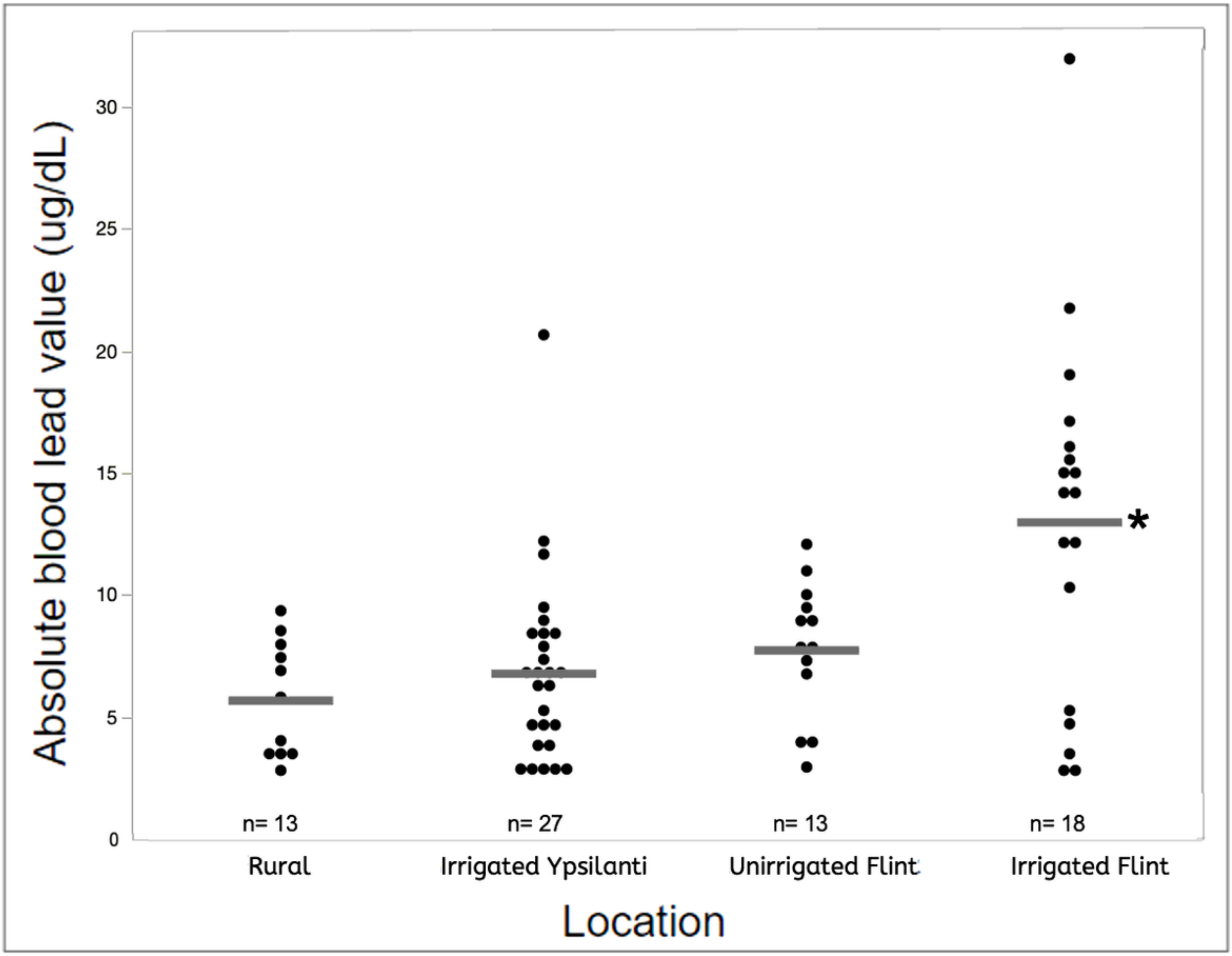

Figure 1

Blood lead levels (ug/dL) by location. BLL was highest in irrigated sites of Flint, MI that were impacted by high lead levels during the Flint Drinking Water Crisis. Horizontal lines indicate median, (*) indicates statistical significance from all other groups by Wilcoxon comparison of means ($p < 0.05$)

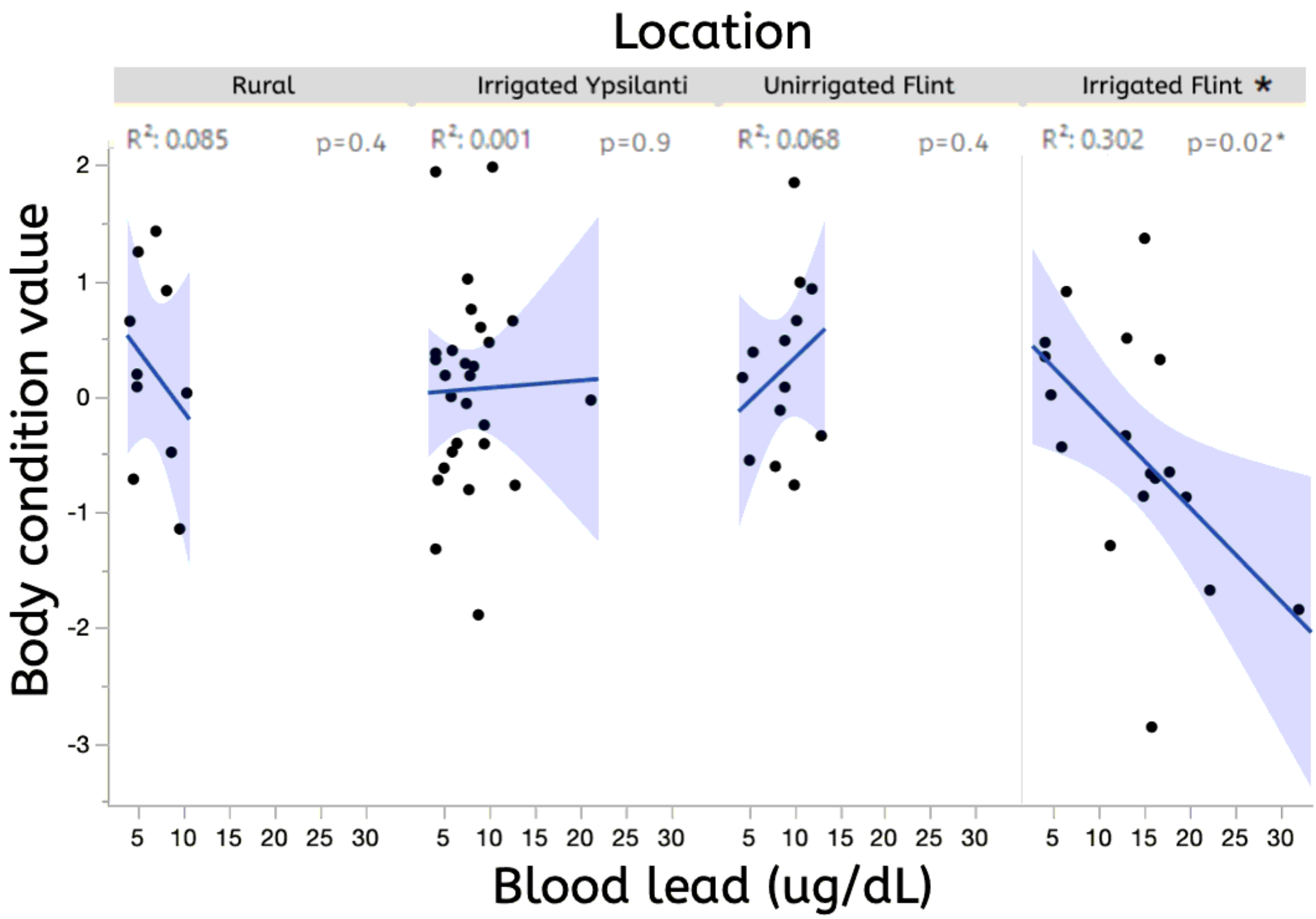

Figure 2

Blood lead levels (ug/dL) and body condition by location. BLL negatively correlates with body condition only at the irrigated Flint sites where BLL was highest. Lines represent the best fit of linear regression, shading represents 95% CI, (*) indicates statistical significance.