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Urban wall lizards are resilient to high levels of blood lead

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ABSTRACT

Living in urban environments presents many challenges to wildlife, including exposure to potentially toxic pollutants. For example, the heavy metal lead (Pb) introduces numerous health problems to all animals, including humans. The little work that has been conducted on lead toxicity in reptiles suggests that lizards may be extraordinarily resilient to very high levels of lead pollution, by either avoiding or mitigating the toxicity. To assess the impact of lead exposure, we measured field blood levels and tested for the effects on ecologically-relevant performance measures in common wall lizards (*Podarcis muralis*) – a small reptile particularly capable of thriving in urban environments. We captured lizards from roadside and park habitats across Cincinnati, Ohio, USA and quantified the concentration of lead in blood samples (n = 71 adult lizards). Lizards from roadside populations had higher blood lead concentrations than lizards from park populations, and females had higher blood lead concentrations than males regardless of habitat type. We then tested two aspects of lizard performance important for survival: (1) balance, a cognitively-demanding task, to assess the effect of lead on cognition (n = 41), and (2) running endurance, an aerobic exercise dependent on oxygen (n = 43), to assess the impact of lead on blood oxygen-carrying capacity. We then used correlation analyses to quantify the relationship between lead levels and these ecologically-relevant performance measures. There was no effect of blood lead levels on running endurance, but contrary to our predictions there was a slight positive effect on balance performance, whereby lizards with higher blood lead concentrations slipped less often than lizards with lower blood lead concentrations. Understanding the effects of lead toxicity and resilience in a particularly resistant animal could help us better respond to public health and environmental pollution concerns.

1. Introduction

Lead (Pb) is a naturally occurring metal in the earth's crust, but its presence in local environments is exacerbated by human activity, such as from atmospheric emissions from fossil fuel combustion (Rauch and Pacyna, 2009). Though its prevalence has decreased in recent decades, it continues to be used globally for a multitude of applications, such as leaded gas, industrial processes, paint, pipes, recycling, and car repair (Wani et al., 2015a,b). Due to its non-biodegradability, lead has become a significant environmental hazard, posing threats to soil conditions, water quality, and wildlife (Buekers, 2009; Khaksar Fasaee et al., 2022; Kushwaha et al., 2018). Further, this toxic heavy metal pollutant induces negative effects on human health, including the renal, reproductive, nervous and cardiovascular systems, embryogenesis, fertility, cognition, behavior, blood physiology, and the gastrointestinal and urinary tracts (Duruibe et al., 2007; Larsen and Sánchez-Triana, 2023; Wani et al., 2015a,b). Impaired postural balance and locomotion, poor motor coordination, and reduced neurodevelopment have been reported as neuromuscular effects of lead in humans from infancy to adulthood (Bhattacharya et al., 2006, 2007; Dietrich et al., 1987; Mansouri and Cauli, 2009). These negative health outcomes extend to other animals. For example, there is evidence suggesting that lead exposure impairs neurogenesis in adult mice, causing abnormalities in cognition and behavior (Wang and Matsushita, 2021). The nervous system of neonatal mice that have been exposed to lead can be damaged, causing apoptotic neurodegeneration (Dribben et al., 2011).

Wildlife are exposed to lead via contaminated food, water, and airborne dust (Lanno et al., 2019; Dar et al., 2019; Levin et al., 2021) and endure similar negative effects to humans, including changes to organ weight, hematocrit levels and hematology, reproduction, growth, and

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physiology (Buekers et al., 2009; Hitt et al., 2023). While the negative consequences of lead exposure are widely understood in taxonomic groups like birds and mammals, there is comparatively little knowledge on the effects of lead on herpetofauna (reptiles and amphibians; Weir et al., 2010). Some ecological risk assessments have resorted to using avian models as proxies for reptilian models, under the assumptions that (1) birds are equally or more susceptible to contaminants and/or (2) that birds undergo greater exposure to contaminants due to endothermy. Weir et al. (2010) refute this and stress the need for more reptilian species in ecological risk assessments because of the specific sensitivities of reptiles to lead toxicity. For example, in reptiles and amphibians lead is toxic to the endocrine system, which can compromise metamorphosis, jeopardize hatchling thyroid function, and reduce female fertility (Croteau et al., 2008). In lizards specifically, lead impacts body weight, organ weight, feeding, hematology, and behavior (Salice et al., 2009).

Notably, in lizards males and females accumulate heavy metals differently (Burger et al., 2004) and sprinting performance was insensitive to lead (Holem, 2006). Further study is thus necessary to unravel the complex interactions that may shape reptile sensitivity to lead toxicity. This is especially important in the context of urban environments, where industrial processes, automobile traffic, and older residential areas where lead paint has been used increase the prevalence of lead (Santoro et al., 2024; Laidlaw et al., 2017; Mielke et al., 2010; Chen et al., 2010; Rubio et al., 2022; Szwalec et al., 2020). The effects of such exposure to environmental lead can be compounded by other challenges faced by wildlife in urban areas. This can include higher temperatures, increased air, noise, and light pollution, and habitat fragmentation (Johnson and Munshi-South, 2017; Alberti, 2015; Diamond and Martin, 2021; Winchell et al., 2023). Nonetheless, some species thrive in such anthropogenically-altered habitats, such as pigeons, racoons, opossums, skunks, rats, and cockroaches (Johnson and Munshi-South, 2017; Lambert and Donihue, 2020).

Cincinnati is a topographically and economically diverse city made up of dense human populations, industrial sites, and natural parks with mixed levels of human activity. The common wall lizard (*Podarcis muralis*) is a small (snout-vent length <75 mm), diurnally active lizard that thrives in urban habitats and has expanded outside its southern European range due to human transport (Speybroeck and Beukema, 2016; Beninde et al., 2018; Oskyrko et al., 2020; Engelstoft et al., 2020). It was introduced to Cincinnati in the early 1950s from source populations in Italy (Davis et al., 2021). Since this single original introduction, lizard populations have thrived and spread across the Cincinnati metropolitan area, inhabiting diverse habitats in the urban matrix.

Our experiment tested the variability of blood lead concentrations in lizards across populations and relates the blood lead concentrations in individual lizards to ecologically relevant performance abilities. Our study tests two primary hypotheses: (1) lizards inhabiting habitats with differing levels of human impact would exhibit different mean blood lead concentrations, and (2) these differences in blood lead concentrations would affect performance ability. Specifically, we predict that (1) lizards from locations in close proximity to roads would exhibit increased lead exposure when compared to populations in city parks removed from automobile traffic, and (2) lizards with higher blood lead concentrations would perform worse than lizards with lower blood lead concentrations. The performance measures we chose to test are balance and running endurance. When choosing a performance measure, it is important that it is ecologically relevant and tests the expected physiological effects produced by the toxin (Amaral et al., 2012).

We chose to study balance because it is associated with cognition (Tangen et al., 2014), and lead exposure is associated with cognitive impairment and decreased balance in humans (Ramírez Ortega et al., 2021; Leasure et al., 2008; Mansouri et al., 2013; Nehru and Sidhu, 2001). Balance is relevant to *P. muralis* fitness because these lizards are climbing specialists. Due to the known neurotoxic effects of lead, it can be assumed that higher blood lead concentrations would negatively impact balance and motor coordination, cognitively demanding tasks (Overmann, 1977).

We chose to study endurance because it is an aerobic exercise that requires oxygen (Beck et al., 2018), and lead reduces hemoglobin production and oxygen carrying capacity (Ilizaliturri-Hernández et al., 2013; Mager and Grosell, 2011). Lead has also been shown to decrease endurance in various taxonomic groups (Yu et al., 1996; Tu et al., 2018; Mansouri and Cauli, 2009; Burger and Gochfeld, 2004). Further, endurance is relevant to fitness through its impacts on predator avoidance, the ability to find food and mates, and territory defense (Clemente et al., 2009; Sinervo et al., 2000). Introduced populations of common wall lizards in Cincinnati, Ohio, USA provide the opportunity to study the effects of lead on a reptile species that has recently become established in urban environments on a new continent, providing relevant data on ecotoxicology at the intersection of several vectors of anthropogenic environmental change: pollution, urban biology, and invasion biology.

2. Materials and methods

2.1. Capture and blood collection

We caught adult male and female common wall lizards (*Podarcis muralis*) at nine sites in Cincinnati, Ohio, USA (Fig. 1, Table 1) during peak activity periods (08h00–17h30; June 2023–July 2023). Site choice was primarily influenced by the City of Cincinnati Head et al., 2024) Lead Report, which provides information about the distribution of lead in Cincinnati and the hazards associated with lead poisoning (Vigran and Rehman, 2022). Populations were classified as either “roadside” or “park” based on habitat characteristics and proximity to city streets. “Roadside” populations were stone walls adjacent to city streets (within 2 m). “Park” populations were a mix of anthropogenic and natural structures in city parks. Lizards were captured via a thread lasso attached to an extendable fishing rod or by hand. Sampling of males and females was not biased between park and roadside habitats (Kruskal--Wallis, $X_1^2 = 0.62$, $p = 0.43$).

Within 5 min from capture, we collected a blood sample (minimum 25 μL) from the retro-orbital sinus (MacLean et al., 1973) using two heparinized glass capillary tubes per individual. We stored blood samples in capillary tubes on ice until processing (see below).

The day of capture, we measured snout-vent length (SVL) as the distance from the tip of the snout to the cloaca with digital calipers (Model CD-6, Mitutoyo, Japan; male mean ± SD: 62.57 ± 5.23 mm, range: 50.96–70.88 mm, female mean ± SD: 57.39 ± 5.54 mm, range: 50.92–69.95 mm). Lizards were weighed to the nearest 0.01 g using a digital scale (Weigh Gram Top-100, Pocket Scale, Tulelake, California,

Fig. 1. Adult common wall lizard (*Podarcis muralis*) found on a stone wall at a park population in Cincinnati, Ohio, USA.

Table 1
Locality and collection data for common wall lizards (*Podarcis muralis*) from Cincinnati, Ohio, USA.

Location	Date	n Total	n Females	n Males	Habitat Type	n individuals in performance trials (Males)	Latitude/Longitude (WGS84)
MT. AIRY (AIR)	May 17, 2023	1	1	0	roadside	0	39.16820, −84.55431
ALMS PARK (ALM)	June 20, 2023 June 21, 2023 October 11, 2023 October 12, 2023 October 13, 2023	13	0	13	park	12	39.15033, −84.44644
AULT PARK (AUL)	May 18, 2023 June 13, 2023 October 11, 2023 October 12, 2023	15	7	8	park	3	39.13526, −84.40672
FAIRVIEW PARK (FAR)	July 10, 2023	11	0	11	park	11	39.12429, −84.52666
MISTLETOE (MIS)	May 17, 2023	1	0	1	roadside	0	39.11237, −84.55140
MT. ECHO PARK (MTE)	June 12, 2023	5	0	5	park	5	39.09418, −84.56498
MT. STORM (MTS)	October 13, 2023	1	0	1	park	0	39.15516, −84.53250
RIVER ROAD (RIV)	June 12, 2023 October 13, 2023	11	1	10	roadside	6	39.09308, −84.56189
SERENDIPITY (SER)	May 16, 2023 May 17, 2023 July 10, 2023	13	4	9	roadside	6	39.11769, −84.57261

USA; male mean ± SD: 6.67 ± 1.60 g, range: 2.88–9.56 g, female mean ± SD: 4.92 ± 1.77 g, range: 2.72–9.16 g). Sex was determined based on head size, palpation for the presence of developing egg follicles in females, and the presence of enlarged femoral pores in males (Žagar et al., 2012; Aleksić et al., 2009; Bülbül et al., 2023; Amer et al., 2023). From length and mass data, we calculated a scaled mass index (SMI) for each sex independently, which represents the mass of an individual relative to its size, following Peig and Green (2009) and Amer et al. (2023). SMI presents a proxy of an individual's muscle mass and/or fat storage relative to its body size, a useful indicator of overall physiological and energetic status. A subset of animals (n = 43) were used in performance assays (see below). These lizards were individually isolated in breathable cloth sacks and kept overnight at room temperature (~20 °C).

A minimum of 25 μL whole blood from the first capillary tube of collected blood was stored in a microcentrifuge tube and flash-frozen in liquid nitrogen for lead testing. We centrifuged the second capillary tube at 5000 *g* for 5 min. We then measured the volume of packed red blood cells and total blood volume with digital calipers (Model CD-6, Mitutoyo, Japan). Hematocrit (Hct) was calculated as the ratio of packed red blood cells to total blood volume measured in millimeters (n = 60) (Amer et al., 2023). Blood samples were stored in a freezer (−20 °C) until shipped overnight to Louisiana Animal Disease Diagnostic Laboratory (Baton Rouge, Louisiana, USA) for quantification of lead concentration in whole blood. Samples (15 μL) were diluted in a solution of 0.1% Triton-X100 acidified to 0.2% with HNO_3, resulting in a 21-fold dilution or, in the case of clotted samples, digested with 600 μL of 35% Trace Metal HNO_3 overnight at 85 °C and diluted to a 1/60 solution with 0.2% HNO_3. Both the control and the samples were analyzed twice. Blood-lead concentration analyses were conducted on a PinAAcle 900Z Graphite Furnace Atomic Absorption Spectrometer (PerkinElmer, Shelton, Connecticut, USA), with a limit of detection of 0.7 ppb and a limit of quantification of 2 ppb. The reference material used for qualification was Seronorm Trace Elements Whole Blood L-2 (Sero, Billingstad Norway) with an average recovery of 103% ± 2.9% RSD.

2.2. Performance measures

We conducted performance assays for balance and endurance one day post-capture and within the animals' natural activity period (08h00–17h30). We measured balance by running lizards on a circular wooden dowel (D = 2.86 cm; L = 121.9 cm) covered with sandpaper (60 grit) to simulate the roughness of rock walls where they are naturally found. Tick marks were made every 5 cm to record distance covered per trial. The balance beam was suspended at a height of 35 cm through holes cut into a plastic bin (52 cm wide × 48.5 cm high × 90.5 cm long). Light-colored towels were placed in the bottom of the bin to reduce the impact of falling and to improve visibility of the lizards in the video recording. We set up the camera (Panasonic AG-AC30; super-slow motion, 120 fps) to view the entire balance beam.

Lizards were placed in individual plastic bins (15 × 5.7 × 14 cm) with holes cut into the lid using a soldering iron and kept under a heat lamp for 4 min or until they reached a body temperature between 34 °C and 36 °C (balance trials mean ± SD: 35.85 ± 1.27 °C), which is close to the optimal temperature for both sprint and aerobic performance (Telemeco et al., 2022; Vaughn et al., 2023). We recorded starting body temperature by inserting a type K thermocouple approximately 0.5 cm into the lizard's cloaca (HH801, Omega Engineering, Norwalk, Connecticut, USA), as well as ambient air temperature and humidity (PTH8708 Digital Temperature & Humidity Pen, General Tools, New York, USA) before each trial. We then started the camera and placed the lizard at the start of the balance beam. We allowed them to run across the beam, gently tapping the tip of the tail and hindlimbs with a paintbrush for motivation. We aimed for three complete, successful trials per individual, meaning we excluded animals from the data if they kept intentionally jumping off the beam before attempting to cross.

Two animals were not cooperative during balance trials (refused to run and/or immediately leapt off balance beam) and were therefore excluded from balance analysis. Once we observed three complete trials per lizard, we stopped the recording and recorded end body temperature via cloacal probe as above. Lizards were then returned to their individual box and allowed to rest between 1 and 3 h before starting endurance trials (see below). One observer (MMM) scored all videos. A subset of videos (n = 5) were scored a second time, and we found high concordance indicating high interrater reliability (Kendall's Coefficient of Concordance = 0.99; Burghardt et al., 2012). We recorded the distance the lizard ran to the nearest 5 cm, noting when both forelimbs crossed the tick mark. Slips were counted when a lizard's hind feet lost traction or when a lizard jumped and clung to the balance beam to stop itself from falling off. For each trial, we quantified the total number of slips and total distance traveled. For analysis, we used the total number of slips per meter traveled.

Endurance trials were intentionally conducted after balance trials as balance trials are less physically taxing. We measured running endurance capacity by running lizards on a treadmill (Columbus Instruments Exer 3/6, Columbus, Ohio, USA; lane dimensions 43.5 x 6.0 × 12.0 cm). To prevent escape, we placed a slip knot lasso with 15 cm of string (same as used for capture) around the pelvic girdle, with the knot on the dorsal side of the animal, and attached the end of the string to a dowel placed perpendicularly directly above the treadmill lane. As in the balance assay, lizards were warmed in plastic bins with heat lamps to a body temperature of 34–36 °C before trials, which is close to the optimal temperature for both sprint and aerobic performance (endurance trials mean ± SD: 35.6 ± 0.98 °C). Ceramic heat bulbs (100 W) were placed above the treadmill to maintain warm ambient conditions during the trial. We set the treadmill to a speed of 10.0 m/min, which lies in the range of speeds used in previous studies (Baxter-Gilbert et al., 2017; Lee et al., 2023; Noble et al., 2014; Sorlin et al., 2022). Preliminary trials with animals not included in this experiment indicated that this speed provided a reasonable time to exhaustion and also provided variation among individuals. Once the lizards reached optimal temperatures, lizards were placed on the moving treadmill and allowed to orient themselves before being gently prodded with a paintbrush on the tip of the tail and hindlimbs to encourage running. Starting body temperature, air temperature, and humidity were recorded as for balance trials above. The duration from the initiation of the trial (when lizards were placed onto the treadmill) to inability to run after being prodded for 5 s was recorded as the measure of endurance (Lee et al., 2023; Baxter-Gilbert et al., 2017). Once exhaustion was determined, end body temperature was recorded immediately then animals were returned to their individual boxes. After data collection, each lizard was returned to its location of capture or brought to our captive colony at Ohio Wesleyan University.

2.3. Statistics

We conducted all data processing and analyses in the programming language R (R Core Team, 2023). To assess the heterogeneity in blood lead concentrations among populations, we used a Kruskal-Wallis Rank Sum test. To test for differences between roadside and park populations and between males and females, we used Wilcoxon Rank Sum tests. Additionally, we tested for correlations between blood lead concentration and two measures of body size (SVL and SMI) as well as hematocrit using Spearman's Rank Correlations. We then assessed the impact of body size and blood lead concentration on two measures of performance, running endurance and balance, using Spearman's Rank Correlations. We also tested whether performance measures differed between roadside and park populations using Wilcoxon Rank Sum tests. Finally, we tested the correlation between performance measures to assess whether lizards with better balance could run longer distances, and vice-versa, using Spearman's Rank Correlations. We used non-parametric analyses because parametric models failed to produce normally distributed residuals. We created data figures using the package *ggplot2* (Wickham, 2016).

3. Results

3.1. Field lead concentrations

Lead concentrations measured in field-caught lizards varied significantly across populations (Kruskal-Wallis, X^2_8 = 22.55, p = 0.0040). Blood lead concentrations were higher in roadside populations compared to those in park populations (Roadside mean ± SD: 44.7 ± 60.0 μg/dL, Park mean ± SD: 34.8 ± 83.0 μg/dL; Wilcoxon Rank Sum test, W = 330, p = 0.0024; Table 2, Fig. 2A). Blood lead concentrations varied between sexes (Wilcoxon Rank Sum test, W = 619, p = 0.00033), where females had higher blood lead concentrations than males (Female mean ± SD: 113.2 ± 146.3 μg/dL, Male mean ± SD: 21.7 ± 29.3 μg/dL; Table 2, Fig. 2B). Smaller animals exhibited marginally higher levels of blood lead concentrations than larger animals (Spearman's Rank Correlation, rho = −0.23, S = 70394, p = 0.05).

Lizards from park habitats were larger (SVL mean ± SD: 63.38 ± 5.69 mm; Mass mean ± SD: 6.93 ± 1.73 g) than lizards from roadside habitats (SVL mean ± SD: = 58.81 ± 4.14 mm; Mass mean ± SD: 5.42 ± 1.35 g; Welch Two-Sample *t*-test, $t_{64.9}$ = 3.88, p = 0.00025), however there was no relationship between SVL and lead within each habitat type (Spearman Rank Correlation, Park: rho = −0.11, S = 15780, p = 0.47; Roadside: rho = −0.021, S = 2986.6, p = 0.92). We found no correlation between SMI and blood lead concentration (Spearman's Rank Correlation, S = 63362, rho = −0.11, p = 0.37). Blood lead concentrations were not correlated with hematocrit values (n = 60; Spearman's Rank Correlation, rho = 0.14, S = 30954, p = 0.29; Fig. 3A).

Table 2
Summary statistics of blood lead concentration (μg/dL) by habitat type and sex for common wall lizards (*Podarcis muralis*) from Cincinnati, Ohio, USA.

	Males	Females	Total
Roadside	median: 19 mean ± sd: 34.5 ± 44.9 range: 8–200	median: 38 mean ± sd: 78.7 ± 92.5 range: 22–260	median: 22 mean ± sd: 42.7 ± 60.0 range: 8–260
Park	median: 11.5 mean ± sd: 14.9 ± 12.0 range: 4–54	median: 48 mean ± sd: 142.9 ± 182.9 range: 10–440	median: 12 mean ± sd: 34.8 ± 83.0 range: 4–440
Total	median: 13 mean ± sd: 21.7 ± 29.3 range: 4–200	median: 46 mean ± sd: 113.2 ± 146.3 range: 10–440	

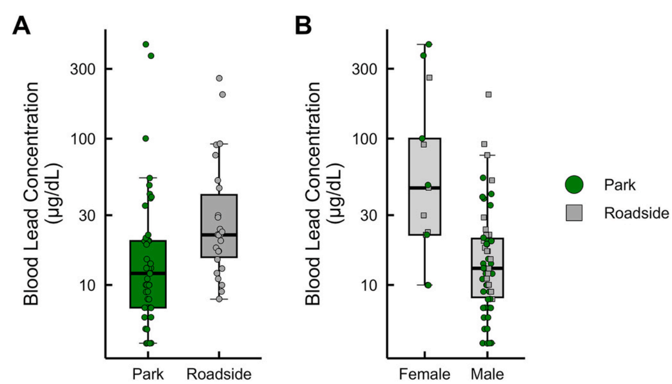

Fig. 2. Blood Lead Concentration (μg/dL) varies between habitat types and sex in common wall lizards (*Podarcis muralis*). A) Populations from roadside habitats had significantly higher blood lead concentrations than populations from park habitats. B) Females had significantly higher blood lead concentrations than males. Data point shapes represent the habitat type in both boxplots. Please note the $\log_{10}$-transformed scale of the y-axis.

3.2. Performance experiments

Lead concentrations did not affect running endurance (Spearman's Rank Correlation: rho = 0.083, S = 12139, p = 0.59; Fig. 3B) nor did endurance differ between roadside and park populations (Kruskal-Wallis, X^2_1 = 1.98, p = 0.16). Blood lead concentration was negatively correlated with slips per meter, where lizards with lower blood lead concentration slipped more than lizards with higher blood lead correlation (S = 16063, rho = −0.40, p = 0.0097; Fig. 3C). Larger animals exhibited greater running endurance (Spearman's Rank Correlation: rho = 0.37, S = 8392, p = 0.016). SVL had no effect on slips per meter covered on the balance beam (S = 12037, rho = −0.048, p = 0.76) nor did balance differ between lizards from roadside and park populations (Kruskal-Wallis, X^2_1 = 0.85, p = 0.36). Finally, there was no among-individual relationship between endurance and slips per meter (S = 11438, rho = 0.0037, p = 0.98).

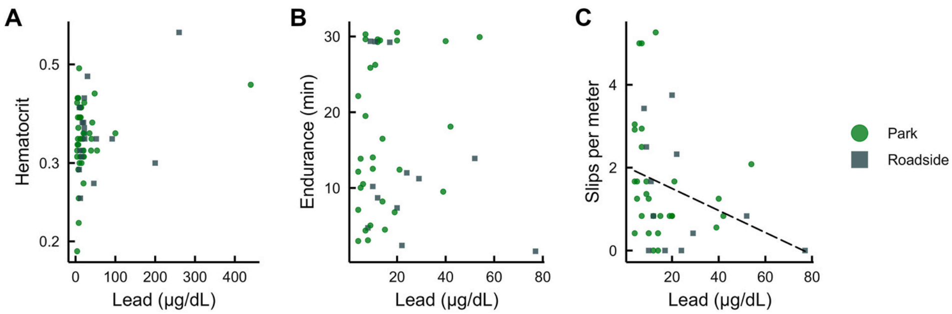

Fig. 3. Effect of lead on performance measures in common wall lizards (*Podarcis muralis*). A) Blood lead concentration had no effect on hematocrit. B) Blood lead concentration had no effect on running endurance. C) Blood lead concentration and balance are inversely related, where individuals with lower blood lead concentrations slip more per meter than individuals with higher blood lead concentrations. Data point shapes represent the habitat type in all plots.

4. Discussion

The concentrations of lead in lizard blood are high across urban populations, with many lizards exhibiting concentrations above those which would be acutely dangerous to humans. According to the Centers for Disease Control and World Health Organization, a human blood lead concentration of 10 µg/dL is concerning, though there is no level of lead that is considered "safe" (Wani et al., 2015a). For mammals and birds, a blood lead concentration above 35 µg/dL and 50–100 µg/dL, respectively, could affect growth or reproduction (Buekers et al., 2009). Of our tested samples, which included both males and females, 53 out of 71 lizards (75%) had a blood lead concentration ≥10 µg/dL. Of the 43 males used for performance trials, 27 (63%) had a blood lead concentration ≥10 µg/dL. Lizards from roadside populations exhibited higher blood lead concentrations than lizards from park populations (Roadside mean ± SD: 44.7 ± 60.0 µg/dL, Park mean ± SD: 34.8 ± 83.0 µg/dL). This suggests that the level of human activity and vehicle traffic may drive variation in this highly toxic heavy metal across the urban landscape.

Despite having a smaller sample size, females had more than five times higher mean blood lead concentrations than males. This is important because maternal transfer of lead to embryos may occur and cause deleterious effects to embryonic and hatchling development, as seen in mallards (Vallverdú-Coll et al., 2015), Sprague-Dawley rats (Bunn et al., 2001), and humans (Mobarak, 2008; Dorea and Donangelo, 2006). This, in turn, could have long-term population-level impacts, as demonstrated by human birth defects caused by maternal lead contamination (Vinceti et al., 2001; Xie et al., 2013), increased susceptibility to lead toxicity with increasing age and prenatal exposure in Wistar rats (Nascimento et al., 2016), and maternal transfer of lead to cubs in Scandinavian free-ranging brown bears (Fuchs et al., 2021). In *P. muralis*, lead can be transferred to eggs (unpublished data: egg yolk lead concentration 2–12 µg/dL, n = 3). The large difference in blood lead concentrations between males and females may be due to differences in diet preferences between the sexes (Vacheva and Naumov, 2024). Further assessment of how lizards acquire lead is necessary to test potential explanations of these observed dimorphisms. Despite the high blood lead levels of *P. muralis*, we found no negative effect of lead on performance measures which indicate cognition and aerobic capacity. This suggests the presence of an unexplored mitigation mechanism utilized by the lizards to avoid the negative effects of lead.

Urban landscapes are not homogenous with respect to heavy metal contamination (delBarco-Trillo and Putman, 2023; Vigran and Rehman, 2022). We sampled lizards from park and roadside sites (1) to test for differences in lead contamination between habitat types, and (2) to investigate how lead exposure across a human-altered landscape affects wildlife. Anthropogenic sources of urban lead contamination include traffic emission (e.g., vehicle exhaust particles, weathered street surface particles), industrial emission (e.g., power plants, coal combustion), and domestic emissions (e.g., buildings built before the mid-1990s, leaded paint) (Wei and Yang, 2010; Levin et al., 2010; Santoro et al., 2024). Urban lead pollution is the result of older homes with leaded paint, car exhaust, and industrial practices like coal combustion and burning municipal waste (Santoro et al., 2024), and it can accumulate in residential soils for long periods of time. From there, lead may be transferred to lizards directly or via their invertebrate prey. *P. muralis* is an opportunistic generalist feeder, with a wide variety of invertebrates making up their diet (Vacheva and Naumov, 2024). Assuming that park sites experience less of this type of human activity than roadside sites, we hypothesized that lizards from park habitats would have lower blood lead concentrations than lizards from roadside habitats. This was supported by our field data, with lizards from roadside populations having on average 28% higher blood lead concentrations than lizards from park populations. This demonstrates the importance of quantifying heterogeneity in blood lead concentrations across urban landscapes. *P. muralis* may serve as a bioindicator of heavy metals in urban environments, and their surprisingly high blood lead concentrations posit the need for better monitoring of environmental health - especially in cities whose economy is supported by industrial processes.

We found no relationship between blood lead concentration and endurance. Opposite of what we expected, there was a slight – but statistically significant – negative relationship between blood lead concentration and the number of slips on a balance beam, suggesting a positive impact of lead on balance. These results demonstrate some resiliency to lead contamination, but the mechanisms of which are unclear. The ability to avoid or endure environmental contamination is crucial for species survival and reproduction (Andrew et al., 2019). Resilience to toxicity reduces the magnitude of negative physiological effects of heavy metal contamination and may explain the lack of an expected correlation between blood lead concentration and performance ability. In our study, lizards from park habitats had larger mean body size (SVL) and greater mass than lizards from roadsides, possibly indicating some negative effects to body condition associated with heavy metal contamination, though the explanation may be confounded by other aspects of habitat differences. We intend to direct future research toward examining such costs of heavy metal exposure in populations of *P. muralis*.

The expected negative effects of heavy metal contamination do not always appear at the whole organismal level. Sublethal effects can take place at lower levels of biological organization but still affect survival (Ilizaliturri-Hernández et al., 2013; Triebskorn et al., 2001). This may explain why we found no relationship between lead - a highly toxic metal - and locomotor performance. Ilizaliturri-Hernández et al. (2013) investigated the blood lead concentrations and hematological parameters in the cane toad (*Rhinella marina*) from highly polluted urban regions and rural regions in Veracruz, Mexico. Across both sites, they found a positive relationship between blood lead concentrations and hematocrit, suggesting a physiological coping mechanism against anemic hypoxia. Interestingly, we found no impact of lead levels on hematocrit levels, which can serve as an indicator of physiological status (for example, Amer et al., 2023). This is in contrast to Salice et al. (2009) who report that lizards exposed to greater concentrations of lead exhibited lower hematocrit values. This suggests that different animal taxa may have different compensatory mechanisms for heavy metal toxicity.

Furthermore, Ilizaliturri-Hernández et al. (2013) found a significant decrease in the enzyme delta-aminolevulinic acid dehydratase (δ-ALAD) activity in toads from urban regions compared totoads from rural regions. The inhibition of δ-ALAD is one of the first indicators of lead poisoning and is extremely concerning due to δ-ALAD's role in the biosynthesis of heme, an important molecule for binding oxygen in the bloodstream (Ilizaliturri-Hernández et al., 2013; Scinicariello et al., 2007; Mager and Grosell, 2011). This points to a potential research avenue in populations of urban lizards. There is also the possibility for selection to shape organismal tolerance of lead toxicity. For example, the ubiquitous urban songbird the house sparrow (*Passer domesticus*), shows signs of genetic adaptation to trace amounts of lead (Andrew et al., 2019). An additional mechanism conferring resilience to the effects of lead on the brain is neurogenesis. Adult neurogenesis is a mechanism by which *P. muralis* may have developed an increased resilience to lead toxicity compared to urban mammalian species. Unlike mammals, lizards possess a widespread ability to regenerate brain cells within their central nervous system (Pérez-Cañellas and García--Verdugo, 1996; González-Granero et al., 2023; McDonald and Vickaryous, 2018; Perez-Sanchez et al., 1989). Nonetheless, little is known about the potential role of neurogenesis in mitigating the negative effects of either acute or chronic lead exposure, a potentially fruitful avenue of future study.

While vertebrate ecotoxicology is a widely studied field, there is a dearth of knowledge on reptilian ecotoxicology (Lettoof et al., 2022). The present study reinforces the importance and validity of using reptiles in environmental monitoring and risk assessments. The results of the present study reveal that there is a great deal of bioavailable lead in the Cincinnati landscape that has the potential to severely affect the population. As a next step to understanding the potential mechanisms underlying lizard resistance to lead toxicity, we recommend controlled laboratory studies testing the impact of blood lead concentrations across the observed range on performance, physiological processes, and reproduction. Further research on the effects of heavy metals on performance measures in lizards should include females because (1) most animal research studies have a disproportionate number of males (Head et al., 2024; Zucker and Beery, 2010), and (2) females in our study had significantly higher levels of lead in their blood than males, which poses developmental concerns for future generations, and thus the population. We also suggest examining the effects of maternal lead contamination on reproductive success in *P. muralis,* as other species have shown reduced reproductive success due to lead exposure, though studies have focused on avian species (Chatelain et al., 2021; Fritsch et al., 2019; Hitt et al., 2023).

A comparison of the lead concentrations and performance measures of *P. muralis* from native populations to this study may also provide some insight into the mechanisms by which the Cincinnati populations are able to combat the lethal effects of lead. The xenobiotic nature of environmental pollutants like lead can be fatal even in the smallest amounts to humans, while research is illuminating the fact that wildlife may be able to tolerate heavy metals much more effectively. When we can determine why some species are so resilient, these findings may then be applicable to human environmental health. Research such as this is essential to inform the 'One Health' approach to achieving optimal and sustainable human, wildlife, and environmental health, which uses systems thinking to address the social-ecological link between humans and wildlife (Zinsstag et al., 2011), and this method will become even more necessary as the human population grows.

CRediT authorship contribution statement

Maya M. Moore: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emma G. Foster:** Writing – review & editing, Investigation. **Ali Amer:** Writing – review & editing, Investigation. **Logan Fraire:** Writing – review & editing, Investigation. **Alyssa Head:** Writing – review & editing, Investigation. **Annelise Blanchette:** Writing – review & editing, Methodology, Conceptualization. **Shala J. Hankison:** Writing – review & editing, Methodology. **Alex R. Gunderson:** Writing – review & editing, Methodology, Conceptualization. **Eric J. Gangloff:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eric Gangloff reports financial support was provided by National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data and analysis code are available from the Dryad Digital Repository (Moore et al, 2024): https://doi.org/10.5061/dryad.0rxwdbs9m.

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