



Effects of lead on avian thermoregulation in the heat: An experimental test with pied crows (*Corvus albus*)

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ABSTRACT

Many of the negative physiological effects of lead involve the hypothalamus, but the possibility that thermoregulation is affected has received little attention. We tested the hypothesis that lead exposure reduces avian thermoregulatory performance under hot conditions in pied crows (*Corvus albus*) experimentally exposed to lead in their diet. Crows in our high lead treatment (blood [Pb] = $87.3 \pm 44.7 \mu\text{g dL}^{-1}$) showed significantly higher air temperature (T_{air}) inflections for evaporative water loss (EWL) and resting metabolic rate (RMR) compared to control ($6.4 \pm 1.8 \mu\text{g dL}^{-1}$) or intermediate ($53.9 \pm 23.7 \mu\text{g dL}^{-1}$) lead groups, which did not differ. EWL, RMR and body temperature (T_b) all increased more rapidly at $T_{\text{air}} > T_b$ in the high lead treatment. In contrast, neither maximum T_{air} tolerated by the crows nor maximum T_b varied with treatment. Our data reveal that water and energy balance during hot weather is affected by lead exposure.

1. Introduction

Human activities have resulted in organisms being increasingly exposed to pollutants, many containing molecules that in concentrations exceeding background levels can have severe negative physiological effects. One such molecule is lead (De Francisco et al., 2003; Demayo et al., 1982; Monclús et al., 2020). The physiological effects of lead are primarily neurological, interfering with neurotransmitter function and synaptogenesis by binding to voltage-gated Ca^{2+} channels (Eisler, 1988; Mukherjee et al., 2022; Virgolini and Aschner, 2021). Avian effects of chronic exposure including reduced foraging ability, lethargy, decreased spatial awareness, loss of coordination and drooping wings as well as compromised immune response and reduced reproductive success (Franson and Pain, 2011a; Haig et al., 2014). Acute exposure to large

quantities of lead can be lethal; for example, there have been several cases in which lead-containing paint or food has resulted in mortality among captive birds (e.g., Naidoo et al., 2012; Pikula et al., 2013).

Although overall environmental levels of lead have declined since the global phase-out of leaded automotive fuel (e.g., Escobar et al., 2013; Wu and Boyle, 1997; Xu et al., 2012), other sources of lead continue to create significant conservation challenges. Prominent among these sources are lead ammunition and fishing tackle (Fisher et al., 2006; Grade et al., 2019; Haig et al., 2014) and mining (Beyer et al., 2013; Henny et al., 1991). Spent ammunition in carcasses is thought to be a major avenue of exposure among scavenging birds (Fisher et al., 2006; Franson and Pain, 2011b; Ganz et al., 2018; Naidoo et al., 2017), with high levels of lead exposure frequently reported among vultures (Finkelstein et al., 2012; van den Heever et al., 2019;

Abbreviations: EHL, evaporative heat loss; EWL, evaporative water loss; M_b , body mass; MHP, metabolic heat production; T_{air} , air temperature; T_b , body temperature; RMR, resting metabolic rate.

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Wiemeyer et al., 2017). Blood lead levels associated with significant subclinical and clinical poisoning in southern African vultures have been reported by several workers (e.g., Garbett et al., 2018; Naidoo et al., 2017; van den Heever et al., 2019), supporting arguments that poisoning in general is a major cause of recent rapid declines in vulture populations in Africa (Ogada et al., 2016; Safford et al., 2019).

Many of the negative effects of lead on avian physiological function occur in the hypothalamus, a region of the central nervous system where many neuroendocrine processes occur. For instance, lead exposure impairs several physiological pathways involved in reproduction that originate in the hypothalamus (He et al., 2020; Sokol et al., 1985; Vallverdú-Coll et al., 2016). The preoptic anterior hypothalamus (POAH) is the site of the major thermoregulatory integration centre, where populations of warm-, cold- and temperature-insensitive neurons regulate a body temperature (T_b) setpoint (Boulant, 2006; Hammel, 1965; Simon, 2000). Somewhat surprisingly, the possibility that lead interferes with avian thermoregulatory control processes in the POAH has not, as far as we are aware, been evaluated beyond Burger and Gochfeld's (1985) experimental test of whether lead exposure affects shade-seeking behaviour in herring gull (*Larus argentatus*) chicks.

An effect of lead exposure on avian thermoregulatory performance would have implications for understanding the physiological effects of environmental lead, particularly in light of current rapid anthropogenic climate change (IPCC, 2021). Limits to thermoregulatory performance during heat exposure result in several effects of increasing temperatures and more frequent and severe heat waves on avian survival and reproduction. These include mass mortality events during extreme heat events (Finlayson, 1932; Holt and Boersma, 2022; McKechnie et al., 2021b; Saunders et al., 2011). Chronic exposure to sustained hot weather is associated with missed opportunity costs arising from behavioural trade-offs that negatively affect several components of fitness related to both adult survival and breeding success (reviewed by Cunningham et al., 2021). These negative impacts of heat waves are not confined to warmer regions; studies of high-latitude species reveal similar temperature-dependent behavioural trade-offs affecting reproduction (Oswald and Arnold, 2012; Oswald et al., 2008) and much lower heat tolerance limits than birds inhabiting warmer regions (O'Connor et al., 2021). If the neurological effects of lead include compromising thermoregulatory performance limits, exposure to lead could exacerbate the negative impacts of global warming.

In this study, we tested the hypothesis that the physiological effects of lead exposure among birds extend to thermoregulatory performance. Specifically, we predicted that lead exposure reduces birds' abilities to defend T_b below lethal limits when environmental temperature exceeds T_b . Control birds with baseline blood lead levels were predicted to tolerate higher environmental temperatures than individuals with levels associated with subclinical levels or clinical poisoning, and we expected more precise thermoregulation (i.e., less variation around a normothermic T_b setpoint) in control birds. We evaluated these predictions using pied crows (*Corvus albus*), a widespread and common southern African corvid. We considered *C. albus* a suitable model species as their consumption of carrion potentially exposes them to lead from spent ammunition, as is the case for vultures in southern Africa (van den Heever et al., 2019, 2023). Corvids seem to be more heat-tolerant than many passerines (Marder, 1973), but the extreme environmental temperatures they experience while incubating in full sun in exposed locations such as utility poles and windmills raises the possibility of deleterious effects of rising temperatures.

2. Material and methods

2.1. Study site and animals

The study took place during September to December 2021 at Vulpro (S 25° 42' 42" E 27° 57' 14"), a vulture conservation and research centre near the town of Hartbeespoort in South Africa's Northwest Province.

During April 2021, 37 pied crows (*Corvus albus* Müller 1776) were caught by placing carrion in a large flight aviary for several weeks. On the days of capture, the aviary entrance was pulled closed when a sufficient number of crows were inside. The crows were then transferred into smaller enclosures where they were held until late August 2021 to ensure no exposure to lead for several months prior to the study. Thereafter, they were transferred into one of two half-cylinder shaped enclosures (16 m long, 5 m wide, 2.5 m high), each constructed from aluminum tubing and shade cloth. Additional 80 % shade cloth was affixed over ~3 m at the far end of each enclosure to provide shade. While in captivity, the crows were provided with *ad libitum* borehole water (known to be free of lead contamination) and food (piglet carcasses and other carrion). Each enclosure was outfitted with 2–3 perches spanning the full width. When the crows were placed in the two enclosures for the main study, birds from each experimental treatment (see below) were randomly allocated so that an equal number was housed in each enclosure. Each crow was outfitted with three spiral colour rings to permit individual identification for the remainder of the study.

2.2. Experimental treatments and measurements of blood lead

2.2.1. Pilot study

We manipulated blood lead in a subset of the crows by dosing them with No. 3 lead shot, with mean \pm SD pellet mass = 0.200 ± 0.006 g ($n = 23$). After trying several approaches, the best way to dose the crows involved opening the beak of each bird while in the hand and placing the pellet(s) at the back of the gape. We then squirted a small amount of water using a syringe barrel to ensure the lead was swallowed. During July - August, we conducted a pilot study with nine randomly selected crows to establish the relationship between the number of pellets dosed and blood lead levels. Three of the birds received two pellets (i.e., ~0.4 g lead) every ten days, three received three pellets (~0.6 g) every ten days, while the remaining three were not given lead. Dosing occurred three times for the 0.4 g and 0.6 g groups. Blood samples were drawn once from the unmanipulated group, and on three occasions from the 0.4 g and 0.6 g groups (4, 8 and 10 days after dosing events). Blood samples (~100 μ L) were collected by brachial venipuncture and analysed within 1 h using a Leadcare 2 (Meridian Bioscience, Cincinnati OH, USA) blood lead analyser calibrated using the two supplied standards. Blood lead values for the three un-dosed birds were 8.4 ± 1.3 μ g dL⁻¹. Values for the three birds dosed with 0.4 g lead varied from 17.3 ± 5.5 μ g dL⁻¹ after 4 days to 12.2 ± 3.1 μ g dL⁻¹ after 10 days, whereas corresponding values for the bird dosed with 0.6 g lead were 55.3 ± 7.4 μ g dL⁻¹ and 26.4 ± 3.6 μ g dL⁻¹, respectively. Following these measurements, however, the Leadcare 2 kit we had used was recalled, so we caution that these pilot study values may have been inaccurate. Six of the pilot study birds were released, with three retained for the main study on account of difficulties in capturing additional birds.

2.2.2. Main study

The main study took place in October and November 2021. Crows were allocated to one of three experimental treatments: a control group with unmanipulated blood lead levels, an intermediate blood lead group and a high blood lead group (hereafter control, intermediate and high groups, respectively). Based on the results of the pilot study, starting on 6 October we dosed birds weekly with either 0.4 g lead (intermediate group) or 0.6 g (high group). Blood samples were drawn from all the birds on 13 October (one week after the start of dosing) and again on 24 November, a week after the last dosing event. In addition, a third blood sample was drawn from each bird in the intermediate and high groups between 2 and 17 November, on the day each individual was used for respirometric measurements during acute heat exposure.

Blood lead analysis was performed by Ampath Laboratories (Pretoria, South Africa) using Graphite Furnace Atomic Absorption Spectrometry (GFAAS), using a matrix-matched calibration curve (calibration range 5–40 μ g dL⁻¹) for quantification of lead

concentrations. Samples were prepared by dilution of blood standards, controls and specimens with an appropriate diluent, prior to analysis. Quality was assured using internal and external control specimens. The method limit of quantification was $4 \mu\text{g dL}^{-1}$, and measurement precision was $1 \mu\text{g dL}^{-1}$.

2.3. Thermoregulation during acute heat exposure

Between 22 October and 24 November, interactions between evaporative heat loss, metabolic heat production and T_b at air temperature (T_{air}) approaching and exceeding normothermic T_b were quantified using flow-through respirometry. The equipment and experimental setup were the same as those described by Czenze et al. (2021), Smit et al. (2018) and Whitfield et al. (2015), with only minor modifications listed below. The respirometry equipment was housed in an off-road caravan modified as a mobile laboratory that was located at Vulpro for the duration of this aspect of the study. The crows were placed individually in a 30-L chamber (62 cm long x 32 cm wide, with a sloping lid such that the height varied from 40 cm to 32 cm) chamber constructed from clear polycarbonate, placed in the same custom-built temperature-controlled box described by Czenze et al. (2021). Using the same approach as these authors, the crows' T_b was measured using intraperitoneally-injected Biotherm passive-integrated transponder (PIT) tags and HPR+ readers (Biomark, Boise ID, USA). To improve PIT tag reception, the crows were restricted to one half of the chamber by placing plastic containers on the mesh platform at the opposite end. Two PIT tag readers were used, with one antenna on either side of the chambers. The PIT tags used here are from the same batch for which a subset was calibrated by Freeman et al. (2020), which deviated from the actual temperature by $0.28 \pm 0.23^\circ\text{C}$ ($n = 23$). Following Czenze et al. (2021), Smit et al. (2018) and Whitfield et al. (2015), a surveillance camera and infrared light source mounted inside the temperature-controlled box allowed us to monitor the crows' behaviour continuously during measurements. For nine individuals, we estimated panting frequency at $T_{\text{air}} \geq 40^\circ\text{C}$ by timing 10–30 breaths with a stopwatch. We also monitored all individuals in the two experimental treatments for behavioural symptoms of lead poisoning, which include weakness, loss of coordination, involuntary eye movement and tremors (Fallon et al., 2017), both while in the aviaries and during respirometric measurements.

In brief, compressed air supplied by an oil-free compressor was scrubbed of water vapour using a membrane dryer (Model SD1N-13, Atlas Copco, Nacka, Sweden) before being supplied to an experimental channel and a baseline channel. Flow rates to the baseline channel were maintained at $1\text{--}2 \text{ L min}^{-1}$ using a needle valve, and flow rates to the chamber were maintained using a mass flow controller (MC50 SLPM, Alicat Scientific Inc., Tucson, AZ, United States), calibrated using a soap-bubble flow meter (Giliblator 2; Sensidyne, St. Petersburg, FL, United States). Flow rates were adjusted between 20 and 50 L min^{-1} to maintain low chamber humidity during measurements, with dewpoints typically below 5°C and never higher than 10°C . Measurements of T_b , carbon dioxide production (\dot{V}_{CO_2}) and evaporative water loss (EWL) and monitoring of birds while in the chambers followed Whitfield et al. (2015).

Prior to each set of measurements, a crow was caught early in the morning before feeding to ensure it was postabsorptive. Retention time of food was predicted as 113 min, using the scaling equation provided by Karasov (1990). Crows were initially placed in the chambers at $T_{\text{air}} = 28^\circ\text{C}$ and allowed 1.5 h to habituate before any data were collected. Thereafter, T_{air} was increased in 4°C increments until $T_{\text{air}} = 40^\circ\text{C}$ was reached, whereafter it was increased in 2°C increments until $T_{\text{air}} = 52^\circ\text{C}$ was reached (or, in a few cases, birds had to be removed early on account of sustained escape behaviour. A few runs continued to $T_{\text{air}} = 54^\circ\text{C}$, and the rapid increases in T_b and/or loss of balance that occurred at this temperature suggest that $T_{\text{air}} = 52^\circ\text{C}$ is near the upper limit of the

crows' heat tolerance. Following (Czenze et al., 2021; Smit et al., 2018; Whitfield et al., 2015) and other studies, once T_{air} was approximately stable at each setpoint value, birds were maintained until traces of $[\text{CO}_2]$ and $[\text{H}_2\text{O}]$ were stable for at least 10 min. Crows typically spent 15–20 min at each T_{air} setpoint. The total duration of measurements per individual was 3.5–4 h, with measurements for either one or two individuals per day. The order in which individuals were measured was random within each treatment, i.e., the first individual tested was randomly selected from one of the three treatments, the second was randomly selected from the second treatment and the third from the remaining treatment.

2.4. Data analyses

Algorithms provided in Expedata software (Sable Systems, Las Vegas NV, USA) were used to correct for analyzer drift and lag, after which we used (Lighton, 2008) equations 9.5 and 9.6 to calculate \dot{V}_{CO_2} and EWL from the lowest stable 5-min periods of CO_2 at each T_{air} setpoint. For EWL, we assumed a density of $0.803 \text{ mg H}_2\text{O mL}^{-1}$ vapour. We calculated resting metabolic rate (RMR; W) from \dot{V}_{CO_2} assuming a respiratory exchange ratio (RER) of 0.71 (i.e., lipid metabolism; (Walsberg and Wolf, 1995) and Joule thermal equivalence of $2.78 \text{ J mL}^{-1} \text{ CO}_2$ (Withers, 1992) as the birds were removed from the aviaries some time before measurements and were likely postabsorptive at the commencement of data collection. Rates of evaporative heat loss were calculated from EWL assuming a latent heat of vaporization of water of 2.406 J mg^{-1} at 40°C (Tracy et al., 2010).

We restricted analyses to $T_{\text{air}} = 28^\circ\text{C} - 52^\circ\text{C}$ and did not include the subset of crows measured at $T_{\text{air}} = 54^\circ\text{C}$ (3 per treatment). Final sample sizes for the control, intermediate and high treatments were 8, 10 and 8, respectively. Respective inflection T_{air} values above which response variables (T_b , EWL, RMR and EHL/MHP) increased rapidly were identified using the package *segmented.lme* (Muggeo, 2016), with individual identity, included as a random predictor. Response variables were analysed at T_{air} above and below inflection points separately using linear mixed-effect models in the R package *nlme* (Pinheiro et al., 2009), allowing for the estimating of relationships (slopes) of thermoregulatory response variables as functions of T_{air} .

Levene's tests implemented in the R package *lawstat* (Gastwirth et al., 2017) confirmed that no significant heteroscedasticity existed in T_b , EWL or RMR data at T_{air} above inflection points. After confirming that no obvious deviations from normality or homoscedasticity were evident in residual plots, we fitted linear mixed-effect models using the *nlme* package (Pinheiro et al., 2009). Our standard mixed-effect model included T_{air} (or $T_{\text{air}} - T_b$), M_b , Pb treatment, and the $T_{\text{air}} \cdot \text{Pb}$ treatment interaction. Where a significant interaction between Pb treatment and T_{air} occurred, we used Type III analysis of variance to determine the significant effects of predictors. Tukey HSD multiple comparison analysis was used to test for differences between maximum values of response variables (T_b , RMR, EWL and EHL/MHP). Maximum values for response variables were compared at $T_{\text{air}} = 52^\circ\text{C}$, the highest T_{air} at which sample sizes for each treatment were large enough to make statistically rigorous comparisons.

3. Results

3.1. Blood lead levels

One week after the start of dosing, blood lead in the control birds averaged $6.4 \pm 1.8 \mu\text{g dL}^{-1}$ ($n = 8$), whereas values for the intermediate and high treatments were $46.8 \pm 26.9 \mu\text{g dL}^{-1}$ ($n = 10$) and $66.4 \pm 27.6 \mu\text{g dL}^{-1}$ ($n = 10$), respectively. Immediately before gas exchange and T_b measurements, the corresponding values were $53.9 \pm 23.7 \mu\text{g dL}^{-1}$ ($n = 9$, range = $22\text{--}99 \mu\text{g dL}^{-1}$) and $87.3 \pm 44.7 \mu\text{g dL}^{-1}$ ($n = 9$, range = $43\text{--}159 \mu\text{g dL}^{-1}$), respectively. Blood [Pb] varied

considerably between individuals within treatments, typically by ~4-fold for each set of blood [Pb] measurements.

3.2. Behaviour

Crows typically remained calm during measurements but shifted position more frequently at $T_{\text{air}} \geq 46^\circ\text{C}$. They often held their beaks open, with some individuals gaping continuously during their time in the chamber, even at the starting $T_{\text{air}} = 28^\circ\text{C}$. Panting at $T_{\text{air}} \geq 40^\circ\text{C}$ occurred at rates of 68–146 breaths min^{-1} ($n=9$). We also observed wing-drooping behaviour in some individuals, as well as copious salivation evident as fluid on the beaks upon removal from the chamber at the end of measurements. In these instances, partial wetting of both the inner and outer beak surfaces occurred. No obvious differences in behaviour occurred between the three treatments while birds were in the chamber. We also detected no behavioural indications of lead toxicity in any of the crows during the study, neither while in chambers nor in the aviaries where they were held.

3.3. Body temperature and maximum air temperature

Crow T_b varied from ~ 41.5°C at $T_{\text{air}} = 28^\circ\text{C}$ to $43\text{--}44^\circ\text{C}$ at the highest T_{air} values at which we obtained data ($T_{\text{air}} = 53\text{--}54^\circ\text{C}$). Inflection T_{air} values for T_b (Table 1) did not vary significantly with Pb treatment (Table 1). Crows in all three Pb treatments reached maximum setpoint air temperature at $T_{\text{air}} = 52^\circ\text{C}$ or $T_{\text{air}} = 54^\circ\text{C}$ (Table 1). At $T_{\text{air}} = 52^\circ\text{C}$, maximum T_b did not differ significantly between the control ($\bar{x} = 43.12 \pm 0.50^\circ\text{C}$) and intermediate ($\bar{x} = 43.43 \pm 0.51^\circ\text{C}$; Tukey: HSD = 0.31, $p=0.71$) or high treatment ($\bar{x} = 43.31 \pm 0.74^\circ\text{C}$; Tukey: HSD = 0.61, $p=0.32$). Our top linear mixed-effect model for T_b at T_{air} above inflections identified T_{air} ($F_{1121} = 409.06$, $p < 0.001$), Pb treatment ($F_{2129} = 3.66$, $p = 0.03$) and the interaction between Pb treatment and T_{air} ($F_{2121} = 3.23$, $p = 0.045$), but not body mass (M_b ; $F_{1,23} = 1.35$, $p = 0.26$), as significant predictors of T_b , evident as steeper increases in T_b with increasing T_{air} in the high treatment compared to the other two treatments (Table 1; Fig. 1).

3.4. Resting metabolic rate

The crows' RMR was significantly predicted by T_{air} ($F_{1105} = 390.86$, $p < 0.001$), M_b ($F_{1,23} = 19.68$, $p < 0.001$), Pb treatment ($F_{2111} = 3.52$, $p = 0.03$) and the interaction between Pb treatment and T_{air} ($F_{2121} = 3.21$, $p = 0.04$; Fig. 2). Minimum values of RMR were ~ 3.5 W at $T_{\text{air}} = 28^\circ\text{C}$, increasing to maxima of ~ 6 W at $T_{\text{air}} = 52\text{--}54^\circ\text{C}$. The inflection T_{air} for RMR (i.e., the upper critical limit of thermoneutrality, T_{uc} ; Table 1) was significantly higher for the high Pb treatment compared to the control group (Tukey: HSD = 4.02, $p = 0.048$), but did not differ between the control and intermediate treatments (Tukey: HSD = -1.76, $p = 0.48$) nor the high and intermediate Pb treatments (Tukey: HSD = 2.26, $p = 0.31$). The slope of RMR above the inflection T_{air} for the high Pb treatment ($263 \text{ mW } ^\circ\text{C}^{-1}$) was 1.4- and 1.2-fold greater than slopes for the control ($189 \text{ mW } ^\circ\text{C}^{-1}$) and intermediate treatment ($220 \text{ mW } ^\circ\text{C}^{-1}$), respectively (Fig. 2). At $T_{\text{air}} = 52^\circ\text{C}$, maximum RMR did not differ significantly between the control group ($\bar{x} = 5.88 \pm 1.19^\circ\text{C}$), intermediate ($\bar{x} = 5.54 \pm 0.83^\circ\text{C}$; Tukey: HSD = 0.34, $p = 0.86$) or high Pb treatments ($\bar{x} = 6.05 \pm 1.23^\circ\text{C}$; Tukey: HSD = 0.17, $p=0.96$).

3.5. Evaporative water loss

Our mixed effect model revealed EWL was significantly predicted by T_{air} ($F_{1116} = 859.71$, $p < 0.001$), M_b ($F_{1,23} = 17.08$, $p < 0.001$), Pb treatment ($F_{2121} = 4.81$, $p < 0.01$) and the interaction between Pb treatment and T_{air} ($F_{2115} = 4.13$, $p = 0.02$; Fig. 3). Across treatments, rates of EWL varied from a minimum of ~ $2\text{--}3 \text{ g h}^{-1}$ at the lower experimental T_{air} values to maxima of ~ 20 g h^{-1} ($T_{\text{air}} = 52\text{--}54^\circ\text{C}$) and $23.08 \pm 5.75 \text{ g h}^{-1}$ ($n=3$) in the high Pb treatment ($T_{\text{air}} = 54^\circ\text{C}$;

Table 1

Summary of thermoregulatory performance as a function of air temperature (T_a) for pied crows (*Corvus albus*) with blood lead levels of $6.4 \pm 1.8 \mu\text{g dL}^{-1}$ (Control), $53.9 \pm 23.7 \mu\text{g dL}^{-1}$ (Intermediate) or $87.3 \pm 44.7 \mu\text{g dL}^{-1}$ (High). T_b = body temperature, T_{air} = air temperature, RMR = resting metabolic rate, T_{uc} = upper critical limit of thermoneutrality, EWL = evaporative water loss, EHL= evaporative heat loss, MHP = metabolic heat production. Means \pm SD are reported, with sample sizes in parentheses. For inflection T_{air} values, 95 % confidence intervals are reported in square brackets.

Variable	Control	Intermediate	High
Body mass (g)	519.94 \pm 30.45 (8)	523.43 \pm 43.01 (10)	492.07 \pm 41.80 (9)
Body temperature			
Min. T_b ($^\circ\text{C}$)	41.5 \pm 0.5 (7)	41.6 \pm 0.5 (9)	41.6 \pm 0.4 (8)
Inflection T_{air} ($^\circ\text{C}$)	40.5 [37.64–43.45]	41.0 [37.99–44.01]	43.3 [41.36–45.28]
T_b vs T_{air} slope ($^\circ\text{C } T_b \text{ } ^\circ\text{C}^{-1} T_{\text{air}}$)	0.13	0.11	0.17
Max. T_b ($^\circ\text{C}$) $T_{\text{air}} = 52^\circ\text{C}$	43.1 \pm 0.5 (5)	43.4 \pm 0.5 (7)	43.3 \pm 0.7 (9)
Max. T_b ($^\circ\text{C}$) $T_{\text{air}} = 54^\circ\text{C}$	43.6 \pm 0.7 (3)	43.9 \pm 0.3 (3)	43.6 \pm 0.2 (3)
Resting metabolic rate			
Min. RMR (W)	3.92 \pm 0.50 (7)	3.51 \pm 0.99 (9)	3.78 \pm 0.57 (8)
T_{uc} ($^\circ\text{C}$)	39.27 [36.96–41.57]	41.03 [38.72 – 43.33]	43.29 [41.83–44.75]
RMR slope (mW $^\circ\text{C}^{-1}$)	188.67	220.01	263.19
Max. RMR (W) $T_{\text{air}} = 52^\circ\text{C}$	5.88 \pm 1.19 (5)	5.54 \pm 0.83 (7)	6.05 \pm 1.23 (9)
Max. RMR (W) $T_{\text{air}} = 54^\circ\text{C}$	6.78 \pm 1.99 (3)	5.71 \pm 0.06 (3)	6.25 \pm 1.16 (3)
Max. RMR/min. RMR	1.73	1.63	1.65
Evaporative water loss			
Min. EWL (g h^{-1})	3.03 \pm 1.15 (7)	1.87 \pm 0.67 (9)	2.89 \pm 1.21 (8)
Inflection T_{air} ($^\circ\text{C}$)	41.22 [39.89–42.55]	43.11 [41.62–44.77]	44.93 [44.18–45.68]
EWL slope (g $\text{h}^{-1} \text{ } ^\circ\text{C}^{-1}$)	1.16	1.34	1.49
Max. EWL (g h^{-1}) $T_{\text{air}} = 52^\circ\text{C}$	19.23 \pm 4.04 (5)	19.01 \pm 2.83 (7)	20.41 \pm 4.71 (9)
Max. EWL (g h^{-1}) $T_{\text{air}} = 54^\circ\text{C}$	21.55 \pm 6.89 (3)	19.59 \pm 0.45 (3)	23.08 \pm 5.75 (3)
Max. EWL/min. EWL	7.11	10.49	7.99
Min. EHL/MHP	0.52 \pm 0.25 (7)	0.35 \pm 0.11 (9)	0.45 \pm 0.10 (8)
EHL/MHP inflection T_{air} - T_b ($^\circ\text{C}$)	-10.69 [-12.05 - -6.09]	-6.54 [-11.16 - -1.93]	-4.65 [-6.47 - -2.84]
EHL/MHP slope	0.09	0.10	0.11
Max. EHL/MHP $T_{\text{air}} = 52^\circ\text{C}$	2.18 \pm 0.05 (5)	2.30 \pm 0.17 (7)	2.25 \pm 0.20 (7)
Max. EHL/MHP $T_{\text{air}} = 54^\circ\text{C}$	2.11 \pm 0.12 (3)	2.30 \pm 0.07 (3)	2.45 \pm 0.21(3)

Table 1). The inflection T_{air} above which rates of EWL increased rapidly varied with Pb treatment (control = 41.22°C , intermediate = 43.11°C , high = 44.93°C , Table 1), with the inflection T_{air} for our control treatment significantly lower than for our high Pb treatment (Tukey: HSD = -3.71, $p = 0.004$). Inflection points were not significantly different between our intermediate Pb treatment and control (Tukey: HSD = 1.89, $p = 0.15$), nor between our intermediate and high Pb treatment (Tukey: HSD = -1.82, $p = 0.17$). At $T_{\text{air}} = 52^\circ\text{C}$, maximum EWL did not differ significantly between the control group ($\bar{x} = 19.31 \pm 3.80^\circ\text{C}$) and the intermediate ($\bar{x} = 19.01 \pm 2.83^\circ\text{C}$; Tukey: HSD = 0.02, $p = 0.99$) or high Pb treatments ($\bar{x} = 20.41 \pm 4.71^\circ\text{C}$; Tukey: HSD = -1.38, $p = 0.81$).

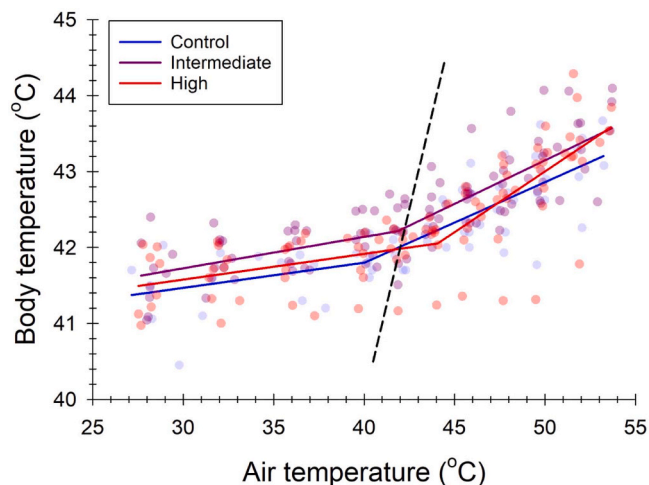


Fig. 1. Relationship between body temperature (T_b) and air temperature (T_{air}) in pied crows (*Corvus albus*) with background blood [Pb] ($6.4 \pm 1.8 \mu\text{g dL}^{-1}$, “Control”), dosed with 0.4 g of Pb every ten days ($53.9 \pm 23.7 \mu\text{g dL}^{-1}$, “Intermediate”) or dosed with 0.6 g of Pb every ten days ($87.3 \pm 44.7 \mu\text{g dL}^{-1}$, “High”). The solid lines represent segmented linear regression models fitted to the data and the dashed line shows $T_b = T_{air}$.

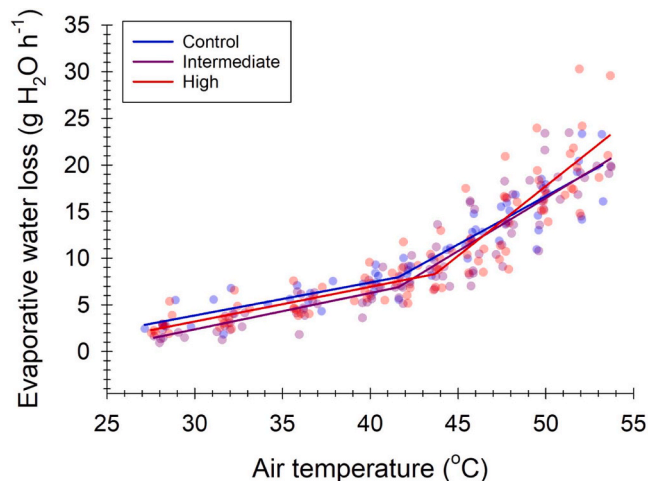


Fig. 3. The inflection air temperature above which evaporative water loss began to increase rapidly of pied crows (*Corvus albus*) was significantly higher among individuals dosed with 0.6 g Pb every ten days ($87.3 \pm 44.7 \mu\text{g dL}^{-1}$, “High”) compared to individuals with background blood [Pb] ($6.4 \pm 1.8 \mu\text{g dL}^{-1}$, “Control”) or dosed with 0.4 g of Pb every ten days ($53.9 \pm 23.7 \mu\text{g dL}^{-1}$, “Intermediate”). The solid lines represent segmented linear regression models fitted to the data.

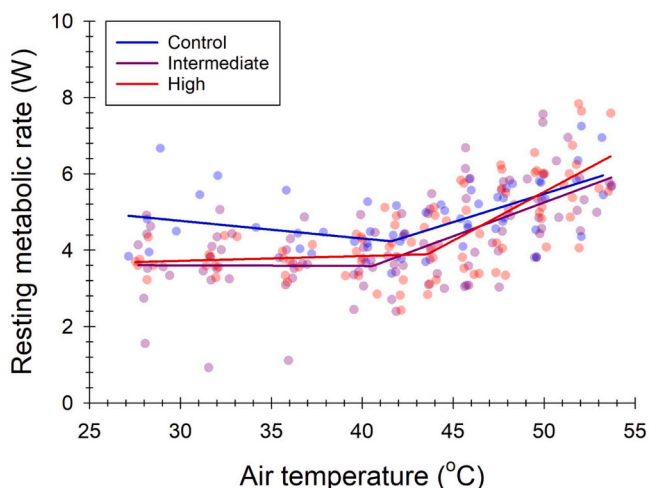


Fig. 2. The inflection air temperature above which resting metabolic rate began to increase (i.e., the upper critical limit of thermoneutrality) of pied crows (*Corvus albus*) was significantly higher among individuals dosed with 0.6 g Pb every ten days ($87.3 \pm 44.7 \mu\text{g dL}^{-1}$, “High”) compared to individuals with background blood [Pb] ($6.4 \pm 1.8 \mu\text{g dL}^{-1}$, “Control”) or dosed with 0.4 g of Pb every ten days ($53.9 \pm 23.7 \mu\text{g dL}^{-1}$, “Intermediate”). The solid lines represent segmented linear regression models fitted to the data.

3.6. Evaporative heat loss/metabolic heat production

Evaporative cooling efficiency (i.e., EHL/MHP) was significantly predicted by T_{air} ($F_{1153} = 1334.78$, $p < 0.001$), Pb treatment ($F_{2161} = 6.49$, $p = 0.002$) and the interaction between Pb treatment and T_{air} ($F_{2153} = 5.46$, $p = 0.005$; Fig. 4). However, M_b ($F_{1,23} = 0.21$, $p = 0.65$) was not a significant predictor of EHL/MHP. Inflection T_{air} values for EHL/MHP increased with Pb treatment (control = -9.07 , intermediate = -6.55 and high = -4.65) but these differences were not significant (Table 2). At $T_{air} = 52^\circ\text{C}$, maximum EHL/MHP did not differ significantly between the control group ($\bar{x} = 2.18 \pm 0.05^\circ\text{C}$) and the intermediate ($\bar{x} = 2.30 \pm 0.17^\circ\text{C}$; Tukey: HSD = -0.12 , $p = 0.44$) or high Pb treatments ($\bar{x} = 2.25 \pm 0.20^\circ\text{C}$; Tukey: HSD = -0.07 , $p = 0.75$).

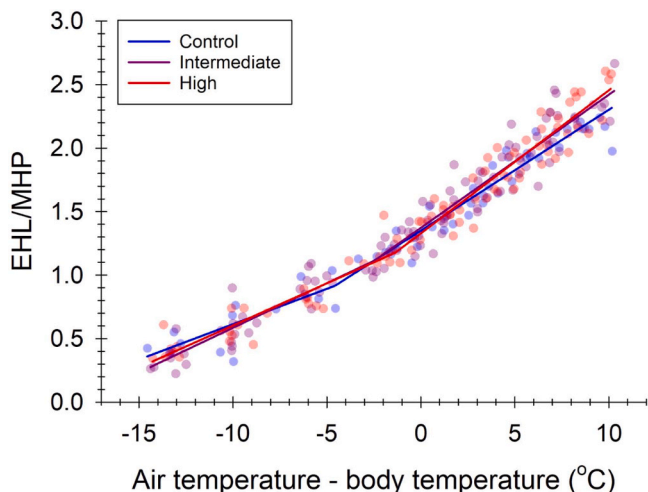


Fig. 4. Relationship between evaporative heat loss / metabolic heat production (EHL/MHP) and the gradient between air temperature and body temperature in pied crows (*Corvus albus*) with background blood [Pb] ($6.4 \pm 1.8 \mu\text{g dL}^{-1}$, “Control”), dosed with 0.4 g of Pb every ten days ($53.9 \pm 23.7 \mu\text{g dL}^{-1}$, “Intermediate”) or dosed with 0.6 g of Pb every ten days ($87.3 \pm 44.7 \mu\text{g dL}^{-1}$, “High”). The solid lines represent segmented linear regression models fitted to the data.

4. Discussion

Our findings did not support our first prediction that lead reduces avian heat tolerance, with no significant effect of lead on maximum T_b at the highest experimental T_{air} achieved during acute heat exposure in pied crows. However, significant effects of Pb treatment on thermoregulation at T_{air} approaching or exceeding normothermic T_b were evident. Increases above baseline levels in both EWL and RMR commenced at T_{air} values $\sim 4^\circ\text{C}$ higher in the high Pb treatment compared to the control treatment, with EWL, RMR and T_b above inflection points increasing more rapidly in the high Pb treatment. The onset of evaporative cooling responses of crows with high Pb exposure at higher T_{air} , as well as the subsequent steeper increases in T_b , EWL and

Table 2

Conventional analysis of variance (ANOVA) stepwise multiple comparisons (Tukey HSD post-hoc test) comparing inflection air temperature values for our control, intermediate and high Pb treatments detected using segmented regression analysis in the relationship between our thermal response variables and air temperature. Bold p-values indicate significance ($p < 0.05$).

	Tukey post hoc			
	Diff.	lower	upper	p-value
Body temperature				
Control - Intermediate	-0.50	-5.41	4.42	0.97
Control - High	-2.80	-7.83	2.23	0.36
Intermediate - High	-2.30	-7.06	2.46	0.46
Resting metabolic rate				
Control - Intermediate	-1.76	-5.54	2.02	0.48
Control - High	-4.02	-8.00	-0.04	0.048
Intermediate - High	-2.26	-6.03	1.52	0.31
Evaporative water loss				
Control - Intermediate	-1.89	-4.34	0.56	0.15
Control - High	-3.71	-6.29	-1.13	0.004
Intermediate - High	-1.82	-4.27	0.63	0.17
Evaporative heat loss/ metabolic heat production				
Control - Intermediate	-2.53	-8.86	3.83	0.59
Control - High	-4.42	-10.94	2.09	0.23
Intermediate - High	-1.90	-8.05	4.26	0.72

RMR with increasing T_{air} , raises the possibility that decreased neural conductance (Dey et al., 2000; Eisler, 1988; Martel et al., 2020) delayed the behavioural response of panting. Although we used a surveillance camera to monitor the crows in the chamber, we were unable to quantify panting occurrence and frequency in sufficient detail to evaluate this possibility further, as the crows frequently shifted position in the chambers and often stood facing away from the camera for extended periods.

The steeper slopes of T_b , EWL and RMR at $T_{air} > \sim 44^\circ\text{C}$ support our prediction of lead exposure causing a decrease in thermoregulatory precision, and suggest that, during hot weather, lead poisoning does indeed have ecologically relevant effects on pied crows' thermal physiology. The experimental T_{air} values to which we exposed crows in this study are higher than they would experience naturally in their southern African range, but likely representative of the operative temperatures [overall thermal environment incorporating radiative, convective and conductive heat fluxes (Bakken, 1976; Robinson et al., 1976)] they experience during summer in sunlit microsites. Pied crows frequently nest in completely exposed sites on powerlines, with increasing availability of electrical infrastructure having been proposed to have facilitated the species' expansion into South Africa's arid southwest in recent decades (Cunningham et al., 2016). Laying dates include the austral summer, during which incubating adults may experience a combination of high summer T_{air} plus intense solar heat loads. The lack of a significant effect of lead on maximum T_b at our highest consistent T_{air} of 52°C suggests that lethal hyperthermia risk is not affected, but the $\sim 30\%$ steeper increase in EWL among crows in the high Pb treatment has implications for water balance and dehydration risk. Increasing T_{air} associated with global warming (IPCC, 2021) will greatly increase the risk of lethal dehydration arid-zone birds face during extreme heat events (Albright et al., 2017; Conradie et al., 2020; McKechnie and Wolf, 2010), and lead-exposed crows may be expected to reach dehydration tolerance limits sooner than unaffected birds.

The $\sim 4^\circ\text{C}$ difference in pied crow T_{uc} between our control and high Pb treatments represents a novel source of variation in this trait, with implications for the use of T_{uc} data in comparative analysis. Avian T_{uc} values have been used to infer cold adaptation (Beaman et al., 2024) and thermal tolerances in analyses of vulnerability to global warming (Araújo et al., 2013; Khaliq et al., 2014), although the latter approach is conceptually flawed (Mitchell et al., 2018). These analyses often implicitly assume that T_{uc} is a fixed, species-specific parameter, but our results here reveal that avian T_{uc} can vary with exposure to

environmental pollutants. Our observed effects of lead on the slopes of T_b , RMR and EWL in the crows suggest that pollutants can similarly affect other variables used in comparative analyses of avian thermoregulation in the heat (e.g., McKechnie et al., 2021a).

Our data provide novel insights into how lead affects thermoregulation in endotherms. The handful of previous investigations in interactions between lead and thermoregulation typically focused on transient reductions in T_b following acute exposure, such as Gordon et al.'s (1987) study in which mice injected with $25\text{--}100\text{ mg kg}^{-1}$ lead acetate showed transient hypothermia of up to 2.6°C at $T_{air} = 20^\circ\text{C}$ and 1.6°C at $T_{air} = 30^\circ\text{C}$, and preferred $T_{air} \sim 8^\circ\text{C}$ lower compared to control individuals, within 1 h of injection. In heat-exposed rats, individuals fed 1% Pb acetate did not differ from control individuals in lethal T_b nor time taken to reach lethal T_b (Wright and Lessler, 1979). Among birds, acute lead nitrate exposure of 100 ug g^{-1} negatively affected shade-seeking behaviour in six-day old herring gull chicks (Burger and Gochfeld, 1995). The need to understand interactions between environmental pollutants and thermoregulation in the heat is, however, gaining urgency, for instance in the context of interacting effects of climate change and urbanisation (Sumasgutner et al., 2023).

Although our data suggest limited effects of lead exposure on the capacity of pied crows to tolerate high environmental temperatures for short periods, it could nevertheless influence other aspects of avian responses to global warming. Lead exposure is associated with negative effects on avian cognition and motor performance (Burger and Gochfeld, 2005; Goodchild et al., 2021), potentially exacerbating declines in neurological function during heat exposure (Danner et al., 2021; Soravia et al., 2023). Reductions in adult body mass or nest provisioning during sustained hot weather have emerged as a major risk associated with global warming (du Plessis et al., 2012; Pattinson et al., 2022; van de Ven et al., 2019, 2020). Although these effects appear to be driven primarily by behavioural trade-offs between foraging and thermoregulation (Cunningham et al., 2021), declines in cognitive performance likely compound the effects of high environmental temperatures on foraging success.

The lack of any obvious behavioural signs of lead poisoning among crows in the intermediate and high treatments suggests they are more tolerant of high lead exposure than many birds. In orders such as Anseriformes and Falconiformes, for instance, blood [Pb] = $50\text{--}100\text{ }\mu\text{g dL}^{-1}$ is indicative of clinical poisoning (Franson and Pain, 2011b). Few blood [Pb] data for corvids have been published, although a maximum individual value of $51.7\text{ }\mu\text{g dL}^{-1}$ has been documented in free-ranging common ravens (*Corvus corax*) in the Yellowstone area (Craighead and Bedrosian, 2009). Values measured in free-ranging corvids are, however, usually much lower (Craighead and Bedrosian, 2009; Van Hemert and Handel, 2016) and we consider it unlikely that the blood [Pb] of free-ranging pied crows reach the levels used in our experimental treatments. Blood [Pb] in the $50\text{--}100\text{ }\mu\text{g dL}^{-1}$ range is sometimes encountered in specialist scavengers such as vultures (van den Heever et al., 2019) and waterbirds exposed to lead shot (Newth et al., 2013; Riecke et al., 2015).

Our data for crows in the control treatment add to the limited data available on thermoregulation in the heat among large passerines, and corvids in particular. Pied Crows tolerated $T_{air} = 52\text{--}54^\circ\text{C}$, values among the highest reported among passerines, as are the maximum EHL/MHP values of 2.1–2.5 (reviewed by McKechnie et al., 2021a). Brown-necked ravens (*Corvus ruficollis*; 610 g) reached a maximum T_b of 44.7°C and EHL/MHP = 1.67 at $T_{air} = 50^\circ\text{C}$ (Marder, 1973). However, the higher T_b and lower EHL/MHP at $T_{air} = 50^\circ\text{C}$ in *C. ruficollis* compared to *C. albus* in our control group (T_b : 44.7°C vs 42.9°C ; EHL/MHP: 1.67 vs 2.11) may reflect methodological differences related to humidity levels within metabolic chambers. Marder (1973) maintained chamber relative humidity below 30%, whereas values in our study never exceeded 9% at $T_{air} = 50^\circ\text{C}$. Elevated humidity impedes evaporative cooling (Lasiewski et al., 1966), and the differences between these congeneric species in terms of thermoregulation at high T_{air}

likely reflect the experimental conditions involved. The salivation and consequent beak wetting we observed in pied crows was similar to that reported in American kestrels (*Falco sparverius*; Bartholomew and Cade, 1957) and more recently in chipping sparrows (*Spizella passerina*; Zuluaga and Danner, 2023).

The high heat tolerance and evaporative cooling capacity of *Corvus* spp. compared to most passerines (albeit lower than those of similar-sized non-passerines that use gular flutter Czenze et al., 2021), is consistent with recent observations that members of this genus seem unusual among bird communities in their responses to climate change. The heat tolerance of these two *Corvus* species contrast strongly with the apparent sensitivity to high T_{air} of another corvid, the range-restricted Ethiopian Bush-crow (*Zavattariornis stresemanni*), whose restricted range is characterised by cooler conditions than surrounding areas and commences heat dissipation behaviours at lower T_{air} than other similarly-sized sympatric passerines (Bladon et al., 2019; Donald et al., 2012). It is possible that the relatively high heat tolerance of *Corvus* spp. has been a factor in positive correlations between warming and population increases reported for Common Ravens (*Corvus corax*) over the last century in North America's Mojave Desert (Iknayan and Beissinger, 2018) and Pied Crows in southwest South Africa between 1987 and 2010 (Cunningham et al., 2016). However, Iknayan and Beissinger (2018) attributed the increases in *C. corax* primarily to the proliferation of anthropogenic food resources, and the potential role of thermal physiology remains to be evaluated.

In conclusion, our data confirm that lead exposure affects avian thermoregulation in the heat. The steeper relationships between environmental temperature and T_b , evaporative water requirements and MHP suggest that, under natural conditions, water balance during very hot weather under current conditions, and increases in evaporative cooling demands associated with climate warming, are influenced by lead exposure. These findings identify a need for studies of a) thermoregulation among free-ranging birds exposed to lead, b) the effects of lead exposure on heat dissipation behaviours such as panting, wing-drooping and shade-seeking, and c) how reductions in cognitive performance associated with lead exposure affect the dynamics of behavioural trade-offs between foraging and thermoregulation and the consequences for the fitness costs of periods of sustained hot weather.

Ethics

The research reported here was approved by the University of Pretoria's Animal Ethics Committee (protocol NAS018/2021) and the Animal Research Ethics and Scientific Committee (protocol P2021-03) of the South African National Biodiversity Institute. Crows were captured and housed under permit from the Northwest Province, and the study complies with South African law.

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CRediT authorship contribution statement

Vinny Naidoo: Writing – review & editing, Methodology, Investigation, Conceptualization. **Kerri Wolter:** Writing – review & editing, Resources, Methodology, Investigation. **Andrew McKechnie:** Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ryno Kemp:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Marc Freeman:** Writing – review & editing, Methodology, Formal analysis, Data curation.

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 will be made available on request.

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References

- Albright, T.P., Mutiibwa, D., Gerson, A.R., Smith, E.K., Talbot, W.A., McKechnie, A.E., Wolf, B.O., 2017. Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proc. Natl. Acad. Sci.* 114, 2283–2288.
- Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F., Chown, S.L., 2013. Heat freezes niche evolution. *Ecol. Lett.* 16, 1206–1219.
- Bakken, G.S., 1976. A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *J. Theor. Biol.* 60, 337–384.
- Bartholomew, G.A., Cade, T.J., 1957. The body temperature of the American Kestrel, *Falco sparverius*. *Wilson Bull.* 69, 149–154.
- Beaman, J.E., White, C.R., Clairbaux, M., Peret, S., Fort, J., Grémillet, D., 2024. Cold adaptation does not handicap warm tolerance in the most abundant Arctic seabird. *Proc. R. Soc. B* 291, 20231887.
- Beyer, W.N., Franson, J.C., French, J.B., May, T., Rattner, B.A., Shearn-Bochsler, V.I., Warner, S.E., Weber, J., Mosby, D., 2013. Toxic exposure of songbirds to lead in the Southeast Missouri Lead Mining District. *Arch. Environ. Contam. Toxicol.* 65, 598–610.
- Bladon, A.J., Donald, P.F., Jones, S.E., Collar, N.J., Deng, J., Dadacha, G., Abebe, Y.D., Green, R.E., 2019. Behavioural thermoregulation and climatic range restriction in the globally threatened Ethiopian Bush-crow *Zavattariornis stresemanni*. *Ibis* 161, 546–558.
- Boulant, J.A., 2006. Neuronal basis of Hammel's model for set-point thermoregulation. *J. Appl. Physiol.* 100, 1347–1354.
- Burger, J., Gochfeld, M., 1995. Behavior effects of lead exposure on different days for gull (*Larus argentatus*) chicks. *Pharmacol. Biochem. Behav.* 50, 97–105.
- Burger, J., Gochfeld, M., 2005. Effects of lead on learning in herring gulls: an avian wildlife model for neurobehavioral deficits. *Neurotoxicology* 26, 615–624.
- Conradie, S.R., Woodborne, S., Wolf, B.O., Pessato, A., Mariette, M.M., McKechnie, A.E., 2020. Avian mortality risk during heat waves will increase greatly in arid Australia during the 21st Century. *Conserv. Physiol.* 8, coaa048.
- Craighead, D., Bedrosian, B., 2009. A relationship between blood lead levels of common ravens and the hunting season in the southern Yellowstone ecosystem. *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, Idaho, USA.
- Cunningham, S.J., Gardner, J.L., Martin, R.O., 2021. Opportunity costs and the response of birds and mammals to climate warming. *Front. Ecol. Environ.* 19, 300–307.
- Cunningham, S., Madden, C., Barnard, P., Amar, A., 2016. Electric crows: powerlines, climate change and the emergence of a native invader. *Divers. Distrib.* 22, 17–29.
- Czenze, Z.J., Freeman, M.T., Kemp, R., van Jaarsveld, B., Wolf, B.O., McKechnie, A.E., 2021. Efficient evaporative cooling and pronounced heat tolerance in an eagle-owl, a thick-knee and a sandgrouse. *Front. Ecol. Evol.* 9, 799302.
- Danner, R.M., Coomes, C.M., Derryberry, E.P., 2021. Simulated heat waves reduce cognitive and motor performance of an endotherm. *Ecol. Evol.* 11, 2261–2272.
- De Francisco, N., Ruiz Troya, J., Agüera, E., 2003. Lead and lead toxicity in domestic and free living birds. *Avian Pathol.* 32, 3–13.
- Demayo, A., Taylor, M.C., Taylor, K.W., Hodson, P.V., Hammond, P.B., 1982. Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants, and livestock. *Crit. Rev. Environ. Sci. Technol.* 12, 257–305.
- Dey, P., Burger, J., Gochfeld, M., Reuhl, K., 2000. Developmental lead exposure disturbs expression of synaptic neural cell adhesion molecules in herring gull brains. *Toxicology* 146, 137–147.
- Donald, P.F., Gedeon, K., Collar, N.J., Spottiswoode, C.N., Wondafrahs, M., Buchanan, G.M., 2012. The restricted range of the Ethiopian Bush-crow *Zavattariornis stresemanni* is a consequence of high reliance on modified habitats within narrow climatic limits. *J. Orn* 153, 1031–1044.
- du Plessis, K.L., Martin, R.O., Hockey, P.A.R., Cunningham, S.J., Ridley, A.R., 2012. The costs of keeping cool in a warming world: implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Glob. Change Biol.* 18, 3063–3070.

- Eisler, R., 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish and Wildlife Service, Patuxent Wildlife Research Center.
- Escobar, J., Whitmore, T.J., Kamenov, G.D., Riedinger-Whitmore, M.A., 2013. Isotope record of anthropogenic lead pollution in lake sediments of Florida, USA. *J. Paleolimnol.* 49, 237–252.
- Fallon, J.A., Redig, P., Miller, T.A., Lanzone, M., Katzner, T., 2017. Guidelines for evaluation and treatment of lead poisoning of wild raptors. *Wildl. Soc. Bull.* 41, 205–211.
- Finkelstein, M.E., Doak, D.F., George, D., Burnett, J., Brandt, J., Church, M., Grantham, J., Smith, D.R., 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. *Proc. Natl. Acad. Sci.* 109, 11449–11454.
- Finlayson, H.H., 1932. Heat in the interior of South Australia – holocaust of bird-life. *S. Aus. Ornithol.* 11, 158–160.
- Fisher, L.J., Pain, D.J., Thomas, V.G., 2006. A review of lead poisoning from ammunition sources in terrestrial birds. *Biol. Conserv.* 131, 421–432.
- Franson, J., Pain, D., 2011a. In: Beyer, W.N., Meador, J.P. (Eds.), *Environmental contaminants in biota: interpreting tissue concentrations*. Chapter 16, Lead in Birds. CRC Press, Boca Raton, FL, USA.
- Franson, J.C., Pain, D.J., 2011b. Lead in birds. USGS Staff – Published Research 974.
- Freeman, M.T., Czenze, Z.J., Schoeman, K., McKechnie, A.E., 2020. Extreme hyperthermia tolerance in the world's most abundant wild bird. *Sci. Rep.* 10, 13098.
- Ganz, K., Jenni, L., Madry, M.M., Kraemer, T., Jenny, H., Jenny, D., 2018. Acute and chronic lead exposure in four avian scavenger species in Switzerland. *Arch. Environ. Contam. Toxicol.* 75, 566–575.
- Garbett, R., Maude, G., Hancock, P., Kenny, D., Reading, R., Amar, A., 2018. Association between hunting and elevated blood lead levels in the critically endangered African white-backed vulture *Gyps africanus*. *Sci. Total Environ.* 630, 1654–1665.
- Goodchild, C.G., Beck, M.L., VanDiest, I., Czesak, F.N., Lane, S.J., Sewall, K.B., 2021. Male zebra finches exposed to lead (Pb) during development have reduced volume of song nuclei, altered sexual traits, and received less attention from females as adults. *Ecotoxicol. Environ. Saf.* 210, 111850.
- Gordon, C.J., Workman, R.A., Highfill, J., 1987. Thermoregulation in mice following acute administration of lead acetate. *Neurotoxicol. Teratol.* 9, 193–196.
- Grade, T., Campbell, P., Cooley, T., Kneeland, M., Leslie, E., MacDonald, B., Melotti, J., Okoniewski, J., Parmley, E.J., Perry, C., 2019. Lead poisoning from ingestion of fishing gear: A review. *Ambio* 48, 1023–1038.
- Haig, S.M., D'Elia, J., Eagles-Smith, C., Fair, J.M., Gervais, J., Herring, G., Rivers, J.W., Schulz, J.H., 2014. The persistent problem of lead poisoning in birds from ammunition and fishing tackle. *Condor: Ornithol. Appl.* 116, 408–428.
- Hammel, H.T., 1965. *Neurons and temperature regulation*, in: Yamamoto, W.S., Brobeck, J.R. (Eds.), *Physiological controls and regulations*. Saunders, Philadelphia, PA, pp. 71–97.
- He, Y., Wang, L., Li, X., Zhao, H., 2020. The effects of chronic lead exposure on the ovaries of female juvenile Japanese quails (*Coturnix japonica*): Developmental delay, histopathological alterations, hormone release disruption and gene expression disorder. *Ecotoxicol. Environ. Saf.* 205, 111338.
- Henny, C.J., Blus, L.J., Hoffman, D.J., Grove, R.A., Hatfield, J.S., 1991. Lead accumulation and osprey production near a mining site on the Coeur d'Alene River, Idaho. *Arch. Environ. Contam. Toxicol.* 21, 415–424.
- Holt, K.A., Boersma, P.D., 2022. Unprecedented heat mortality of Magellanic Penguins. *Ornithol. Appl.* 124, 1–12.
- Iknayan, K.J., Beissinger, S.R., 2018. Collapse of a desert bird community over the past century driven by climate change. *Proc. Natl. Acad. Sci.* 201805123.
- IPCC, 2021. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, in: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Karasov, W.H., 1990. Digestion in birds: chemical and physiological determinants and implications. In: Morrison, M.L., Ralph, C.J., Verner, J., Jehl, J.R. (Eds.), *Studies in Avian Biology No. 13*. Cooper Ornithological Society, Los Angeles, pp. 391–415.
- Khalig, I., Hof, C., Prinzinger, R., Böhning-Gaese, K., Pfenninger, M., 2014. Global variation in thermal tolerances and vulnerability of endotherms to climate change. *Proc. R. Soc. Lond. B: Biol. Sci.* 281, 20141097.
- Lasiewski, R.C., Acosta, A.L., Bernstein, M.H., 1966. Evaporative water loss in birds - I. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability. *Comp. Biochem Physiol.* 19, 445–457.
- Lighton, J.R.B., 2008. *Measuring metabolic rates: a manual for scientists*. Oxford University Press, Oxford.
- Marder, J., 1973. Body temperature regulation in the brown-necked raven (*Corvus corax ruficollis*)—I. Metabolic rate, evaporative water loss and body temperature of the raven exposed to heat stress. *Comp. Biochem Physiol.* A 45, 421–430.
- Martel, A.K., Doss, G.A., Mans, C., 2020. Suspected peripheral neuropathy secondary to lead intoxication in three psittacine birds. *J. Exot. Pet. Med.* 32, 13–17.
- McKechnie, A.E., Gerson, A.R., Wolf, B.O., 2021a. Thermoregulation in desert birds: scaling and phylogenetic variation in heat tolerance and evaporative cooling. *J. Exp. Biol.* 224, jeb229211.
- McKechnie, A.E., Rushworth, I.A., Myburgh, F., Cunningham, S.J., 2021b. Mortality among birds and bats during an extreme heat event in eastern South Africa. *Austral Ecol.* 46, 687–691.
- McKechnie, A.E., Wolf, B.O., 2010. Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. *Biol. Lett.* 6, 253–256.
- Mitchell, D., Snelling, E.P., Hetem, R.S., Maloney, S.K., Strauss, W.M., Fuller, A., 2018. Revisiting concepts of thermal physiology: predicting responses of mammals to climate change. *J. Anim. Ecol.* 87, 956–973.
- Monclús, L., Shore, R.F., Krone, O., 2020. Lead contamination in raptors in Europe: a systematic review and meta-analysis. *Sci. Total Environ.* 748, 141437.
- Muggeo, V.M.R., 2016. Segmented mixed models with random change-points in R. Working Paper. Università di Palermo, Palermo.
- Mukherjee, A., Kar, I., Patra, A., 2022. Lead toxicity in livestock and poultry: illuminating the molecular pathomechanism, bioaccumulation and effective mitigation strategies. *Indian J. Anim. Health.* 61, 103–118.
- Naidoo, V., Wolter, K., Botha, C.J., 2017. Lead ingestion as a potential contributing factor to the decline in vulture populations in southern Africa. *Environ. Res.* 152, 150–156.
- Naidoo, V., Wolter, K., Espie, I., Kotze, A., 2012. Lead toxicity: consequences and interventions in an intensively managed (*Gyps coprotheres*) vulture colony. *J. Zoo. Wildl. Med.* 43, 573–578.
- Newth, J., Cromie, R.L., Brown, M., Delahay, R.J., Meharg, A.A., Deacon, C., Norton, G. J., O'Brien, M., Pain, D.J., 2013. Poisoning from lead gunshot: still a threat to wild waterbirds in Britain. *Eur. J. Wildl. Res.* 59, 195–204.
- O'Connor, R.S., Le Pogam, A., Young, K.G., Robitaille, F., Choy, E.S., Love, O.P., Elliott, K.H., Hargreaves, A.L., Berteaux, D., Tam, A., 2021. Limited heat tolerance in an Arctic passerine: Thermoregulatory implications for cold-specialized birds in a rapidly warming world. *Ecol. Evol.* 11, 1609–1619.
- Ogada, D., Shaw, P., Beyers, R.L., Buij, R., Murn, C., Thiollay, J.M., Beale, C.M., Holdo, R. M., Pomeroy, D., Baker, N., 2016. Another continental vulture crisis: Africa's vultures collapsing toward extinction. *Conserv. Lett.* 9, 89–97.
- Oswald, S.A., Arnold, J.M., 2012. Direct impacts of climatic warming on heat stress in endothermic species: seabirds as bioindicators of changing thermoregulatory constraints. *Integr. Zool.* 7, 121–136.
- Oswald, S.A., Bearhop, S., Furness, R.W., Huntley, B., Hamer, K.C., 2008. Heat stress in a high-latitude seabird: effects of temperature and food supply on bathing and nest attendance of great skuas *Catharacta skua*. *J. Avian Biol.* 39, 163–169.
- Pattinson, N.B., Van De Ven, T.M., Finnie, M.J., Nupen, L.J., McKechnie, A.E., Cunningham, S.J., 2022. Collapse of Breeding Success in Desert-Dwelling Hornbills Evident Within a Single Decade. *Front. Ecol. Evol.* 10.
- Pikula, J., Hajkova, P., Bandouchova, H., Bednarova, I., Adam, V., Beklova, M., Kral, J., Ondracek, K., Osickova, J., Pohanka, M., 2013. Lead toxicosis of captive vultures: case description and responses to chelation therapy. *BMC Vet. Res.* 9, 1–10.
- Pinheiro, J., Bates, D., Deb Roy, S., Sarkar, D., Development Core Team, R., 2009. *nlme*: linear and nonlinear mixed effects models. R package 3.57.
- Riecke, T.V., Conway, W.C., Haukos, D.A., Moon, J.A., Comer, C.E., 2015. Baseline blood Pb concentrations in black-necked stilts on the upper Texas coast. *Bull. Environ. Contam. Toxicol.* 95, 465–469.
- Robinson, D.E., Campbell, G.S., King, J.R., 1976. An evaluation of heat exchange in small birds. *J. Comp. Physiol. B* 105, 153–166.
- Safford, R., Andevski, J., Botha, A., Bowden, C.G., Crockford, N., Garbett, R., Margalida, A., Ramirez, I., Shobrak, M., Tavares, J., 2019. Vulture conservation: the case for urgent action. *Bird. Conserv. Int.* 29, 1–9.
- Saunders, D.A., Mawson, P., Dawson, R., 2011. The impact of two extreme weather events and other causes of death on Carnaby's Black Cockatoo: a promise of things to come for a threatened species? *Pac. Conserv. Biol.* 17, 141–148.
- Simon, E., 2000. The enigma of deep-body thermosensory specificity. *Int. J. Biometeorol.* 44, 105–120.
- Smit, B., Whitfield, M.C., Talbot, W.A., Gerson, A.R., McKechnie, A.E., Wolf, B.O., 2018. Avian thermoregulation in the heat: phylogenetic variation among avian orders in evaporative cooling capacity and heat tolerance. *J. Exp. Biol.* 221, jeb174870.
- Sokol, R.Z., Madding, C.E., Swerdloff, R.S., 1985. Lead toxicity and the hypothalamic-pituitary-testicular axis. *Biol. Reprod.* 33, 722–728.
- Soravia, C., Ashton, B.J., Thornton, A., Ridley, A.R., 2023. High temperatures are associated with reduced cognitive performance in wild southern pied babblers. *Proc. R. Soc. B* 290, 20231077.
- Sumasgutner, P., Cunningham, S.J., Hegemann, A., Amar, A., Watson, H., Nilsson, J.F., Andersson, M.N., Isaksson, C., 2023. Interactive effects of rising temperatures and urbanisation on birds across different climate zones: A mechanistic perspective. *Glob. Change Biol.* 29, 2399–2420.
- Tracy, C.R., Welch, W.R., Pinshow, B., Porter, W.P., 2010. *Properties of air: a manual for use in biophysical ecology*. 4th Ed. The University of Wisconsin Laboratory for Biophysical Ecology: Technical Report.
- Vallverdú-Coll, N., Mougeot, F., Ortiz-Santaliestra, M.E., Castañó, C., Santiago-Moreno, J., Mateo, R., 2016. Effects of lead exposure on sperm quality and reproductive success in an avian model. *Environ. Sci. Technol.* 50, 12484–12492.
- van de Ven, T., McKechnie, A., Cunningham, S., 2019. The costs of keeping cool: behavioural trade-offs between foraging and thermoregulation are associated with significant mass losses in an arid-zone bird. *Oecologia* 191, 205–215.
- van de Ven, T.M., McKechnie, A.E., Er, S., Cunningham, S., 2020. High temperatures are associated with substantial reductions in breeding success and offspring quality in an arid-zone bird. *Oecologia* 193, 225–235.
- van den Heever, L., Elburg, M.A., Iaccheri, L., Naidoo, V., Ueckermann, H., Bybee, G., Smit-Robinson, H.A., Whitecross, M.A., McKechnie, A.E., 2023. Identifying the origin of lead poisoning in white-backed vulture (*Gyps africanus*) chicks at an important South African breeding colony: a stable lead isotope approach. *Environ. Sci. Pollut. Res.* 30, 15059–15069.
- van den Heever, L., Smit-Robinson, H., Naidoo, V., McKechnie, A.E., 2019. Blood and bone lead levels in South Africa's Gyps vultures: Risk to nest-bound chicks and comparison with other avian taxa. *Sci. Total Environ.* 669, 471–480.
- Van Hemert, C., Handel, C.M., 2016. Elements in whole blood of NorthWestern crows (*Corvus caurinus*) in Alaska, USA: no evidence for an association with beak deformities. *J. Wildl. Dis.* 52, 713–718.

- Virgolini, M.B., Aschner, M., 2021. Molecular mechanisms of lead neurotoxicity. *Advances in Neurotoxicology* 5, 159–213.
- Walsberg, G.E., Wolf, B.O., 1995. Variation in the respirometry quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. *J. Exp. Biol.* 198, 213–219.
- Whitfield, M.C., Smit, B., McKechnie, A.E., Wolf, B.O., 2015. Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* 218, 1705–1714.
- Wiemeyer, G.M., Pérez, M.A., Bianchini, L.T., Sampietro, L., Bravo, G.F., Jácome, N.L., Astore, V., Lambertucci, S.A., 2017. Repeated conservation threats across the Americas: High levels of blood and bone lead in the Andean Condor widen the problem to a continental scale. *Environ. Pollut.* 220, 672–679.
- Withers, P.C., 1992. *Comparative animal physiology*. Saunders College Publishing, Fort Worth.
- Wright, G.L., Lessler, M.A., 1979. Interaction of lead and high ambient temperatures in the rat. *Environ. Res.* 18, 377–384.
- Wu, J., Boyle, E.A., 1997. Lead in the western North Atlantic Ocean: completed response to leaded gasoline phaseout. *Geochim. Et. Cosmochim. Acta* 61, 3279–3283.
- Xu, H., Cao, J., Ho, K., Ding, H., Han, Y., Wang, G., Chow, J., Watson, J., Khol, S., Qiang, J., 2012. Lead concentrations in fine particulate matter after the phasing out of leaded gasoline in Xi'an, China. *Atmos. Environ.* 46, 217–224.
- Zuluaga, J.D., Danner, R.M., 2023. Novel approaches for assessing acclimatization in birds reveal seasonal changes in peripheral heat exchange and thermoregulatory behaviors. *J. Exp. Biol.* 226, jeb245772.