

1 **Exposure to lead (Pb) contamination paradoxically heightens predator**
2 **avoidance behaviours in an urban bird**

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21 **Abstract**

22 To survive, prey animals must correctly assess and respond to predation, by vigilantly
23 scanning their environment for threats, assessing predation risk through gaze aversion
24 (responding fearfully to predator gaze), and escaping efficiently. As these anti-predatory
25 behaviours are integrated through the nervous and motor systems, they could be disrupted by
26 neurotoxic contaminants, such as lead (Pb), which is ubiquitous in the Anthropocene. Here,
27 we examined the effects of Pb on anti-predatory behaviours of free-living house sparrows
28 (*Passer domesticus*), in the mining city of Broken Hill, Australia, where birds have been
29 exposed to elevated Pb levels for many generations. We found that sparrows in higher-Pb
30 contaminated areas spent more time scanning their surroundings and were more reluctant to
31 approach a feeder under direct, experimentally introduced human gaze, than sparrows in
32 lower-Pb contaminated areas. Higher-Pb birds had slightly worse (though not statistically
33 different) escape flight performance than their lower-Pb counterparts. Our results suggest that
34 greater exposure to Pb is associated with increased fear, which may or may not be linked to
35 Pb-compromised escape performance. We highlight the importance of considering multi-
36 faceted, integrated effects of environmental pollution on the behaviours of urban wildlife.

37 **Research highlights**

- 38 • We investigated the effects of chronic lead exposure on three dynamically linked
39 predator avoidance behaviours in an urban house sparrow population
- 40 • Wild house sparrows are sensitive to the differing threat posed by a direct vs averted
41 eye-gaze of a potential human predator
- 42 • This aversion to eye-gaze and vigilance is heightened in higher-leaded sparrows that
43 should be cognitively impaired
- 44 • Escape flight performance was slightly worse in higher-leaded sparrows, suggesting
45 heightened vigilance as a compensatory behavioural response

46

47 **Keywords:** Animal behaviour, avian flight, gaze aversion, lead pollution, urban ecology,
48 vigilance

49 **Introduction**

50 For prey animals, predator avoidance is a key behavioural trait as it directly influences the
51 probability of survival (McDonnell & Hahs, 2015). Effective predator avoidance comprises
52 several related behaviours: First, to detect threats and attacks, an animal must be vigilant (i.e.,
53 be alert to the presence of potential predators) and be readily able to sense predatory threats,
54 usually through visual, acoustic, and/or chemical cues (Lima & Bednekoff, 1999). Second,
55 beyond detection, the animal also needs to correctly assess the risk of attack by a predator.
56 This can be achieved through gaze sensitivity (i.e., the ability to distinguish the head or eye-
57 gaze orientation of a predator) (Davidson & Clayton, 2016), and subsequent gaze aversion
58 (i.e., responding fearfully to the direct gaze of a predator). Head or eye-gaze orientation is an
59 effective cue, because most predators rely on binocular, front-facing vision to focus on prey
60 (Davidson & Clayton, 2016). Indeed, many species, in particular birds, show gaze aversion
61 against potential predators such as humans: birds are more reluctant to approach a food
62 source, or are more likely to flee when under direct human eye gaze, compared to when eye-
63 gaze was either averted or obstructed (Carter et al., 2008; Clucas et al., 2013; Garland et al.,
64 2014; Goumas et al., 2019; Hampton, 1994). Third, beyond this risk assessment, the prey
65 animal also needs to escape efficiently from a potential predator attack. This escape response
66 is largely influenced by biomechanical performance (e.g., escape velocity). Importantly, if an
67 animal is less able to escape quickly from a predatory event, it may assess risk differently to
68 an animal that can escape more quickly. Therefore, these anti-predator behaviours (i.e.,
69 vigilance, gaze aversion, escape response) are likely non-independent, and should be
70 examined as an integrated suite of responses.

71 In natural settings, predator avoidance should be optimised, as responding incorrectly to
72 potential threats could have negative fitness consequences. For example, under-reacting could

73 result in immediate injury or death, while over-reacting could result in reduced foraging
74 efficiency (Carter et al., 2008), which might impact long-term reproduction, breeding success,
75 and survival. However, the optimisation of predator avoidance could be compromised by
76 environmental toxicants introduced by anthropogenic activities, one of which – lead (Pb) –
77 has the potential to adversely affect cognitive and physiological attributes that contribute to
78 effective anti-predator responses.

79 Pb contamination is ubiquitous, especially in the urban environment, because of its broad use
80 in manufacturing, construction, plumbing and automotive industries (Rocha & Trujillo, 2019).
81 Once ingested, Pb enters the blood stream and soft tissues, and is eventually deposited in
82 bone, where it accumulates and induces a wide range of toxic effects various systems (Mason
83 et al., 2014). Even sublethal Pb exposure has been associated with reduced overall organismal
84 health, including altered erythrocyte health, immune function, and bone formation (Cid et al.,
85 2018; Ishii et al., 2018; Vallverdú-Coll et al., 2019). The neurological impacts of Pb are also
86 well known. Notably, exposure to Pb degrades vertebrate neural tissue (Zou et al., 2020),
87 interferes with neural health during development (Rocha & Trujillo, 2019), and with synaptic
88 processes (Zou et al., 2020), all of which may influence downstream cognitive abilities.
89 Specifically, this may result in impaired or altered reaction time, motor control, spatial
90 memory, and coordination (Rocha & Trujillo, 2019; Zou et al., 2020). Pb is also known to
91 compromise the development of neurons in the brain during development, leading to impaired
92 cognitive functions, such as reduced reaction time, motor control, and hand-eye coordination,
93 and is linked with cognitive impairment, such as Alzheimer's disease in humans and impaired
94 memory in rodents (Rocha & Trujillo, 2019). In addition, exposure to Pb alters personality
95 traits in both humans and animals, e.g., increased aggression and delinquency in human
96 teenagers, increased aggression and slower exploration in captive birds, and increased anxiety

Lead effects on predator avoidance in house sparrows

97 in captive rhesus monkeys (Grunst et al., 2019; Lasky & Laughlin, 2001; McClelland et al.,
98 2019; Rocha & Trujillo, 2019).

99 Given that Pb affects many biological pathways and organismal functions, it is likely that Pb
100 could alter the ability of exposed wildlife to detect and react to predators, or alter their
101 perception of risk and associated fear responses, resulting in changes in predator avoidance.
102 To the best of our knowledge, however, no study has reported the effects of Pb exposure on
103 predator avoidance behaviours in prey animals under natural settings.

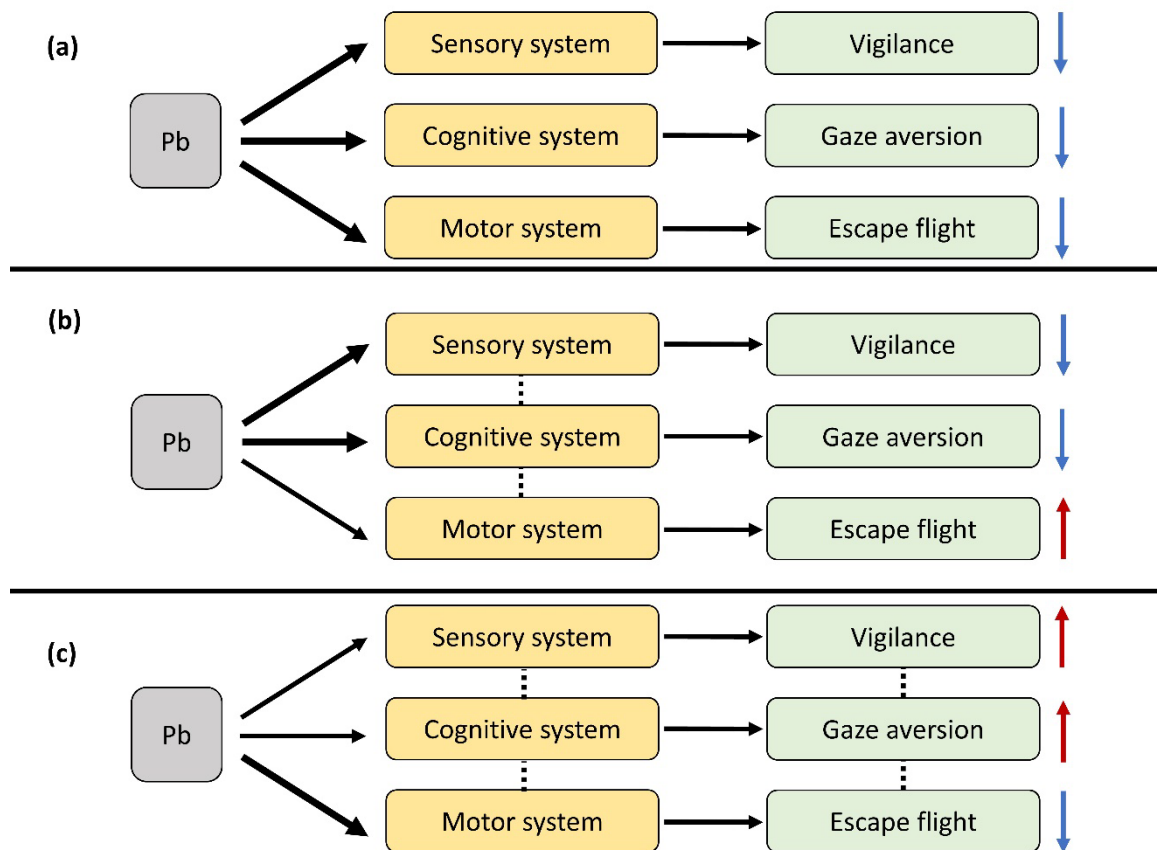
104 In this study, we tested multiple components of predator avoidance in an urban population of
105 house sparrows (*Passer domesticus*) exposed to different levels of environmental Pb. The
106 house sparrow is a synanthropic species highly integrated into urban areas, and is largely
107 sedentary (home range < 300 m radius or 0.28 km²; (Vangestel et al., 2010)). This means that
108 the blood Pb level of sparrows is strongly determined by the level of contamination in the
109 local environment (Gillings, Ton, Harris, Swaddle, et al., 2024), therefore making it a suitable
110 species to examine the effect of anthropogenic disturbances and pollution across gradients of
111 contamination (Havlíček et al., 2022; Swaileh & Sansur, 2006). The house sparrow has also
112 previously been shown to demonstrate gaze aversion against humans (Hampton, 1994).

113 Accordingly, we conducted a series of field measurements with house sparrows in the Pb-
114 contaminated city of Broken Hill, New South Wales, Australia, and measured three
115 components of an integrated predator avoidance sequence: vigilance, gaze aversion, and
116 escape flight performance, to examine whether these behaviours differ between areas with
117 higher and lower Pb levels.

118 Given the deleterious effects of Pb on cognitive processes (Rocha & Trujillo, 2019), motor
119 impairment (Mason et al., 2014), and the potential interaction between anti-predator

120 behaviours, we proposed several alternative predictions (Fig. 1). In Prediction A, we proposed
121 that these behaviours are independent from one another, and Pb could directly compromise all
122 three sensory, cognitive and motor systems. This would be demonstrated through lower
123 vigilance, a reduced capacity to assess the gaze of a predator (gaze aversion), and worse
124 escape flight performance in higher-Pb birds than in lower-Pb birds (Fig. 1a). Alternatively,
125 we propose that predator avoidance behaviours are dynamically linked, and Pb has differential
126 effects on these behaviours, leading to different compromising strategies. In Prediction B, Pb
127 exerts stronger detrimental effects on the sensory and cognitive system, inducing reduced
128 vigilance and gaze aversion levels in higher-Pb birds, and in turn heightened escape
129 responses, to compensate for reduced risk perception abilities (Fig. 1b). In Prediction C, Pb
130 impairs motor function more strongly than other behaviours, leading to a reduced flight
131 response in higher-Pb birds, and in turn a heightened sense of fear against predators to
132 compensate. This would manifest as higher-Pb birds showing lower escape flight energetics
133 but paradoxically higher levels of cognitive processes such as vigilance and gaze aversion
134 (Fig. 1c).

Lead effects on predator avoidance in house sparrows



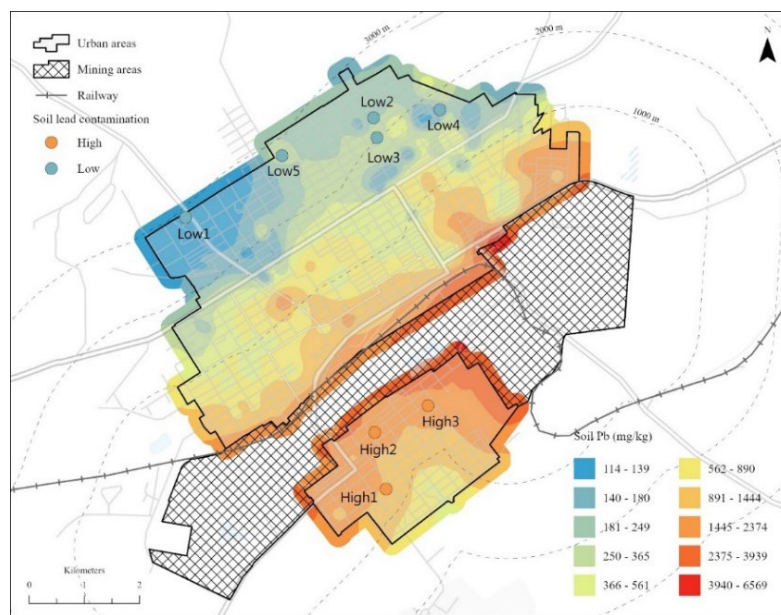
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136 Fig. 1. Schematic diagram of alternative predictions of the links between exposure to lead
 137 (Pb), its effects on physiology (yellow boxes) and predator avoidance behaviours (green
 138 boxes). Arrow thickness represents differential magnitudes of the effects of Pb, and red and
 139 blue arrows refer to respectively higher and lower levels of behaviours in higher-Pb birds
 140 compared to lower-Pb birds. Black dotted lines represent dependence between different
 141 systems and behaviours. (a) Predator avoidance behaviours are independent, and Pb directly
 142 negatively impact three behaviours. (b) Pb has stronger negative effects on the sensory and
 143 cognitive system, leading to reduced vigilance and gaze aversion ability, and compensatory
 144 increases in escape flight performance. (c) Pb has stronger negative effects on escape flight
 145 performance, causing compensatory elevation of fear responses, and consequently heightened
 146 vigilance and gaze aversion.

147 **Methods**

148 *Study site and system*

149 We conducted this study in Broken Hill (31.57°S, 141.27°E), a lead-zinc-silver mining city in
150 New South Wales, Australia. The urban landscape of Broken Hill is largely homogenous,
151 consisting of mostly residential areas and parks, with the exception of mining operations
152 located in the city centre and dividing the city into northern and southern sides. Emissions
153 from these mining operations have created a gradient of soil and dust Pb contamination
154 throughout residential areas in Broken Hill (Gillings et al., 2022). The most severely
155 contaminated areas are immediately adjacent to the mining facilities, with soil and dust Pb
156 levels highest in the southern residential district (Gillings et al., 2022) (Fig. 2).



157

158 Fig. 2. Map of Broken Hill depicting the distribution of surface soil lead (Pb) across the city
159 and the assay sites. Checkered area indicates the location of mining operations. Red shading
160 indicates higher soil Pb levels, while blue shading indicates lower soil Pb levels. Orange dots
161 indicate higher-Pb assay locations, while blue dots indicate lower-Pb assay locations.

162

163 The house sparrow (*Passer domesticus*) population in Broken Hill arrived in 1897, originating
164 from sparrows introduced from Europe into Adelaide in the 1850's (Andrew & Griffith,
165 2016). As a result, the Broken Hill population belongs to the genetic cluster of sparrow
166 populations from South Australia (Andrew et al., 2018). However, due to the geographical
167 isolation of Broken Hill in the Australian arid zone, and the sparrow's sedentary nature, we
168 expect minimal mixing of Broken Hill sparrows with those from other populations, and hence
169 behavioural modifications observed in this study would be inherent to Pb exposure in Broken
170 Hill.

171 Monitoring of soil and dust Pb levels, and the sparrow population in Broken Hill, began in
172 2020, with birds captured using mist nets and banded with uniquely coded metal leg rings
173 under the Australian Bird and Bat Banding Scheme (ABBBS).

174 *Lead content characterisation*

175 Blood Pb content has been assayed in this population of sparrows at numerous sites across
176 town, and strongly and positively correlates with soil Pb contamination (Gillings, Ton, Harris,
177 Swaddle, et al., 2024). To contextualise the behavioural data collected for this study, we
178 describe here the procedures to characterise blood Pb data from the eight sites where we
179 conducted behavioural assays (see below).

180 Whole blood samples (50 μ L) were collected from the brachial vein of each bird, and
181 transferred to sample tubes containing a dilute solution of hydrochloric acid. Following
182 dilution, we measured blood Pb concentrations (μ g/dL) using a Meridian Bioscience
183 LeadCare Plus blood Pb analyser, and adjusted obtained values with paired measurements
184 from inductively coupled plasma mass spectrometry (ICP-MS), following established

185 protocols (Gillings, Ton, Harris, Swaddle, et al., 2024). The mean measured sparrow blood Pb
186 concentrations were used to characterise levels of Pb exposure at each site. At one assay
187 location (Low3), we did not measure blood Pb content, and the mean of blood Pb
188 measurements from the nearest three sites were used instead. In total, we characterised Pb
189 exposure levels from 164 birds across the eight sites involved in this study.

190 In addition to blood Pb levels, we characterized surface soil Pb concentrations (in mg/kg) at
191 each site, using X-ray fluorescence (XRF) spectrometry, following established procedures
192 (Gillings, Ton, Harris, Taylor, et al., 2024). Depending on the level of suspected soil Pb
193 contamination, we collected between three to nine soil samples around each site and sieved
194 them to <250 μm before transferring to mylar film analysis cups for measurement. We then
195 characterised levels of soil Pb contamination as the mean of soil Pb concentrations taken at
196 each site. As above, at one location (Low3) soil samples were not collected, and so the mean
197 of measurements from the closest three sites were used instead. In total, measurements from
198 42 soil samples (21 from higher-Pb sites, 21 from lower-Pb sites, see below) were used to
199 characterise soil Pb contamination across the eight sites involved in this study.

200 To enable pairwise (higher vs lower) comparisons of behavioural data, we divided our sites
201 based on their relative levels of soil Pb contamination. Sites classified as having lower levels
202 of Pb had mean soil Pb concentrations of 59–209 mg/kg, which overlapped the median
203 background soil Pb concentration in Broken Hill of 100 mg/kg, based on previous
204 measurements of uncontaminated subsoil samples (>30 cm deep) (Kristensen & Taylor,
205 2016). Sites classified with higher levels of Pb contamination had soil Pb concentrations
206 ranging from 965–3577 mg/kg. The mean soil and blood Pb levels at all experimental
207 locations are summarized in Table S1, with a map of the assay locations provided in Fig. 2.

208 *Behavioural assay 1: Gaze aversion*

209 We conducted gaze aversion assays, which tested the reluctance of house sparrows to feed
210 from an experimental food source when under direct or averted simulated human gaze, at two
211 higher-Pb locations ('High1', and 'High2') and two lower-Pb locations ('Low1' and 'Low2').
212 Beginning in June 2022, we tagged birds of both sexes at these locations with subcutaneous
213 radio frequency identification (RFID) tags (Micro Products Australia Mini Microchips, 1.4 ×
214 8.5 mm, 0.52 g), and set up a feeding station fitted with an RFID antenna at each location to
215 record the presence/absence of birds at the feeder (Fig. 3a). Each feeding station was
216 constructed from a metal cage (approx. 100 × 60 × 50 cm), with a seed tray inside. The RFID
217 antenna was fitted around an entrance hole with a 16 cm diameter on the front side of the cage
218 and recorded the time when tagged sparrows entered and exited the cage. We lined the sides
219 of the cage with shade cloth to prevent seeds from scattering, and to stop sparrows from
220 foraging without entering the feeder. We also fitted an external wire mesh cover over the
221 entrance to prevent larger birds (e.g. pigeons) and predators (e.g. feral cats) from accessing
222 the feeder (Fig. 3a). All feeding stations were operational for at least one month before the
223 gaze aversion assay began. At the start of the experiment, the number of tagged birds at
224 High1, High2, Low1 and Low2 were 181, 104, 101, and 87 respectively, and the total number
225 of unique tagged birds that attended the feeder on the days when the gaze aversion assay was
226 conducted (see below) were 110, 55, 48, and 51 respectively.



227

228 Fig. 3. Experimental set-up for the gaze aversion assay. Panel (a) displays the field set up: A
229 wooden board, attached with the image of a human male face at varying head orientations,
230 was erected in front of a feeding station fitted with a radio frequency identification (RFID)
231 antenna which detected the presence of birds entering and exiting. Panel (b) displays the three
232 head orientation treatments presented to the sparrows (from left to right: 0°, 45°, 90°; image
233 credit: Yaro Jackson).

234 We conducted the gaze aversion assay from late November to early December 2022. At each
235 feeding station, we erected a wooden board 1.2 m away from the wire mesh guard, 0.9 m off
236 the ground (Fig. 3a). One day before the experiment, we presented a blank sheet of paper
237 attached to the wooden board to allow for birds to habituate to the novel object. On the
238 following day, we presented an image of the face of a human male on the wooden board, with
239 the head of the model oriented in the order of 0° (i.e., looking directly at the feeder), 45°, and
240 90° to the feeder (hereafter three ‘treatments’ constituting one ‘round’) (Fig. 3b). We
241 presented one head orientation treatment per day, for one hour during the evening feeding

242 bout (typically 5 to 6 pm). We avoided experimentation during the morning feeding bouts, as
243 we did not wish to prevent birds from replenishing their energy reserves after night-time
244 starvation (McNamara et al., 1994). At the start of the experimental hour, we topped up the
245 feeding tray to ensure that birds' reluctance to approach was not due to shortage of food,
246 swapped the blank sheet of paper with the appropriate face image, marked the start of the
247 experiment by tapping a marker RFID tag on the coil, and departed the location. After one
248 hour, we returned to the location and again tapped the marker tag on the coil to record the end
249 of the experiment and replaced the face image with a blank sheet of paper. To ensure gaze
250 aversion is not random, we repeated the assay for a total of three rounds, where round 2 was
251 carried out 24 hours after round 1, and round 3 was carried out five days after round 2.

252 We extracted the RFID records during the experimental hour, to quantify gaze aversion with
253 three metrics: 1) First-bird latency, defined as the time taken for the first tagged bird to enter
254 the feeder; 2) Median latency, defined as the median of the time taken for all tagged birds that
255 used the feeder during the experimental hour to enter the feeder. We opted to use the median
256 instead of the mean latency, because the latter could be skewed by very aversive individuals
257 with high latencies. We also recorded 3) the number of unique tagged birds that visited the
258 feeder during the experimental hour, divided by the total number of unique tagged birds
259 recorded at the feeder on the days when the assay was conducted (hereafter the 'proportion of
260 birds visited'), to adjust for difference in flock sizes among feeder locations.

261 *Behavioural assay 2: Vigilance*

262 We quantified vigilance (i.e., heads-up scanning behaviour) of sparrows at the four feeder
263 locations described above, by analysing videos recorded outside of the gaze aversion assay
264 experimental hours, typically in the morning. Note that this assay is independent of the gaze

265 aversion assay, as we aimed to test for baseline levels of vigilance under different Pb
266 contamination levels, without interference from a potential predator (i.e., the human face
267 image). Therefore, vigilance was not necessarily conducted on tagged birds involved in the
268 gaze aversion assay. To record vigilance behaviour, we attached a GoPro Hero 8 camera to
269 the wooden board used in the gaze aversion assay, and recorded footage of the feeder and its
270 surrounding area (approximately 1.2 m radius around the feeder) for approximately one hour
271 at each location. As the shade cloth around the feeder could obstruct observation, we scattered
272 a handful of seeds outside of the feeder so the birds could be seen. From these videos we
273 conducted focal observations of the first 20 sparrows that arrived. Because birds might linger
274 off screen once they arrived, selecting the first 20 sparrows minimizes the probability of a
275 bird re-entering the screen and being scored twice. Using the software BORIS 8.21.8 (Friard
276 & Gamba, 2016), we recorded the start time and end time of bouts of scanning, defined as a
277 heads-up position lasting >0.5 s, for each individual, starting from when the individual
278 appeared on screen, until it exited out of the screen. We also recorded the duration in which
279 the bird was obstructed from view ('unseen'), e.g., when a bird moved behind the feeder. We
280 ended the observation when a bird was unseen for >30 s. As vigilance could be influenced by
281 group size (Bednekoff & Lima, 1998), we recorded the flock size at the start of each scanning
282 bout as the number of sparrows on screen. We also noted the sex of the focal sparrows. Sex
283 could not be determined for three sparrows due to suboptimal lighting.

284 From these behavioural data we first quantified the total duration that each bird was observed,
285 defined as the duration from when a bird entered the screen until the bird exited, or the
286 observation was ended, minus the total duration of the bird being unseen. We then quantified
287 vigilance of each bird using three metrics: 1) percentage of time spent scanning over total

288 observed time ('percentage time scanning'); 2) mean duration of each scanning bout ('mean
289 scanning duration'); and 3) the total number of scanning bouts.

290 *Behavioural assay 3: Escape flight performance*

291 We conducted escape flight assays from May to July 2022, at three higher-Pb locations and
292 five lower-Pb locations, including the four sites with the RFID feeders (in the gaze aversion
293 assay; Table S1). Here we provide a summary of the experimental procedures, whereas
294 detailed methods are provided in the supplementary text. Briefly, after banding and
295 morphometric measurements, we kept sparrows individually in small holding bags until they
296 were released in an escape flight arena, which consisted of three walls and a small wooden
297 platform that served as the release point and was designed to standardize the flight trajectories
298 of the birds (Fig. S1). Using three GoPro Hero 7 cameras (60 frames per second, 1440
299 resolution, Fig. S1) in the arena, synchronized with light and sound cues, we recorded the
300 escape flight of each sparrow. Birds were released one by one in batches of six to ten, at
301 intervals of ≥ 30 s between releases. An escape flight was considered successful if the bird
302 took off from the wooden platform and escaped the arena following the standardized flight
303 trajectory without stopping, touching the ground, or touching the structure of the arena itself.
304 We recorded one successful flight per bird, for a total of 204 birds (99 from higher-Pb sites,
305 105 from lower-Pb sites, 87 females and 117 males, Table S1).

306 We analysed the video recordings of successful flights using Argus (Jackson et al., 2016),
307 focusing on the take-off phase, defined as the first 15 frames of the bird's flight, starting from
308 the frame in which the bird's feet left the platform (frame 1) as the bird became airborne. We
309 then followed the calibration and computational procedures detailed in (Jackson et al., 2016)

310 to generate three-dimensional coordinates of the centroid of each sparrow in each frame,
311 allowing us to calculate the following escape flight metrics.

312 First, we calculated instantaneous velocity v , by subtracting the centroid coordinates (in x , y ,
313 and z dimensions) in the previous frame ($n - 1$) from those in the current frame (n), and
314 multiplying the resultant vector magnitude by 60 (i.e. the frame rate of recordings) (Equation
315 1; (Di Liberto et al., 2024)).

$$316 \quad (\text{Eq 1}) \quad v = \sqrt{(x_n - x_{n-1})^2 + (y_n - y_{n-1})^2 + (z_n - z_{n-1})^2} * 60$$

317 Second, we used the resulting v , along with the mass of each bird (m), the vertical height
318 achieved in each frame (z), and the gravity constant (g), to calculate both the instantaneous
319 kinetic and potential energy. These were summed to compute the total energy (TE) expended
320 by the bird in each frame (Equation 2 (Di Liberto et al., 2024)), which we then averaged over
321 the 15 frames to generate the average energy expended during take-off.

$$322 \quad (\text{Eq 2}) \quad \text{TE} = \frac{1}{2}(m + v^2) + (m * z * g)$$

323 Third, we calculated the initial take-off leap force (TOF) to quantify the force generated by
324 the bird's legs as it left the platform over the first two frames (Provini et al., 2012). This was
325 calculated by multiplying the acceleration of the bird in the second frame (v_{sf}^2) by the mass m
326 of each bird (Equation 3, (Di Liberto et al., 2024)).

$$327 \quad (\text{Eq 3}) \quad \text{TOF} = v_{sf}^2 * m$$

328 *Ethical note*

329 All house sparrows were captured, banded, sampled and RFID-tagged by personnel with
330 ABBBS permits, and all animal procedures were conducted under an NSW Scientific License

331 (SL100378), and approval by ABBBS, and the Macquarie University Animal Ethics
332 Committee (AEC #2020/011). Mist-netting was conducted only in mild conditions with
333 limited wind, rain and heat, and nets were checked approximately every ten minutes.
334 Extracted birds were kept in individual cloth bags until handling. Banding, sampling and
335 tagging were conducted swiftly and handling time did not exceed ten minutes, after which
336 birds were released immediately, except for the escape flight assay. For this assay, birds were
337 returned to their individual cloth bag until release into the flight arena, which released them
338 directly into the wild. These birds were typically held for 15 to 30 additional minutes, and
339 their flight and conditions were monitored closely to ensure no abnormal behaviour due to
340 handling. In addition, subcutaneous RFID-tagging has no negative fitness impact on house
341 sparrows (Schroeder et al., 2011).

342

343 *Statistical analyses*

344 We conducted all statistical analyses in R 4.1.2 (R Core Team, 2021). To test whether
345 sparrows exhibited gaze aversion, and whether gaze aversion differed between higher- and
346 lower-Pb areas, we built three generalized linear mixed models, one for each gaze aversion
347 metric, using the package *lme4* 1.1-27.1 (Bates et al., 2015). Model assumptions and
348 convergence were checked visually using trace plots and Q-Q plots. We fitted head
349 orientation (0° - 90°) as a linear continuous predictor, and Pb level (higher vs lower) as an
350 interacting two-level factor. Fitting head orientation as a factor did not qualitatively alter the
351 results. We log-transformed first-bird latency and median latency to fit model assumptions of
352 residual normality. For the proportion of birds that visited the feeder, we fitted the model
353 assuming a binomial distribution using a logit link function, and the total number of birds at

354 each site as weights. We used data from all three experimental rounds, fitting round as a
355 random predictor. On two occasions, the feeding station did not detect any tagged birds: On
356 round 2 of the 45° orientation treatment at High2, and on round 3 of the 90° treatment at
357 Low1. As we could not attribute the absence of records to faulty equipment, or the real
358 behaviour of the birds, we excluded these two treatments from our analysis.

359 We tested whether vigilance was influenced by flock size, using individual scanning bout
360 data. We fitted a linear mixed model with *lme4*, with the duration of each scanning bout as the
361 response, log-transformed to fit model assumptions, and added flock size and sex as fixed
362 predictors, and bird identity and feeder location as random variables. We found no effect of
363 flock size on scanning bout duration (see results), and hence it was excluded from subsequent
364 analysis.

365 To test whether soil Pb level was associated with differences in vigilance, we fitted three
366 (generalized) linear models, one for each vigilance metric. Both percentage time scanning and
367 mean scanning duration were log-transformed to fit model assumptions, while the number of
368 scanning bouts was fitted assuming a Poisson error distribution using a log link. For all three
369 models we fitted Pb (higher vs lower) and sex of the bird as fixed categorical predictors. To
370 examine differences in vigilance at the feeder location level, we also reran these models,
371 replacing Pb level with feeder location codes (High1, High2, Low1, Low2) as a four-level
372 fixed factor.

373 To test whether escape flight metrics were associated with soil Pb levels, we built linear
374 mixed models in *lme4*, using each of velocity, mean TE, and TOF as responses respectively.
375 For all three models, we fitted Pb level (higher vs lower) and sex as categorical fixed
376 predictors, the time spent by each bird in the holding bag ('bag time', in mins) as a continuous

377 predictor, and location as a random variable. Fitting an interaction between lead level and sex
378 did not improve model fit and hence interaction terms were not included in the final models.
379 As flight velocity could be influenced by body condition, we further fitted body condition,
380 derived from the residuals of a mass-against-tarsus linear regression, into the velocity model,
381 as a continuous predictor. We did not fit body condition into the other two models, as mass
382 was already accounted for in the calculation of mean total energy expenditure and take-off
383 force. For all linear mixed models, we determined statistical significance at $|t| > 2$.

384

385 **Results**

386 *Soil and blood Pb levels*

387 Across the eight sites, mean soil Pb levels ranged between 59 mg/kg and 3,577 mg/kg (Table
388 S1). The mean sparrow blood Pb of the five lower-Pb sites ranged from 8.1 to 20.0 µg/dL. At
389 the three higher-Pb sites, this range was 48.7 to 79.5 µg/dL (Table S1). There was no overlap
390 between lower and higher lead sites in either blood or soil Pb concentrations.

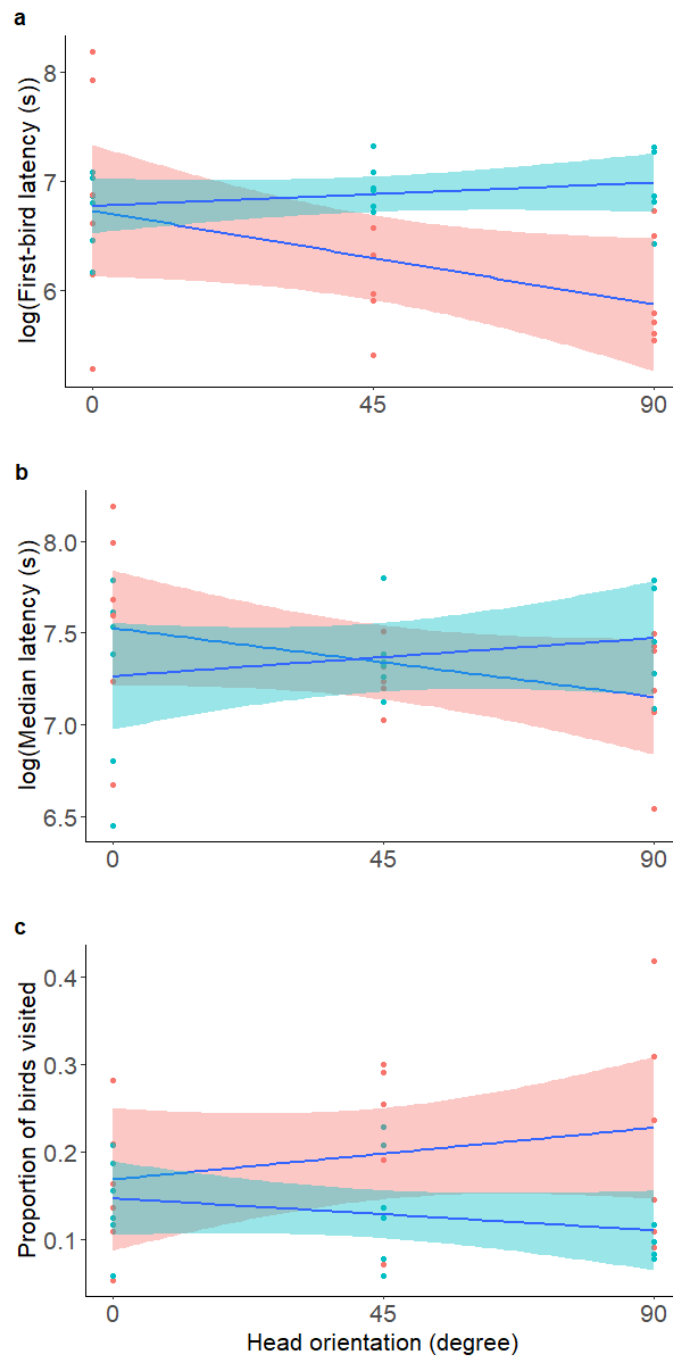
391 *Behavioural assay 1: Gaze aversion*

392 Across the four feeder locations (High1, High2, Low1 and Low2), we recorded a total of 497
393 RFID reads from 155 unique birds: 75 from High1, 29 from High2, 32 from Low1, and 19
394 from Low2. The mean first-bird latency was 909 s (s.d. = 694, range = 198 – 3610) after the
395 presentation of the face image, the mean median latency was 1,668 s (s.d. = 628, range = 634
396 – 3,610), and the mean proportion of birds that visited the feeder was 0.16 (s.d. = 0.09, range
397 = 0.05 – 0.42).

Lead effects on predator avoidance in house sparrows

398 We found statistically significant effects of head orientation of the human image stimulus on
399 both first-bird latency and the proportion of birds, as well as an interaction between head
400 orientation and Pb level (Table S2, Fig. 4a & 4c). At higher-Pb locations, as the angle of
401 orientation of the face increased (i.e., as the gaze was turning away from the feeder), the first
402 bird entered the feeder earlier and more birds entered the feeder in total. No such trend was
403 observed in lower-Pb locations. In other words, birds from higher-Pb locations demonstrated
404 gaze aversion behaviour, while those from lower-Pb locations did not. We did not find a
405 statistically significant effect of head orientation on median latency, nor an interaction
406 between head orientation and Pb on median latency (Table S2, Fig. 4b).

Lead effects on predator avoidance in house sparrows



407

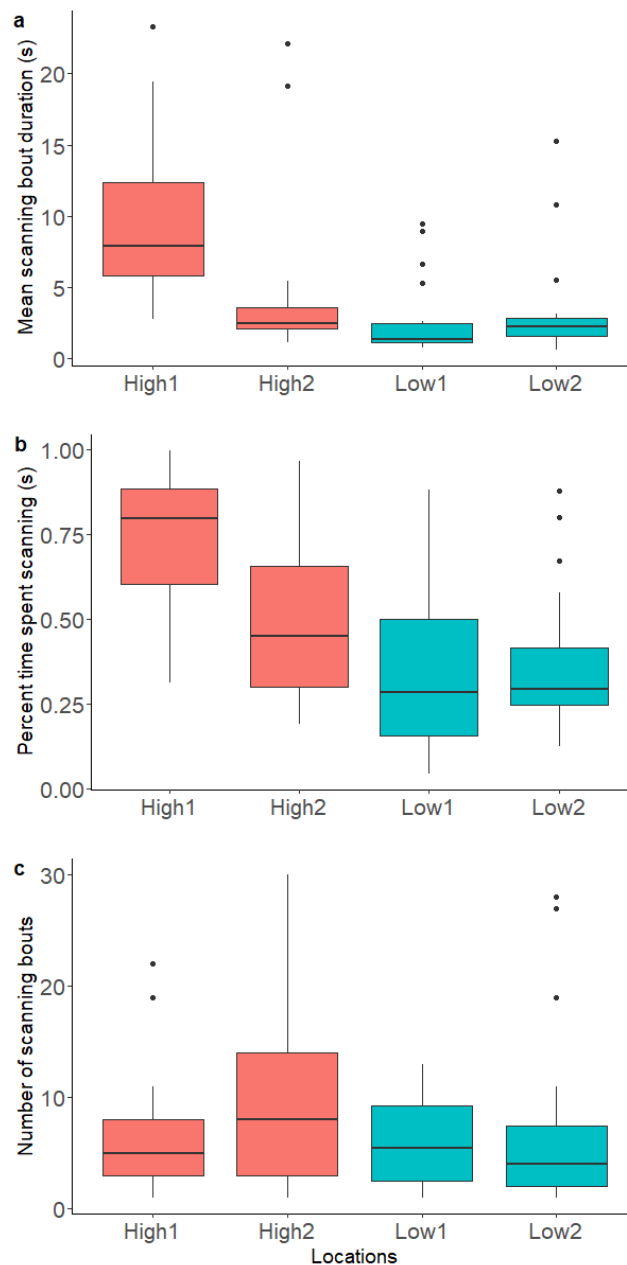
408 Fig. 4. Changes in (a) first-bird latency, (b) median latency, and (c) the proportion of birds
409 that visited the feeder (to the total number of birds) with the head orientation of the human
410 face image in higher- and lower-Pb areas in the Broken Hill house sparrow population. Red
411 dots and line represent higher-Pb locations, while blue dots represent lower-Pb locations.
412 Shaded areas around the lines represent 95 % confidence intervals.

413 *Behavioural assay 2: Vigilance*

414 At the four sites where vigilance assays were conducted (High1, High2, Low1 and Low2),
415 there was no effect of flock size on the duration of each scanning bout (estimate = -0.006, s.d.
416 = 0.006, $t = -1.06$). Scanning bout duration of males and females did not differ (estimate = -
417 0.063, s.d. = 0.094, $t = 0.668$).

418 We found statistically significant and negative effects of Pb on all measures of vigilance, such
419 that birds at lower-Pb locations had on average 60.5% shorter scanning bouts, spent 23.2%
420 less time scanning, and had 17.4% fewer scanning bouts than birds at higher-Pb locations
421 (Table S3). When we further examined vigilance metrics among locations, we found that the
422 greater vigilance in higher-Pb locations was largely explained by increased vigilance at
423 High1, which had significantly higher mean scanning bout duration and percentage time spent
424 scanning than the other three locations (Fig. 5a & 5b). For the number of scanning bouts,
425 there was no difference among High1, Low1 and Low2, while we observed more scanning
426 bouts at High2 (Fig. 5c).

Lead effects on predator avoidance in house sparrows



427

428 Fig. 5. Box-and-whisker plots depicting the difference in (a) mean scanning bout duration, (b)

429 percentage time spent scanning, and (c) the number of scanning bouts among higher-Pb (red)

430 and lower-Pb (blue) locations in the Broken Hill sparrow population. The box represents data

431 within the 25th and the 75th percentile, while the line within the box shows the median.

432 Vertical lines from the box represents data falling within 1.5 times the interquartile range

433 from the lower and higher percentile, while dots represent potential outliers.

434

435 *Behavioural assay 3: Escape flight performance*

436 We found weak evidence that lower-Pb birds had higher escape velocity, higher mean total
437 energy expended (TE), and higher take-off leap force (TOF) than higher-Pb birds, though the
438 effects did not quite reach our criterion for statistical significance (coefficient for escape
439 velocity = 0.214, s.e. = 0.129, $t = 1.655$; coefficient for mean TE = 0.015, s.e. = 0.008, $t =$
440 1.876; coefficient for TOF = 0.498, s.e. = 0.289, $t = 1.719$; Table S4). There was no effect of
441 bag time on any of the three metrics, and no effect of body condition on escape velocity. We
442 found a significant sex effect in velocity and mean TE, where males took off at higher speeds
443 and expended more energy during take-off flight than females, but take-off force did not
444 differ between the sexes. The summaries of all three linear models are provided in Table S4.

445

446 **Discussion**

447 In this study, we examined the effects of environmental lead (Pb) exposure on three predator
448 avoidance behaviours: vigilance, gaze aversion, and escape flight, using a free-living
449 population of house sparrows in a mining city that has historical heavy metal pollution. We
450 found that sparrows from higher-Pb locations displayed heightened vigilance and gaze
451 aversion, compared to their counterparts in lower-Pb locations – they scanned their
452 surroundings more often, and were more reluctant to approach an experimental food source
453 when they were under an experimental, direct human gaze. This gaze aversion behaviour was
454 consistently demonstrated through multiple rounds of testing.

455 The fact that house sparrows demonstrated gaze aversion against a human face stimulus is
456 perhaps indicative of the long commensal relationship that sparrows have with humans, which

457 likely started roughly 10,000 years ago with the development of agricultural societies (Sætre
458 et al., 2012). While the sensitivity to the gaze of humans by animals, in particular birds, has
459 been demonstrated before (Carter et al., 2008; Hampton, 1994), this study is among the few
460 that observed gaze sensitivity in wild urban birds in a natural setting, with one previous study
461 identifying a similar sensitivity in herring gulls (*Larus argentatus*; (Goumas et al., 2019)), and
462 another one in American crows (*Corvus brachyrhynchos*; (Clucas et al., 2013)).

463 The gaze perception task we employed involved complex cognitive processes, and it is
464 paradoxical that only Pb-affected sparrows demonstrated this behaviour, given that one of the
465 widely known effects of Pb poisoning in humans and animals is compromised neural and
466 cognitive capacity (Mason et al., 2014; Rocha & Trujillo, 2019). The differences in
467 performance between higher-Pb and lower-Pb sparrows found here lend support to the idea,
468 that Pb-affected sparrows are prioritising some components of their anti-predation repertoire
469 because of deficiencies elsewhere. Existing research has demonstrated that house sparrows
470 could indeed prioritise certain anti-predatory behaviours as a compensatory response under
471 anthropogenic disturbances – sparrows breeding in noisier environments had greater flushing
472 distances to make up for compromised predator detection due to noise masking (Meillere et
473 al., 2015). Here, the heightened predator awareness (increased scanning and gaze aversion)
474 would make sense if sparrows' escape flight was compromised (Prediction C, Fig. 1c).
475 Though effect sizes were small and marginal, we found a tendency for birds from higher-Pb
476 locations to perform worse at escape flight, with decreased instantaneous velocity, total
477 energy expenditure, and take-off force, compared to sparrows from lower-Pb locations. These
478 findings align with a captive study where house sparrows exposed to Pb in their drinking
479 water showed slower and less energetic escape flight take-off (Di Liberto et al., 2024).

480 A reduced escape performance in sparrows with higher levels of blood lead is consistent with
481 a range of well-known symptoms of Pb poisoning in birds such as anaemia, lethargy, and
482 muscle and fat loss (Pain et al., 2019). In particular, Pb decreases haemoglobin levels in blood
483 (Berglund & Nyholm, 2011) and thus the negative effect of Pb on oxygen-carrying capacity
484 of red blood cells could be reflected in declined flight velocity and take-off force. In addition,
485 Pb could also exert small effects on bone structure and integrity, as well as muscular
486 functioning, to decrease flight performance (Gangoso et al., 2009), because of the ability of
487 Pb ions to mimic and replace calcium ions (Handlogten et al., 2000). Impaired flight ability
488 could mean that higher-Pb birds would take longer and expend more energy to escape, and as
489 such may need to compensate or mitigate with heightened predator awareness, allowing them
490 to initiate an escape more quickly than individuals that have greater motor abilities, similar to
491 the way that heavier birds react faster to predators (Jones et al., 2009). A trade-off like this
492 would result in the pattern observed here, that higher-Pb birds exhibit higher vigilance and
493 gaze aversion, whereas lower-Pb birds lacked a gaze aversion response. To explicitly test for
494 this trade-off, an important next step would be to measure behavioural links among vigilance,
495 gaze aversion, and escape flight performance in the same set of individuals under
496 experimental Pb exposure, for example in a captive study.

497 Alternatively, the elevated rate of scanning, and the aversion to human eye gaze in the higher-
498 Pb sparrows could be considered a result of an altered perception of risk and/or a heightened
499 level of fear, and do not necessarily depend on an impaired take-off response. This
500 interpretation is consistent with existing behavioural research on animals exposed to Pb or
501 similar neurotoxic metals. For example, in free-living great tits (*Parus major*), individuals
502 with higher blood Pb and heavy metal content displayed slower exploration in a novel
503 environment (Grunst et al., 2018, 2019). Though not directly comparable to vigilance or gaze

504 aversion, slower exploration could be interpreted as an indicator of risk aversion in birds, thus
505 in line with our findings here (Grunst et al., 2018; Van Oers et al., 2004). Similarly, in rhesus
506 monkeys (*Macaca mulatta*), Pb-exposed individuals exhibited higher fearfulness and agitation
507 compared to controls in a novel environment (Lasky & Laughlin, 2001), and showed
508 increased latency to enter an open field (Ferguson & Bowman, 1990). In a study of
509 domesticated zebra finches (*Taeniopygia castanotis*), individuals exposed to methylmercury,
510 another heavy metal neurotoxin, demonstrated heightened risk aversion, and waited longer to
511 feed when under higher predation risk (Kobiela et al., 2015), similar to our findings.

512 Though we provided empirical evidence for associations between exposure to Pb and the
513 perception of predation risk, the neurological pathways of such a link is unclear. One part of
514 the brain that could be susceptible to Pb-induced damage is the hippocampus, which regulates
515 fear-based behaviour by interacting with the amygdala, and by mediating the hypothalamic-
516 pituitary-adrenocortical (HPA) axis which controls stress hormones release (Ferguson &
517 Bowman, 1990; Lasky & Laughlin, 2001; Toyoda et al., 2011). If this pathway is responsible
518 for heightened predator sensitivity in the sparrows, future research could examine whether
519 changes in oxidative stress levels, after exposure to threats in the environment, differ between
520 higher- and lower-Pb conditions. We acknowledge that vigilance and gaze aversion could also
521 be influenced by other extrinsic factors, such as habitat structure or availability of shade or
522 cover (Lima & Dill, 1990; Van Oers et al., 2004). We did partly account for this by having
523 replicate higher- and lower-Pb sites, which showed similar variation in vigilance and gaze
524 aversion behaviours, providing some confidence in the differences we demonstrated.

525 Furthermore, although we did not measure the blood Pb levels of all of the individuals tested
526 here, we are confident that birds in the higher- and lower-Pb sites will have had significantly
527 different levels of blood Pb, with those in the higher-Pb area having blood Pb levels of

528 approximately 49-80 $\mu\text{g/dL}$ (Table S1). In passerine birds, a blood Pb level of 50 $\mu\text{g/dL}$ is
529 believed to be the threshold for clinical Pb poisoning (Franson & Pain, 2011) and hence the
530 sparrows in the higher-Pb areas were expected to have been adversely affected. Indeed, other
531 studies of the adverse effects of Pb on house sparrows have detected deleterious effects at
532 lower blood Pb levels (e.g., $<20 \mu\text{g/dL}$ (Cid et al., 2018; Di Liberto et al., 2024)).

533 We compared anti-predatory behaviours of highly localised house sparrows in different parts
534 of a city that are contaminated with varying levels of Pb. These apparently altered anti-
535 predatory strategies could lead to further trade-offs. By increasing the perceived risk of
536 predation, and hence the time spent avoiding potential attacks, sparrows in higher-Pb areas
537 could allocate less time on foraging (Lima & Dill, 1990), either for themselves, or for
538 offspring during breeding. Decreased foraging could produce negative physiological effects,
539 such as decreased muscle mass and fat storage, and ultimately impact long term survival. In
540 the aforementioned study of zebra finch anti-predatory behaviour, authors found that birds
541 dosed with methylmercury lost more body mass as a result of spending less time foraging in
542 the open (Kobiela et al., 2015). Decreased foraging time, coupled with potentially higher risk
543 of predation due to impaired flight, could lead ultimately to decreased survival and
544 reproduction in wild birds inhabiting higher-Pb areas. Future studies should therefore examine
545 survival (e.g. through mark-recapture analyses) and reproductive output across urban soil Pb
546 gradients, to elucidate the long-term fitness effects of predator avoidance behaviours mediated
547 by exposure to Pb.

548 In conclusion, this study demonstrated that house sparrows are sensitive to the gaze of
549 humans, which is perhaps not too surprising given the long association between house
550 sparrows and people. Although this behaviour is a relatively sophisticated cognitive task and
551 might have been expected to be compromised by the deleterious effects of Pb on neural or

552 physiological processes, surprisingly this adaptive behavioural response was only exhibited in
553 the areas where sparrows would have suffered from clinical levels of Pb poisoning. These
554 sparrows in higher-Pb areas were also more wary, scanning for predators at a higher
555 frequency. In contrast, there was weaker evidence that escape flight performance was
556 predicted by Pb contamination levels. Our results indicate that the effects of Pb on wildlife
557 can be complex and interactive, and are likely influenced by a behavioural trade-off between
558 risk detection and escape performance, with Pb-affected birds investing more in behaviours
559 that will increase early detection or avoidance of predators than individuals in lower-Pb areas.
560 In addition to highlighting the appropriate responses that urban wildlife can take to improve
561 their survival chances, even in highly modified or contaminated environments, our findings
562 demonstrate that the effects of common environmental pollutants can be multi-faceted. Not
563 only could toxicants have direct impact on physiology and mortality, but they can also alter a
564 series of linked behaviours and physiological traits, which are likely to impact mortality
565 indirectly. Our study highlights some of the components through which toxic metal exposure
566 may impact predator avoidance in birds and provides insight into the less-explored effects of
567 environmental pollutants on behaviour.

568

569 **Data availability**

570 The data files and R script used to produce the results in this study will become publicly
571 available upon publication of the manuscript.

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744 **Supplementary text: Detailed methods for escape flight assay**

745 We conducted escape flight assays from May to July 2022, at three high-leaded locations and
746 five low-leaded locations, including the four RFID feeder locations (Table S1). Upon capture,
747 we banded each bird and collected maximum wing length to the nearest mm, tarsus length to
748 the nearest 0.1 mm, and body mass to the nearest g, using techniques as described in (1).
749 Sparrows were then kept individually in small holding bags until they were released in the
750 escape flight studio. This three-sided studio consisted of two upright wooden boards (180 x
751 120 cm) on the sides (the ‘walls’), and an upright but shorter wooden barrier (120 x 80 cm) at
752 one end (the ‘barrier’). This arrangement created a gap towards which the bird was
753 encouraged to fly, in order to standardise the trajectory of each flight. On the remaining open
754 side of the studio, we fixed a small wooden platform at the mid-point between both walls, at
755 ground level, which served as the release point. To record escape flight, we placed three
756 GoPro Hero7 cameras (60 frames per second, 1440 resolution) on a fixed PVC structure
757 behind the release platform. We put an L-shaped PVC structure at the centre of the studio on
758 the ground. This structure allowed us to determine the spatial orientation of the x (left-right),
759 y (close-far), and z (up-down) axes for the airspace through which the bird was flying. To
760 prevent birds from flying backwards from the release platform, we hung a heavy drop-cloth to
761 the right extending from the right-hand wall, and positioned ourselves to the left of the
762 platform during release (see Fig. S1).

763 Sparrows were released one by one in batches of six to ten, at intervals ≥ 30 seconds. At the
764 start of each batch release, we activated the cameras, and synchronised them using ambient
765 sound cues, and a flash of light from an experimenter’s phone that was simultaneously visible
766 on all cameras. To help calibrate the airspace in the chamber, we then slowly moved a 46 cm
767 wand with two brightly coloured polystyrene orbs on each end through the flight area. This

768 allowed us to generate paired points with a known distance between them for video
769 calibrations (see below). To release each bird, we placed the focal bird onto the platform, and
770 held it in place with a loose bander's grip until the bird was in a stable upright position, at
771 which time the experimenter released the grip. A flight was considered successful if the bird
772 took off from the platform and escaped the chamber directly over the barrier without stopping
773 or touching the ground or wooden sides of the chamber.

774 We analysed the video recordings in Python 3.8.3 using the software Argus (2). We focused
775 on the take-off phase, defined as the first 15 frames of the bird's flight, starting from the
776 frame in which the bird's feet left the platform (frame 1) as the bird became airborne. We first
777 aligned the frames from each camera using the sound and light cues, and digitized the three-
778 dimensional air space of the chamber, using the L-shaped structure for orientation of the x, y,
779 and z axes, and the paired points from the calibration wand for distance. Following these
780 calibrations, we manually digitized the position of the approximate centroid of each bird in
781 the first 15 frames of their flight. Argus generated three-dimensional coordinates of the
782 centroid in each frame, allowing us to calculate the escape flight metrics described in the main
783 text.

Supplementary Tables and figures

Table S1. The distribution of sample sizes across three higher-Pb and five lower-Pb sites in the gaze aversion and escape flight assay.

<i>Site code</i>	<i>Mean soil Pb content (mg/kg)</i>	<i>Mean blood Pb content (µg/dL)</i>	<i>Total number of RFID-tagged sparrows</i>	<i>Number of visiting sparrows during gaze aversion experimental days</i>	<i>Number of assayed RFID-tagged sparrows</i>	<i>Number of successful flights</i>
High1	965	48.7	181	110	75	36
High2	2908	50.3	104	55	29	43
High3	3577	79.5				20
Low1	59	8.1	101	48	32	30
Low2	208	15.3	87	51	19	30
Low3	167	20.0				35
Low4	172	19.6				5
Low5	209	10.1				5

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Table S2. Summary of the (generalized) linear mixed models testing for the effects of head orientation of a human face image, and soil lead levels (high/low), on three gaze aversion metrics: (a) log-transformed first-bird latency, (b) log-transformed median latency, and (c) the proportion of birds that visited the feeder during the experimental hour, out of the total number of birds that visited the feeder on the experimental days, in the Broken Hill house sparrow population. Statistically significant effects are highlighted in bold.

(a) Log-transformed first-bird latency				
Fixed effects				
	<i>Estimate</i>	<i>s.e.</i>	<i>t-value</i>	
(Intercept)	6.729	0.231	29.058	
Head direction	-0.010	0.004	-2.667	
Lead (low)	0.049	0.296	0.167	
Head direction x Lead (low)	0.012	0.005	2.258	
Random effect				
	<i>Variance</i>	<i>No. of groups</i>		
Round	0.028	3		
(b) Log-transformed median latency				
Fixed effects				
	<i>Estimate</i>	<i>s.e.</i>	<i>t-value</i>	
(Intercept)	7.525	0.153	49.270	
Head direction	-0.004	0.002	-1.786	
Lead (low)	-0.259	0.193	-1.342	
Head direction x Lead (low)	0.006	0.003	1.887	
Random effect				
	<i>Variance</i>	<i>No. of groups</i>		
Round	0.013	3		
(c) Proportion of birds				
Fixed effects				
	<i>Estimate</i>	<i>s.e.</i>	<i>z-value</i>	<i>p-value</i>

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(Intercept)	-1.513	0.154	-9.800	<0.001
Head direction	0.005	0.002	2.918	0.004
Lead (low)	-0.266	0.186	-1.436	0.151
Head direction x Lead (low)	-0.008	0.003	-2.466	0.014
Random effect				
	<i>Variance</i>	<i>No. of groups</i>		
Round	0.038	3		

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Table S3. Summary of the (generalized) linear models testing for the effects of lead levels (high/low) on three vigilance metrics in the Broken Hill sparrow population: (a) log-transformed mean scanning bout duration, (b) percentage time spent scanning, and (c) the number of scanning bouts. Statistically significant effects are highlighted in bold.

	<i>Estimate</i>	<i>s.d.</i>	<i>t-value</i>	<i>p-value</i>
(a) Log-transformed mean scanning bout duration				
(Intercept)	1.626	0.149	10.935	<0.001
Lead (Low)	-0.928	0.183	-5.065	<0.001
Sex (Male)	-0.047	0.185	-0.255	0.800
Sex (Unknown)	0.915	0.485	1.885	0.063
(b) Percentage time spent scanning				
(Intercept)	0.584	0.044	13.248	<0.001
Lead (Low)	-0.264	0.054	-4.864	<0.001
Sex (Male)	0.055	0.055	1.003	0.319
Sex (Unknown)	0.307	0.144	2.132	0.036
(c) Percentage time spent scanning				
(Intercept)	2.251	0.062	36.130	<0.001
Lead (Low)	-0.191	0.083	-2.309	0.021
Sex (Male)	-0.275	0.086	-3.208	0.001
Sex (Unknown)	-2.059	0.581	-3.541	<0.001

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Table S4. Summary of the linear mixed models testing for the effects of lead on (a) escape take-off velocity, (b) mean energy expended during escape take-off, and (c) escape take-off force, in the Broken Hill house sparrow system. Statistically significant effects are highlighted in bold.

(a) Escape take-off velocity			
Fixed effects			
	<i>Estimate</i>	<i>s.e.</i>	<i>t-value</i>
(Intercept)	2.220	0.116	19.157
Body condition	-0.034	0.023	-1.511
Lead (low)	0.214	0.129	1.655
Sex (Male)	0.177	0.077	2.299
Bag time	-0.000	0.001	-0.591
Random effect			
	<i>Variance</i>	<i>No. of groups</i>	
Location	0.018	8	
(b) Mean energy expended			
Fixed effects			
	<i>Estimate</i>	<i>s.e.</i>	<i>t-value</i>
(Intercept)	0.091	0.008	11.007
Lead (low)	0.015	0.008	1.876
Sex (Male)	0.017	0.006	2.573
Bag time	0.000	0.000	0.118
Random effect			
	<i>Variance</i>	<i>No. of groups</i>	
Location	0.000	8	
(c) Escape take-off force			
Fixed effects			
	<i>Estimate</i>	<i>s.e.</i>	<i>t-value</i>
(Intercept)	3.049	0.246	12.393
Lead (low)	0.498	0.289	1.719
Sex (Male)	0.111	0.130	0.856

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Bag time	-0.003	0.002	-1.803
Random effect			
	<i>Variance</i>	<i>No. of groups</i>	
Location	0.116	8	

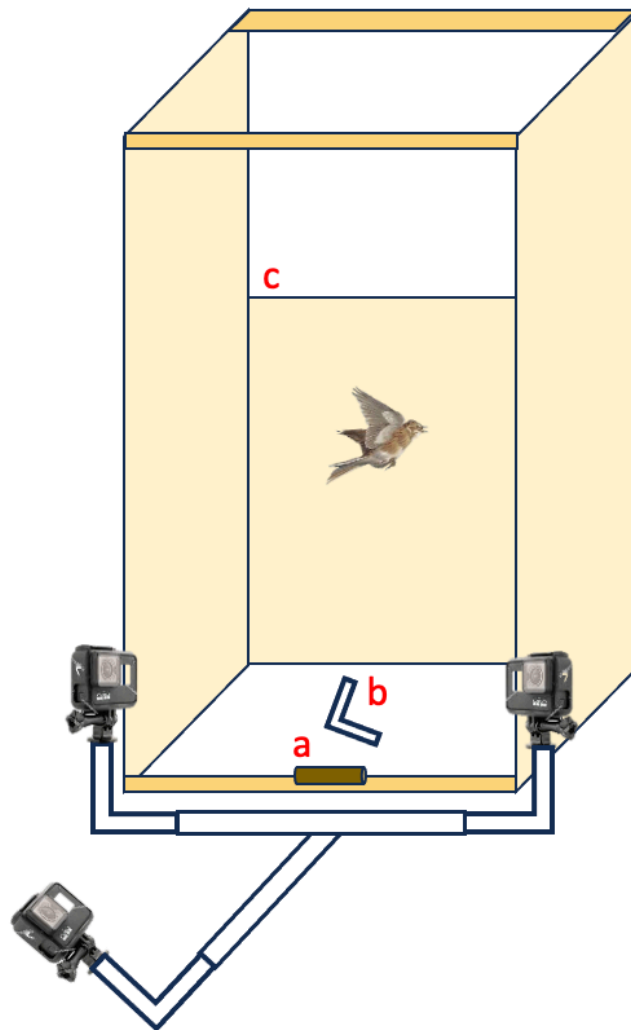


Fig. S1 Diagram of the escape flight studio. (a) indicates the position of the wooden release platform; (b) is a small “L”-shaped PVC structure that aided in determining the axes of digitized flights; and (c) signifies the end barrier that birds flew over. Flights were recorded on three fixed GoPro cameras.

Supplementary references

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