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3	Aggression and multimodal signaling in noise in a common urban songbird
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Abstract

21 Anthropogenic noise may disrupt signals used to mediate aggressive interactions, leading 22 to more physical aggression between opponents. One solution to this problem is to switch 23 signaling effort to a less noisy modality (e.g., the visual modality). In the present study we 24 investigate aggressive behaviors and signaling in urban and rural male European robins 25 (*Erithacus rubecula*) in response to simulated intrusions with or without experimental noise. 26 First, we predicted that urban birds, living in noisier habitats, would be generally more 27 aggressive than rural birds. We also predicted that during simulated intrusions with experimental 28 noise, robins would increase their physical aggression and show a multi-modal shift, i.e., respond 29 with more visual threat displays and sing fewer songs. Finally, we expected the multi-modal shift 30 in response to noise to be stronger in urban birds compared to rural birds. The results showed 31 that urban birds were more aggressive than rural robins, but an increase in aggression with 32 experimental noise was seen only in the rural birds. Urban but not rural birds decreased their 33 song rate in response to noise. Contrary to the multi-modal shift hypothesis, however, there was 34 no evidence of a concurrent increase in visual signals. These results point to a complex role of 35 immediate plasticity and longer-term processes in affecting communication during aggressive 36 interactions under anthropogenic noise.

37

38 Keywords:

39 Multi-modal signaling, territoriality, anthropogenic noise, multi-modal shift, European robin

40 Significance Statement

41 Human activity has an enormous effect on wildlife, including on their social behavior. 42 Animals living in urban areas often tend to be more aggressive than those living in rural areas, 43 which may be due to urban acoustic noise making communication between individuals more 44 difficult. In a study with a common songbird, the European robin, we investigated the role of 45 urban acoustic noise in aggression and territorial communication. Urban robins were more aggressive than rural robins, and additional noise in the territory increased aggression in rural but 46 47 not urban robins. While urban robins decreased their singing effort with additional noise, they 48 did not increase visual signals concurrently. These results suggest that noise can indeed make 49 animals behave more aggressively although the effect may depend on how noisy it is already. 50 These results further our understanding of how human-made noise changes animal communication and social behavior. 51

52 Introduction

53 Urban habitats are polluted with anthropogenic noise, often in multiple modalities, which 54 creates challenges for urban-living wildlife (Brumm and Slabbekoorn 2005). Many species rely 55 heavily on signals for communication in contexts such as mate attraction and territorial defense, 56 and noise from vehicles, buildings and other human activities often interferes with these signals 57 (Francis et al. 2009; Halfwerk and Slabbekoorn 2015; Lee and Thornton 2021). A well-studied 58 example of the effect of anthropogenic noise on communication is vocal signaling in urban birds: 59 in response to anthropogenic noise commonly found in cities, many species of birds may 60 increase repetition rates, amplitude, or frequency characteristics of their acoustic signals (Gil and 61 Brumm 2014; Roca et al 2016; Duquette et al 2021). 62 Urban living also leads to increased aggressiveness of individuals in urban habitats 63 compared to the rural habitats (Evans et al. 2010; Scales et al 2011; Davies and Sewall 2016; 64 Hardman and Dalesman 2018; Phillips and Derryberry 2018). The reasons for increased 65 aggression in urban habitat are not yet fully understood. It may result from several factors, 66 including selection for bolder individuals (Evans et al. 2010), increased food resources (Foltz et 67 al. 2015), increased exposure to harmful chemicals such as lead (McClelland et al. 2019) and less 68 stable social environment due to high rates of territory turnover in urban habitats (Davis et al. 69 2013). Anthropogenic noise in urban habitats may also be responsible for increased aggression. 70 Animals often use signals in aggressive interactions (e.g., during territory defense) to 71 resolve conflicts with opponents. Use of signals is often beneficial for both parties if they can 72 avoid costly physical fights in this way (Maynard Smith and Price 1973). Consequently, if 73 signaling is prevented or the signals are rendered ineffective, individuals may need to resort to 74 higher levels of physical aggression (Logue et al. 2010). Applied to urban habitats, this

hypothesis suggests that the high levels of urban noise may render long-distance aggressive
signals less effective, which in turn may lead to higher levels of aggression (Phillips and
Derryberry 2018). Consistent with this hypothesis, some studies reported a positive correlation
between ambient noise levels and aggressive behaviors (Phillips and Derryberry 2018; Akçay et
al. 2020; but see Kleist et al. 2016).

Signalers employ various strategies to overcome interference from anthropogenic noise. We focus here on the flexibility afforded by having signals in more than one modality (Partan and Marler 1999; Bro-Jørgensen 2010; Halfwerk and Slabbekoorn 2015). When animals have signals in more than one modality, they may shift their signaling effort from the noisy modality to a less noisy modality to increase the likelihood that the message of the signals gets through to the receivers (Partan et al. 2010; Partan 2017). This hypothesis is termed the multi-modal shift hypothesis.

87 Few studies tested the multi-modal shift hypothesis in signals used in territorial 88 interactions. In one study, Ríos-Chelén et al. (2015) found that male red-winged blackbirds 89 (Agelaius phoeniceus) did not use more intense visual signaling in noisier territories although 90 they modified their acoustic signals (e.g., decreased their song rate). Another study on song 91 sparrows (Melospiza melodia) found that males in noisier urban habitats were both more 92 aggressive and used proportionally more visual threat signals (wing waves) during territory 93 defense compared to the males in rural habitats, consistent with a multi-modal shift (Akçay et al. 94 2020). In a further experiment, however, individual song sparrows did not increase their visual 95 signaling effort when experimentally presented with noise, suggesting that multi-modal shift 96 seen in urban song sparrows was not due to immediate plasticity (Akçay and Beecher 2019).

97 Here we investigate the responses of European robins (*Erithacus rubecula*) living in urban and rural habitats in Istanbul, Turkey, to simulated territorial intrusions with or without 98 99 experimental noise playback. European robins have both visual and acoustic signals that are used 100 in agonistic interactions (Lack 1943). Previous studies have found that robins respond both to 101 song and visual signals during territorial intrusions (Chantrey and Workman 1984). Territory 102 holders sing in response to the song of an intruder, while visual signals are used when the 103 intruding male is within range of vision. Their most prominent visual signal is the neck display, 104 which has been observed in response to the sight of a rival male's red neck, or indeed even a ball 105 of red feathers (Lack 1943). Other visual threat signals include wing flutters, pricking the tail up, 106 and swaying, where the resident male moves his head from one side to the other (Lack 1943).

107 Signaling and aggressive behaviors of robins have been investigated in multiple studies. A 108 study by Mclaughlin and Kunc (2013) found that robins, after being lured by playback of a robin 109 song from a speaker, tended to move away from the speaker when the speaker switched to 110 playback of low-frequency noise mimicking typical traffic noise, particularly when the amplitude 111 of noise was high (90 dB at 1m). In response increasing amplitude of noise, the robins sang 112 shorter songs with fewer notes and increased the minimum frequency of their songs. In another 113 study, experimental presentation of wind turbine noise during simulated territorial intrusions led 114 to a decrease in low frequency elements in the songs, at the same time leading to an increase in 115 flight rates (Zwart et al. 2016). Song rates did not significantly differ between the noise and no-116 noise treatments. Interestingly, fewer robins used visual threat postures under experimental 117 presentation of noise compared to no noise, although the difference was not significant (Zwart et 118 al. 2016).

119 The presence of both acoustic and visual signals in territorial defense makes the European 120 robin a suitable candidate for testing the multimodal shift hypothesis but to our knowledge no 121 previous study compared visual signaling between urban and rural robins. We predict that robins 122 in urban habitats will exhibit higher levels of visual signaling. Additionally, if such a multi-123 modal shift is due to phenotypic plasticity, robins should increase their visual signaling under 124 experimental noise. We also predicted that urban birds would exhibit higher levels of plasticity 125 in signaling than rural birds, because they more frequently experience significant anthropogenic 126 noise levels (Lazerte et al. 2016; Gentry et al. 2017). Finally, in accordance with earlier studies, 127 we also expect to see a greater level of aggression from urban robins compared to rural robins. If 128 increased aggression is due to individual plasticity in response to noise, we also expect higher 129 levels of aggression from robins in response to experimental noise, particularly in urban birds 130 who have more experience with noise.

131 Methods

132 *Study sites* and species

133 We carried out playback experiments with male European robins that held territories in 134 rural areas (forests around Sariyer, Istanbul, 41° 9' 50.73971"N, 29° 0' 32.25243"E) and urban 135 parks and green areas in Sariyer, Istanbul, Turkey in April and May 2021 (urban: n=9; rural: 136 n=12). Robin territories were detected by the presence of an already-singing male robin before 137 the first playback or during recording sessions prior to playback. We determined a central 138 location by observing the robin's flights for about 5 minutes, although we did not attempt to map 139 the entire territory. In all trials reported below, only a single bird responded to the playback (for 140 one subject, not included in the final sample, we aborted the trial when a second male came to 141 within 10 m of the speaker). It was not possible to record data blind as our study involved

observing focal individuals in urban or rural habitats and noise manipulation was audible to allobservers.

144 Stimuli

145 Playback stimuli were generated on the software Syrinx (John Burt, Portland, OR) from 146 male European robin songs recorded in March 2021 in four of the nine study sites. We generated 147 stimulus files by extracting high quality songs from each recording and filtering out low 148 frequency noise below 1000 Hz. We added a silent period after each song so that stimuli were 149 presented at a rate of one song per seven seconds. The songs lasted on average (\pm SD) 2.36 150 (± 0.49) . We created one-minute stimuli (consisting of nine different songs) which were repeated 151 three times to make up three-minute stimuli to be played during the trials. In total, we used 17 152 files created from the songs of 17 different robins. The stimuli played for each subject came from 153 a robin whose territory was separated by at least one km from that of the subject's territory. 154 Subjects received the same song stimulus in both trials. As a visual stimulus, we used a 3-D 155 printed bird model (dimensions, height: 8 cm, length: 12 cm, width: 4.5 cm) which was hand-156 painted to resemble an adult robin (Fig. S6).

We generated the experimental acoustic noise stimuli by filtering white noise (created with Audacity) with the average amplitude spectrum from a 1-minute recording (made with a Marantz PMD660 and ME66/K6 microphone) of constant car traffic noise on a road in Sarıyer, Istanbul using the package *seewave* in R (Sueur et al. 2008). The power spectrum of the noise stimulus can be found in the supplementary materials (Fig. S1).

162 *Experimental procedure and design*

163 We started each trial by placing the robin model attached to a speaker (Anker Soundcore 164 Bluetooth Speaker, Anker, Inc.) on a natural perch at the estimated center of the resident male's 165 territory, approximately 1.5 m above the ground. A second Bluetooth speaker (same model as 166 above) was placed on the ground, face-up below the first, for noise playback. In the control 167 treatment, the second speaker was placed but not turned on, so the resident male received only 168 song playback. In the noise treatment, in addition to the song playback, traffic noise was played 169 at 75 dB SPL at 1 m. The noise playback lasted for the entire duration of the song playback, 170 simulating an acute but transient increase in car traffic noise.

171 Each subject received two 3-minute trials, one with experimental noise and one without 172 noise. The order of the treatments was counterbalanced, and the two trials were separated by 173 approximately an hour. Two observers, about 10 m away from the experimental setup, recorded 174 the songs and calls of the resident robin. The observers also narrated the trials, verbally noting 175 flights (any airborne movement by the bird), distance with each flight, and visual displays 176 described in Table 1 onto the recording. We continued recording songs for 3 minutes after the 177 end of each trial. Recordings were made on a Marantz PMD660 with a Sennheiser ME66/K6 178 microphone, or on a Zoom H5 handheld recorder with a Zoom SGH6 shotgun microphone.

In 31 of the trials, the bird was already singing when we started the trial. For these birds, the 3-minute playback period started with the first song played. In 11 of the trials, where the subject was quiet when the playback started, the 3-minute trial period began with their first response (song or approach). The average duration of pretrial playback for these 11 trials was 64.9 seconds (SD = 40.8). After each trial, we measured the ambient noise with a VLIKE VL6708 sound-level meter with the method described in (Brumm 2004). We took eight measurements (two in each cardinal direction) within a minute period, which were then averaged. For three subjects, we only had noise measurements from a single trial.

188 *Response variables*

189 We scanned and annotated the narrated trial recordings using the Syrinx software. The 190 number of songs and visual displays (number of neck displays and wing flutters as well as the 191 start and end times for swaying in seconds) were extracted from the verbal notes made on the 192 recordings. We only analyzed song rates and durations, as overlapping stimulus and subject 193 songs made it impossible to determine with certainty the note compositions for most songs, 194 precluding frequency measurements. Only 8 subjects used any visual displays during the 195 experimental period, we therefore coded visual displays as a binomial variable (visual signal 196 present vs. absent during a trial). From the recordings, we also extracted the number of flights, 197 closest approach to the model/speaker and proportion of time spent within 5m of the speaker. 198 These three spatial variables were taken as aggressive behaviors.

Because the spatial variables of aggression were significantly correlated with each other (all p< 0.05), we carried out a principal component analysis (PCA) using the *principal* function in package *psych* (Revelle 2021). The first component of PCA (PCA1) explained 61% of variance and was taken as our primary measure of aggression (see Table 2 for loading coefficients). The aggression scores thus calculated have been shown to be valid measures of territorial aggression in songbirds (Akçay et al. 2013). We report the analyses using raw spatial measures in the supplementary materials. All analyses were carried out in R version 4.1.0 (R Core Team 2021). We first checked whether urban territories had higher ambient noise with a linear mixed model (LMM) using habitat type (urban vs. rural) as the predictor variable and territory ID as the random variable. We also assessed whether noise levels were repeatable using the *rptR* package (Stoffel et al. 2017).

212 We then checked whether the order of trials had a significant effect on aggression scores, 213 song rates and visual signaling. The order of trials did not have a significant effect on song rate 214 (LMM, coefficient = -0.68, SE = 0.60, p = 0.28) or aggression score (LMM, coefficient = -0.22, 215 SE = 0.15, p = 0.16). However, there was a significant order effect on the incidence of visual 216 displays (GLMM, estimate = -15.32, SE = 6.22, p = 0.01). Eight subjects used visual displays in 217 the first trial, compared to two in the second trial (both subjects also used visual displays in the 218 first trial). Because of this order effect, we only used the first trial for each subject in models 219 including visual displays.

220 We analyzed song rates and aggression scores with linear mixed models (LMM), using the 221 *lme* function in the package *nlme* (Pinheiro et al. 2022). We took habitat type (urban vs. rural) 222 and experimental treatment (noise vs. control), and their interaction as the predictor variables, 223 and male ID as the random variable. We applied a generalized linear model (with log-link, using 224 the "glm" function in base R) with visual displays as binomial response variable, and habitat and 225 treatment as predictor variables, using only the first trials for each subject. Since only two rural 226 subjects used visual displays, we also carried out this analysis with the subset of only urban birds 227 (see Supplementary Materials).

Results

Urban habitats had significantly higher levels of ambient noise than rural habitats (urban: M = 49.0, SD = 7.1; rural: M = 39.9, SD = 3.6) and noise measurements were highly repeatable between the two trials (intra-class correlation coefficient; r=0.96, standard error: 0.02; p < 0.0001).

233 Urban birds were significantly more aggressive than rural birds. There was no main effect 234 of noise treatment but there was a significant interaction effect of habitat and noise treatment 235 (Table 3, Fig. 1a). To understand this interaction effect, we carried out paired t-tests on rural and 236 urban birds with noise treatment as predictor variable. Rural birds were more aggressive under 237 the noise compared to no-noise treatment (paired t-test; t(11)=2.44, p=0.033), whereas there was 238 no effect of experimental noise on aggression in urban birds (paired t-test: t(8)=-1.23, p=0.25; 239 Fig. 1a). Looking at the spatial variables separately, this interaction effect seems to be driven 240 mostly by the closest approach measure (see Supplementary Materials).

241 Song rates did not differ significantly between urban and rural birds and the noise 242 treatment had no main effect. The interaction effect of habitat and noise treatment however 243 approached significance (Table 3, Fig. 1b). When we analyzed song rates for urban and rural 244 birds separately there was a significant effect of noise treatment in urban birds, with lower song 245 rates under experimental noise compared to without noise (paired t-test; t(9)=3.15, p=0.014); 246 while there was no effect of noise treatment for rural birds (paired t-test; t(11)=1.01, p=0.33; 247 Fig. 1b). There was no significant main or interaction effect of habitat or noise treatment on song 248 duration (Table 3).

249	Urban birds used visual threat displays in the first trials significantly more than their rural
250	counterparts (GLM; χ^2 = 9.75, p=0.0018; Fig. 2). While there was no main effect of noise
251	treatment (χ^2 = 0.00, p=1.0), the interaction effect of treatment and habitat approached
252	significance (χ^2 = 3.72, p=0.053). This interaction effect was driven by a tendency in urban birds
253	to use more visual signals in the no-noise treatment compared to noise treatment, although the
254	effect was not significant (GLM within the subset of urban birds; χ^2 = 3.22, p=0.07). Out of the
255	five urban birds that received the no-noise treatment in the first trial, all five used visual signals,
256	compared to only one out of the four birds which received the noise treatment first (Fig. 2). Half
257	of the eight subjects that used visual signals displayed more than one type of signal (e.g., neck
258	display and tail up display), while five out of eight used visual signals more than once.

259 Discussion

260 In the present study, we examined the role of acute anthropogenic noise in determining aggressiveness and aggressive signaling. We predicted that urban robins, living in noisier 261 262 territories, would be more aggressive compared to rural robins in simulated territorial intrusions 263 and experimental noise during simulated intrusions will change both their aggressive behaviors 264 and signaling behaviors. Particularly, we expected that experimental acoustic noise should 265 increase aggression during intrusions and lead to an increase in using signals in the visual 266 modality, particularly in urban birds who would have more experience dealing with fluctuating 267 levels of noise.

In line with our first prediction, we found that urban robins responded with significantly more aggressive behaviors (particularly close approach) to simulated intrusions than rural robins. The effect of experimental noise treatment on aggressive approach was dependent on the habitat: contrary to our hypothesis, experimental noise led to increased aggression in the (comparatively quiet) rural habitats, but it had no effect in the noisy urban habitats. Experimental noise led to a decrease in song rates in urban and not rural birds. No change was observed in song duration (see Table 3). Finally, visual signals were more common in urban habitats (consistent with the fact that urban birds are more aggressive) and tended to be less common under experimental noise in the urban habitats, albeit not significantly.

277 Noise and aggressive behaviors in territory defense

278 Our results on the effect of habitat on aggression replicates earlier findings that urban-279 living birds are more aggressive than rural birds (Evans et al. 2010; Davies and Sewall 2016; 280 Hardman and Dalesman 2018; Phillips and Derryberry 2018). We also extend previous findings 281 by showing that the effect of experimental noise on aggression was dependent on habitat: urban 282 males showed no further increases in aggression with experimental noise while rural males 283 showed a significant increase in aggression. This is opposite of our expectation that urban birds 284 would show higher levels of phenotypic plasticity in response to experimental noise treatment 285 (LaZerte et al. 2016; Gentry et al. 2017). The lack of an effect of noise on aggressive behaviors 286 in urban habitats may be due to several reasons: First, for urban males that are already living in 287 noisy territories, additional noise may not have as much as an effect as in rural habitats. Urban 288 birds may also be more habituated to acute increases of noise than rural birds, although they did 289 show a plastic response in their singing rate as discussed below. Finally, urban birds may not be 290 able to increase their already high levels of aggression in response to noise playback.

The increased aggression with experimental noise in rural habitats is consistent with the idea that urban noise has a causal role in increasing aggression. Only a small number of studies experimentally manipulated noise levels to examine a causal role of noise in increased aggression. These studies yielded mixed results. Grabarcyzk and Gill (2019) found that house 295 wrens (*Troglodytes aedon*) males attacked the simulated intruder more frequently when playback 296 was accompanied with experimental noise than when it wasn't, consistent with the hypothesis 297 that noise induces higher levels of aggression. Another study in song sparrows however, found 298 no effect of experimental noise on aggression levels, measured as time spent within one meter of 299 the speaker, or attacks (Akçay and Beecher 2019). In the latter study, the noise playback started 300 only when subjects approached to within five meters, which all subjects did within a short period 301 of time (< 1 minute). Thus, lack of an effect in physical proximity may be due the fact that 302 subjects already were close to the speaker when the noise playback started.

303 In another study, Zwart et al. (2016) found that European robins did not show a statistically 304 significant increase in aggressive behaviors in response to experimental wind turbine noise 305 during simulated intrusions, although some variables like flights did show a trend consistent with 306 higher aggression with experimental noise. A more recent study by Reed et al. (2021) in lazuli 307 buntings (Passerina amoena) and spotted towhees (Pipilo maculatus) found that experimental 308 presentation of natural noise (such as noise from a river, ocean surf or cicadas) at the landscape 309 level led to slower detection of a simulated intruder and consequently weaker approach 310 responses (see also Kleist et al. 2016). Finally, a study conducted with saffron finches (Sicalis 311 *flaveola*) found birds displayed lower agonistic behaviors under experimental traffic noise, 312 although this study is more difficult to interpret in the present context as the experiment was 313 done with captive birds in small cages (Passos et al. 2020).

Together these studies point to two apparently contradictory effects of noise on territorial aggression. On one hand, noise may make localization of the simulated intruder and perception of stimulus features more difficult, leading to slower or weaker approach behaviors (Kleist et al. 2016; Templeton et al. 2016; Reed et al. 2021). On the other hand, assuming the simulated intruder is located, noise may interfere with the signaling behaviors of subjects which may induce them to resort to higher physical aggression such as closer approach (e.g., Grabarcyzk and Gill 2019). Thus, the differences in the findings may be due in part to differences in experimental designs, particularly with respect to the presentation of the noise stimulus (e.g., type of noise, location of noise relative to the conspecific stimulus etc.).

323 The experimental noise in our study represents a transient increase in noise to a high 324 amplitude that coincides with the need to confront a territorial intruder. Thus, the effects we see 325 are responses to acute noises, while urban birds would experience varying noise levels due to 326 cars passing as well as daily patterns of human activity (Gill et al. 2017). Our study was 327 explicitly designed to study plastic responses to acute increases in noise and therefore the results 328 may not apply to chronic but varying amounts of noise. Nevertheless, the situation we simulated 329 is a realistic one: urban wildlife must deal with transient increases in noise such as this regularly 330 (e.g., when a park worker uses a leaf blower to clean trails or when a lawnmower works nearby).

331

Change in multi-modal signals with noise

332 We found that European robins changed their signaling behaviors in response to noise. In 333 the acoustic modality, urban but not rural robins decreased their song rates, while birds in neither 334 habitat changed their song duration. From the perspective of the multi-modal shift hypothesis, a 335 decrease in signals in auditory modality was expected to coincide with an increase in visual 336 signals. We did not find this second effect. Two caveats are worth mentioning here: First, we 337 were not able to carry out the experiment blindly with respect to habitat or noise treatment, 338 which may have biased our observations of visual displays. Clearly, we could not blind observers 339 regarding which type of habitat they were in, and "blinding" observers with respect to noise 340 treatment (e.g., by using noise-canceling headphones) would have made keeping track of the

341 vocal behaviors almost impossible. In any case, given the pattern of results that tend to the 342 opposite direction of our expectations however, we believe observer bias is unlikely to be an 343 issue here.

344 Second, we could not examine individual-level plasticity in visual signaling, because of a 345 significant order effect in visual signals: visual displays were mostly used in the first trials only. 346 We do not have a good hypothesis as to why we found such an order effect. It is possible that our 347 stationary 3D model ceased to be a good visual stimulus by the time of the second trial, leading 348 to a decrease in visual signaling. It is also possible that the second trials may have represented a 349 lesser threat to the territory owner (given that it simulates the return of a previously retreating 350 individual) thus eliciting lower threat signals. Lack (1939) noted that repeated presentations of a 351 taxidermic mount quickly leads the lowered responses which he interpreted as the lack of 352 realistic response of the immobile taxidermic mount. While something similar may be happening 353 in our experiment, we note that there was no order effect in other aggressive or vocal 354 behaviors.

355 Whatever the reason, the order effect meant that we could only analyze the visual signals 356 in the first trials for each subject, halving our sample size and precluding a within-subject 357 comparison. The between-individual comparison of visual signals among urban birds in the first 358 trials yielded evidence in the opposite direction of what we expected: there was more visual 359 signaling in trials without experimental noise than with experimental noise, although the 360 difference was not significant, likely due to the small sample size. Nevertheless, this finding 361 suggests that while urban birds decrease their acoustic signaling effort, they do not necessarily 362 depend on visual signals as a back-up as predicted by the multi-modal shift hypothesis.

363 Our finding is similar to that of a study on European robins by Zwart et al (2016) which 364 reported slightly lower rates of visual threat signals with experimental noise than without noise. 365 Another study on song sparrows found no effect of experimental noise during territorial defense 366 on overall rates of wing waves, a visual threat signal (Akçay and Beecher 2019), even though 367 urban song sparrows show higher rates of wing waves than rural birds when controlling for the 368 total number of acoustic and visual threat signals (Akçay et al. 2020). These studies suggest that 369 if urban noise causes a multi-modal shift in visual threat signals, it is unlikely to be due to 370 immediate phenotypic plasticity.

Note that the fact that urban robins used visual displays more frequently than rural robins is consistent with a multimodal shift due to noise. This finding, however, is also consistent with the hypothesis that urban birds are simply more aggressive and therefore use visual threat signals more than rural birds (cf. Akçay et al. 2020). A valid comparison of the use of visual signals between urban and rural birds would need to correct for aggressiveness. Thus, currently the evidence for a multi-modal shift in this species is relatively weak.

377 *Behavioral plasticity of urban vs. rural birds in response to noise.*

378 We had expected that urban birds would show a higher level of plasticity in their responses 379 to experimentally presented noise compared to rural birds. This prediction was based on studies 380 that showed prior experience with noise (as urban birds would have) leads to more directional 381 plasticity in acoustic parameters of their song, by e.g. increasing the minimum frequency of their 382 song in noise (LaZerte et al. 2016; Gentry et al. 2017). Instead, we found contrasting patterns of 383 response to noise depending on the behavior measured: while urban birds showed lower 384 directional plasticity than rural birds in aggression scores, (specifically, rural birds increased 385 their approach distance in noise, while urban birds showed no difference), the opposite was true

in song rates (urban birds decreased song rates in noise while rural birds did not show adifference between treatments).

388 These findings suggest that if there is a role of learning in determining responses, it may 389 take different forms depending on the behavior. It is possible for instance that urban birds in 390 general have learned to "sit out" transient increases in noise (such as the situation we simulated 391 here) by reducing song rates and not increasing their approach towards opponents and visual 392 signaling. Such a strategy may be adaptive, given that closely approaching an opponent and 393 increasing visual threat signals likely would escalate an aggressive interaction to a fight (Searcy 394 et al. 2006). In contrast, rural birds may show less plasticity in signing in noise and instead 395 approach the opponent more closely during noise, because they haven't had the opportunity to 396 learn how to deal with transient increases in noise. Thus, the contrasting patterns found in urban 397 and rural birds may still indicate the role of prior experience with noise, although clearly more 398 controlled experiments are needed to further test this hypothesis.

399 *Conclusion*

In summary, our results showed important differences in how urban and rural robins
respond to noise during aggressive interactions. These results suggest that the ambient noise
levels experienced by animals is an important factor in determining their responses to transient
increases in noise.

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405	Commens	or interest

406 The authors declare that they have no conflicts of interest.

407 Ethics approval

408 This study did not require an ethics committee approval. All procedures used in this study

409 follow the ASAB/ABS guidelines for the treatment of animals in behavioral research and

410 teaching. Subjects were not captured or handled before, during or after any of the trials. Time

411 spent within a territory did not exceed 15 minutes per trial, and 30 minutes per day.

412 Data availability

413 The raw data and the R-code to reproduce the analyses reported in the manuscript are414 available in the supplementary materials.

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419 References

420	Akçay Ç, Beck ML, Sewall KB (2020) Are signals of aggressive intent less honest in urban
421	habitats? Behav Ecol 31:213-221. https://doi.org/10.1093/beheco/arz179
422	Akçay Ç, Beecher MD (2019) Multi-modal communication: song sparrows increase signal
423	redundancy in noise. Biol Lett 15:20190513. https://doi.org/10.1098/rsbl.2019.0513
424	Akçay Ç, Porsuk YK, Avşar A, Çabuk D, Bilgin CC (2020) Song overlapping, noise, and
425	territorial aggression in great tits. Behav Ecol 31:807-814.
426	https://doi.org/10.1093/beheco/araa030
427	Akçay Ç, Tom ME, Campbell SE, Beecher MD (2013) Song type matching is an honest early
428	threat signal in a hierarchical animal communication system. Proc R Soc B
429	280:20122517. https://doi.org/10.1098/rspb.2012.2517
430	Bro-Jørgensen J (2010) Dynamics of multiple signalling systems: animal communication in a
431	world in flux. Trends Ecol Evol 25:292–300. https://doi.org/10.1016/j.tree.2009.11.003
432	Brumm H (2004) The impact of environmental noise on song amplitude in a territorial bird. J
433	Anim Ecol 73:434–440. https://doi.org/10.1111/j.0021-8790.2004.00814.x
434	Brumm H, Slabbekoorn H (2005) Acoustic communication in noise. Adv Stud Behav 35:151-
435	209
436	Chantrey DF, Workman L (1984) Song and plumage effects on aggressive display by the
437	European robin Erithacus rubecula. Ibis 126:366–371. https://doi.org/10.1111/j.1474-
438	919X.1984.tb00257.x

439	Davies S, Sewall KB (2016) Agonistic urban birds: elevated territorial aggression of urban song
440	sparrows is individually consistent within a breeding period. Biol Lett 12:20160315.
441	https://doi.org/10.1098/rsbl.2016.0315
442	Davis A, Major RE, Taylor CE (2013) Housing shortages in urban regions: Aggressive
443	interactions at tree hollows in forest remnants. PLoS ONE 8:e59332.
444	https://doi.org/10.1371/journal.pone.0059332
445	Duquette CA, Loss SR, Hovick TJ (2021) A meta-analysis of the influence of anthropogenic
446	noise on terrestrial wildlife communication strategies. J Appl Ecol 58:1112-1121.
447	https://doi.org/10.1111/1365-2664.13880
448	Evans J, Boudreau K, Hyman J (2010) Behavioural syndromes in urban and rural populations of
449	song sparrows. Ethology 116:588-595. https://doi.org/10.1111/j.1439-0310.2010.01771.x
450	Foltz SL, Ross AE, Laing BT, Rock RP, Battle KE, Moore IT (2015) Get off my lawn: increased
451	aggression in urban song sparrows is related to resource availability. Behav Ecol
452	26:1548–1557. https://doi.org/10.1093/beheco/arv111
453	Francis CD, Ortega CP, Cruz A (2009) Noise pollution changes avian communities and species
454	interactions. Curr Biol 19:1415–1419. https://doi.org/10.1016/j.cub.2009.06.052
455	Gentry KE, Derryberry EP, Danner RM, Danner JE, Luther DA (2017) Immediate signaling
456	flexibility in response to experimental noise in urban, but not rural, white-crowned
457	sparrows. Ecosphere 8:e01916. https://doi.org/10.1002/ecs2.1916

459	Ecology. Oxford University Press, Oxford, pp 69–83
460	Gill SA, Grabarczyk EE, Baker KM, Naghshineh K, Vonhof MJ (2017) Decomposing an urban
461	soundscape to reveal patterns and drivers of variation in anthropogenic noise. Sci Total
462	Environ 599:1191-1201. https://doi.org/10.1016/j.scitotenv.2017.04.229
463	Grabarczyk EE, Gill SA (2019) Anthropogenic noise affects male house wren response to but
464	not detection of territorial intruders. PLoS ONE 14:e0220576.
465	https://doi.org/10.1371/journal.pone.0220576
466	Halfwerk W, Slabbekoorn H (2015) Pollution going multimodal: the complex impact of the
467	human-altered sensory environment on animal perception and performance. Biol Lett
468	11:20141051. https://doi.org/10.1098/rsbl.2014.1051
469	Hardman SI, Dalesman S (2018) Repeatability and degree of territorial aggression differs among
470	urban and rural great tits (Parus major). Sci Rep 8:5042. https://doi.org/10.1038/s41598-
471	018-23463-7
472	Kleist NJ, Guralnick RP, Cruz A, Francis CD (2016) Anthropogenic noise weakens territorial
473	response to intruder's songs. Ecosphere 7:e01259. https://doi.org/10.1002/ecs2.1259
474	Lack D (1939) The behaviour of the robin. — Part I. The life-history, with special reference to
475	aggressive behaviour, sexual behaviour, and territory. Part II. A partial analysis of
476	aggressive and recognitional behaviour. J Zool A109:169–219.
477	https://doi.org/10.1111/j.1096-3642.1939.tb03362.x

Gil D, Brumm H (2013) Acoustic communication in the urban environment. In: Avian Urban

478 Lack D (1943) The life of the robin, 1st edn. Witherby, London

479	LaZerte SE, Slabbekoorn H, Otter KA (2016) Learning to cope: vocal adjustment to urban noise
480	is correlated with prior experience in black-capped chickadees. Proc R Soc B
481	283:20161058. https://doi.org/10.1098/rspb.2016.1058
482	Lee VE, Thornton A (2021) Animal cognition in an urbanised world. Front Ecol Evol 9:633947.
483	https://doi.org/10.3389/fevo.2021.633947
484	Logue DM, Abiola IO, Rains D, Bailey NW, Zuk M, Cade WH (2010) Does signalling mitigate
485	the cost of agonistic interactions? A test in a cricket that has lost its song. Proc R Soc
486	Lond B 277:2571-2575. https://doi.org/10.1098/rspb.2010.0421
487	Maynard Smith J, Price GR (1973) The logic of animal conflict. Nature 246:15–18.
488	https://doi.org/10.1038/246015a0
489	McClelland SC, Durães Ribeiro R, Mielke HW, Finkelstein ME, Gonzales CR, Jones JA,
490	Komdeur J, Derryberry E, Saltzberg EB, Karubian J (2019) Sub-lethal exposure to lead is
491	associated with heightened aggression in an urban songbird. Sci Total Environ 654:593-
492	603. https://doi.org/10.1016/j.scitotenv.2018.11.145
493	McLaughlin KE, Kunc HP (2013) Experimentally increased noise levels change spatial and
494	singing behaviour. Biol Lett 9:20120771. https://doi.org/10.1098/rsbl.2012.0771
495	Partan SR (2017) Multimodal shifts in noise: switching channels to communicate through rapid
496	environmental change. Anim Behav 124:325-337.
497	https://doi.org/10.1016/j.anbehav.2016.08.003

498	Partan SR, Fulmer AG, Gounard MAM, Redmond JE (2010) Multimodal alarm behavior in
499	urban and rural gray squirrels studied by means of observation and a mechanical robot.
500	Curr Zool 56:313-326. https://doi.org/10.1093/czoolo/56.3.313
501	Partan SR, Marler P (1999) Communication goes multimodal. Science 283:1272–1273.
502	https://doi.org/10.1126/science.283.5406.1272
503	Passos MFO, Beirão MV, Midamegbe A, Duarte RHL, Young RJ, de Azevedoa CS (2020)
504	Impacts of noise pollution on the agonistic interactions of the saffron finch (Sicalis
505	flaveola Linnaeus, 1766). Behav Process 180:104222.
506	https://doi.org/10.1016/j.beproc.2020.104222
507	Phillips JN, Derryberry EP (2018) Urban sparrows respond to a sexually selected trait with
508	increased aggression in noise. Sci Rep 8:7505. https://doi.org/10.1038/s41598-018-
509	25834-6
510	Pinheiro J, Bates D, R Core Team (2022) nlme: Linear and nonlinear mixed effects models. R
511	package version 3.1-157, https://CRAN.R-project.org/package=nlme
512	R Core Team (2021) R: a language and environment for statistical computing. R Foundation for
513	Statistical Computing, Vienna, Austria, http://www.R-project.org/
514	Revelle W (2021) psych: procedures for psychological, psychometric, and personality research
515	(2.1.9), https://CRAN.R-project.org/package=psych

516	Reed VA, Toth CA, Wardle RN, Gomes DGE, Barber JR, Francis CD (2021) Natural noise
517	affects conspecific signal detection and territorial defense behaviors in songbirds. Behav
518	Ecol 32:993–1003
519	Ríos-Chelén AA, Lee GC, Patricelli GL (2015) Anthropogenic noise is associated with changes
520	in acoustic but not visual signals in red-winged blackbirds. Behav Ecol Sociobiol
521	69:1139-1151. https://doi.org/10.1007/s00265-015-1928-7
522	Roca IT, Desrochers L, Giacomazzo M, Bertolo A, Bolduc P, Deschesnes R, Martin CA,
523	Rainville V, Rheault G, Proulx R (2016) Shifting song frequencies in response to
524	anthropogenic noise: a meta-analysis on birds and anurans. Behav Ecol 27:1269–1274.
525	https://doi.org/10.1093/beheco/arw060
526	Scales J, Hyman J, Hughes M (2011) Behavioral syndromes break down in urban song sparrow
527	populations. Ethology 117:887-895. https://doi.org/10.1111/j.1439-0310.2011.01943.x
528	Searcy WA, Anderson RC, Nowicki S (2006) Bird song as a signal of aggressive intent. Behav
529	Ecol Sociobiol 60:234-241. https://doi.org/10.1007/s00265-006-0161-9
530	Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance
531	decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8:1639-
532	1644. https://doi.org/10.1111/2041-210X.12797
533	Sueur J, Aubin T, Simonis C (2008) Seewave, a free modular tool for sound analysis and
534	synthesis. Bioacoustics 18:213-226. https://doi.org/10.1080/09524622.2008.9753600

535	Templeton CN, Zollinger SA, Brumm H (2016) Traffic noise drowns out great tit alarm calls.
536	Curr Biol 26:R1173-R1174. https://doi.org/10.1016/j.cub.2016.09.058
537	Wolfenden AD, Slabbekoorn H, Kluk K, Kort SR (2019) Aircraft sound exposure leads to song
538	frequency decline and elevated aggression in wild chiffchaffs. J Anim Ecol 88:1720-
539	1731. https://doi.org/10.1111/1365-2656.13059
540	Zwart MC, Dunn JC, McGowan PJK, Whittingham MJ (2016) Wind farm noise suppresses
541	territorial defense behavior in a songbird. Behav Ecol 27:101–108.
542	https://doi.org/10.1093/beheco/arv128

544 Table 1. Visual displays of European robins during territorial interactions (Lack (1943); see S7)

Behavior	Description
Neck display	The robin raises his head, displaying his neck.
Wing flutter	The robin flutters his wings.
Swaying	The robin rhythmically sways his body from one side to the other.
Tail up	The robin perks his tail up.
-	

548 Table 2. Loading coefficients of the Principal Component Analysis

Factor	Loading coefficient to PC1
Flight rate	0.65
Proportion of time spent within 5 meters	0.83
Closest approach distance	-0.85
SS loadings	1.83
%Variance	61%

- Table 3. Coefficients (SE) from the linear mixed models and the p-values from Wald t tests,
- examining the effect of habitat and experimental noise treatment. Statistically significant valuesare shown in bold type

	Aggression Score		Song Rate		Song Duration	
Predictors	Estimates (SE)	р	Estimates (SE)	р	Estimates (SE)	р
(Intercept)	-0.58 (0.26)	0.035	8.44 (0.59)	<0.001	1.86(0.14)	<0.001
Treatment	1.32 (0.39)	0.003	0.61 (0.65)	0.36	0.04(0.21)	0.8454
Habitat	0.32 (0.19)	0.105	0.81 (0.90)	0.38	0.03(0.11)	0.7890
Treatment*Habitat	-0.68 (0.29)	0.031	-2.02 (1.00)	0.059	0.11(0.17)	0.5376
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- Fig. 1 The relationship of aggression scores (A) and song rate (B) with habitat and noise treatment. The boxes
- 557 558 559 indicate interquartile ranges, the middle line indicates median, and whiskers indicate 95% confidence intervals. Dots connected by dotted lines represent data from individual subjects



- 562 Fig. 2 Proportion of first trials where the resident male used visual signals, grouped by habitat and noise treatment.
- The numbers at the bottom of each bar indicates the total number of subjects for each combination

