1   Higher songs of city birds may not be an individual response to noise

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9   **Abstract**

10   It has been observed in many songbird species that populations in noisy urban areas sing

11   with a higher minimum frequency than do matched populations in quieter, less developed

12   areas. However, why and how this divergence occurs is not yet understood. We

13   experimentally tested whether chronic noise exposure during vocal learning results in songs

14   with higher minimum frequencies in great tits (*Parus major*), the first species for which a

15   correlation between anthropogenic noise and song frequency was observed. We also

16   tested vocal plasticity of adult great tits in response to changing background noise levels by

17   measuring song frequency and amplitude as we changed noise conditions. We show that

18   noise exposure during ontogeny did not result in songs with higher minimum frequencies. In

19   addition, we found that adult birds did not make any frequency or song usage adjustments

20   when their background noise conditions were changed after song crystallization. These

21   results challenge the common view of vocal adjustments by city birds, as they suggest that

22   either noise itself is not the causal force driving the divergence of song frequency between

23   urban and forest populations, or that noise induces population-wide changes over a time

24   scale of several generations rather than causing changes in individual behaviour.

Introduction

The past decade has seen a dramatic increase in research into the effects of anthropogenic noise on the lives of animals, and much of that research has focused on vocal behaviour and communication. Anthropogenic noise from street, air and boat traffic is dominated by low frequency sounds, and associated upward changes in the frequency components of acoustic signals have been reported in half of the songbird species tested on 5 continents [1], some whale species [2], frogs [3] and even insects [4], suggesting that this is a global phenomenon. However, there are few studies examining the mechanisms underlying the observed changes.

Vocal differences have been found between individuals in noisy urban centres and conspecifics in quieter areas in more than 25 species of songbirds [1]. One trait that has been the focus of most studies is minimum song frequency. The first study to describe an increase in song frequency was a study of great tits (*Parus major*), one of the commonest songbirds across Europe and Asia, and a successful urban colonizer [5]. Urban great tits have been found to sing with a higher minimum frequency than those in nearby forest populations in over 30 city-forest paired locations in Great Britain, Continental Europe, and Japan [6-8]. Some studies found that even within a city, birds in noisier areas sang with higher minimum frequencies than those in quieter areas [5, 9]. Three mechanisms have been proposed as to how noise may lead to the observed changes: ontogenetic effects including vocal learning, adult song plasticity, and microevolutionary changes [10].

Noise impacts on vocal development

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There are several processes by which low-frequency noise exposure during vocal learning might lead to upward changes in song frequency. In continuous noise at levels above 93 dB (re. 20μPa) songbirds are likely to experience a temporary hearing threshold shift, and above 110 dB to experience permanent ear damage [11]. Although noise levels in cities or near motorways may have transient peaks above 90 dB, it is rare that such events are sustained long enough to induce hearing damage in birds [11] . In juvenile songbirds, noise loud enough to cause temporary threshold shifts, resulted in severe impairment of song development, and in the formation of the underlying song control circuits in the brain [12, 13]. That noise disrupting hearing impairs vocal learning or song production is not surprising, however the effects of intermediate noise levels, like those commonly experienced in urban habitats, on song development are less known.

Noise at more moderate levels is unlikely to result in physical damage to the ears, but still can impact the process of song learning in multiple ways. In cities and along motorways, anthropogenic noise is predominantly low frequency, with most of the sound energy concentrated in the frequency range below 3 kHz. It is possible that such low frequency noise masks the lower frequency parts of songs. Young birds listening to adult tutors may therefore hear the higher frequency components of songs better, and hence be more likely to learn those aspects of the tutor songs [14, 15]. Young songbirds go through periods of sensory and sensorimotor plasticity during the song learning process. During the sensorimotor phase, vocal output is compared with memorized "templates" based on tutor songs [16]. Noise may also interfere with this self-assessment of a juvenile's own song, and thus bias song output towards higher pitches [17].

68   Alternatively, noise could disrupt vocal learning by a more indirect effect, such as by

69   inducing physiological stress responses that interfere with learning behaviour or cortical

70   development. In rats, it has been shown that chronic exposure to intermediate (~70 dB SPL)

71   levels of noise causes a delay in the development of the auditory cortex [18]. Rats exposed

72   to higher levels of noise (90 dB SPL) during development grew more slowly and performed

73   poorly in learning tasks [19]. In human children, noise has been found to disrupt learning

74   and memory, especially language comprehension tasks [e.g. 20, 21], as well as accuracy of

75   vocal production [22]. Whether traffic noise leads to learning or developmental delays in

76   birds is not known, but experimental exposure to chronic noise has been shown to increase

77   embryo mortality and delay somatic growth in zebra finches [23], which suggests that birds

78   may suffer some of the same physiological consequences of noise pollution that have been

79   found in mammals.

80   *Noise, adult plasticity and microevolutionary changes*

81   Traffic noise might not influence song learning or song features during ontogeny, but rather

82   may trigger changes in the songs of individual adults, or influence song features on longer,

83   microevolutionary time scales. Adult birds across a broad taxonomic range have been

84   shown to have a degree of vocal plasticity in response to changes in background noise

85   levels. Even phylogenetically basal birds exhibit the Lombard effect [24], a vocal

86   phenomenon whereby vocal amplitude increases as background noise increases [25]. The

87   Lombard effect in humans is often, but not always, associated with a concurrent increase in

88   vocal frequency or a shift in spectral tilt [reviewed in 25]. One hypothesis, therefore, is that

89   birds in noisy urban areas sing louder due to the Lombard effect, and this, in turn, results in

90   a rise in pitch [26].

5

91   While it is generally assumed that the primary driving force behind the upward shift in song

92   frequency is the high level of low-frequency noise, experimental evidence in support of this

93   assumption is limited. In the house finch *Haemorhous mexicanus*, individuals sang with a

94   higher minimum frequency when exposed temporarily to noise [27]. Silvereyes (*Zosterops*

95   *lateralis*), immediately lowered the minimum frequency of their calls in response to high-

96   frequency noise playback, but did not raise minimum frequency in response to low-

97   frequency noise [28]. In another study, long-term experimental traffic noise exposure in

98   zebra finches *Taeniopygia guttata*, led adult males to sing with *lower* minimum frequencies

99   after a 5 month long period of noise exposure [29], an adjustment opposite of what would

100   be predicted by the hypothesis that birds shift songs up to gain a release from masking

101   noise, and opposite of what would be predicted by the Lombard hypothesis.

102   In great tits, the response at the individual level is unclear. When exposed to experimental

103   noise playback in the field, fewer than half (41%) of the individuals switched to new song

104   types, while the rest (59%) did not [30]. Of those individuals that did switch song types, the

105   switch was made to a song type with a higher minimum frequency than the song type they

106   were singing when low-frequency noise presentation began, or to a lower song type when

107   high-frequency noise began. Additionally, many of the birds switched song types only after

108   the noise exposure ended [30], calling into question whether the noise exposure induced

109   the song change.

110   Finally, it may be that the differences in frequency between the vocalizations of urban and

111   rural populations are not the result of individual plasticity, but of gradual changes over

112   many generations. As in birds, upwards trends in frequency have been reported in

113   grasshoppers living near noisy motorways [4]. Lampe and colleagues [31]demonstrated that

114 this signal divergence is the outcome of both cross-generational effects of environmental

115 noise and developmental plasticity. In songbirds, such cross-generational effects could be

116 mediated by genetic or cultural changes. Although birds have much longer generation times

117 than grasshoppers, precluding similar experimental investigations, Luther and Derryberry

118 [32] used historical records to reconstruct changes in song over a 36 year period in a city

119 population of songbirds in San Francisco, and found that song frequencies have changed

120 over time as noise levels have risen. Since the songs of most oscine songbirds are learned

121 [33], it is hypothesized that their vocalizations could adapt more quickly to environmental

122 changes, through cultural evolution [34]. In line with this notion, Rios-Chelen et al. [35]

123 found that oscines diverged more in minimum song frequency between noisy and quiet

124 habitats than suboscines, which are not thought to acquire their song through vocal

125 production learning. Thus, although urban noise pollution is a relatively recent

126 phenomenon, it is possible that the trends of vocal divergence of urban birds across the

127 globe are indicators not of many individuals responding individually to fluctuating noise

128 conditions, but rather the result of cultural or microevolutionary shifts at the population

129 level.

130 We present here data from two behavioural domains: vocal learning and adult song

131 plasticity. In particular, we present 1) the first experimental study of the effects of chronic

132 exposure on song frequency in a songbird that is a frequent inhabitant of noise-polluted

133 habitats and 2) tests of adult vocal plasticity in response to changing background noise

134 conditions.

135 **Materials and methods**

7

136   ***Vocal ontogeny study***

137   *Birds and rearing conditions*

138   We collected 20 male nestling great tits 8-12 dph (days post hatch) in forests around

139   Starnberg, Germany. We used molecular markers to sex nestlings before collection.

140   Nestlings were then hand-raised in the laboratory in two acoustically-isolated groups and

141   exposed to one of two noise treatments during their entire first year. Siblings were divided

142   between the two treatment groups (1-2 siblings per treatment group, depending on the

143   number of males in a nest box). After birds were fledged and feeding independently (~50

144   dph) they were housed in single cages (125 x 44 x 40 cm) visually, but not acoustically,

145   isolated from other birds in their group.

146   *Noise exposure and song tutoring*

147   The two noise treatments were city-like ("CITY") noise, consisting of filtered white noise 0 –

148   3 kHz with a 500 Hz linear roll-off. The CITY noise was designed to simulate the average

149   noise profile found in a busy urban area, while at the same time being more extreme both in

150   spectral shape and temporal consistency (see Fig S1). Further details describing the degree

151   of masking of the minimum frequency of each tutor song in each noise treatment is

152   provided in the electronic supplement, Table S1. The control noise group was exposed to

153   noise with the same bandwidth as the CITY group, but covering high, rather than low

154   frequencies (3-6 kHz band-pass filtered white noise, 500 Hz linear fade-in, 100Hz linear roll-

155   off). The CONTROL noise treatment was designed to control for non-auditory effects of

156   noise exposure, but with noise in a different bandwidth than that of most traffic or urban

157   noise sources. The sound pressure level (SPL) of the noise was 60-63 dB SPL (re. 20μPa) at

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158   the position of the middle perch in each cage. In addition to the noise playback, both groups

159   of birds were tutored with 9 great tit songs (Fig S2) that were recorded the previous year

160   from nine adult males in the quiet forested area where our nestlings were collected. Typical

161   great tit song consists of several repetitions of identical song phrases (motifs). Phrases or

162   motifs often contain 2 to 4 different notes or "syllables", and the bird alternates between

163   high and low pitched syllables to produce the characteristic great tit "teacher teacher" song

164   (Fig. S2). Tutor songs were selected to include both the lowest and highest minimum

165   frequency song types recorded in the population. As great tits probably learn songs from

166   tutors during both their pre-dispersal time near their natal area and post-dispersal from

167   neighbours during territory formation in the late winter/early spring the following year , we

168   continued tutor playback until the birds were a full year old, when song is thought to

169   crystallize and then remain constant throughout life [36]. Each tutor file was 30 seconds

170   long and included 10-24 motifs in total. Playback consisted of 1 hour blocks of the 9 tutor

171   files in randomized order.   These 1 hour tutor playback blocks occurred 1-4 times per day

172   during the first year post-hatch.

173   *Song recording*

174   When the birds were one year old (340-370 dph), they were moved individually into wire

175   cages inside custom built sound-isolating recording chambers that measured 70×50x50 cm

176   inside. Cages were equipped with two wooden perches approximately 35 cm below a

177   microphone (Behringer C-2), mounted above the centre of the cage. Song was recorded

178   using Sound Analysis Pro version 2.063 [37], to a computer hard drive through an M-Audio

179   Delta 44 external sound card (44.1 kHz, 16 bit). Birds were first recorded for 1-2 weeks in

180   the same background noise condition in which they were reared. Noise playback in the

9

181   recording chambers was through a Pioneer A-109 stereo amplifier and Kenwood JVC Pro-III

182   loudspeakers.

183   *Song analyses*

184   From the 20 nestling birds, 9 CITY and 10 CONTROL birds survived to adulthood. Some birds

185   did not sing enough for statistical analyses in the recording chambers, so were excluded

186   from the analysis, leaving 7 birds in the CITY group and 6 birds in the CONTROL group.

187   To determine whether mean minimum frequency of our noise-exposed birds differed from

188   the minimum frequency of the tutor songs, we measured a mean minimum for each song

189   type in each bird's repertoire. When possible we took these measurements from 50 or more

190   repetitions of the song type, but included songs only if there were at least 12 repetitions.

191   Minimum frequency was measured in all three studies (this and the two below) at a set

192   threshold below the peak frequency [38]. To this end, we used a script written in Igor Pro v.

193   5 (Wavemetrics Inc.) that determined the peak frequency, and the frequency -22 dB below

194   that peak in a power spectrum (f_s 22 kHz, 1024 point FFT, Hamming window, 22 Hz

195   frequency resolution) for each syllable. We used the -22 dB cut-off because this was the

196   lowest threshold at which the minimum frequency could be measured given the signal-to-

197   noise ratio of the quietest syllable type in noise for our set of recordings.

198   *Statistical analyses*

199   For each bird we took the mean minimum frequency of each song type in the bird's

200   repertoire. The average number of syllables measured per song type was 41.7 (range 17-61)

201   in the CITY noise condition, and 41.8 (range 14-60) in the CONTROL condition. For each bird,

202   an overall mean minimum frequency was calculated by taking the average of all these mean

203   minimum frequencies for all the song types in their recorded repertoire. We then tested

204   whether the mean of the minimum frequencies of all the songs in each bird's repertoire

205   were different from those of tutor songs with a Kruskal-Wallis test.

206   ***Adult plasticity study 1***

207   *Syllable frequency plasticity – Birds and noise treatments, song analysis*

208   We tested whether the hand-raised birds from our Vocal Ontogeny study would modify the

209   frequency content of their songs when background noise conditions changed after song

210   crystallization (>365 days post hatch). We compared the minimum frequency of songs of

211   birds in different noise conditions with the same song types sung in the noise condition in

212   which they were reared. After birds were recorded in their "home" noise condition, we

213   changed the noise playback to either the opposite noise treatment (i.e. CITY birds were

214   exposed to CONTROL noise, and CONTROL birds were exposed to CITY noise), or to a no-

215   noise treatment. Birds were housed in the second noise treatment for 1-2 weeks, with noise

216   playback at the same intensity levels as the previous noise condition (60-63 dB SPL) or, in

217   the no-noise treatment, with no playback during the 1-2 week period (ambient noise levels

218   in the sound recording chambers was 35-40 dB SPL).

219   *Syllable frequency plasticity – Statistical analyses*

220   We compared the mean minimum frequency of each syllable type that each individual sang

221   in more than one noise condition. We only included a syllable if the individual sang it at least

222   10 times in each noise treatment. Syllables were given unique identifiers, so that even if

223   more than one individual sang the same syllable type, these were only compared within a

11

224   bird, not between individuals. We compared differences in minimum frequency between

225   noise treatments (CITY, CONTROL and NO-NOISE) using a linear mixed model with syllable

226   nested inside bird ID as a random factor, the difference in min frequency as predicted value,

227   and pairs of background noise treatments as a fixed factor (CITY vs. CONTROL, CITY vs. NO-

228   NOISE, and CONTROL vs. NO-NOISE). The test was performed independent of noise

229   presentation order. Because of low sample sizes, we have included effect sizes for each test

230   in the three Adult plasticity studies (Fig S3 & S4). In significant models we calculated

231   adjusted r^2 for GLMs according to [39]. In non-significant models we plotted effect sizes

232   with confidence intervals. We calculated significance using an Analysis of Deviance test with

233   2 degrees of freedom. Statistical analyses were done with R 3.2.1 and the packages lme4

234   and MuMIn.

235

236   ***Adult plasticity study 2***

237   *Syllable type usage – Birds, treatment, and song analysis*

238   To test whether birds might selectively use certain song types from within their repertoires

239   to minimize overlap with current noise profiles, we recorded adult birds from our vocal

240   ontogeny study (see above) in both their "home noise" and in the "opposite noise" (i.e. CITY

241   noise for CONTROL birds, and CONTROL noise for CITY birds). We then compared the

242   average minimum frequencies of all song types sung during 3 randomly selected 5-minute

243   intervals in each background noise condition. If birds selectively sing song types with higher

244   minimum frequencies in low frequency noise than they do in high noise or no noise, then

245   these differences should be detected in the mean minimum frequencies sung during these

246   randomly chosen intervals in each noise condition.

247   *Syllable type usage – Statistical analyses*

248   We tested the influence of rearing conditions on the minimum frequency of songs during

249   three 5-minute intervals, and whether the minimum song frequency changed when noise

250   conditions changed, with linear mixed models (LMMs). Noise treatment during rearing was

251   a fixed effect, and individual was a random effect. Songs were taken as repeated

252   measurements of one individual without distinguishing different syllables. First we

253   compared the minimum frequency of song of seven birds raised in the CITY-noise condition

254   with four birds raised in CONTROL-noise (1553 songs total, 18 to 296 songs per bird). We

255   tested with a Log-Likelihood test whether the model was better than a null-model without

256   treatment.

257   Second, we investigated whether birds switched to using song types with different minimum

258   frequencies in a changed noise condition (five CITY birds exposed to CONTROL noise, and

259   four CONTROL birds first exposed to CITY noise; in total 1538 differences in minimum

260   frequency, with 43 to 271 measured songs per individual). Rearing condition was a fixed

261   effect, and individuals were random effects. Here the tested value was the difference of the

262   minimum frequency in the changed condition to the average minimum frequency of the

263   same individual in the condition it had been raised in (means calculated from 36 to 296

264   songs per individual). Again the model was compared to a null-model with no influence of

265   the raising and corresponding testing condition.

266   **Adult Plasticity Study 3**

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267   *Lombard Effect - Animals, housing, noise playback and song recording*

268   Finally, we tested whether noise-induced increases in song amplitude correspond with

269   increases in song frequency in adult birds. We caught 6 adult male great tits from quiet

270   forest areas around Starnberg, Germany. Birds were housed singly in cages 125 x 44 x 40

271   cm, which were placed on tables in a sound-shielded room and provided with food and

272   water ad libitum. Birds were visually, but not acoustically isolated from each other, although

273   the sounds of neighbouring birds were dampened by panels of sound-absorbing foam.

274   We exposed singing birds to filtered white noise (1-10 kHz band pass Butterworth filter) at

275   levels between 65-70 dB(A) SPL, measured at the position of the perches. Noise was played

276   from a computer with an external digital sound card (Edirol UA-25EX), fed through a Pioneer

277   A-109 stereo amplifier to two JBL Control 1 Pro loudspeakers. During recording sessions, all

278   but two perches were removed from the cages, with the 2 remaining perches placed at the

279   same height and 15 cm apart. Sennheiser ME62 microphones were positioned 50 cm above

280   the cage, equidistant between the two perches. We calibrated the recording microphones

281   using a calibration microphone (Behringer ECM8000) and acoustic calibrator (Casella CEL-

282   184). When birds were habituated to the room and singing regularly, we recorded their song

283   in the quiet room (average ambient room noise 35 dB(A) SPL) and then again during noise

284   playback. Recordings were made using Sound Analysis Pro version 2.063 at a sampling rate

285   of 44.1 kHz and 16 bit resolution.

286   *Lombard effect - Song analysis*

287   Using calibrated song recordings in Avisoft SASLab Pro v. 5.2, we measured the root mean

288   square (RMS) amplitude of each individual syllable in each motif in each song type that birds

289   sang in both no-noise and noise conditions. We then subtracted the noise using logarithmic

290   computational rules [25] to determine the RMS values for the syllables themselves in both

291   noise treatments.

292   For measuring the peak frequency of each syllable, we resampled the song recordings at

293   22.05 kHz, and created power spectra for each syllable (1024 pt FFT, Hamming window, 22

294   Hz frequency resolution). Because of the signal-to-noise ratios in these recordings and

295   because of the wideband spectrum of the noise used to elicit the Lombard effect, minimum

296   frequency would be difficult to measure reliably.

297   *Lombard effect - Statistical analyses*

298   Amplitude and frequency of the same syllables between the noise-exposed and control

299   treatments were analysed with LMMs, with noise or no-noise as fixed effects, and individual

300   and syllable type nested in individuals as random factors. We tested for differences in the

301   mean values between conditions of 55 syllables of 6 individuals (3 to 23 syllables each),

302   which were sung at least 20 times in each treatment. The significance of the models was

303   tested by comparing them to null models with a Log-Likelihood test.

304   The observed change in amplitude was investigated in additional LMMs with birds as

305   random effects. Here we used amplitude in the no-noise condition, bandwidth of the

306   syllable and the distance of the peak frequency to the median peak frequency of all syllables

307   in silent conditions as predictor variables for the rise in amplitude. We used the Akaike

308   Information Criterion (AIC) to include or exclude variables in a model [40, 41] and calculated

309   the explained variance R^2 for fixed effects in linear mixed models [39].

310

Results

Vocal Ontogeny Study

Birds exposed to the CITY-noise did not sing with higher minimum frequencies than the tutors or than the CONTROL-noise birds, when singing in their "home" noise condition (Fig 1) (Kruskal-Wallis chi-squared = 2.57, df = 2, p-value = 0.28).

We further examined the specific songs that birds in each group learned from tutors and found that city-noise birds did not preferentially learn or sing song types with the highest minimum frequencies. In fact, 3 of the 4 birds that copied tutor songs in the city-noise group learned and sang the tutor song with the lowest minimum frequency, even though this song type had the greatest degree of overlap with the background noise (Table S1). The control-noise birds also did not selectively copy tutor song types that would have minimized overlap between song and noise.

Not all birds copied the tutor songs. Songs that could not be identified as imitations of tutor songs were also included in the minimum frequency analysis. That birds in both noise groups "invented" songs rather than only copying tutors provided an interesting perspective on noise avoidance in song frequency. Even when birds invented new song types, they did not produce songs with higher or lower minimum frequencies in different noise conditions (Mann Whitney U Test, n_{city} = 6 and $n_{control}$ = 5, W = 10, p=0.42).

Adult Plasticity Study 1- Syllable frequency plasticity

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331 When background noise was turned off, the mean minimum frequency did not differ from

332 that produced during noise playback. In this comparison we again took the average

333 minimum frequency of all the different song types in the bird's repertoire.

334 Birds sang less often in the second noise condition (either no-noise or "opposite noise")

335 than they did in their "home" noise condition. We presume that this decline in song output

336 had to do with the advancing date in the season, as great tits sing most early in the spring

337 and song output decreased overall with each successive week. Despite the smaller sample

338 size of song types, we did not find a shift in frequency of individual syllable types as

339 background noise conditions changed (X^2 = 0.98, Df = 2, P = 0.6125).

340 *Adult Plasticity Study 2 – Syllable type usage*

341 For each bird that sang in both noise conditions, we compared minimum frequency in three

342 5-minute intervals during which the bird was actively singing..

343 We found no significant differences in the minimum frequency between birds raised in CITY

344 noise and birds raised in CONTROL noise when they were singing in their "home" noise

345 conditions (Fig 2) (Maximum Likelihood Test, model with treatment vs. model without,

346 logLikNull-Model = -598.56, Df = 1, X^2 = 1.76, P < 0.19). In the model, 71 % of the variance

347 can be attributed to the individuals (here we did not further distinguish between individual

348 and syllable variation). When we tested whether average minimum frequency of songs

349 used changed in a new noise condition , we again found no significant difference for either

350 CITY or CONTROL birds singing in the opposite noise condition (Fig 2B)(Maximum Likelihood

351 Test, model with treatment vs. model without, logLikNull-Model = -275.98, logLik-Model = -

17

352   275.81, Df = 1, X^2 = 1.76, P < 0.58)(Fig 2). Forty-two percent of the variation in the

353   differences in minimum frequency could be attributed to individual.

354   *Adult Plasticity Study 3 - Lombard effect*

355   All birds exhibited the Lombard effect when exposed to increased background noise levels

356   (Fig 3A). Song amplitude was significantly higher in noise (Maximum Likelihood Test, model

357   with treatment vs. model without, $logLik_{Null-Model}$ = -381.03, $logLik_{Model}$ = -344.12, Df = 1, X^2 =

358   73.82, P < 0.0001), rising, on average, by 10 dB (LMM, random factors 6 birds, 55 syllables;

359   fixed effect, estimate for noise treatment is 9.9 ± 0.8 dB, t-value = 12.20, r^2 for fixed factors ,

360   r^2_{GLIMM} = 0.41). In contrast, peak frequencies did not change significantly in noise (LMM, 6

361   birds, 55 syllables; estimated noise effect: -43 ± 27 Hz, t-value = -1.575), and the model was

362   not significantly different from the null hypothesis (Maximum Likelihood Test, model with

363   treatment vs. model without, $logLik_{Null-Model}$ = -837.65, $logLik_{Model}$ = -836.42, Df = 1, X^2 = 2.47,

364   p= 0.12).

365   **Discussion**

366   We found that birds collected as nestlings from quiet forested areas, and reared in the

367   laboratory with chronic exposure to noise did not sing songs that differed in frequency from

368   those of the tutors from quiet forested areas. The minimum song frequencies were the

369   same as those of the tutors both when we considered all the song types within their tutees'

370   repertoires, and if we considered which song types they used in different noise conditions.

371   While the commonest suggested reason for the higher frequency songs observed in urban

372   birds has been that these pitch shifts are a response to low frequency noise, our

373   experiments indicate that chronic exposure to city-like noise did not induce frequency shifts

374   within individual birds. Although there have only been a few studies that have examined the

375   effects of noise exposure on individual song frequency shifts [27, 29, 30], our findings are

376   contrary to those previous studies, but support the prediction of Slabbekoorn and den Boer-

377   Visser that urban-forest population differences are likely to result from differences in song

378   repertoire composition rather than individuals modifying the same song types [8].

379

380   The birds in our ontogeny study were exposed to tutor songs that varied in minimum

381   frequency as well as overall bandwidth and song structure. While not all birds copied tutors

382   from the playback, some birds in both the CITY and CONTROL noise groups sang both the

383   lowest and highest tutor song types, even though the lowest song was most heavily masked

384   by the CITY noise, and the highest was most heavily masked by the CONTROL noise. In

385   addition, we did not find that exposure to higher frequency noise resulted in songs with

386   lower frequencies than those of tutors, or of CITY-noise birds, as might be predicted from

387   the "masking release" hypothesis.

388

389   A recent study found that young zebra finches that were exposed to low-frequency noise

390   during early vocal ontogeny did not develop adult songs with higher minimum frequencies

391   [42]. While this finding is important for a general understanding of the auditory feedback

392   mechanisms during vocal production learning, it is not clear whether wild zebra finch

393   populations differ at all in song pitch. Our data show that even in a species in which the

394   urban upward shift of song frequencies is well documented, this pattern cannot be

395   explained by developmental plasticity during vocal learning. Moreover, unlike zebra finches,

396   great tits typically have a repertoire of several different song types and our results show

397   that males not only stayed on pitch when they copied songs from their tutors in noise, but

398   also did not selectively acquire those song types into their repertoires that are particularly

399   well-suited to the noise conditions under which they are memorized.

400   We also tested whether adult birds would flexibly adjust either the minimum frequency of

401   their songs or the song types they chose to sing when background noise conditions were

402   changed. Neither our hand-reared birds, nor wild-caught adult birds, changed the frequency

403   of their songs when background noise conditions were changed, either to a different noise

404   profile or to a no-noise condition.

405

406   Previous studies reported individual frequency shifts related to noise exposure in several

407   songbird species [e.g. 30-33]. Based on an experiment on free-ranging great tits, Halfwerk

408   and Slabbekoorn suggested that males selected those song types from their repertoire that

409   are particularly well suited for certain noise conditions [30]. However, the birds in our

410   experiment did not show any tendency towards differential song type usage dependent on

411   current noise conditions, as they neither sang higher frequency song types in low frequency

412   noise nor lower frequency song types in high frequency noise. Can this conflicting evidence

413   be explained by different methodology? Our noise treatment was different from that of

414   Halfwerk & Slabbekoorn [30] and similar studies in other songbird species [27, 28], in that

415   the period of noise exposure was of a much longer duration (2-3 weeks vs. several minutes).

416   It may be that song type switching is a viable strategy only when dealing with transient

417   increases in background noise level, but when faced with chronic noise, the need to display

418   a large repertoire size, or to use song-type matching in territorial encounters with

419   neighbours, overrides any potential benefits that might be gained by using only the higher

420   frequency subset of the song repertoire. However, if a switch in song types only occurs in

421   response to short-term fluctuations in noise, this cannot account for the observed

422   divergence in minimum frequency between birds in noisy and quiet habitats.

423

424   Great tits in our study responded to increased background noise levels with a rise in

425   amplitude, as predicted by the Lombard effect [25]; however, this increase in amplitude was

426   not accompanied by an increase in frequency. In humans, vocal frequency often increases

427   along with amplitude in Lombard-induced speech, independent of a potential release from

428   masking [43]. However, the frequency rise and spectral tilt often observed during Lombard

429   speech may be controlled independently [44, 45]. That the great tits in our study did not

430   show an upward shift in frequency when their songs got louder suggests that frequency and

431   amplitude are not strictly coupled in song production, just as in call production in this

432   species[46]. In contrast, call frequency varies with amplitude in elegant-crested tinamous

433   (*Eudromia elegans*) [24] and budgerigars (*Melopsittacus undulatus*) [47]. This diversity may

434   reflect phylogenetic differences in vocal control, with songbirds having greater flexibility in

435   their vocalizations because of independent regulation of frequency and amplitude.

436   The change in song frequency observed in urban populations is often assumed to be an

437   adaptive response to the low-frequency noise typical of these areas [but see 48]. However,

438   environmental acoustics of cities differ from the original habitats of birds in more than just

439   noise profile [49], and the combined effects of noise and altered habitat acoustics may

440   impose a stronger selective force on vocal signalling than does traffic noise alone [50].

441   While several studies reported positive correlations between breeding bird species

442   richness/abundance and proximity to noisy roads [reviewed in 1, 51], a recent study

443   attempted to disentangle the effects of traffic noise from the traffic itself and found that

444   roads and vehicles on them explained the negative effects better than the noise per se [51].

445   Male density may also contribute to changes in song [52] as has been found in urban

446   Japanese great tits [6] and Northern cardinals (*Cardinalis cardinalis*) [53]. However, no

447   correlation between male density and minimum frequency was found in cardinals [53] or

448   Eurasian blackbirds [54]. Little is known about the impact of air or water pollution on the

449   behaviour of wild animals, but as air pollutants such as ozone and nitrogen oxides

450   irreversibly damage birds' lungs [55], and chronic exposure to hydrocarbons emitted by

451   traffic is correlated with reduced growth rates [56] and body weight [57] in birds, it is not

452   hard to imagine that air pollution could indirectly lead to changes in vocalizations.

453

454   Our data suggest that, while higher minimum frequencies of songs may be found in areas of

455   higher noise levels, a causal link between noise and pitch shifts is not clear. Changes in

456   minimum or peak song frequency did not occur in great tits that were exposed to chronic

457   traffic-like noise during song ontogeny, nor did it occur as a result of individual adult

458   plasticity. Our birds consistently sang on pitch and with the same mean minimum

459   frequencies in all noise conditions, which suggests that observed changes between

460   populations of rural and forest birds are the not the result of individual responses, but may

461   instead be the outcome of slower, population-wide changes. Such changes may be

462   mediated by selection for songs that transmit particularly well in noisy habitats, and the

463   subsequent spread of these songs through cultural transmission. This scenario would favour

464   loud songs, which at the same time are often higher pitched   due to vocal production

465   dynamics [58].

466

Ethics

468 All procedures described in this manuscript were conducted in accordance with appropriate

469 German regulations (licence 55.2-1-54-2532.3-13-09 by the government of Upper Bavaria)

470 and the guidelines for the treatment of animals in behavioural research and teaching of the

471 Association for the Study of Animal Behaviour (ASAB).

Competing Interests

473 We have no competing interests

Author's contributions

475 H.B., P.J.B.S. and S.Z. designed the study. S.Z. carried out the experiments. S.Z. and E.N. analysed the

476 data. S.Z. led the writing of the manuscript with critical input from all authors.

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483

485   **Figure 1**. Mean minimum frequencies of songs produced by great tits reared in either low

486   frequency (CITY) noise or high frequency (CONTROL) noise did not differ from the mean

487   minimum frequency of the tutors (stars), which were recorded in the quiet forested areas

488   where the experimental birds were collected as nestlings. Error bars indicate 95%

489   confidence intervals of the means.

490   **Figure 2**. Neither the birds reared in low frequency (CITY) noise (A), nor those reared in high

491   frequency (CONTROL) noise (B) switched to song types that differed in minimum frequency

492   when the background noise conditions changed. Mean minimum frequencies for three, 5-

493   minute long randomly selected song bouts for each bird are shown. Means for each bird in

494   each background noise condition are shown; error bars indicate 95% confidence intervals

495   for the data. Colour denotes individuals.

496   **Figure 3**. Wild-caught adult male great tits sang with higher amplitudes when background

497   noise levels increased (A), but did not increase song frequency as vocal amplitudes

498   increased (B). Means, plus 95% confidence intervals for each individual are shown. Colour

499   denotes individuals.

500

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