Manchester Metropolitan University

e-space

Manchester Metropolitan University's Research Repository

Please cite the Published Version

Zollinger, Sue Anne, Slater, Peter JB, Nemeth, Erwin and Brumm, Henrik (2017) Higher songs of city birds may not be an individual response to noise. Proceedings of the Royal Society B: Biological Sciences, 284 (1860). ISSN 0962-8452

DOI: https://doi.org/10.1098/rspb.2017.0602

Publisher: Royal Society, The

Version: Accepted Version

Downloaded from: https://e-space.mmu.ac.uk/623377/

Additional Information: This is an Author Accepted Manuscript of an article in Proceedings of the Royal Society B: Biological Sciences published by The Royal Society.

Enquiries:
If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines)

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

2

3 Sue Anne Zollinger[1], Peter J. B. Slater[2], Erwin Nemeth[1,3] and Henrik Brumm[1]

4

5 [1]Communication and Social Behaviour Group, Max Planck Institute for Ornithology, 82319

6 Seewiesen, Germany

7 [2]School of Biology, University of St Andrews, St Andrews KY16 9TH, Scotland

8 [3]BirdLife Austria, A-1070 Vienna, Austria

Abstract

It has been observed in many songbird species that populations in noisy urban areas sing with a higher minimum frequency than do matched populations in quieter, less developed areas. However, why and how this divergence occurs is not yet understood. We experimentally tested whether chronic noise exposure during vocal learning results in songs with higher minimum frequencies in great tits (*Parus major*), the first species for which a correlation between anthropogenic noise and song frequency was observed. We also tested vocal plasticity of adult great tits in response to changing background noise levels by measuring song frequency and amplitude as we changed noise conditions. We show that noise exposure during ontogeny did not result in songs with higher minimum frequencies. In addition, we found that adult birds did not make any frequency or song usage adjustments when their background noise conditions were changed after song crystallization. These results challenge the common view of vocal adjustments by city birds, as they suggest that either noise itself is not the causal force driving the divergence of song frequency between urban and forest populations, or that noise induces population-wide changes over a time 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

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].

While it is generally assumed that the primary driving force behind the upward shift in song frequency is the high level of low-frequency noise, experimental evidence in support of this assumption is limited. In the house finch *Haemorhous mexicanus*, individuals sang with a higher minimum frequency when exposed temporarily to noise [27]. Silvereyes (*Zosterops lateralis*), immediately lowered the minimum frequency of their calls in response to high-frequency noise playback, but did not raise minimum frequency in response to low-frequency noise [28]. In another study, long-term experimental traffic noise exposure in zebra finches *Taeniopygia guttata*, led adult males to sing with *lower* minimum frequencies after a 5 month long period of noise exposure [29], an adjustment opposite of what would be predicted by the hypothesis that birds shift songs up to gain a release from masking noise, and opposite of what would be predicted by the Lombard hypothesis.

In great tits, the response at the individual level is unclear. When exposed to experimental noise playback in the field, fewer than half (41%) of the individuals switched to new song types, while the rest (59%) did not [30]. Of those individuals that did switch song types, the switch was made to a song type with a higher minimum frequency than the song type they were singing when low-frequency noise presentation began, or to a lower song type when high-frequency noise began. Additionally, many of the birds switched song types only after the noise exposure ended [30], calling into question whether the noise exposure induced the song change.

Finally, it may be that the differences in frequency between the vocalizations of urban and rural populations are not the result of individual plasticity, but of gradual changes over many generations. As in birds, upwards trends in frequency have been reported in 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**

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

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

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

Song analyses

From the 20 nestling birds, 9 CITY and 10 CONTROL birds survived to adulthood. Some birds did not sing enough for statistical analyses in the recording chambers, so were excluded from the analysis, leaving 7 birds in the CITY group and 6 birds in the CONTROL group.

To determine whether mean minimum frequency of our noise-exposed birds differed from the minimum frequency of the tutor songs, we measured a mean minimum for each song type in each bird's repertoire. When possible we took these measurements from 50 or more repetitions of the song type, but included songs only if there were at least 12 repetitions.

Minimum frequency was measured in all three studies (this and the two below) at a set threshold below the peak frequency [38]. To this end, we used a script written in Igor Pro v. 5 (Wavemetrics Inc.) that determined the peak frequency, and the frequency -22 dB below that peak in a power spectrum (f_s 22 kHz, 1024 point FFT, Hamming window, 22 Hz frequency resolution) for each syllable. We used the -22 dB cut-off because this was the lowest threshold at which the minimum frequency could be measured given the signal-to-noise ratio of the quietest syllable type in noise for our set of recordings.

Statistical analyses

For each bird we took the mean minimum frequency of each song type in the bird's repertoire. The average number of syllables measured per song type was 41.7 (range 17-61) 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

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

these differences should be detected in the mean minimum frequencies sung during these randomly chosen intervals in each noise condition.

Syllable type usage – Statistical analyses

We tested the influence of rearing conditions on the minimum frequency of songs during three 5-minute intervals, and whether the minimum song frequency changed when noise conditions changed, with linear mixed models (LMMs). Noise treatment during rearing was a fixed effect, and individual was a random effect. Songs were taken as repeated measurements of one individual without distinguishing different syllables. First we compared the minimum frequency of song of seven birds raised in the CITY-noise condition with four birds raised in CONTROL-noise (1553 songs total, 18 to 296 songs per bird). We tested with a Log-Likelihood test whether the model was better than a null-model without treatment.

Second, we investigated whether birds switched to using song types with different minimum frequencies in a changed noise condition (five CITY birds exposed to CONTROL noise, and four CONTROL birds first exposed to CITY noise; in total 1538 differences in minimum frequency, with 43 to 271 measured songs per individual). Rearing condition was a fixed effect, and individuals were random effects. Here the tested value was the difference of the minimum frequency in the changed condition to the average minimum frequency of the same individual in the condition it had been raised in (means calculated from 36 to 296 songs per individual). Again the model was compared to a null-model with no influence of the raising and corresponding testing condition.

Adult Plasticity Study 3

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

Finally, we tested whether noise-induced increases in song amplitude correspond with increases in song frequency in adult birds. We caught 6 adult male great tits from quiet forest areas around Starnberg, Germany. Birds were housed singly in cages 125 x 44 x 40 cm, which were placed on tables in a sound-shielded room and provided with food and water ad libitum. Birds were visually, but not acoustically isolated from each other, although the sounds of neighbouring birds were dampened by panels of sound-absorbing foam.

We exposed singing birds to filtered white noise (1-10 kHz band pass Butterworth filter) at levels between 65-70 dB(A) SPL, measured at the position of the perches. Noise was played from a computer with an external digital sound card (Edirol UA-25EX), fed through a Pioneer A-109 stereo amplifier to two JBL Control 1 Pro loudspeakers. During recording sessions, all but two perches were removed from the cages, with the 2 remaining perches placed at the same height and 15 cm apart. Sennheiser ME62 microphones were positioned 50 cm above the cage, equidistant between the two perches. We calibrated the recording microphones using a calibration microphone (Behringer ECM8000) and acoustic calibrator (Casella CEL-184). When birds were habituated to the room and singing regularly, we recorded their song in the quiet room (average ambient room noise 35 dB(A) SPL) and then again during noise playback. Recordings were made using Sound Analysis Pro version 2.063 at a sampling rate of 44.1 kHz and 16 bit resolution.

Lombard effect - Song analysis

Using calibrated song recordings in Avisoft SASLab Pro v. 5.2, we measured the root mean square (RMS) amplitude of each individual syllable in each motif in each song type that birds

sang in both no-noise and noise conditions. We then subtracted the noise using logarithmic computational rules [25] to determine the RMS values for the syllables themselves in both noise treatments.

For measuring the peak frequency of each syllable, we resampled the song recordings at 22.05 kHz, and created power spectra for each syllable (1024 pt FFT, Hamming window, 22 Hz frequency resolution). Because of the signal-to-noise ratios in these recordings and because of the wideband spectrum of the noise used to elicit the Lombard effect, minimum frequency would be difficult to measure reliably.

Lombard effect - Statistical analyses

Amplitude and frequency of the same syllables between the noise-exposed and control treatments were analysed with LMMs, with noise or no-noise as fixed effects, and individual and syllable type nested in individuals as random factors. We tested for differences in the mean values between conditions of 55 syllables of 6 individuals (3 to 23 syllables each), which were sung at least 20 times in each treatment. The significance of the models was tested by comparing them to null models with a Log-Likelihood test.

The observed change in amplitude was investigated in additional LMMs with birds as random effects. Here we used amplitude in the no-noise condition, bandwidth of the syllable and the distance of the peak frequency to the median peak frequency of all syllables in silent conditions as predictor variables for the rise in amplitude. We used the Akaike Information Criterion (AIC) to include or exclude variables in a model [40, 41] and calculated the explained variance R^2 for fixed effects in linear mixed models [39].

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

When background noise was turned off, the mean minimum frequency did not differ from that produced during noise playback. In this comparison we again took the average minimum frequency of all the different song types in the bird's repertoire.

Birds sang less often in the second noise condition (either no-noise or "opposite noise") than they did in their "home" noise condition. We presume that this decline in song output had to do with the advancing date in the season, as great tits sing most early in the spring and song output decreased overall with each successive week. Despite the smaller sample size of song types, we did not find a shift in frequency of individual syllable types as background noise conditions changed ($X^2 = 0.98$, Df = 2, P = 0.6125).

Adult Plasticity Study 2 – Syllable type usage

For each bird that sang in both noise conditions, we compared minimum frequency in three 5-minute intervals during which the bird was actively singing..

We found no significant differences in the minimum frequency between birds raised in CITY noise and birds raised in CONTROL noise when they were singing in their "home" noise conditions (Fig 2) (Maximum Likelihood Test, model with treatment vs. model without, logLikNull-Model = -598.56, Df = 1, $X^2 = 1.76$, $P < 0.19$). In the model, 71 % of the variance can be attributed to the individuals (here we did not further distinguish between individual and syllable variation). When we tested whether average minimum frequency of songs used changed in a new noise condition , we again found no significant difference for either CITY or CONTROL birds singing in the opposite noise condition (Fig 2B)(Maximum Likelihood Test, model with treatment vs. model without, logLikNull-Model = -275.98, logLik-Model = -

275.81, Df = 1, X^2 = 1.76, P < 0.58)(Fig 2). Forty-two percent of the variation in the differences in minimum frequency could be attributed to individual.

Adult Plasticity Study 3 - Lombard effect

All birds exhibited the Lombard effect when exposed to increased background noise levels (Fig 3A). Song amplitude was significantly higher in noise (Maximum Likelihood Test, model with treatment vs. model without, $logLik_{Null\text{-}Model}$ = -381.03, $logLik_{Model}$ = -344.12, Df = 1, X^2 = 73.82, P < 0.0001), rising, on average, by 10 dB (LMM, random factors 6 birds, 55 syllables; fixed effect, estimate for noise treatment is 9.9 ± 0.8 dB, t-value = 12.20, r^2 for fixed factors , r^2_{GLIMM} = 0.41). In contrast, peak frequencies did not change significantly in noise (LMM, 6 birds, 55 syllables; estimated noise effect: -43 ± 27 Hz, t-value = -1.575), and the model was not significantly different from the null hypothesis (Maximum Likelihood Test, model with treatment vs. model without, $logLik_{Null\text{-}Model}$ = -837.65, $logLik_{Model}$ = -836.42, Df = 1, X^2 = 2.47, p= 0.12).

Discussion

We found that birds collected as nestlings from quiet forested areas, and reared in the laboratory with chronic exposure to noise did not sing songs that differed in frequency from those of the tutors from quiet forested areas. The minimum song frequencies were the same as those of the tutors both when we considered all the song types within their tutees' repertoires, and if we considered which song types they used in different noise conditions. While the commonest suggested reason for the higher frequency songs observed in urban birds has been that these pitch shifts are a response to low frequency noise, our 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

that males not only stayed on pitch when they copied songs from their tutors in noise, but also did not selectively acquire those song types into their repertoires that are particularly well-suited to the noise conditions under which they are memorized.

We also tested whether adult birds would flexibly adjust either the minimum frequency of their songs or the song types they chose to sing when background noise conditions were changed. Neither our hand-reared birds, nor wild-caught adult birds, changed the frequency of their songs when background noise conditions were changed, either to a different noise profile or to a no-noise condition.

Previous studies reported individual frequency shifts related to noise exposure in several songbird species [e.g. 30-33]. Based on an experiment on free-ranging great tits, Halfwerk and Slabbekoorn suggested that males selected those song types from their repertoire that are particularly well suited for certain noise conditions [30]. However, the birds in our experiment did not show any tendency towards differential song type usage dependent on current noise conditions, as they neither sang higher frequency song types in low frequency noise nor lower frequency song types in high frequency noise. Can this conflicting evidence be explained by different methodology? Our noise treatment was different from that of Halfwerk & Slabbekoorn [30] and similar studies in other songbird species [27, 28], in that the period of noise exposure was of a much longer duration (2-3 weeks vs. several minutes). It may be that song type switching is a viable strategy only when dealing with transient increases in background noise level, but when faced with chronic noise, the need to display a large repertoire size, or to use song-type matching in territorial encounters with 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

attempted to disentangle the effects of traffic noise from the traffic itself and found that roads and vehicles on them explained the negative effects better than the noise per se [51]. Male density may also contribute to changes in song [52] as has been found in urban Japanese great tits [6] and Northern cardinals (*Cardinalis cardinalis*) [53]. However, no correlation between male density and minimum frequency was found in cardinals [53] or Eurasian blackbirds [54]. Little is known about the impact of air or water pollution on the behaviour of wild animals, but as air pollutants such as ozone and nitrogen oxides irreversibly damage birds' lungs [55], and chronic exposure to hydrocarbons emitted by traffic is correlated with reduced growth rates [56] and body weight [57] in birds, it is not hard to imagine that air pollution could indirectly lead to changes in vocalizations.

Our data suggest that, while higher minimum frequencies of songs may be found in areas of higher noise levels, a causal link between noise and pitch shifts is not clear. Changes in minimum or peak song frequency did not occur in great tits that were exposed to chronic traffic-like noise during song ontogeny, nor did it occur as a result of individual adult plasticity. Our birds consistently sang on pitch and with the same mean minimum frequencies in all noise conditions, which suggests that observed changes between populations of rural and forest birds are the not the result of individual responses, but may instead be the outcome of slower, population-wide changes. Such changes may be mediated by selection for songs that transmit particularly well in noisy habitats, and the subsequent spread of these songs through cultural transmission. This scenario would favour loud songs, which at the same time are often higher pitched due to vocal production dynamics [58].

Ethics

All procedures described in this manuscript were conducted in accordance with appropriate German regulations (licence 55.2-1-54-2532.3-13-09 by the government of Upper Bavaria) and the guidelines for the treatment of animals in behavioural research and teaching of the Association for the Study of Animal Behaviour (ASAB).

Competing Interests

We have no competing interests

Author's contributions

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

Acknowledgements

We thank two reviewers and editors for their thoughtful comments, Luke Rendell for MATLAB scripts, and Brian S. Nelson for IgorPro scripts.

Funding

Funding was provided by the BBSRC (award BB/E003494/1)) to PJBS and HB and the DFG (awards BR 2309/7-1, BR 2309/8-1) to HB.

484

Figure 1. Mean minimum frequencies of songs produced by great tits reared in either low frequency (CITY) noise or high frequency (CONTROL) noise did not differ from the mean minimum frequency of the tutors (stars), which were recorded in the quiet forested areas where the experimental birds were collected as nestlings. Error bars indicate 95% confidence intervals of the means.

Figure 2. Neither the birds reared in low frequency (CITY) noise (A), nor those reared in high frequency (CONTROL) noise (B) switched to song types that differed in minimum frequency when the background noise conditions changed. Mean minimum frequencies for three, 5-minute long randomly selected song bouts for each bird are shown. Means for each bird in each background noise condition are shown; error bars indicate 95% confidence intervals for the data. Colour denotes individuals.

Figure 3. Wild-caught adult male great tits sang with higher amplitudes when background noise levels increased (A), but did not increase song frequency as vocal amplitudes increased (B). Means, plus 95% confidence intervals for each individual are shown. Colour denotes individuals.

References

1. Brumm H., Zollinger S.A. 2013 Avian vocal production in noise. In *Animal Communication and Noise* (ed. Brumm H.), pp. 187-227. Berlin Heidelberg, Springer-Verlag.
2. Tyack P., Janik V.M. 2013 Effects of Noise on Acoustic Signal Production in Marine Mammals. In *Animal Communication and Noise* (ed. Brumm H.), pp. 251-271. Berlin, Springer-Verlag.
3. Schwartz J.J., Bee M.A. 2013 Anuran Acoustic Signal Production in Noisy Environments. In *Animal Communication and Noise* (ed. Brumm H.), pp. 91-132. Berlin, Springer-Verlag.
4. Lampe U., Schmoll T., Franzke A., Reinhold K. 2012 Staying tuned: grasshoppers from noisy roadside habitats produce courtship signals with elevated frequency components. *Funct Ecol* **26**(6), 1348-1354.

5. Slabbekoorn H., Peet M. 2003 Birds sing at a higher pitch in urban noise - Great tits hit the high notes to ensure that their mating calls are heard above the city's din. *Nature* **424**(6946), 267-267.
6. Hamao S., Watanabe M., Mori Y. 2011 Urban noise and male density affect songs in the great tit Parus major. *Ethol Ecol Evol* **23**(2), 111-119.
7. Mockford E.J., Marshall R.C. 2009 Effects of urban noise on song and response behaviour in great tits. *P Roy Soc B-Biol Sci* **276**(1669), 2979-2985.
8. Slabbekoorn H., den Boer-Visser A. 2006 Cities change the songs of birds. *Curr Biol* **16**(23), 2326-2331.
9. Salaberria C., Gil D. 2010 Increase in Song Frequency in Response to Urban Noise in the Great Tit Parus Major as Shown by Data from the Madrid (Spain) City Noise Map. *Ardeola* **57**(1), 3-11.
10. Brumm H., Slabbekoorn H. 2005 Acoustic communication in noise. In *Advances in the Study of Behavior* (pp. 151-209. San Diego, Elsevier Academic Press Inc.
11. Dooling R.J., Blumenrath S.H. 2013 Avian sound perception in noise. In *Animal Communication and Noise* (ed. Brumm H.), pp. 229-250. Berlin, Springer-Verlag.
12. Marler P., Konishi M., Lutjen A., Waser M.S. 1973 Effects of continuous noise on avian hearing and vocal development. *P Natl Acad Sci USA* **70**(5), 1393-1396.
13. Iyengar S., Bottjer S.W. 2002 The role of auditory experience in the formation of neural circuits underlying vocal learning in zebra finches. *J Neurosci* **22**(3), 946-958.
14. Brumm H. 2006 Animal communication: City birds have changed their tune. *Curr Biol* **16**(23), R1003-R1004.
15. Hansen P. 1979 Vocal learning: its role in adapting sound structures to long-distance propagation and a hypothesis on its evolution. *Anim Behav* **27**, 1270-1271.
16. Brainard M.S., Doupe A.J. 2000 Auditory feedback in learning and maintenance of vocal behaviour. *Nat Rev* **1**, 31-40.
17. Kuebrich B.D., Sober S.J. 2015 Variations on a theme: Songbirds, variability, and sensorimotor error correction. *Neurosci* **296**, 48-54.
18. Chang E.F., Merzenich M.M. 2003 Environmental noise retards auditory cortical development. *Science* **300**(5618), 498-502.
19. Zheng Y., Meng M., Zhao C., Liao W., Zhang Y., Wang L., Wen E. 2014 Impact of Environmental Noise on Growth and Neuropsychological Development of Newborn Rats. *Anat Record* **297**(5), 949-954.
20. Cohen S., Evans G.W., Stokols D., Krantz D.S. 1986 Environmental Stress and Cognitive Performance. In *Behaviour, Health, and Environmental Stress* (eds. Cohen S., Evans G.W., Stokols D., Krantz D.S.), pp. 143-183. Berlin, Springer-Verlag.
21. Evans G.W., Lepore S.J. 1993 Nonauditory effects of noise on children. *Children's Environment* **10**, 31-51.
22. Riley K.G., McGregor K.K. 2012 Noise Hampers Children's Expressive Word Learning. *Lang, speech, and hearing serv schools* **43**(3), 325-337.
23. Potvin D.A., Macdougall-Shackleton S.A. 2015 Traffic Noise Affects Embryo Mortality and Nestling Growth Rates in Captive Zebra Finches. *J Exp Zool Part A* **323**(10), 722-730.
24. Schuster S., Zollinger S.A., Lesku J.A., Brumm H. 2012 On the evolution of noise-dependent vocal plasticity in birds. *Biol Lett* **8**(6), 913-916.
25. Brumm H., Zollinger S.A. 2011 The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour* **148**, 1173-1198.
26. Brumm H., Naguib M. 2009 Environmental acoustics and the evolution of bird song. In *Advances in the Study of Behavior* (eds. Naguib M., Zuberbühler K., Clayton N.S., Janik V.M.), pp. 1-33. Cambridge, MA, Academic Press.

27. Bermudez-Cuamatzin E., Rios-Chelen A.A., Gil D., Garcia C.M. 2011 Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird. *Biol Lett* **7**(1), 36-38.
28. Potvin D.A., Mulder R.A. 2013 Immediate, independent adjustment of call pitch and amplitude in response to varying background noise by silvereyes (Zosterops lateralis). *Behav Ecol* **24**(6), 1363-1368.
29. Potvin D.A., MacDougall-Shackleton S.A. 2015 Experimental chronic noise exposure affects adult song in zebra finches. *Anim Behav* **107**, 201-207.
30. Halfwerk W., Slabbekoorn H. 2009 A behavioural mechanism explaining noise-dependent frequency use in urban birdsong. *Anim Behav* **78**(6), 1301-1307.
31. Lampe U., Reinhold K., Schmoll T. 2014 How grasshoppers respond to road noise: developmental plasticity and population differentiation in acoustic signalling. . *Funct Ecol* **28**, 660-668.
32. Luther D.A., Derryberry E.P. 2012 Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. *AnimBehav* **83**(4), 1059-1066.
33. Catchpole C.K., Slater P.J.B. 2008 *Bird Song*. 2 ed. Cambridge, England, Cambridge University Press.
34. Lachlan R.F., Servedio M.R. 2004 Song learning accelerates allopatric speciation. *Evolution* **58**, 2049–2063.
35. Rios-Chelen A.A., Salaberria C., Barbosa I., Macias Garcia C., Gil D. 2012 The learning advantage: bird species that learn their song show a tighter adjustment of song to noisy environments than those that do not learn. *J Evol Biol* **25**, 2171-2180.
36. McGregor P.K., Krebs J.R. 1982 Song Types in a Population of Great Tits (Parus major): Their Distribution, Abundance and Acquisition by Individuals. *Behaviour* **79**(2/4), 126-152.
37. Tchernichovski O., Lints T.J., Deregnaucourt S., Cimenser A., Mitra P.P. 2004 Studying the song development process rationale and methods. In *Behavioral Neurobiology of Birdsong* (eds. Zeigler H.P., Marler P.), pp. 348-363. New York, New York Acad Sciences.
38. Zollinger S.A., Podos J., Nemeth E., Goller F., Brumm H. 2012 On the relationship between, and measurement of, amplitude and frequency in birdsong. *Anim Behav* **84**(4), e1-e9.
39. Nakagawa S., Schielzeth H. 2013 A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol* **4**, 133-142.
40. Burnham K.P., Anderson D.R. 2002 *Model selection and multimodel inference : a practical information-theoretic approach.* New York, Springer.
41. Crawley M.J. 2007 *The R Book*. NewYork, John Wiley & Sons.
42. Potvin D.A., Curcio M.T., Swaddle J.P., MacDougall-Shackleton S.A. 2016 Experimental exposure to urban and pink noise affects brain development and song learning in zebra finches (Taenopygia guttata). *PeerJ* **4**, e2287.
43. Lu Y., Cooke M. 2008 Lombard speech: Effects of task and noise type. *J Acoust Soci Am* **123**, 3072.
44. Eliades S.J., Wang X.Q. 2012 Neural Correlates of the Lombard Effect in Primate Auditory Cortex. *J Neurosci* **32**(31), 10737-10748.
45. Eliades S.J., Wang X. 2008 Neural substrates of vocalization feedback monitoring in primate auditory cortex. *Nature* **453**(7198), 1102-1106.
46. Templeton C.N., Zollinger S.A., Brumm H. 2016 Traffic noise drowns out great tit alarm calls. *Current Biology* **26**(22), R1173-R1174.
47. Osmanski M.S., Dooling R.J. 2009 The effect of altered auditory feedback on control of vocal production in budgerigars (*Melopsittacus undulatus*). *J Acoust Soci Am* **126**(2), 911-919.
48. Nemeth E., Zollinger S.A., Brumm H. 2012 Effect sizes and the integrative understanding of urban bird song *Am Nat* **180**, 146-152.
49. Mockford E.J., Marshall R.C., Dabelsteen T. 2011 Degradation of Rural and Urban Great Tit Song: Testing Transmission Efficiency. *PLoS ONE* **6**(12).

50. Nemeth E., Brumm H. 2010 Birds and anthropogenic noise: Are urban songs adaptive? *The Am Nat* **176**(4), 465-475.
51. Summers P.D., Cunnington G.M., Fahrig L. 2011 Are the negative effects of roads on breeding birds caused by traffic noise? *J Appl Ecol* **48**(6) 1527–1534.
52. Nemeth E., Brumm H. 2009 Blackbirds sing higher-pitched songs in cities: adaptation to habitat acoustics or side-effect of urbanization? *Anim Behav* **78**(3), 637-641.
53. Narango D.L., Rodewald A.D. 2016 Urban-associated drivers of song variation along a rural-urban gradient. *Behavi Ecol* **27**(2), 608-616.
54. Ripmeester E.A.P., Mulder M., Slabbekoorn H. 2010 Habitat-dependent acoustic divergence affects playback response in urban and forest populations of the European blackbird. *Behav Ecol* **21**(4), 876-883.
55. Rombout P.J., Dormans J.A., van Bree L., Marra M. 1991 Structural and biochemical effects in lungs of Japanese quail following a 1-week exposure to ozone. *Environmental research* **54**(1), 39-51.
56. Albers P.H. 2006 Birds and polycyclic aromatic hydrocarbons. *Avian Poult Biol Rev* **17**(4), 125-140.
57. Llacuna S., Gorriz A., Riera M., Nadal J. 1996 Effects of air pollution on hematological parameters in passerine birds. *Arch Environ Con Tox* **31**(1), 148-152.
58. Zollinger S.A., Brumm H. 2015 Why birds sing loud songs and why they sometimes don't. *Anim Behav* **105**, 289-295.