Immediate spectral flexibility in singing chiffchaffs during experimental exposure to highway noise

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SUMMARY

Sound plays an important role in the life of many animals, including many bird species. Typically, male birds sing to defend a territory and to attract mates. Ambient noise may negatively affect the signal efficiency of their songs, which may be critical to reproductive success. Consequently, anthropogenic noise may be detrimental to individual birds and to populations in cities and along highways. Several bird species that are still common in urban areas have been shown to sing at higher frequency at locations where there is more low-frequency traffic noise. Here we show that chiffchaffs along noisy highways also sing with a higher minimum frequency than chiffchaffs nearby at a quiet riverside. Furthermore, through experimental exposure to highway noise we show that these birds are capable of making such adjustments over a very short time scale. The first 10 songs sung during the noise exposure revealed an immediate shift to higher frequencies, with a return to pre-exposure levels in recordings without noise the following day. In a transmission re-recording experiment we tested the impact of a potential measurement artifact by recording playback of the same songs repeatedly under different controlled noise conditions. We found an upward shift in the minimum frequency measurement associated with more noisy recordings of the same song, but this artifact was not of a scale that it could explain the noise-dependent spectral shifts in chiffchaffs.

Key words: anthropogenic noise, birdsong, experimental exposure, frequency shift, masking avoidance.

INTRODUCTION

Birds living in and around cities often experience high levels of ambient noise originating from all sorts of human activities (Slabbekoorn and Peet, 2003; Patricelli and Blickley, 2006; Warren et al., 2006). Many bird species depend on acoustic signals for communication as they use singing and calling to defend a territory against competing conspecifics and to attract potential mates (Collins, 2004; Catchpole and Slater, 2008). Ambient noise may affect whether birds are heard and whether all information encoded in acoustic details can be recognized (Brumm and Slabbekoorn, 2005). Male birds that are less efficient in deterring competitors or attracting mates acoustically because of masking noise will probably suffer from energetic and reproductive costs. As a consequence, successful breeding in cities and along highways may at least partly depend on whether and how individual birds can adjust their singing behaviour to high levels of anthropogenic noise (Slabbekoorn and Ripmeester, 2008).

Patterns of lower species diversity and breeding density close to roads are rather common (Reijnen and Foppen, 2006) and are probably directly related to a negative impact of anthropogenic noise on bird breeding activities (Bayne et al., 2008; Francis et al., 2009). For example, male ovenbirds (*Seiurus aurocapillus*) of lower quality have to accept territories with more noisy conditions and, independent of individual quality, males in noisy territories suffer from lower mate attraction rates (Habib et al., 2007). Masking noise may also be detrimental to females if they are no longer able to select high-quality mates based on specific acoustic features (Swaddle and Page, 2007) as has been shown in tree frogs (*Hyla ebraccata*) (Wollerman and Wiley, 2002). Such negative consequences of anthropogenic noise for individual reproductive

success are likely to accumulate eventually into negative consequences at the population level.

Avian biodiversity is at stake, especially as a result of homogenization of bird communities in the context of urbanization (Clergeau et al., 2006; McKinney, 2006). Typically, the same set of urban survivors is doing well in urban areas everywhere, irrespective of the original habitat and pre-existing bird community. Although a variety of factors may explain which species do relatively well in cities, the ability to communicate and reproduce under noisy conditions could play a critical role in the success of the urban survivors. It is therefore important to gain insight into the behavioural changes that can help birds to make themselves heard above the city din.

Masking depends on spectral overlap and therefore detrimental effects are most severe for signals of relatively low frequency and for the lower parts of signals of a wide frequency bandwidth. There are several behavioural changes that have been observed in birds that may improve signal efficiency of songs sung under masking pressure from anthropogenic noise. It has been shown for example that urban nightingales respond to higher noise levels by singing louder (Brumm and Todt, 2002) and urban robins at noisy city locations seem to shift temporally towards more nocturnal singing (Füller et al., 2007). Another acoustic change concerns a spectral shift to the use of higher frequency notes that results in masking avoidance from typically low-frequency traffic noise (Slabbekoorn and Peet, 2003).

Several species seem capable of a spectral adjustment of their song away from masking noise. Great tit (*Parus major*) populations in noisy cities were found to sing with higher minimum frequencies on average than populations in more quiet forest locations

(Slabbekoorn and de Boer-Visser, 2006). Furthermore, the same correlation was found at the individual level, with birds in more noisy territories singing higher pitched songs than birds in quieter territories (Slabbekoorn and Peet, 2003). This correlation between noise level and minimum song frequency has now been found for multiple urban species in the context of anthropogenic noise (reviewed in Slabbekoorn and Ripmeester, 2008). There is also a similar example in chaffinches (*Fringilla coelebs*) for natural low-frequency noise from rivers (Brumm and Slater, 2006), which suggests that the flexibility is not a novel adaptation to anthropogenic noise, but rather that some species have evolved the ability under natural noise conditions and as such are pre-adapted to city life.

Although noise-dependent patterns of frequency use are repeatedly found, there is little insight yet into the time scale at which these behavioural changes take place (Brumm and Slabbekoorn, 2005; Patricelli and Blickley, 2006). The associations between frequency use and noise level at the level of individual territories make genetic differentiation over evolutionary time an unlikely explanation. An alternative time scale for spectral modification could be noise-dependent development of the song repertoire over ontogenetic time (Slabbekoorn and Peet, 2003; Slabbekoorn and den Boer-Visser, 2006). An even faster mechanism concerns immediate vocal adjustment based on perceptual feedback. The patterns within and between populations of great tits appear to be due to a tendency of individual males to continue singing song types for longer when they fit current noise conditions well (Halfwerk and Slabbekoorn, 2009). It is currently unclear whether such noise-dependent spectral adjustment through song-type switching is something specific to great tits, or whether other species also have the ability to rapidly modify song frequencies in response to fluctuating noise levels.

In this study we first assessed acoustic divergence in minimum frequency between territorial chiffchaffs (*Phylloscopus collybita* Vieillot 1817) in a transect close to a noisy highway and in a parallel transect away from this highway (riverside). Subsequently, we tested experimentally whether birds in the quiet riverside transect were capable of an immediate spectral shift in response to playback of highway noise recorded at the noisy transect. We found a divergence in minimum frequency between birds along the highway and those along the riverside, with chiffchaffs singing at higher frequencies in the noisy transect. Furthermore, we found experimental evidence for the ability to shift frequency on a highly similar scale in the immediate response to a rise in highway noise. Finally, we checked whether song measurements show noise-dependent errors with a 'playback and re-recording transmission' experiment with the same songs recorded under different noise conditions. We found that minimum frequency measurements tended to be higher when recordings were noisier. However, this effect was not significant for our measurement technique and the scale of the measurement error was small in comparison to the observed spectral shifts in original song recordings.

MATERIALS AND METHODS
Chiffchaff song and noise measurements

The common chiffchaff is a widespread leaf-warbler, breeding in woodland habitat across northern and temperate Eurasia (Helbig et al., 1996). It is a migratory species flying south for the winter to southern Europe, southern Asia and northern Africa. Male chiffchaffs sing a distinctive onomatopoeic song (Fig. 1), consisting of a few introductory notes followed by syllables referred to as 'chiffs' and 'chaffs'. Although birds seem to alternate between relatively high and low frequency syllables, hence their name, this

pattern is not obvious on sonograms. They often sing three or four (not just two) differently shaped syllable types within the series of a song bout, and individuals may have more than four syllable types in their repertoire which they rotate within and across songs. The spectral distribution of sound within syllables often exhibits energy concentrations which may be relatively high or low. However, categorization of syllables into chiffs and chaffs seems not possible without reference to neighbouring syllables in a sequence. There is also no simple declining pattern in frequency use within a song such as is typical for willow warblers (*Phylloscopus trochilus*) (Gil and Slater, 2000).

Singing activity in chiffchaffs in The Netherlands peaks in spring from late March until the end of June. They are active throughout the day, but activity is highest from around sunrise until 2 h after, and in the hour prior to sunset. Fieldwork took place on weekdays between 31st May and 8th June 2007, between 06.00 and 11.00 h. Traffic activity was high throughout the period of observation and recording, with a pronounced peak between 07.00 and 08.00 h. Our study site concerned two transects of woodland on both sides of the wetland area 'De Vlietlanden', located just outside an urban area, between the cities of Leiden and The Hague (52°12′N; 4°46′E). Each transect was about 4 km long, separated by about 1 km of open water, reed beds and some woods. The transects were oriented, parallel to each other, from southwest to northeast. One transect on the southeast side was bordered by a busy highway (named E19 and A4), while the second transect on the northwest side was adjacent to a quiet river and polder landscape, which we labelled 'riverside'. We measured sound pressure levels with a Cesva SC-30 sound analyser (www.cesva.com) at eight points along the highway, and eight points along the riverside, pair-wise with respect to time of day and location. Overall A-weighted decibel [dB(A)]-values and dB-values per octave band of territories along the highway and the riverside were compared with Wilcoxon's rank sum tests in R (R development core team 2008; www.r-project.org).

We identified 11 territories of chiffchaffs in each of the two transects: noisy highway territories within 100 m of the shoulder of the road and quiet riverside territories more than 1 km from the highway. Each territory along the highway corresponded roughly with a territory along the riverside, on a line perpendicular to the highway. We visited the 22 territories in a pair-wise simultaneous fashion, such that two corresponding territories would be recorded on the same day and at the same time. This design reduces possibly confounding variation in singing motivation related to time of day or weather conditions on a particular day. Song recordings were made with a Sennheiser ME67 directional microphone (Almere, The Netherlands) and a Marantz PMD670 digital recorder (www.marantz.com). Recordings were saved as wave files at a 44.1 kHz sampling rate. We visited each territory in the highway transect once and each riverside territory three times, except for one riverside territory, because that singing male was not observed again after the first visit. In both the highway and riverside territories, we collected song recordings ~8–16 m from the focal bird, while precisely pointing our highly directional microphone at the bird. This guaranteed optimal recording quality given the poor noise conditions, which still allowed accurate spectral song measurements.

Experimental noise exposure on the riverside

We collected song recordings at the riverside territories in the same way as on the highway side, but in addition, on a subsequent day, we recorded the same birds while playing back highway noise. The noise stimuli were seven recordings from between 08.00 and 10.00 h (three recordings were used twice) made 25 m from the
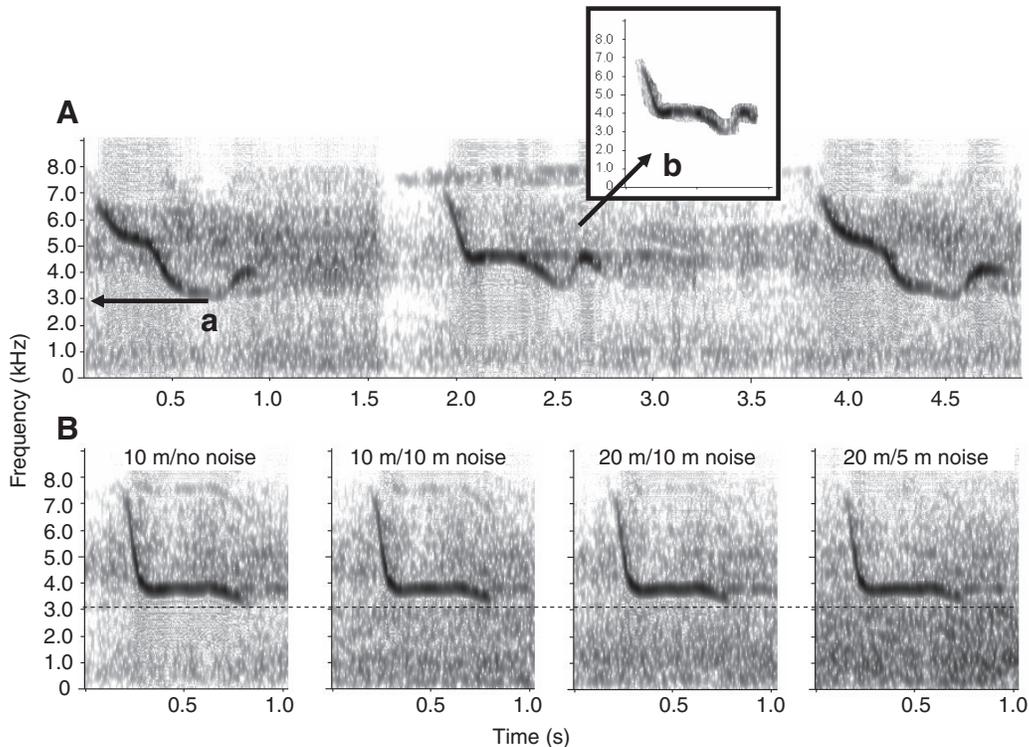
Fig. 1. Sonographic representations of chiffchaff song syllables. (A) A natural sequence of three syllables against a naturally noisy background is depicted in the upper panel. The first and third syllable have the same shape and are referred to as being of the same type. The arrow under the first syllable, labelled 'a', indicates the bottom-end of the visible sound trace for this element, which we refer to as the minimum frequency. The arrow pointing upward to the inset from the second syllable, labelled 'b', indicates the measurement technique used within the acoustic software program Luscinia, version 1.0 (see Materials and methods). The cursor is used to demarcate in detail an area which the investigator considers to circumvent the sound trace to be measured. Subsequently, the program digitally determines the minimum frequency, or any other measurement of interest. (B) The four short sonograms illustrate re-recordings of the very same syllable under four different conditions, with least noise on the left (10 m/no noise), most noise on the right (20 m/5 m noise) and intermediate noise levels in the middle (10 m/10 m noise and 20 m/10 m noise). The syllable is taken from the same syllable sequence as those in the top panel and concerns a third syllable type for this individual. The dashed line helps to check spectral consistency across noise conditions: the minimum frequency of the syllable remains clearly visible, although there is a slight measurement artifact in terms of an upward shift in frequency with more noise on the sonogram (see text).

shoulder of the highway, at locations spread out over the 4 km transect. These recordings were cut into 5 min tracks, played back in a loop, and normalized to equal amplitude with Audacity software (http://audacity.sourceforge.net/). For playback we used a Sansa Express wav-player and a Blaupunkt 100 W speaker. The volume of the playback was adjusted using the Cesva SC-30 sound analyser. We aimed at playing back noise such that it resembled the noise level in a territory within 100 m of the highway, for which pilot measurements indicated a noise level of around 60 dB(A). The speaker was placed 8–16 m from the bird and the volume was set to 86 dB(A) measured 1 m from the speaker to get the desired noise level at the position of the bird of 60–66 dB(A).

Playback of noise started when the bird was singing and lasted until we had recorded at least 10 songs. The next day, we returned to the same location for the third time for a third sample of songs sung at the same time in the morning without playing back noise. Songs were analysed with Luscinia software, version 1.0 (R. F. Lachlan, retrieved from www.lusciniasound.org on 30th June 2007), using a fast Fourier transformation size of 1024, which led to a spectral resolution of 14 Hz and a temporal resolution of 27 ms. We excluded the introductory notes, and selected 10 songs of high recording quality per bird per treatment (one time for the highway transect and three times for the riverside transect: pre-, during and post-noise exposure). We measured the minimum

frequencies of all syllables in the whole song, of which we used the three lowest measurements to assess the minimum frequency per song. We nested these three measurements per song, and the 10 songs per bird, so that the unit of analysis was always the bird. We processed our spectral measurements in this way to reduce the impact of accidental measurement errors and to get a reliable value for low-frequency use. However, the results based on the means of the lowest three measurements were not different from those using measurements of all or just a single (lowest) syllable. We also determined the song duration by counting the number of syllables in a song. All statistical analyses were done in R (R development core team 2008), using linear models fitted with maximum likelihood.

Playback and re-recording transmission experiment
Measurements of minimum frequency may be affected by sonographic grey-scale settings and the amount of noise in the background. Within Luscinia, as in most sound analysis software, the grey-scale is adjusted to the loudest sound in the recording, which is typically the bird song. This was also true for both our highway and riverside recordings, and thus was not a potential cause for measurement bias. Although in the higher frequencies, sonograms were noisy to a variable degree at all locations due to wind and rustling leaves, the highway and experimental exposure recordings

were often relatively noisy in the lower frequencies, potentially affecting the measurement of minimum frequency. Theoretically, we could mistake background noise for bird song and cursor placements would yield a measurement bias towards lower frequencies. Alternatively, in the case that we could not visually discriminate the minimum frequency from the spectrographic trace because of masking noise, this would lead to error in cursor placements causing a measurement bias towards higher frequencies. This artifact would be problematic as this is the direction in which we expect songs to change behaviourally in response to noisy conditions.

Within Luscinia, syllables are measured by demarcating an area by cursor movements within which the subsequent digital measurements will take place on what the investigator considers to be part of the bird song sound trace (Fig. 1A). Consequently, a subjective step of cursor placement by visual inspection of the sonogram by the observer is followed by a more objective step of digital frequency assessment, which also adds some potential error up and down. More accurate or more objective measurement techniques on noisy field recordings such as in the current data set are not possible as computers do not recognize the distinction between bird song and background.

However, in order to test for a noise-dependent measurement error, we conducted a playback and re-recording transmission experiment in which we re-recorded the same songs under different noise conditions. We used a replicate set of eight song recordings from eight riverside territories on quiet days. We played back the songs repeatedly at a constant level of 86 dB(A), measured 1 m from a Visaton SC4ND loudspeaker, which was fixed at a pole at 2.5 m height. Each song was played four times at a unique outdoor location, with some nearby shrubs and trees, in the surroundings of the Sylvius Laboratory, home to the Institute of Biology in Leiden, on 11th and 12th February 2010. The songs were recorded with the same recording equipment as the original recordings without and with experimental noise exposure. The noise was played back through a Blaupunkt 100 W speaker at ground level, also at 86 dB(A) measured 1 m from the speaker. Each song received a unique noise playback loop using one of the seven different highway noise recordings (at two locations we used a different part of the same noise recording).

We kept playback of both song and noise at the same amplitude, to avoid spectral changes related to speaker capacities, but varied noise conditions by adjusting the distance of the microphone from both speakers. We recorded songs at a distance of 10 m without noise (10 m/no noise); at 10 m with noise playback also at 10 m (10 m/10 m noise); at 20 m with noise playback left at 10 m (20 m/10 m noise); and the most noisy condition at 20 m with noise playback at 5 m from the microphone (20 m/5 m noise). This implies that we tested recordings of chiffchaff song amplitude levels at the microphone of about 60 and 66 dB(A), with experimental traffic noise levels at the microphone of about 66 and 73 dB(A). These values reflect realistic song levels and go well beyond the majority of noise levels for original highway and experimental exposure recordings. We measured minimum frequencies of the re-recordings and compared the no-noise condition with the other three more noisy conditions, using the same measurement technique in Luscinia as used for the original song recordings (and done by the same person, M.N.V.). Furthermore, the same measurements were also obtained by on-screen readings from cursor placements by another person (V.R.O.), in Luscinia and in another acoustic software program, PRAAT (http://www.praat.org).

RESULTS

Noise and song differences between highway and riverside

Noise levels were 57.69 ± 1.45 dB(A) for the highway transect and 46.21 ± 1.85 dB(A) for the riverside transect (means ± s.e.). The difference of about 10 dB in overall dB(A) values was significant ($W=59$, $N_{riverside}=8$, $N_{highway}=8$, $P=0.005$), which was also true for similar differences in the separate low-frequency octave bands of 36.5 Hz, 63 Hz, 125 Hz, 500 Hz, 1.0 kHz and 2.0 kHz ($N_{riverside}=8$, $N_{highway}=8$, $P<0.05$), except for the 250 Hz band, which only showed a tendency ($W=50.5$, $N_{riverside}=8$, $N_{highway}=8$, $P=0.06$). The high-frequency octave bands, 4.0 kHz, 8.0 kHz and 16.0 kHz, were only about 3 dB louder along the highway than at the riverside, and variability probably relating to wind and rustling leaves made this difference non-significant ($N_{riverside}=8$, $N_{highway}=8$, $P>0.1$).

Despite the contrast in ambient noise levels, male chiffchaffs sang their typical territorial songs in both transects. There were no obvious differences audible to the human ear, but the minimum frequency of songs in highway territories was significantly higher than the minimum frequency of songs at the riverside ($F_{1,10}=17.1$, $P<0.001$). The mean difference between individuals in territory pairs was 253 ± 61 Hz. Songs also had significantly fewer syllables in the highway territories than in the riverside territories ($F_{1,20}=9.1$, $P=0.007$). The number of syllables in a song correlated with the minimum song frequency, in both highway and riverside recordings (linear regression: $F_{1,189}=50.9$, $P<0.0001$). However, there was a significant shift between the regression lines of the two transects ($F_{1,20}=22.1$, $P<0.001$) (Fig. 2), indicating that songs with the same number of syllables had a lower minimum frequency on the riverside than along the highway.

Experimental noise exposure on the riverside

The first 10 songs recorded during a session before, during and after the experimental exposure, each on different days, revealed spectral differences that were very similar to the differences between highway and riverside territories (Fig. 3A). The mean difference in minimum frequency between songs recorded before and during experimental exposure in the same territories was 296 ± 34 Hz, while the minimum frequency of the songs was 171 ± 35 Hz lower the day after the noise exposure than during the exposure. The number of syllables again significantly correlated with the minimum frequency in a song ($F_{1,160}=13.72$, $P<0.001$) and the regression line was again significantly higher for songs recorded under noisy conditions than for songs recorded the day after ($F_{1,8}=10.71$, $P=0.011$). We used independent contrasts to test whether the minimum frequency differed significantly between: (1) highway and riverside with noise playback ($t_{37}=-0.02$, $P=0.99$); (2) riverside before and after playback ($t_{37}=-1.97$, $P=0.06$); and (3) noisy (highway and playback) and silent conditions (riverside before and after playback) ($t_{37}=5.17$, $P<0.001$) (Fig. 3A). We also used independent contrasts to test whether the number of syllables in a song differed significantly between: (1) highway and riverside with noise playback ($t_{37}=10.37$, $P<0.001$); (2) riverside before and after playback ($t_{37}=-0.49$, $P=0.63$); and (3) highway and all riverside recordings ($t_{37}=3.13$, $P<0.01$) (Fig. 3B). In addition, songs recorded on the quiet riverside during exposure to highway noise had a significantly higher minimum frequency when tested against those recorded on the day after the experiment ($F_{1,8}=11.5$, $P=0.009$ – which is a conservative test as the contrast between exposure and the day before exposure was more pronounced). Songs also had fewer syllables when recorded during noise exposure compared with those recorded the day after ($F_{1,8}=17.9$, $P=0.0028$).
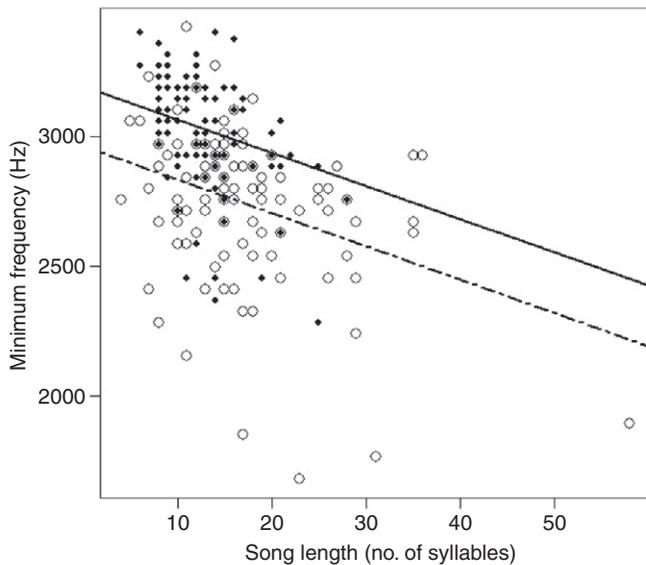
Fig. 2. The relationship between song length and minimum frequency of a song. Songs with more syllables have lower minimum frequencies; however, when comparing songs of equal duration, those recorded in highway territories (solid diamonds, solid line) have significantly higher minimum frequencies than those recorded in riverside territories (open circles, dashed line).

Fig. 3. Chiffchaff song measurements of natural recordings along the highway (N=11) and the riverside transect, the latter without (N=11), with (N=10) and again without (N=10) experimental noise exposure. (A) The mean (±s.e.) minimum frequency. (B) The mean (±s.e.) song length. a and b indicate membership of groups that differ significantly (see text).

Playback and re-recording transmission experiment

The same songs recorded outdoors after playback under different noise conditions remained clearly visible on sonographic representations on the monitor (Fig. 1B). Nevertheless, we also saw slight differences in the appearance of the birdsong sound traces as well as gradual changes in the background noise level, inherent to variability in transmission effects and noise conditions during outdoor recordings. We found small, usually upward, shifts in the minimum frequency of a syllable, depending on the measuring technique. For the Luscinia demarcation method, we detected an upward shift (means ± s.e.) of 11±15 Hz, a downward shift of 16±10 Hz and an upward shift of 22±10 Hz, respectively, for increasingly more noisy recordings (10 m/10 m noise, 20 m/10 m noise and 20 m/5 m noise, all compared with 10 m/no noise). For the more direct cursor measurements in Luscinia the variability was smaller, but the mean shifts were larger: all upward shifts of 22±3 Hz, 32±6 Hz and 49±6 Hz, respectively, for increasingly more noisy conditions. In PRAAT, these values were lower again using the same type of direct cursor measurement: all upward shifts of 4±3 Hz, 22±6 Hz and 29±5 Hz, respectively, for increasingly more noisy conditions. We used paired t-tests to test whether these measurement sets differed significantly between the no-noise and the experimentally raised noise conditions for each technique. None of the differences were significant for the Luscinia demarcation and digital assessment method (used for the current data set) and the PRAAT direct cursor placement method (all tests: N=8; P>0.1). Only for the Luscinia direct cursor placement method were differences significant (all N=8; t=–2.65, P<0.05; t=–2.04, P=0.08; and t=–3.81, P<0.01, respectively).

DISCUSSION

We found a clear noise-dependent frequency use in chiffchaffs, with males singing with higher minimum frequencies alongside a noisy highway compared with males singing alongside a quiet riverside.
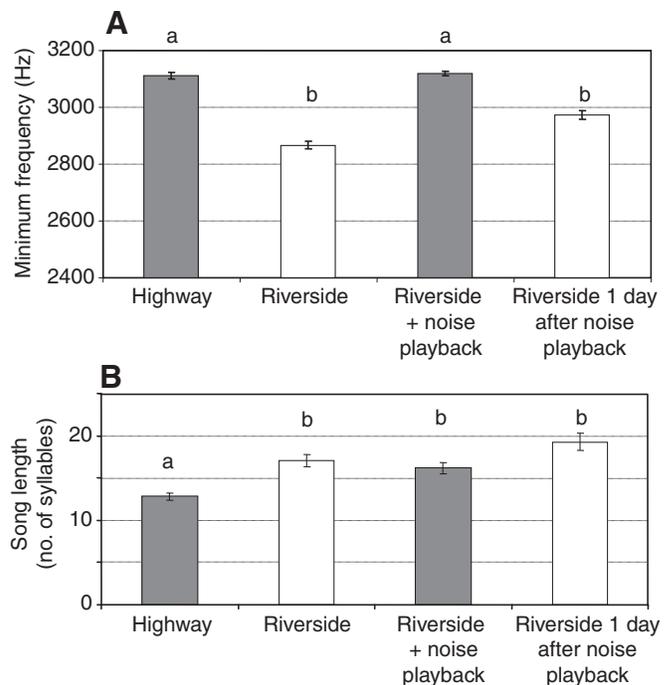
More importantly, by means of experimental noise exposure at the quiet riverside, we were able to show that chiffchaffs can shift in frequency use within a period at least as short as singing a set of 10 songs. There are several examples of bird species making rapid spectral adjustments during social interactions (reviewed in Ripmeester et al., 2007), but we believe this is the first experimental evidence for such an immediate response to fluctuating noise levels. We also provide new insights into a methodological issue; the playback and re-recording transmission experiment showed that it is possible that background noise may affect frequency measurements, depending on the method of measuring. We found upward shifts that were a noise-dependent measurement artifact, but for our measurement technique they were non-significant. The scale of the artifact was also too small to explain the spectral shifts in songs between highway and riverside birds or between recordings from the same territory with and without experimental exposure.

Frequency shifts and behavioural mechanisms

Several bird species that are common in urban areas have been shown to exhibit noise level-dependent minimum frequencies, with higher pitched songs in more noisy locations (for a review, see Slabbekoorn and Ripmeester, 2008). For one of these, the great tit, the actual behavioural mechanism was revealed through noise exposure experiments (Halfwerk and Slabbekoorn, 2009). Male great tits repeatedly sing one song type before switching to another, which may be a song type with a considerably different frequency range. Experimental noise exposure resulted in extended repetition especially of those song types for which masking through spectral overlap was relatively low: this accounted for song types with a high minimum frequency when city noise was played back, but a

similar pattern was found for song types with a low maximum frequency when so-called inverse city noise was played back (Halfwerk and Slabbekoorn, 2009).

The chiffchaff may represent a species with a more common singing style than the specific great tit singing style of song-type switching with a small repertoire of distinct song types (e.g. Franco and Slabbekoorn, 2009). Despite the fact that chiffchaffs cannot use discrete shifts in frequency range by switching song types like great tits, they were able to rapidly move up in their minimum frequency range during experimental exposure with low-pitched traffic noise. They also sang shorter songs in terms of the number of syllables when noise levels were high and this may result in a more restricted frequency use. However, independent of and in addition to this sampling effect, chiffchaff males sang at higher frequencies in noisy conditions, both in the controlled exposure experiment and in the highway territories. The immediate spectral shift may be realized by selectively leaving out those syllables that reach the lowest frequencies or leaving out the bottom part of wide-band notes. However, these speculations go beyond the current data set and more extensive sampling and more detailed analyses are needed for a thorough understanding of the mechanisms underlying the observed frequency changes.

Inherent by-product of the Lombard effect?

The rapid adjustment of the song spectrum in response to noise conditions is a remarkable example of an auditory feedback mechanism. A similar phenomenon has been known for a long time and is taxonomically widespread: the Lombard effect, which involves raising vocal volume with increasing noise levels (Brumm, 2004; Brumm and Slabbekoorn, 2005). Interestingly, when humans raise their voice they also speak at higher frequencies (Titze, 1989). This effect could be due to a general performance constraint that also applies to other taxa. Therefore, we hypothesize that singing at a higher frequency under more noisy conditions may be an inherent by-product of birds singing louder, although it may not explain the whole shift and may not apply to all species. During our previous experimental exposure study (Halfwerk and Slabbekoorn, 2009), great tits may also have sung louder (which was not measured), but those males that continued singing the same song type did not increase any of the note frequencies. Nevertheless, as mentioned above, chiffchaffs have a different style of singing and there is probably another mechanism underlying the noise-dependent spectral shift in their songs. Although it is probable that the chiffchaffs singing at higher frequencies in our study also sang louder in the noisy conditions (again not measured here), we cannot determine yet whether, and to what extent, an amplitude adjustment to noise explains the current findings. Further studies are needed, preferably with birds singing under laboratory conditions in which noise levels can be controlled in detail and song amplitude measurements are more practical.

Adaptive response to fluctuating noise levels?

Independent of the underlying mechanism, it is important to question whether the effect-size in chiffchaffs of about 170–250 Hz with respect to the upward shift, in the frequency range around 3.0 kHz, leads to any benefits for birds in noisy territories. This applies to the general assumption of positive correlations between song frequency and noise level being an adaptive response to fluctuating noise levels (Brumm and Slabbekoorn, 2005; Patricelli and Blickley, 2006; Slabbekoorn and Ripmeester, 2008). Critical for a potentially positive impact on signal efficiency through masking release is the shape of the spectrum in noisy territories (Slabbekoorn and Smith,

2002). Traffic noise spectra typically decline in amplitude with increasing frequency across the entire frequency range [see figure 3 of Halfwerk and Slabbekoorn (Halfwerk and Slabbekoorn, 2009) and figure 3 of Pohl et al. (Pohl et al., 2009)]. This negative correlation reflects a declining potential for masking with increasing frequency. In addition, an increase in overall noise level was found to be related to a steeper decline in amplitude with increasing frequency in the relevant range for urban great tits (Slabbekoorn and Peet, 2003). Consequently, upward shifts in song frequency can be more advantageous in more noisy territories because of the level and spectrum of the potentially masking noise.

In the current study, ambient noise differences between highway and riverside territories were not significant in the 4.0 kHz and the 8.0 kHz octave bands, which cover most of the song frequency range of chiffchaffs. However, the lower limits of chiffchaff songs fall in the 2.0 kHz octave band, which had significantly higher noise levels along the highway than at the riverside. This suggests that for this species too an upward shift in frequency will lead to an improvement in the overall signal-to-noise ratio of the song, especially for birds along the highway. Any inferred increase in signal efficiency caused by masking release, or even just due to a reduction in the energy wasted on frequencies that are not heard properly, could yield a higher breeding success for the individuals singing those songs. At the moment, however, this is just a theory (Rheindt, 2003; Slabbekoorn and Ripmeester, 2008), because there is as yet no clear empirical evidence showing an impact of spectral overlap between traffic noise and birdsong on breeding success within or among species.

Potential for noise exposure experiments

In general, we envisage noise exposure experiments to be a fruitful avenue for future research on the mechanistic basis of vocal adjustments to natural and artificial noise fluctuations, as well as on the detrimental impacts of masking. Hitherto, experimental noise exposure experiments have been used to explore the impact of natural noise conditions in a variety of taxa [e.g. mammals (Sales, 1991; Gillam and McCracken, 2007); birds (Pytte et al., 2003; Leonard and Horn, 2008); and frogs (Penna et al., 2005; Wong et al., 2009)]. The focus of several recent experimental exposure studies has been on testing the impact of anthropogenic noise in particular. Under laboratory conditions, anthropogenic noise has been tested for an impact on sound production (Brumm, 2004; Egnor et al., 2007), but also on sound perception (Lohr et al., 2003; Pohl et al., 2009). Under natural field conditions, as far as we know, only an impact on vocal activity and signal changes has been experimentally explored (Sun and Narins, 2005; Lengagne et al., 2006; Halfwerk and Slabbekoorn, 2009; Parris et al., 2009).

In conclusion, our study reports on the behavioural flexibility of an avian species that is common in places with high anthropogenic noise levels. Acoustic flexibility was revealed both in the correlative pattern between birds in a noisy and quiet transect and in an experimental exposure study. The latter result in particular is novel in methodology and conceptual implications, as it strongly suggests the general ability to rapidly respond to fluctuating noise conditions. Although the underlying causal mechanism may vary, such an immediate spectral shift may also explain correlative patterns in other studies on other species (Slabbekoorn and Ripmeester, 2008; Bermudez-Cuamatzin et al., 2009; Parris and Schneider, 2009; Ripmeester et al., 2010). Without experimental data we are currently unable to ascribe those correlative patterns in other species to an evolutionary, ontogenetic or immediate adjustment of singing (Patricelli and Blickley, 2006) (but see Halfwerk and Slabbekoorn,

2009). Furthermore, despite the growing number of correlative examples and the expanding conceptual framework, we also still lack solid proof of the adaptive value of an increase in song frequency under noisy conditions. Our approach using controlled noise exposure in the field may provide a useful tool for further explorations.

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