bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Song overlapping, ambient noise and territorial aggression in great tits

Çağlar Akçay[1,2*]; Y. Kağan Porsuk[1]; Alican Avşar[3]; Dilan Çabuk[1]; C. Can Bilgin[3]

1. Department of Psychology, Koç University, Istanbul, Turkey
2. Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
3. Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

*corresponding author: akcay.caglar@gmail.com
Çağlar Akçay
Department of Psychology
Koc University
Rumelifeneri Kampusu, Sariyer 34450
Istanbul, Turkey

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Abstract

Animals often communicate with each other in noisy environments where interference from the ambient noise and other signallers may reduce the effectiveness of signals. Signallers also may evolve behaviours to interfere with signals of their opponents, e.g. by temporally overlapping them with their own, such as the song overlapping behaviour that is seen in some songbirds during aggressive interactions. Song overlapping has been proposed to be a signal of aggressive intent although direct evidence for this hypothesis has been lacking. In the present paper we examined the question of whether song overlapping is correlated with aggressive behaviours in a population of great tits (*Parus major*) living in an urban-rural gradient. We also examined whether aggressive behaviours are correlated with the ambient noise levels. We found that overlapping was associated negatively while ambient noise levels were associated positively with aggressive behaviours males displayed against a simulated intruder. Ambient noise levels did not correlate with song rate, song duration or song overlapping. These results suggest that song overlapping of male great tits is not a signal of aggression and great tits in noisy urban habitats may display higher levels of aggression due to either interference of noise in aggressive communication or another indirect effect of noise.

Keywords: song overlapping, urbanization, noise, aggressive signalling, great tits

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Introduction

The environment through which signals are sent and received is open to interference by either sources of noise or other signals traveling through the medium (Bee and Micheyl, 2008; Brumm, 2006; Gil and Brumm, 2014; Wiley, 2006). Signals should therefore evolve to reduce the level of interference from noise and other signals present in the environment (Endler, 1992; Endler and Basolo, 1998; Ey and Fischer, 2009). At the same time, signallers should evolve behaviours that would reduce the interference from noise or other signals, such as adjusting the timing of signalling to minimize overlapping with other signals (Brumm, 2006; Ficken et al., 1974; Gochfeld, 1978; Wasserman, 1977) or acoustic noise (Gil et al., 2014), adjusting the distance to the receivers (Halfwerk et al., 2012).

Generally speaking, signallers tend to space their signals temporally to minimize interference from other signalling individuals (Brumm, 2006; Ficken et al., 1974; Wasserman, 1977; Wilson et al., 2016). In some circumstances, however, actively overlapping the signal of another individual by starting to signal before the other signal has ended, may be a deliberate behaviour used as a signal itself. Indeed, overlapping has been proposed to be a signal of aggressive intent in songbirds (Dabelsteen et al., 1997; Mennill and Ratcliffe, 2004; Naguib and Mennill, 2010). Signals of aggressive intent (also called threat signals) are signals that are not costly to produce but carry information about the likelihood of escalation by the signaller (Caryl, 1979; Searcy et al., 2006). Under this hypothesis, overlapping and therefore interfering with the transmission of an opponent's song may carry information to the opponent that the overlapping bird is ready to escalate an aggressive interaction.

Although song overlapping has been assumed to be an aggressive signal by a large number of studies (Naguib and Mennill, 2010), a review by Searcy and Beecher (2009, 2011) questioned whether song overlapping was a signal at all. Searcy and Beecher (2009) argued that a signalling behaviour should satisfy three empirical criteria to be considered a signal of aggressive intent: 1) the behaviour should occur more often in agonistic interactions than other non-agonistic interactions (context criterion); 2) the behaviour should correlate with other aggressive behaviours or predict a subsequent escalation (predictive criterion) and 3) the behaviour should elicit a differential reaction (either stronger or weaker) in receivers (response criterion).

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Most studies of song overlapping have focused on the perspective of receivers, asking whether birds respond differentially when their songs being overlapped experimentally. These studies generally find changes in singing behaviours such as song rate and duration that are consistent with the singers attempting to avoid the interference due to being overlapped. Changes in aggressive behaviours such as attack or approach due to being overlapped however, are less consistent across studies (Naguib and Mennill, 2010; Searcy and Beecher, 2009).

Very few studies addressed the question whether song overlapping is correlated with aggressive behaviours or subsequent escalation, i.e. the predictive criterion (Baker et al., 2012; Fitzsimmons et al., 2008; van Dongen, 2006; Vehrencamp et al., 2007). Only one of these studies found potential support for overlapping being an aggressive signal: golden whistler males (*Pachycephala pectoralis*) were closer to the speaker when they overlapped the speaker than when they did not (van Dongen, 2006). Two other studies in black-capped chickadees (*Poecile atricapillus*) however, found no correlation between overlapping and concurrent aggressive behaviours or subsequent attack (Baker et al., 2012; Fitzsimmons et al., 2008). Finally a study in banded wrens (*Thryophilus pleurostictus*) found a negative correlation such that overlapping was correlated with lower levels of aggression (Vehrencamp et al., 2007).

In the present study we aim to assess the predictive criterion for song overlapping by examining the relationship between song overlapping and aggressive behaviours in great tits (*Parus major*). Although song overlapping has long been assumed to be an aggressive signal in great tits (Dabelsteen et al., 1996; Langemann et al., 2000; Peake et al., 2001), no previous study has asked whether this behaviour is correlated with the overlapping individual's aggressive behaviours in this species.

A second aim of our study is to ask whether interference due to ambient noise also has an effect on aggressive behaviours. Although noise is a feature of natural habitats as well, the impact of noise in animal communication has garnered special attention due to the increasing levels of anthropogenic noise that affects social behaviour of wildlife (Gil and Brumm, 2014; Johnson and Munshi-South, 2017; Shannon et al., 2016). Anthropogenic noise may make signals less detectable (Kleist et al., 2016; Templeton et al., 2016) or less effective (Halfwerk et al., 2011). In response, animals may change the frequency, amplitude or length characteristics of their signals (Brumm and Todt, 2002; Slabbekoorn and den Boer-Visser,

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

2006; Wood and Yezerinac, 2006) or change their signalling or social behaviours (Halfwerk et al., 2012; Halfwerk and Slabbekoorn, 2009).

If noise interferes with effective signalling during aggressive interactions than we may expect signallers to display higher levels of aggression in noisier habitats. Consistent with this, in several species of songbirds birds living in noisier urban habitats display higher levels of aggression than birds living in rural habitats (Davies and Sewall, 2016; Evans et al., 2010; Fokidis et al., 2011; Foltz et al., 2015; Hardman and Dalesman, 2018). Multiple recent studies found more direct evidence for this hypothesis. In the first study male white-crowned sparrows (*Zonotrichia leucophrys*) holding territories with greater ambient noise displayed higher levels of aggression in response to song playback (Phillips and Derryberry, 2018). In another study, male house wrens (*Troglodytes aedon*) responded to male song playback near the nests with higher levels of aggression (Grabarczyk and Gill, 2019). Another recent study in chiffchaffs (*Phylloscopus collybita*) found that birds that were exposed to airport noise also responded more aggressively to conspecific playbacks compared to birds that were not exposed to noise. In contrast to these studies, a study examining territorial response in chipping sparrows (*Spizella passerina*) and spotted towhees (*Pipilo maculatus*) found that ambient noise was negatively correlated with approach latency to speaker in both of these species (Kleist et al., 2016). This effect may come about if ambient noise makes playbacks less detectable or harder to locate.

Noise also has an effect on singing behaviours, most notably in change of amplitude and frequency (Brumm and Slabbekoorn, 2005; Brumm and Zollinger, 2013), but also song rate (Brumm and Slater, 2006) and song duration (Ríos-Chelén et al., 2013). These changes may be adaptive in terms of ensuring the transmission of the signal to the receivers. Song overlapping may also change as a result of ambient noise: individuals may be less likely to overlap their opponent's songs in noisy environments if ambient noise already interferes with the transmission of the signals. To the best of our knowledge, no previous study examined whether song overlapping varied with ambient noise levels.

In the present study, we ask whether song overlapping and ambient noise levels are associated with territorial aggression in a population of great tits that live in habitats that range from urban to forest. As noted above, overlapping has been proposed to be an aggressive signal but evidence for the predictive criterion has been lacking. Previous research on this species showed that male great tits respond to anthropogenic noise by changing their song and behaviours: for instance, great tits in noisy urban areas sing songs with higher

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

minimum frequencies than great tits in quieter rural areas, which may benefit them as low-frequency songs are more prone to interference by anthropogenic noise (Hamao et al., 2011; Mockford and Marshall, 2009; Slabbekoorn and den Boer-Visser, 2006; Slabbekoorn and Peet, 2003). Urban-living great tits have also been found to be more aggressive than rural-living great tits (Hardman and Dalesman, 2018).

We studied great tits in an urban-forest gradient in Ankara, Turkey. In our population, located on the campus of Middle East Technical University, great tits hold territories throughout built-up areas as well as the forested areas adjoining the populated areas. Background noise in these areas vary significantly depending on proximity to buildings and roads, allowing us to ask whether ambient noise is correlated with aggression. We asked three specific questions: 1) Is song overlapping positively correlated with aggression across males? 2) Does ambient noise on territory predict aggressive behaviours? 3) Is ambient noise correlated with singing behaviours such as song overlapping, song rate, and song duration?

Methods

Study site and subjects

We studied great tits holding territories on the campus of Middle East Technical University, in suburban Ankara, Turkey (39°53'32"N, 32°47'03"E). Although the campus is located in a steppe habitat, it includes a large area that has been afforested mostly with conifers in the last six decades. Great tits nest in human structures on campus (e.g. access ports left open in telephone and electricity poles or cavities on the side of buildings) as well as nest boxes provided in the forested parts and natural cavities spread throughout the campus grounds. We located 42 territories of great tits by observation of singing posts or locating nests in the boxes or human structures. The birds were not captured at any time and therefore were not banded. The trials were carried out between 7 and 13 April 2019 at the start of the nesting period in the morning hours between 0600 and 1130. We avoided testing neighbouring males on the same day and carried out consecutive trials in locations that are outside of earshot of each other.

Playback Stimuli

We recorded male songs from great tits on campus in Spring 2018 and 2019 using a Marantz PMD660 or 661 recorder and a Sennheiser ME66/K6 shotgun microphone. Using the software Syrinx (John Burt, Seattle, WA) we viewed and selected high quality recordings of songs to create playback stimuli. After manually filtering out low-frequency noise (<~1000

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Hz), we added a silent period to create a 7-second wave file. The average (±SD) playback song duration was 2.99 (±0.42) seconds (range= 2.01-3.55 seconds). We created 24 playback stimuli. For each subject we used a stimulus song that was recorded at least 1km away from the subject's territory. This constraint meant we used some stimulus songs multiple times for different males (11 stimuli were used in two trials, 2 stimuli were used in three trials, and one stimulus was used in 4 trials- the rest of the songs were used once).

Procedure

We placed a wireless speaker (Anker SoundCore, Anker, Inc) was placed face-up at a natural perch inside the territory of the male (either 5 m from the nest if the nest was known or at a location that was central to the various singing posts the male was observed to be singing). We then set up flagging at 1m, 3m and 5m from the speaker on either side of the speaker to help with distance estimation during the trial. The observers then stepped back to about 15 m from the speaker. The playbacks were controlled from a smartphone connected to the speaker via Bluetooth.

The trials were recorded with the same equipment as above. We started the trials by playing back the stimulus song until the subject responded either by singing a song or approaching the playback. After this first response, we continued the playback for another 3 minutes and we narrated the behaviour of the subject by noting each flight, distance to the speaker and singing behaviours. The behaviours during this 3-minute period are the main response variables of the study.

Noise measurements

After the trial, we removed the flagging, took a GPS reading (Garmin eTrek, Garmin Inc.) of the trial location and carried out the ambient noise measurements using the method described by Brumm (2004). Briefly, we took two measurements in each cardinal direction at the location of the playback using a sound level meter (VLIKE VL6708, VLIKE Inc.) with A weighting and fast response (125 msec) settings. In a subset of territories (n=32 out of 42) we took a second measurement of ambient noise later in the day to assess how consistent variation in the ambient noise was. For logistical reasons we could not take second noise measurements in 10 territories. The first and second noise measurements were highly correlated with each other (linear regression: β=0.94, SE= 0.17, t=5.46, p= 6.4 x 10^{-6}). We used the first noise measurement in the analyses as this measurement was available for all territories.

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

198 *Response measures*

199 Viewing the trial recordings in Syrinx, we extracted the following behaviours: flights,
200 distance to speaker with each flight, and songs. Because the trial durations slightly varied
201 across trials (ranging between 179 seconds to 192 seconds), we converted the counts of
202 flights and songs into rates by dividing it with the trial duration. We also calculated the
203 proportion of the trial subjects spent within 1 m of the speaker and noted the closest approach
204 of the distance. For each song the subject sang, we determined whether the song overlapped
205 with the playback song and calculated the proportion of the songs that the subject sang that
206 overlapped the playback song. This measure therefore corrects for variation in song rate, but
207 it is undefined for subjects that did not sing any songs (n=7). Because of variation in duty
208 cycle (duration of stimulus with song/entire duration of stimulus) between stimulus songs, we
209 also classified subjects based on whether the observed song overlapping (as defined above)
210 was higher (n=11) or lower (n=24) than the duty cycle. We classified the subjects who did
211 not sing any songs as a third category in this variable. Finally, for each subject we measured
212 duration of each of their song and calculated the average song duration for each subject.

213 *Data Analysis*

214 The rates of flights, closest approach distance and proportion of time spent within 1m of
215 speaker were all highly correlated with each other (Table 1). We therefore used a principle
216 component analysis (PCA) to arrive at a single measure of aggressive response. The first
217 component of the PCA (PCA1) explained 68% of variation and was taken as the aggressive
218 score. The first component was positively correlated with flight rates and time spent within
219 1m, and negatively correlated with closest approach distance (Table 1).

220 We first ran two linear mixed models (LMM) with aggression scores (PCA1) as the
221 response variable and stimulus song as a random factor. In the first model we entered the
222 ambient noise levels measured after the trial as the fixed factor while in the second model we
223 entered song overlapping as the fixed factor. We ran two separate models because song
224 overlapping (as a proportion of subject songs that overlapped) was not defined for 7 subjects
225 who did not sing any songs during the trial, thus reducing the sample size.

226 We then ran a parallel LMM to the first set of models with aggression scores again as
227 response variable and stimulus song as random factor. In this model we entered ambient
228 noise levels and the categorical overlapping variable as the predictor. This variable had three
229 categories high-overlapping (overlapping rates higher than the stimulus duty cycle), low-

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

overlapping (overlapping rates lower than the stimulus duty cycle) or no song. This model included all subjects.

We additionally asked whether ambient noise was related to singing behaviours. To answer that question we ran LMMs on song rate, song overlapping and average song duration as response variables, and ambient noise levels as predictor variables, with stimulus song as a random factor, to determine whether ambient noise had an effect on other singing behaviours. Finally, we compared the observed level of song overlapping with the expected levels (represented by the duty cycle), by carrying out a paired t-test. The analyses were carried out in R using the package nlme (Pinheiro et al., 2017; R Core Team, 2012).

Results

Contrary to the hypothesis that song overlapping is a signal of aggressive intent, overlapping (proportion of subject songs that overlapped the playback songs) was negatively related to the aggression scores: high overlapping was indicative of lower aggression scores (Table 2, Figure 1). Ambient noise was positively related to aggression scores: birds in noisier territories were more aggressive in response to the playback (Table 2, Figure 2). In the model with the categorical overlapping variable (Table 3), both overlapping and ambient noise significantly predicted attack: birds that overlapped the playback less than expected were more aggressive compared to high-overlapping birds (unpaired t test: $t(33)=2.78$, $p=0.0088$). The most aggressive birds were birds who did not sing any songs who were more aggressive than low-overlapping birds (unpaired t-test: $t(29)=2.10$, $p=0.044$; Figure 3).

Ambient noise was not related to song overlapping, song rate or average song duration (Table 4). The observed levels of overlapping (mean ± SD: 0.33 ± 0.29) was lower than the duty cycle of stimulus songs (0.42 ± 0.06) although not significantly so; unpaired t-test unequal variances: $t(34)=-1.77$, $p=0.085$.

Discussion

In this study we asked whether song overlapping and ambient noise was related to territorial aggression in male great tits in a population that spans an urban-rural gradient. We found that song overlapping was negatively related to aggressive behaviours suggesting that high levels of overlapping is not a signal males use to threaten opponents. We also found that ambient noise levels were positively related to aggression scores, providing support for the hypothesis that high ambient noise levels may select for higher aggression levels. Nonetheless, ambient

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

noise levels were not related to singing behaviours and parameters such as song rate, duration or overlapping.

Is song overlapping an aggressive signal?

The present results suggest that song overlapping is not necessarily a reliable signal of aggressive intent in great tits. Our finding of a negative correlation between aggression and song overlapping is similar to what Vehrencamp and colleagues (2007) found in banded wrens. In another species, the black-capped chickadees, song overlapping was not associated with aggressive behaviours or attack (Baker et al., 2012). Taken together, these results imply that song overlapping does not necessarily carry information regarding high likelihood of escalation to the receivers although it remains to be seen whether the negative conclusion will apply generally to other species. Additionally, song overlapping tended to happen slightly lower than expected based on the duty cycle of stimulus songs but this difference was not significant, indicating that overlapping may not be a deliberate behaviour in any case.

It is worth noting that there is some debate recently about what the correct null expectation should be in studies of song overlapping (Masco et al., 2016; Naguib and Mennill, 2010; Searcy and Beecher, 2009, 2011). We used stimulus duty cycle (the ratio of the stimulus song duration over the sum of the stimulus song duration and silent period between two stimulus songs) as the expected levels simply because it does not rely on any additional assumptions. Choosing a different "null" model, with varying assumptions, may yield a result indicating a significant difference between expected and observed levels of overlapping (Masco et al., 2016). Nonetheless, whatever the null model, if song overlapping is a deliberate behaviour that individuals use to signal their intent to escalate, individual variation in song overlapping should correlate positively with aggressive behaviours.

If song overlapping is not an aggressive signal in great tits, what does it indicate? One possible answer is that high levels of overlapping may simply indicate that the overlapping bird is not engaged with the intruder, whereas low levels of overlapping may indicate a closer engagement. In fact, birds who approached the speaker more closely and flew around the speaker more in search of the opponent may be avoiding overlapping the opponent as an assessment strategy (Arnott and Elwood, 2009). Great tit song has been found to contain information regarding the singer's traits such as age and condition (Rivera-Gutierrez et al., 2010). Thus, it may be advantageous for receivers that intend to engage the intruder at close range to listen carefully to the song features of the opponent. This listening strategy may

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

explain the low rates of overlapping among aggressive individuals as well as the fact that birds that do not sing are the most aggressive group.

It is also possible that non-overlapping the intruder may simply be due to a search strategy to locate the opponent acoustically while the opponent is singing. Under this hypothesis, the males are avoiding overlapping the intruder because they are trying to figure out where the intruder is. Indeed, singing while the opponent is also singing is likely to interfere with the processing of spatial information that can be obtained from the song of the intruder. If that is the case, then birds that are aggressively responding to their opponents and therefore more motivated to locate them would be less likely to overlap their opponent's songs. This hypothesis is essentially an extension of the "readiness hypothesis" that was proposed for explaining the occurrence of low amplitude "soft" songs that is used as a signal of aggressive intent in several songbirds (Akçay and Beecher, 2012; Akçay et al., 2011). The readiness hypothesis posits that for soft songs, the low amplitude is simply a by-product of the fact that the beak and body movements required to sing loudly might interfere with visual tracking of an opponent. Therefore, in an aggressive interaction birds that intend to escalate might be signing with their beak closed, which leads to lower amplitudes (Akçay and Beecher, 2012; Goller et al., 2004). Similarly, singing while the intruder is also singing (i.e. overlapping) may interfere with localizing of the opponent acoustically and birds that intend to escalate the interaction may therefore avoid overlapping the opponent's song. This hypothesis can be tested with an experiment in which the simulated intruder can be either located by the subject either easily or with difficulty.

Another possibility is that overlapping, instead of being a signal of aggressive intent, may be correlated with more permanent traits of the signallers, such as some aspect of genetic quality (Helfer and Osiejuk, 2015). In their recent review, Helfer and Osiejuk (2015) presented several hypotheses to that positing that song overlapping is a signal of male quality due to some a physical or physiological constraint or a handicapping mechanism. However, given that song overlapping is a rather flexible behaviour that primarily depends on the chosen timing of the singing it seems unlikely that it would be subject to a physical or physiological constraint or handicapping mechanism. Interestingly, a study on great tits found that great tits that were exposed to an ectoparasite as nestlings were found to be less likely to overlap the song playback carried out in the territory after they were recruited to the population (Bischoff et al., 2009). Song overlapping however, was not correlated with the condition of the male as a nestling, and there was only a trend towards a positive association

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

between overlap and male condition as an adult. Thus, although these data suggest a potential developmental constraint on song overlapping, the mechanism is unclear. Bischoff et al. (2009) do not present any information on aggressive behaviours such as approach, thus it is not clear whether the developmental handicap was associated with aggressive behaviours as well. In summary, while it remains possible that overlapping reflects either male quality, condition or developmental history, more data are clearly needed to test these possibilities.

Noise and aggressive behaviours

Ambient noise was positively correlated with aggressive behaviours in the present study, consistent with the hypothesis that noise may play a significant role in determining aggressive phenotypes observed in urban populations of birds (Davies and Sewall, 2016; Evans et al., 2010; Hardman and Dalesman, 2018). The effect of noise on aggression may come about via multiple mechanisms. First, noise may disrupt effective communication (McMullen et al., 2014; Ríos-Chelén et al., 2015). When effective communication is disrupted, aggressive behaviours may increase in prevalence (Kareklas et al., 2019; Logue et al., 2010). Interestingly, unlike other species (Brumm and Slater, 2006; Ríos-Chelén et al., 2013) great tits in our population did not show any evidence that they change their song rate, average song duration or overlapping depending on ambient noise levels. Given that previous research showed that songs in great tits and other songbirds become less effective in urban noise (Halfwerk et al., 2011; Luther et al., 2015), this finding suggests communication may indeed be hampered in noisier territories.

A second, non-mutually exclusive possibility is that noise may lead to higher levels of physiological arousal or stress which may increase aggression, as has been documented in humans (Baron and Richardson, 2004). In particular, exposure to noise may elevate baseline levels of glucocorticoids (Creel et al., 2002; Crino et al., 2011; Davies et al., 2017; but see Kleist et al., 2018) which in turn may lead to a higher level of aggression in individuals living in noisier areas. Interestingly, in a recent study on urban and rural song sparrows (*Melospiza melodia*), Davies and colleagues (2018) found that baseline levels of corticosterone (the main avian glucocorticoid) in blood samples after a simulated territorial intrusion did not differ between urban and rural birds, and the levels of corticosterone were positively related to territorial aggression males displayed. For a given level of plasma corticosterone however, urban birds displayed more aggression than rural birds, suggesting that urban birds potentially show differences in glucocorticoid signalling from rural birds. Thus, the exact physiological mechanisms that determines the relationship between urbanization and noise on

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

the one hand, and aggression on the other is yet unclear (Bonier, 2012). Further experimental studies are needed to quantify the effect of noise on plasticity at behavioural and physiological levels to tests hypotheses on the physiological mechanisms of aggression under noisy conditions.

Conclusion

In summary our findings on song overlapping suggest that this signal is unlikely to be a signal of aggressive intent, and researchers should pursue new hypotheses for this interesting behaviour. Our findings on ambient noise meanwhile, corroborate the recent studies linking noise and aggression and suggest new avenues to investigate the effect of anthropogenic noise on animal social behaviour and the physiological mechanisms underlying it. Together, the current study highlights how animals may manage interference from noise or other signallers in their social interactions.

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

References

Akçay Ç, Beecher MD, 2012. Signalling while fighting: further comments on soft song. Anim Behav 83:e1-e3.

Akçay Ç, Tom ME, Holmes D, Campbell SE, Beecher MD, 2011. Sing softly and carry a big stick: signals of aggressive intent in song sparrows. Anim Behav 82:377- 382.

Arnott G, Elwood RW, 2009. Assessment of fighting ability in animal contests. Anim Behav 77:991-1004.

Baker TM, Wilson DR, Mennill DJ, 2012. Vocal signals predict attack during aggressive interactions in black-capped chickadees. Anim Behav 84:965-974.

Baron RA, Richardson DR, 2004. Human aggression: Springer Science & Business Media.

Bee MA, Micheyl C, 2008. The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it? Journal of comparative psychology 122:235.

Bischoff LL, Tschirren B, Richner H, 2009. Long-term effects of early parasite exposure on song duration and singing strategy in great tits. Behav Ecol 20:265-270. doi: 10.1093/beheco/arp012.

Bonier F, 2012. Hormones in the city: endocrine ecology of urban birds. Horm Behav 61:763-772.

Brumm H, 2004. The impact of environmental noise on song amplitude in a territorial bird. J Anim Ecol 73:434-440. doi: doi:10.1111/j.0021-8790.2004.00814.x.

Brumm H, 2006. Signalling through acoustic windows: nightingales avoid interspecific competition by short-term adjustment of song timing. Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology 192:1279-1285.

Brumm H, Slabbekoorn H, 2005. Acoustic communication in noise. Advances in the Study of Behavior 35:151-209.

Brumm H, Slater P, 2006. Ambient noise, motor fatigue, and serial redundancy in chaffinch song. Behav Ecol Sociobiol 60:475-481.

Brumm H, Todt D, 2002. Noise-dependent song amplitude regulation in a territorial songbird. Anim Behav 63:891-897.

Brumm H, Zollinger SA, 2013. Avian vocal production in noise. Animal communication and noise: Springer. p. 187-227.

Caryl PG, 1979. Communication by agonistic displays: what can game theory contribute to ethology? Behaviour 68:136-169.

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

404 Creel S, Fox JE, Hardy A, Sands J, Garrott B, Peterson RO, 2002. Snowmobile activity and
405 glucocorticoid stress responses in wolves and elk. Conserv Biol 16:809-814.
406 Crino O, Van Oorschot BK, Johnson E, Malisch J, Breuner C, 2011. Proximity to a high traffic
407 road: glucocorticoid and life history consequences for nestling white-crowned
408 sparrows. Gen Comp Endocrinol 173:323-332.
409 Dabelsteen T, McGregor PK, Holland J, Tobias JA, Pedersen SB, 1997. The signal function of
410 overlapping singing in male robins. Anim Behav 53:249-256.
411 Dabelsteen T, McGregor PK, Shepherd M, Whittaker X, Pedersen SB, 1996. Is the Signal
412 Value of Overlapping Different from That of Alternating during Matched Singing in
413 Great Tits? J Avian Biol 27:189-194. doi: 10.2307/3677221.
414 Davies S, Beck ML, Sewall KB, 2018. Territorial aggression in urban and rural Song Sparrows
415 is correlated with corticosterone, but not testosterone. Horm Behav 98:8-15. doi:
416 https://doi.org/10.1016/j.yhbeh.2017.11.010.
417 Davies S, Haddad N, Ouyang JQ, 2017. Stressful city sounds: glucocorticoid responses to
418 experimental traffic noise are environmentally dependent. Biol Lett 13:20170276. doi:
419 doi:10.1098/rsbl.2017.0276.
420 Davies S, Sewall KB, 2016. Agonistic urban birds: elevated territorial aggression of urban song
421 sparrows is individually consistent within a breeding period. Biol Lett 12:20160315.
422 Endler JA, 1992. Signals, signal conditions, and the direction of evolution. The American
423 Naturalist 139:S125-S153.
424 Endler JA, Basolo AL, 1998. Sensory ecology, receiver biases and sexual selection. Trends
425 Ecol Evol 13:415-420.
426 Evans J, Boudreau K, Hyman J, 2010. Behavioural Syndromes in Urban and Rural Populations
427 of Song Sparrows. Ethology 116:588-595. doi: 10.1111/j.1439-0310.2010.01771.x.
428 Ey E, Fischer J, 2009. The “acoustic adaptation hypothesis”—a review of the evidence from
429 birds, anurans and mammals. Bioacoustics 19:21-48.
430 Ficken RW, Ficken MS, Hailman JP, 1974. Temporal pattern shifts to avoid acoustic
431 interference in singing birds. Science 183:762-763.
432 Fitzsimmons LP, Foote JR, Ratcliffe LM, Mennill DJ, 2008. Frequency matching, overlapping
433 and movement behaviour in diurnal countersinging interactions of black-capped
434 chickadees. Anim Behav 75:1913-1920.
435 Fokidis HB, Orchinik M, Deviche P, 2011. Context-specific territorial behavior in urban birds:
436 no evidence for involvement of testosterone or corticosterone. Horm Behav 59:133-
437 143.

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

438 Foltz SL, Ross AE, Laing BT, Rock RP, Battle KE, Moore IT, 2015. Get off my lawn:
439 increased aggression in urban song sparrows is related to resource availability. Behav
440 Ecol 26:1548-1557.
441 Gil D, Brumm H, 2014. Acoustic communication in the urban environment: patterns,
442 mechanisms, and potential consequences of avian song adjustments. In: Gil D, Brumm
443 H, editors. Avian Urban Ecology Oxford: Oxford University Press.
444 Gil D, Honarmand M, Pascual J, Pérez-Mena E, Macías Garcia C, 2014. Birds living near
445 airports advance their dawn chorus and reduce overlap with aircraft noise. Behav Ecol
446 26:435-443.
447 Gochfeld M, 1978. Intraspecific Social Stimulation and Temporal Displacement of Songs of
448 the Lesser Skylark, Alauda gulgula. Zeitschrift für Tierpsychologie 48:337-344. doi:
449 10.1111/j.1439-0310.1978.tb00264.x.
450 Goller F, Mallinckrodt MJ, Torti SD, 2004. Beak gape dynamics during song in zebra finch. J
451 Neurobiol 59:289-303.
452 Grabarczyk EE, Gill SA, 2019. Anthropogenic noise affects male house wren response to but
453 not detection of territorial intruders. PLOS ONE 14:e0220576. doi:
454 10.1371/journal.pone.0220576.
455 Halfwerk W, Bot S, Buikx J, van der Velde M, Komdeur J, ten Cate C, Slabbekoorn H, 2011.
456 Low-frequency songs lose their potency in noisy urban conditions. Proceedings of the
457 National Academy of Sciences 108:14549-14554.
458 Halfwerk W, Bot S, Slabbekoorn H, 2012. Male great tit song perch selection in response to
459 noise-dependent female feedback. Funct Ecol 26:1339-1347.
460 Halfwerk W, Slabbekoorn H, 2009. A behavioural mechanism explaining noise-dependent
461 frequency use in urban birdsong. Anim Behav 78:1301-1307.
462 Hamao S, Watanabe M, Mori Y, 2011. Urban noise and male density affect songs in the great
463 tit Parus major. Ethol Ecol Evol 23:111-119.
464 Hardman SI, Dalesman S, 2018. Repeatability and degree of territorial aggression differs
465 among urban and rural great tits (Parus major). Scientific Reports 8:5042.
466 Helfer B, Osiejuk TS, 2015. It takes all kinds in acoustic communication: a new perspective
467 on the song overlapping phenomenon. Ethology 121:315-326.
468 Johnson MT, Munshi-South J, 2017. Evolution of life in urban environments. Science
469 358:eaam8327.

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Kareklas K, Wilson J, Kunc HP, Arnott G, 2019. Signal complexity communicates aggressive intent during contests, but the process is disrupted by noise. Biol Lett 15:20180841. doi: doi:10.1098/rsbl.2018.0841.

Kleist NJ, Guralnick RP, Cruz A, Francis CD, 2016. Anthropogenic noise weakens territorial response to intruder's songs. Ecosphere 7:e01259.

Kleist NJ, Guralnick RP, Cruz A, Lowry CA, Francis CD, 2018. Chronic anthropogenic noise disrupts glucocorticoid signaling and has multiple effects on fitness in an avian community. Proceedings of the National Academy of Sciences 115:E648-E657.

Langemann U, Tavares JP, Peake TM, McGregor PK, 2000. Response of great tits to escalating patterns of playback. Behaviour 137:451-471.

Logue DM, Abiola IO, Rains D, Bailey NW, Zuk M, Cade WH, 2010. Does signalling mitigate the cost of agonistic interactions? A test in a cricket that has lost its song. Proceedings of Royal Society of London, B doi:10.1098/rspb.2010.0421.

Luther DA, Phillips J, Derryberry EP, 2015. Not so sexy in the city: urban birds adjust songs to noise but compromise vocal performance. Behav Ecol 27:332-340.

Masco C, Allesina S, Mennill DJ, Pruett-Jones S, 2016. The Song Overlap Null model Generator (SONG): a new tool for distinguishing between random and non-random song overlap. Bioacoustics 25:29-40.

McMullen H, Schmidt R, Kunc HP, 2014. Anthropogenic noise affects vocal interactions. Behav Processes 103:125-128. doi: https://doi.org/10.1016/j.beproc.2013.12.001.

Mennill DJ, Ratcliffe LM, 2004. Overlapping and matching in the song contests of black-capped chickadees. Anim Behav 67:441-450.

Mockford EJ, Marshall RC, 2009. Effects of urban noise on song and response behaviour in great tits. Proceedings of the Royal Society of London B: Biological Sciences:rspb20090586.

Naguib M, Mennill DJ, 2010. The signal value of birdsong: empirical evidence suggests song overlapping is a signal. Anim Behav 80:e11-e15.

Peake TM, Terry AM, McGregor PK, Dabelsteen T, 2001. Male great tits eavesdrop on simulated male-to-male vocal interactions. Proc R Soc Lond, Ser B: Biol Sci 268:1183-1187.

Phillips JN, Derryberry EP, 2018. Urban sparrows respond to a sexually selected trait with increased aggression in noise. Scientific Reports 8:7505. doi: 10.1038/s41598-018-25834-6.

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

503 Pinheiro J, Bates D, DebRoy S, Sarkar D, Heisterkamp S, Van Willigen B, Maintainer R, 2017.
504 Package 'nlme'. R package:3-1.
505 R Core Team, 2012. R: A language and environment for statistical computing. Vienna, Austria:
506 R Foundation for Statistical Computing.
507 Ríos-Chelén AA, Lee GC, Patricelli GL, 2015. Anthropogenic noise is associated with changes
508 in acoustic but not visual signals in red-winged blackbirds. Behav Ecol Sociobiol
509 69:1139-1151.
510 Ríos-Chelén AA, Quirós-Guerrero E, Gil D, Garcia CM, 2013. Dealing with urban noise:
511 vermilion flycatchers sing longer songs in noisier territories. Behav Ecol Sociobiol
512 67:145-152.
513 Rivera-Gutierrez HF, Pinxten R, Eens M, 2010. Multiple signals for multiple messages: great
514 tit,< i> Parus major</i>, song signals age and survival. Anim Behav 80:451-459.
515 Searcy WA, Anderson RC, Nowicki S, 2006. Bird song as a signal of aggressive intent. Behav
516 Ecol Sociobiol 60:234-241.
517 Searcy WA, Beecher MD, 2009. Song as an aggressive signal in songbirds. Anim Behav
518 78:1281-1292.
519 Searcy WA, Beecher MD, 2011. Continued scepticism that song overlapping is a signal. Anim
520 Behav 81:e1-e4.
521 Shannon G, McKenna MF, Angeloni LM, Crooks KR, Fristrup KM, Brown E, Warner KA,
522 Nelson MD, White C, Briggs J, 2016. A synthesis of two decades of research
523 documenting the effects of noise on wildlife. Biological Reviews 91:982-1005.
524 Slabbekoorn H, den Boer-Visser A, 2006. Cities change the songs of birds. Curr Biol 16:2326-
525 2331.
526 Slabbekoorn H, Peet M, 2003. Ecology: Birds sing at a higher pitch in urban noise. Nature
527 424:267.
528 Templeton CN, Zollinger SA, Brumm H, 2016. Traffic noise drowns out great tit alarm calls.
529 Curr Biol 26:R1173-R1174.
530 van Dongen WF, 2006. Variation in singing behaviour reveals possible functions of song in
531 male golden whistlers. Behaviour 143:57-82.
532 Vehrencamp SL, Hall ML, Bohman ER, Depeine CD, Dalziell AH, 2007. Song matching,
533 overlapping, and switching in the banded wren: the sender's perspective. Behav Ecol
534 18:849-859.
535 Wasserman FE, 1977. Intraspecific acoustical interference in the white-throated sparrow
536 (Zonotrichia albicollis). Anim Behav 25:949-952.

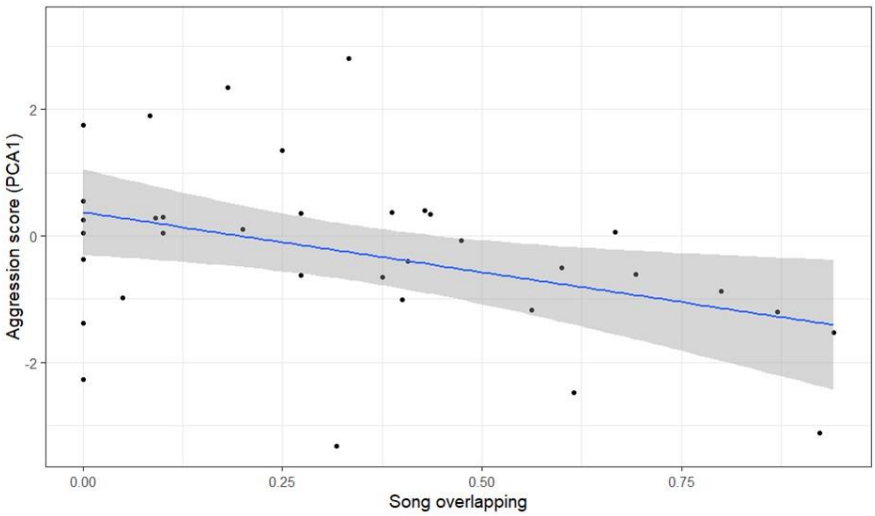
bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

537 Wiley RH, 2006. Signal detection and animal communication. Advances in the study of
538 behavior 36:217-247.
539 Wilson DR, Ratcliffe LM, Mennill DJ, 2016. Black-capped chickadees, Poecile atricapillus,
540 avoid song overlapping: Evidence for the acoustic interference hypothesis. Anim Behav
541 114:219-229.
542 Wood WE, Yezerinac SM, 2006. Song sparrow (Melospiza melodia) song varies with urban
543 noise. Auk 123:650-659.
544
545

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

546 **Figures.**

547 Figure 1. Scatterplot of song overlapping (proportion of the subject's songs that started while
548 the playback song was playing), and aggression scores (PCA 1).



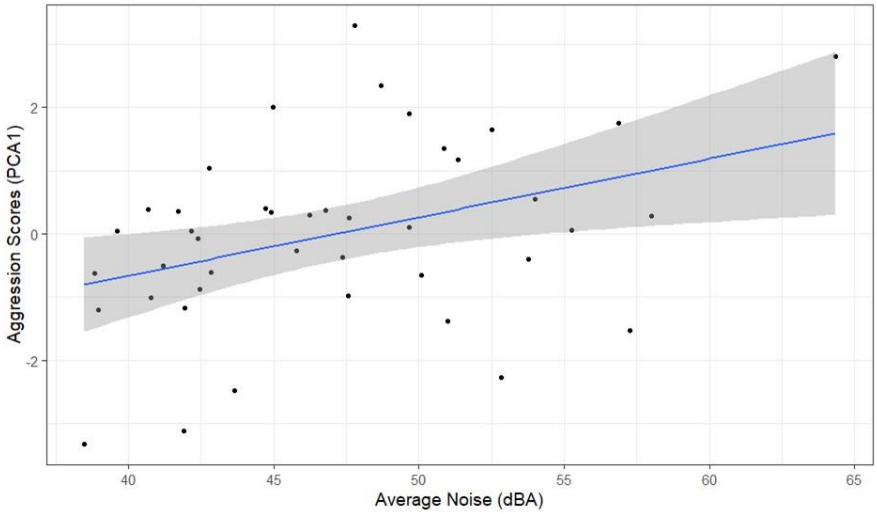
549

550

551

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

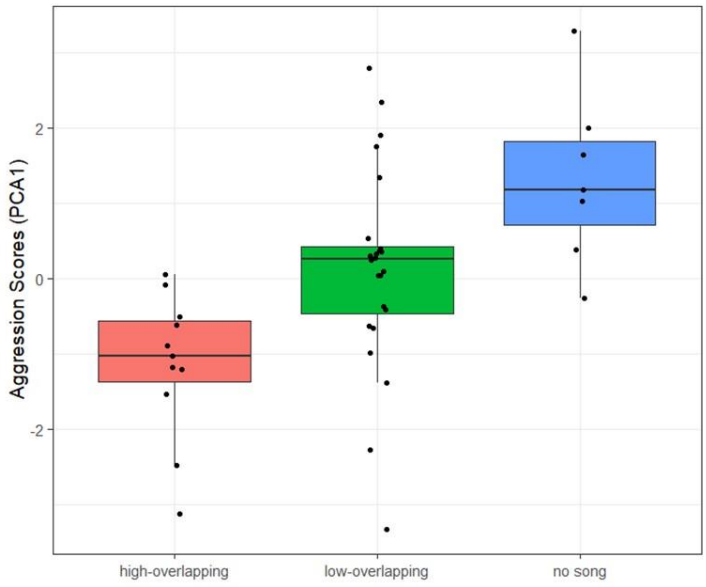
552 Figure 2. Scatterplot of ambient noise levels (average of 8 measurements with A settings, dB)
553 and aggression scores (PCA1)



554
555

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

556 Figure 3. Aggression scores of subjects a) overlapping the playback songs at more than the
557 duty cycle (n=11), less than the duty cycle (n=24) , singing no song (n=7).



558
559

bioRxiv preprint doi: https://doi.org/10.1101/808733. this version posted October 17, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. It is made available under a CC-BY-NC 4.0 International license.

Table 1: Pearson correlation coefficients (p-values) for the correlation between the aggressive response variables and their loadings to the first component of principle component analysis (PCA).

	Flight rate	**Proportion of trial spent within 1m**	**Closest approach**
Flight rate	_	0.60 ($2x10^{-5}$)	-0.57 ($7x10^{-5}$)
Proportion of trial spent within 1m	_	_	-0.42 (0.005)
Loading Coefficient	0.614	0.564	-0.551

Table 2. Linear mixed models (LMM) with stimulus song as random factor and aggression scores as response variable. The first model has song overlapping as the fixed factor (n=35 subjects, 7 subjects are excluded because they did not sing a song, and proportion of their songs that overlapped the stimulus song was therefore undefined). The second model has ambient noise as the fixed factor (n=42 subjects).

Model	**Coefficient (SE)**	χ^2	**p-value**
Song overlapping	-1.89 (0.76)	6.27	0.012
Ambient noise	0.09 (0.035)	6.94	0.0083

Table 3. Linear mixed model on aggression scores with the categorical overlap variable and ambient noise as predictor variables. Stimulus song is a random factor.

	χ^2	**p-value**
Overlap	16.41	0.00027
Ambient noise	6.01	0.0083

Table 4. Linear mixed models on song rate, average song duration and song overlapping as response variables and ambient noise as predictor variable. Stimulus song is a random factor in all models.

Model	χ^2	**p-value**
Song rate	0.063	0.80
Song duration	0.69	0.79
Song overlapping	2.18	0.13