

Anthropogenic noise disrupts mate choice behaviors in female *Gryllus bimaculatus*

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Data Accessibility

Analyses reported in this article can be reproduced using the data published by Bent, Ings & Mowles (2020).

Conflict of interest

We have no competing interests.

Author Contributions

A.M.B. and S.L.M. conceived and designed the study; A.M.B. conducted the experiments and statistical analysis with input from S.L.M. and T.C.I. All authors wrote the manuscript.

Ethics

There are no legal requirements for studies involving orthopteran research subjects in the United Kingdom and Northern Ireland. The subject and methods of this research were approved by Anglia Ruskin University, Faculty of Science and Engineering, departmental research ethics panel committee.

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Abstract

By assessing the sexual signals produced by conspecifics, individuals can make informed decisions on the best choice of mate, which can lead to reproductive fitness benefits. However, these communication systems are often vulnerable to disruption by conflicting with stimuli present in the environment. Anthropogenic noise may act as one such disruptive stimulus, leading to inefficient mate choice decisions, and thus reductions to an animal's fitness. In this study, the mate choice behaviors of female *Gryllus bimaculatus* were tested when presented with artificial male courtship songs of differing 'quality' under different acoustic conditions. In ambient noise conditions, females significantly preferred mates paired with higher quality songs, indicated by increased mating rates and reduced latency to mate. However, this mate selection pattern was disrupted in both traffic and white noise conditions. Additionally, 'high quality' courtship songs had an increased mounting latency in traffic and white noise conditions, when compared to ambient noise conditions. Making non-optimal mating decisions, such as the ones seen here, can lead to deleterious fitness consequences, alter population dynamics and weaken sexual selection, unless individuals adapt to cope with anthropogenic interference.

Keywords: Mate Choice, Anthropogenic Noise, *Gryllus bimaculatus*, Acoustic Signals, Courtship Display

49 **Background**

50 The occurrence of natural variation in fitness-related traits among individuals means
51 that there will be differences in the quality of potential mates and that individuals that
52 show preferences for mates of a higher quality can thus gain considerable direct
53 (resources based) and indirect (genetic based) benefits that may lead to increased fitness
54 (Andersson 1994). However, a crucial element of making such mate choice decisions is
55 the ability of the choosing individual to detect some measure of quality in the potential
56 mates they encounter. To accomplish this, many species assess mate quality by
57 attending to the signals and cues produced by conspecifics, and then respond
58 accordingly. Beneficial traits can be assessed either through direct assessment of the
59 trait (an index; Maynard-Smith and Harper 1995), or through indirect honest signalling
60 (Zahavi 1975). Signals that are used in mate choice decisions are known to occur in
61 many different modalities and reflect various aspects of a signaller's quality, such as
62 pheromonal signals reflecting sperm load (Ruther et al. 2009), visual displays of
63 weapon size (Oliveira and Custódio 1998), and acoustic signals indicating
64 immunocompetence (Rantala and Kortet 2003). As responding appropriately to these
65 signals can offer sizeable benefits to an individual's fitness, it is of no surprise that the
66 assessment of courtship signals is widespread throughout many taxa (Zuk et al. 1992;
67 Censky 1997; Wagner and Reiser 2000; Amundsen and Forsgren 2001; Clutton-Brock
68 and McAuliffe 2009; Henneken et al. 2015).

69 Issues may arise from using courtship signals to make mate choice decisions
70 when transmission or perception of the signal is disrupted. Such disruptions can occur
71 due to natural variation in the signalling environment. For example, visual signals used
72 in mate choice decisions may be disrupted if visibility is reduced. Such an effect is seen

in the broad-nosed Pipefish (*Syngnathus typhle*), where increased water turbidity reduced the time males spent assessing potential mates. As a result, males were less likely to choose larger (and thus higher quality) females (Sundin et al. 2010). Alternatively, high densities of conspecifics using acoustic signals may disrupt assessment of potential mates. This can be seen in chorus signing anurans, such as Tree Frogs in the genus *Hyla*. In these conditions, females suffer a reduced ability to discriminate attractive songs when there are more males signalling (Gerhardt 1982), or signalling males are closer together (Richardson and Lengagne 2010). These disruptive environmental factors often lead to the adaptation or evolution of signalling behavior, so that signals are not disrupted and transmission can occur successfully (Ord et al. 2007; Preininger et al. 2013). However, these strategies may not evolve quickly enough to combat an evolutionarily recent selection pressure.

Through the development and growth of human society, we are continually changing the characteristics of environments on a global scale. This includes the production of anthropogenic noise throughout many different environments, which is known to have various deleterious consequences on both human and non-human animals (Kaiser et al. 2015; Hammersen et al. 2016). Whilst noise can be classified as unwanted signal disruptions in any modality (i.e. visual noise, chemical noise) we are here, and throughout this manuscript, referring to human generated acoustic noise unless otherwise stated. A significant part of the literature on anthropogenic noise is concerned with the effect this acoustic pollutant has on the detection of signals and cues (Kern and Radford 2016; Damsky and Gall 2017; Walsh et al. 2017; Gurule-Small and Tinghitella 2018) and the behavioral alterations that occur in response to this conflict (Cunnington and Fahrig 2010; Díaz et al. 2011; Shieh et al. 2012; Lampe et al. 2014;

Luther et al. 2016). Acoustic signals and cues are most often reported as being affected by the presence of anthropogenic noise, likely due to signal masking or divided attention reducing or altering acoustic perception (Naguib 2013). This body of evidence suggests that anthropogenic noise has the potential to disrupt acoustic signals used in mate choice decisions (Candolin and Wong 2019). As mate choice is a powerful driving force for evolution, via sexual selection, disruptions in this system may result in individual fitness consequences and a decline in population viability (Tanaka 1996).

The aim of this study was to assess whether anthropogenic noise disrupts mate selection behaviors, and to identify which specific acoustic characteristics are necessary to cause this disruption. To accomplish this, we observed the mate choice behaviour of female Mediterranean field crickets, *Gryllus bimaculatus*, under different acoustic conditions. Field crickets are well known for their conspicuous acoustics and sexual behaviors, and they are well studied in this regard (Alexander 1961; Alexander 1962). By stridulating their forewings, males produce a number of different acoustic signals, including a calling song (a long-distance signal for attracting receptive females), a courtship song (a short-distance signal to encourage nearby females to mate) and an aggressive signal (performed during, or after, intraspecific male contests). Previously, we have shown that disruptive acoustic conditions can influence the production of courtship songs in males, and disrupt the detection of calling songs in females (Bent et al. 2018; Bent et al. 2020). Here, we focus on how the mate choice behaviors of female *G. bimaculatus* may be affected by anthropogenic noise. To investigate this, we observed how females attended the courtship songs produced by males. The courtship song in this species (Fig. 1) differs from the other types of song produced by the males as it possesses higher frequency ‘ticks’ (12 – 18 kHz; Shestakov and Vedenina 2015)

and is only performed once a female has been detected nearby. This acoustic display is well suited for studying differences in mate choice as it is known to be used by females to make mate choice decisions (Shestakov and Vedenina 2015), has been shown to correlate with aspects of male quality (Gray and Eckhardt 2001; Rantala and Kortet 2003), and is thought to be costly to the performer (Zuk and Kolluru 1998; Mowles 2014). Additionally, the courtship signal is thought to be more important in detailing male quality than other acoustic produced by the male (Fitzpatrick and Gray 2001; Gray 2005).

The experiments described here involved manually silencing mature male crickets and playing pre-edited acoustic recordings when they attempted to signal in staged courtship interactions. We then observed whether females chose to mount the male, and their latency to do so, as a sign of mate preference; a common practice in mate choice experiments (Shackleton et al. 2005; Kostarakos et al. 2008; Shestakov and Vedenina 2015; Loranger and Bertram 2016). As anthropogenic noise does not generally affect mounting latency (Bent et al. 2020), any difference in this experiment due to the presence of anthropogenic noise will be as a result of differences in the perception of the male's quality.

Methods

Study Organisms

Animals used in this experiment were from an existing *G. bimaculatus* colony (established in 2016) at Anglia Ruskin University (Cambridge, UK). Individuals were kept in multiple plastic terraria (30 x 17 x 20cm) in a temperature-controlled room (25 – 30°C) with a 12:12h light cycle (06:00 sunrise – 18:00 sunset, local time) and at an

ambient noise levels (44dBa, measured through a DT-8852 IEC 61672-1 class 2 compliant handheld sound level meter). All individuals were provided with fresh food (vegetables and rabbit food pellets) and water *ad libitum*. As both age and mating experience is known to affect the sexual behavior of field crickets (Cade and Wyatt 1984; Lickman et al. 1998; Tanner et al. 2019), animals were separated into single sex containers prior to eclosion and individuals were only used when between 7-10 days post-eclosion. All experiments were conducted between September and December 2018.

Male crickets were silenced by anaesthetising them at -20°C for 5 minutes, followed by cutting both their forewings anterior to the stridulatory file with fine dissecting scissors (Electronic Supplementary Material S1). Males were operated on at least 24 hours before they were used in a behavioral trial. By silencing males in this way, they were unable to produce any sounds (although it was clear when males attempted to signal through the movement of their remaining forewings) and could still exhibit other courtship behaviors. We chose this method of silencing males over others (such as using adhesives on the wings; Rillich et al. 2009) as it was necessary to see the movement of the tegmina, and amputation has been shown to work previously, with minimal impact on male sexual behavior (Balakrishnan and Pollack 1996; Shestakov and Vedenina 2015).

Acoustic Stimuli

All acoustic recordings were conducted using a RØDE NTG4+ shotgun microphone connected to a TASCAM DR-07 MKII linear PCM recorder (.wav format, 16-bit resolution and 48 kHz sampling rate). Additionally, the sound pressure level (SPL) of all recordings were measured using a DT-8852 IEC 61672-1 class 2 compliant handheld

sound level meter. All editing of audio files was conducted using Audacity 2.1.2 (<https://www.audacityteam.org/>, last accessed: 29th May 2020).

For this experiment, we created two different courtship songs to be played when males attempted to court. We created two songs that differed in tick rate (TR; ticks min⁻¹), and thus tick period (TP; the time between the start of one tick and the start of the next), as females are known to prefer songs with a higher TR, and it has also been linked to beneficial phenotypic traits (Rantala and Kortet 2003). An original courtship song was recorded from a single singing male in the same temperature range as used in the behavioral trials, as song performance is known to fluctuate due to temperature (Hedrick et al. 2002). The microphone was positioned above the arena (~12cm away from the male) and the male was encouraged to court by presenting him with a tethered female, which meant she was unable to mount the male and the courtship song could be recorded for ~5 minutes (46 ± 2.6 dBA). From this recording, we took a subset of the most active part of the song and removed extended periods of silence, as well as occurrences of chirps, resulting in a 1-minute sample. This sample was then looped a number of times to create a 15-minute ‘high quality’ courtship song (Fig. 1.A). The ‘low quality’ courtship song was created from the same 1-minute sample, but additional periods of silence were added after each tick period, approximately the same duration as the low amplitude pulses. This was then looped a number of times to create a 15-minute ‘low quality’ courtship song (Fig. 1.B). Producing the courtship songs in this way means they only differed in their TR and TP (High quality song: average TP = 341ms, TR = 168 ticks min⁻¹; Low quality song: average TP = 561ms, TR = 108 ticks min⁻¹). Both the high quality and low quality courtship songs were played back to ensure that the average tick amplitude (46 dBA) was the same as the original recording.

To test for differences in mate selection behaviors between different acoustic conditions, we also created two different types of acoustics; traffic noise and white noise (Fig. 2). The ambient noise of the observation room was also utilised as a control condition (44.3 ± 3.8 dBA). Transportation noise, including road traffic noise, is a common form of acoustic pollution (Lee et al. 2014), is known to be present in certain *G. bimaculatus* habitats (Gallego-Abenza et al. 2020), and shares acoustic traits with many other types of anthropogenic noise (i.e. low frequency, high amplitude, temporal trait fluctuations). As such we selected traffic noise as a suitable representation of anthropogenic noise. A 30-minute traffic noise recording was taken at a 5m distance from the A14 road (south Cambridgeshire) at the level of ground vegetation, and at 16:00 - 18:00 local time. This traffic noise recording had an amplitude of $79.1 (\pm 3.5)$ dBA, which is similar to the amplitude faced by individuals in their natural habitat (Gallego-Abenza et al. 2020). We removed extended periods of silence from this recording (5+ seconds) and cut the file down, resulting in a 20-minute playback stimulus (Fig. 2.A). A 20-minute white noise stimulus was created using Audacity 2.1.2. so that it could be played back at the same average amplitude as the traffic noise (79.1 dBA; Fig. 2.B). By utilizing two different types of acoustic conditions that differ in specific spectral traits, we are able to discuss the acoustic characteristics necessary for conflicting with courtship signalling in this system.

Courtship Interactions

Courtship interactions were staged in a transparent plastic arena (15 x 8 x 10cm) with a substrate of sand and an opaque plastic partition (Electronic Supplementary Material S2). The two speakers used for condition playback (Veho® 360° capsule speakers, frequency range: 100Hz – 20kHz) were positioned at each end of the arena, 20cm above

the arena and pointing towards the centre. An additional speaker was also placed 15cm directly above the centre of the arena for the playback of the artificial courtship song. The acoustic stimuli used in this experiment were played back as .wav files (16-bit depth, 48 kHz sampling rate) and was confirmed to match the average of the original recordings (traffic and white noise: 79.1 dBa; artificial courtship song: 46dBa) from the centre of the arena. All interactions were staged during the day phase of the day:night cycle (specifically between 09:00 - 11:00 local time) and between 25 - 29°C .

Prior to the start of the interaction, a muted male was placed into one half of the behavioral arena to acclimate overnight (16 hours minimum). Following this period, a female was placed into the other half of the arena, and left to acclimatize for 20 minutes. After this, the interaction commenced by simultaneously removing the opaque partition and broadcasting the acoustic condition (ambient, traffic or white noise). These interactions continued until the female mounted the male (but before the male transferred a spermatophore), or for a period of 15 minutes. We selected 15 minutes as a “no choice” cut off point, as courtship interactions rarely last longer than this because males will stop signalling or mating will have been successful (personal observation). When the males attempted to court the female, which was noticeable from the movement of the wing stubs (Electronic Supplementary Material S1), either an artificial courtship song was broadcast (‘high quality’ or ‘low quality’, Fig. 1) or nothing was broadcast (‘no song’ trials). As male courtship motivation is known to be affected by acoustic condition (Bent et al. 2020), and because we aimed to observe female responses to this display, the trials were repeated until there were 20 occurrences of male courtship in each set of conditions (9 separate conditions, 221 trials total). Females were only used for one experiment (n = 221), but males were used for up to four (at

least 24 hours between each and randomly spread between acoustic and quality conditions) to reduce the number of males that needed muting ($n = 110$). All interactions were recorded visually using as SONY HDR-CX625 HANDYCAM® positioned directly above the arena, creating “bird’s eye view” footage (Electronic Supplementary Material S2).

Behavioral Analysis

Footage of the behavioral interactions were event coded using the software B.O.R.I.S. (Behavioral Observation Research Interactive Software; Friard and Gamba 2016). Courtship interactions were scored on multiple events, including the number of males that attempted to signal (noticeable by the movement of the remaining tegmina) and the number of females that mounted males. Latency measurements were also taken for overall movement (time until individual moves after the start of trial), male signalling attempt (time until males attempted to signal) and female mounting behavior (time until the female mounted the male).

Statistical Analyses

All statistical analyses were carried out in R studio (Rstudio Team 2016; R Development Core Team 2017), with the packages ‘dunn.test’ (Dinno 2015), ‘multcomp’ (Hothorn et al. 2008), and ‘hmisc’ (Harrell Jr. 2006). All graphs and plots were created using base R and with the package ‘ggplot2’ (Wickham 2016), and proportions and percentages are shown to reflect the size of the effect. Where GZLMs were used, we also visually assessed plots of residuals vs. fitted values to ensure that models fit the data well, and that the data did not violate the model’s assumptions. We

tested scale data for normality where necessary, using a Shapiro-Wilk test, and used non-parametric tests where appropriate.

We used Generalized linear models (GZLM) using a binary logistic function to see if amputated males were less likely to sing than those treated similarly in a previous experiment (Bent et al. 2020). Binary GZLMs were also used to check if the amount of trials males performed in affected the likelihood that they would sing and be mounted. We also used binary GZLMs to test for the effect of acoustic condition and quality condition on the number of females that mounted males, and the effect of acoustic condition on the number of males that attempted to signal. We used Kruskal-Wallis tests to analyse differences in movement latency, male signal attempt latency (from conspecific contact), and female mounting latency (from the onset of courting) among both acoustic and quality conditions. When a statistically significant result was found ($P < 0.05$), we used suitable post-hoc tests (TukeyHSD multiple comparison analysis for binary GZLMs, Dunn's tests with Bonferroni corrections for Kruskal-Wallis tests) to conduct pairwise analyses of groups.

Results

Courtship and Mounting Occurrence

Muted males, in ambient no song trials, showed no reduction in their motivation to court when compared to ambient noise conditions utilised in our previous study (GZLM(b): Wald $X^2_2 = 1.589$, $n = 44$, $P = 0.208$). Males showed a reduction in courtship likelihood in white noise conditions (60/85) when compared to other acoustic conditions (GZLM(b): Wald $X^2_2 = 11.397$, $n = 221$, $P = 0.003$, Fig. 3). Individuals in white noise conditions were less likely to attempt to signal than those in traffic noise

conditions (60/66, $n = 151$, $P = 0.01$), but no effect was found between white and ambient noise conditions (60/70, $n = 155$, $P = 0.07$), or ambient and traffic noise conditions ($n = 136$, $P = 0.616$). The amount of trials that the males had been in did not affect the likelihood that they would sing (GZLM(b): Wald $X^2_2 = 2.116$, $n = 221$, $P = 0.549$) or the likelihood that they would be mounted (GZLM(b): Wald $X^2_2 = 1.245$, $n = 180$, $P = 0.742$).

Females did not differ in their choice to mount males between acoustic conditions in high quality trials (GZLM(b): Wald $X^2_2 = 4.145$, $n = 60$, $P = 0.126$). In ambient noise conditions, there was a reduction in the occurrence of mountings in no song conditions when compared to other quality conditions (GZLM(b): Wald $X^2_2 = 8.845$, $n = 60$, $P = 0.012$, Fig. 4). Females were less likely to mount males in no song trials (50% success rate) than those in high quality trials (90% success rate, $n = 40$, $P = 0.03$), but no effect was found between high quality and low quality trials (80 % success rate, $n = 40$, $P = 0.656$) or low quality and no song trials ($n = 40$, $P = 0.127$). However, this difference between quality conditions was not detected under either traffic noise (GZLM(b): Wald $X^2_2 = 1.18$, $n = 60$, $P = 0.554$) or white noise conditions (GZLM(b): Wald $X^2_2 = 0.959$, $n = 60$, $P = 0.619$). Under traffic noise conditions, 70% of encounters were successful in high quality trials, 80% were in low quality trials, and 65% were in no song trials. Under white noise conditions, 75% of encounters were successful in high quality trials, 55% were in low quality trials, and 50% were in no song trials.

Behavioral Latencies

Male signal attempt latency, male movement latency and female movement latency were not affected by quality conditions or acoustic conditions (Table 1). However, female latency to mount males differed between quality trials under ambient noise

conditions (Fig. 5, Table 2). Females were quicker to mount in high quality trials than they were in low quality or no song trials, but there was no difference between low quality and no song trials. This difference was not detected under traffic noise or white noise conditions (Table 2). Female latency to mount differed between acoustic conditions in high quality trials (Table 2, Fig. 5), where females were quicker to mount in ambient noise trials when compared to white noise trials, but showed no difference between ambient and traffic noise conditions or traffic and white noise conditions. No similar difference in mounting latency was found when comparing low quality trials or no song trials between acoustic conditions (Table 2).

Discussion

The differences in courtship behavior between acoustic and quality conditions described here highlight the disruptive influence of anthropogenic noises on mate choice decisions in animals. In traffic and white noise conditions, we detected no differences in the number of females that choose to mount males or their latency to do so between different quality trials. In contrast, we noted significant differences in these comparisons in the absence of anthropogenic noise, where females mounted males paired with high quality songs sooner and more frequently. Our research suggests that the presence of anthropogenic noise alters how females perceive males when making mate choice decisions, which in turn can have consequences on individual fitness and population viability.

The observed differences in courtship success in ambient noise conditions work as a foundation on which to compare the differences, or lack thereof, seen in other acoustic conditions. In ambient noise conditions, high quality and low quality trials maintained a higher success rate (female mounted) when compared to no song trials.

This is not an unusual result, as both high quality and low quality signals should yield a successful encounter, either through a reduction in a female's selection threshold (variable threshold strategy; Janetos 1980) or by the cumulative effects of the repetitive display (Mowles and Ord 2012). On the other hand, a complete removal of a sexual signal would significantly reduce any mate-choice behavior that is dependent upon it (Gray et al. 2014; Shestakov and Vedenina 2015), although the higher than expected success rate in the no song conditions suggest an element of multimodal signalling maybe in effect (Candolin 2003; Stoffer and Walker 2012), with females potentially attending to visual, olfactory or vibratory cues. In comparison, we found no significant difference in success rates between quality trials in both traffic and white noise conditions. As we have shown here (in high quality conditions), females are not less likely to mate under traffic or white noise conditions, so we can conclude that this difference is likely due to the perception of male quality. This result indicates that mate choice in this species has been disrupted by the presence of anthropogenic noise as females in these conditions show no differentiation between mating with individuals accompanied by high quality songs, low quality song or no song.

When we consider the differences, or lack thereof, seen in mounting latency, this further supports the evidence that anthropogenic noise is disrupting the decision-making mechanisms associated with mate choice. Again, an expected response is seen in ambient noise conditions, where males are mounted sooner when accompanied with a high quality courtship song, than with a low quality song or no song at all. In most no-choice tests, the assumption is that females take different amounts of time to mate with males of differing qualities as they need to exceed a threshold (through falling expectations or additive quality perception) before they are deemed viable mates

(Shackleton et al. 2005; Shestakov and Vedenina 2015; Backwell and Passmore 2016). Thus, we can conclude that, in ambient noise trials, females preferred mating with males coupled with a high quality song, than those with a low quality song, or no song at all. However, this difference is again not observed in either traffic noise or white noise conditions, where there was no difference in mounting latency between different quality trials. Previously, we have shown that females do not differ in their mounting latency between acoustic conditions generally (Bent et al. 2020), suggesting that the differences observed here are due to a disruption in mate quality perception. Additionally, the finding that individuals which were presented with the high quality song mounted more quickly in ambient noise conditions, coupled with the lack of differences in low quality and no song trials between acoustic conditions, suggests that high quality males appear less attractive under unfavorable acoustic conditions. On the other hand, the seemingly similar responses in traffic and white noise conditions may mask some biological relevant differences. Different quality trials in white noise conditions shared a similar mean, but their amount of deviation was comparable to those seen in ambient noise conditions. Conversely, mean mounting latency in traffic noise conditions were noticeably different to each other, and the amount of deviation was not consistent with the other two acoustic conditions. This may indicate that different noise conditions affect mate perception in different ways, despite the similar lack of significant differences between quality conditions in traffic and white noise conditions.

Anthropogenic noise, and the effect it has on biological systems, has been well studied throughout different taxa, with research highlighting the consequences this acoustic pollutant can have on juvenile development (Nedelec et al. 2015; Injaian et al. 2018), species abundance or diversity (Clinton D. Francis et al. 2011; Bunkley et al.

2017) and greater ecological processes (Francis et al. 2012; Wale et al. 2019), for instance. In field crickets alone, research has shown that anthropogenic noise can disrupt the phonotactic behaviour of females (Schmidt et al. 2014; Bent et al. 2018) and alter the sexual signalling behaviour of males (Gallego-Abenza et al. 2020; Bent et al. 2020). Additionally, the life history and behavioural development of traits has also been shown to be disrupted when crickets are exposed to anthropogenic noise (Gurule-Small and Tinghitella 2019; Bowen et al. 2020). Our study adds to this body of research, showing that anthropogenic noise can also reduce female perception of mate quality based on acoustic signals (Huet des Aunay et al. 2013; Candolin and Wong 2019). Immediate implications of this may result in changes to an individual's fitness prospects due to altered or missed mating opportunities. From a female's perspective, they risk mating with a less than preferable male, or avoid mating with a high quality male, when they are unable to detect differences in mate quality. This may lead to a reduction or complete loss of offspring viability (Funk and Tallamy 2000). Signalling males, on the other hand, which are producing high quality, and likely costly, signals are receiving no benefit over individuals that may be investing less into their signals. As a result, males may have to increase the costs they are investing to overcome the signal disruptions (Díaz et al. 2011; Bent et al. 2020) in order to gain mating advantages, which would disrupt the distribution of resources leading to a potential reduction in survivability (Hunt et al. 2004). However, obvious fitness implications of a disrupted mate choice system are just the start, as consequences may also extend to the population level and can weaken sexual selection (Candolin and Wong 2019). For example, weakened mate preferences through anthropogenic disruption may lead to reduced speciation and thus species diversity (Seehausen et al. 2008). On the other hand, if mate preferences don't

adjust with changing sensory conditions, sexually selected traits may need to become more exaggerated (Cunnington and Fahrig 2010), a runaway process that can lead to species extinction (Moen et al. 1999). However, as anthropogenic disturbances are an evolutionarily recent selection pressure, it is difficult to predict how species may adapt (Francis et al. 2011), meaning new evolutionary equilibriums may still be reached. Many species have adapted to signal more efficiently when non-anthropogenic abiotic factors disrupt signalling systems. For example, anole lizards have been shown to alter their visual signal when visibility is disrupted by windblown vegetation (Ord et al. 2007), and tropical anurans alter the spectral characteristics of their acoustic signals when calling near noisy streams (Vargas-Salinas and Amézquita 2013). Additionally, many species adopt multi-modal signals when environmental conditions changes to mitigate any disruption (Partan 2017). Given time, many species may also be able efficiently adapt their acoustic signalling behaviour when anthropogenic noise is present.

The inclusion of both a traffic noise stimulus and a white noise stimulus in the present study allows for discussion on the required acoustic characteristics that can lead to the effects reported here. As there appears to be a disruption of mate preference in both traffic and white noise conditions, when compared to ambient noise conditions, this suggests that it is a shared characteristic of the two noise stimuli that is responsible for this disruption. The main shared characteristic between the two stimuli is their average amplitude, for which they are matched. Neither the difference in frequency or signal fluctuations led to a significant difference between these two acoustic conditions, although noticeable differences in averages and data variation were still present. This is an interesting result as we have previously concluded that amplitude alone is not enough

to disrupt acoustic communication in field crickets (Bent et al. 2018; Bent et al. 2020). Previously, we discussed the likelihood that the effect observed in those experiments was due to frequency masking, based on Naguib's work (Naguib 2013). As masking is mostly based on shared frequency bands, we can conclude that this is not the effect seen here, as traffic noise does not contain frequencies similar to the important ticks in *G. bimaculatus* courtship song. Instead, this result may be caused by distractions from other signals or a lack of attention to the signal.

Limited attention in animals, defined as a restricted rate of information processing by an individual, is likely to cause fitness consequences when prey/predator detection is concerned (Dukas 2004). Indeed, anthropogenic stimuli have been shown to reduce attentional capabilities, leading to altered responses to cues produced by predators (Chan et al. 2010) and food sources (Purser and Radford 2011). Unfortunately, the effects of limited attention has been seldom studied in regards to courtship and sexually selected signals (Dukas 2002). However, attention has been recognised in field crickets, both from a behavioural (Campbell and Clarke 1971) and neurophysiological perspective (Pollack 1988), so the alterations in female mate choice reported here may be the result of limited attention. Naguib (2013) notes that auditory attention based issues may occur when individuals are tasked with the processing of more subtle acoustic information, even if signal detection is unaltered. We have shown here, and in a previous study (Bent et al. 2020) that courtship signal detection is not reduced in these acoustic conditions as females still mount males that court. Additionally, as courtship song preference in *G. bimaculatus* is known to be based on the subtle fine scale timing of ticks in the signal (Rantala and Kortet 2003; Shestakov and Vedenina 2015), an attention deficit may indeed be the effect seen here. In other

species, lower-quality males may exploit this effect of reduced attention by signalling in leks, where females have reduced attentional capabilities and thus altered signal discrimination (e.g. Wollerman and Wiley 2002). Further studies on the affect of anthropogenic noise on animal attention is essential for understanding the behavioral consequences and to aid conservation efforts (Chan and Blumstein 2011).

Finally, this study also adds to the understanding of mate choices based on courtship songs in *G. bimaculatus*. Calling song attractiveness and preference has been studied extensively in field crickets (Simmons and Ritchie 1996; Wagner Jr and Hoback 1999; Simmons et al. 2005; Meckenhäuser et al. 2011; Trobe et al. 2011; Verburgt et al. 2011; Hirtenlehner et al. 2013; Samuel et al. 2013; Zhemchuzhnikov and Knyazev 2015; Hedwig and Sarmiento-Ponce 2017; Zhemchuzhnikov et al. 2017), but by comparison courtship song preference has remained largely understudied (Rantala and Kortet 2003; Shestakov and Vedenina 2015). This is despite suggestions that calling songs in *Gryllus* species allow for species identification, whilst courtship songs are more likely to contain information relating to a male's quality (Fitzpatrick and Gray 2001; Gray 2005). More research is necessary to understand the evolution behind courtship songs in field crickets, which will in turn better reveal the function of the song and what information females gain from attending to the signal.

Conclusions

The experiment presented here has revealed that anthropogenic noise can lead to alterations in mate choice behaviors, when mate quality is advertised through acoustic signals. In this case, both traffic noise and white noise reduced the selection preference observed in females in ambient no-noise conditions, both in terms of mounting success and latency. This effect does not seem to be due to masking of the dominant frequency

477 of the song, but rather due to the amplitude of the presented stimuli, which has led to
478 potential reductions in attentiveness from the females assessing the courtship signal.
479 This result not only helps to further the work on the consequences of anthropogenic
480 noise, but also highlights the importance of courtship song selectivity in *Gryllus*
481 *bimaculatus*, a currently understudied topic.

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	X ² ₂	N n	\bar{x}	S.E.	H n	\bar{x}	S.E.	L n	\bar{x}	S.E.	P
Male movement latency (s)											
Ambient	2.251	24	77.276	18.853	21	89.537	17.625	25	88.191	12.526	0.324
Traffic	0.631	21	107.086	23.376	22	113.398	32.908	23	80.197	16.733	0.73
White	1.53	28	61.319	8.928	29	73.565	13.376	28	81.253	13.023	0.465
Between noise conditions	2.505	70	85.397	9.3724	66	99.664	13.421	85	71.9	6.820	0.286
Female movement latency (s)											
Ambient	0.4	24	81.663	19.445	21	57.421	8.828	25	61.622	12.118	0.819
Traffic	1.452	21	76.425	14.685	22	65.484	8.094	23	73.247	17.813	0.484
White	4.567	28	44.792	7.715	29	51.678	7.463	28	67.002	10.333	0.102
Between noise conditions	1.788	70	66.134	7.803	66	70.549	7.775	85	54.409	5.017	0.41
Signal attempt latency (s)											
Ambient	0.78	20	20.481	5.167	20	56.588	34.762	20	31.323	9.011	0.677
Traffic	0.209	20	26.012	5.862	20	18.486	4.04	20	33.191	10.228	0.901
White	0.857	20	35.599	19.949	20	47.423	17.58	20	58.711	23.143	0.652
Between noise conditions	0.371	60	36.131	12.049	60	26.072	4.029	60	47.245	11.615	0.83

752 *Table 1*

	X^2_2		\bar{x}	S.E.		\bar{x}	S.E.		\bar{x}	S.E.	P
Quality trial differences		N <i>n</i>			H <i>n</i>			L <i>n</i>			
Ambient	8.847	8	143.914	38.596	17	69.094	9.24	15	103.72	12.341	0.012
High - Low					17			15			0.026
Low - No Song		8						15			0.825
No Song - High		8			17						0.015
Traffic	2.062	12	130.119	41.827	14	161.828	52.68	16	91.823	23.013	0.357
White	0.789	10	146.261	62.694	13	148.974	30.356	11	151.971	44.806	0.674
Acoustic trial differences		A <i>n</i>			T <i>n</i>			W <i>n</i>			
High quality	6.688	17	69.094	9.24	15	161.828	52.68	13	148.974	30.356	0.035
Ambient - Traffic		17			15						0.079
Traffic - White					15			13			0.957
White- Ambient		17						13			0.025
Low quality	3.382	15	103.72	12.341	16	91.823	23.013	11	151.971	44.806	0.184
No Song	1.892	8	143.914	38.596	13	130.119	41.827	10	130.119	41.827	0.388

753 *Table 2*

Figure Legends

Figure 1. Frequency spectrograms showing temporal structural and frequency aspects of (a) the ‘high quality’ and (b) ‘low quality’ courtship songs created for this experiment. High quality average TP = 0.341s, average TR = 168 ticks min⁻¹. Low quality average TP = 0.561s, average TR = 108 ticks min⁻¹. Spectrograms were created using Praat with the following properties: window length: 0.005s, time range as shown (0 – 5s); frequency range: 0 – 20000Hz

Figure 2. Frequency spectrograms showing the temporal structural and frequency aspects of the (a) traffic noise and (b) white noise stimuli used in this experiment. Spectrograms were created using Praat with the following properties: window length: 0.005s, time range as shown (0–3s); frequency range: 0–20000Hz.

Figure 3. The number of males who courted or did not in courtship interactions between ambient ($n = 70$), traffic ($n = 66$) and white noise ($n = 85$) conditions. Brackets with an asterisk show a significant result from pairwise analyses.

Figure 4. The number of females who mounted males or did not in courtship interactions between High quality, low quality and no song conditions, in ambient noise trials ($n = 60$). Brackets with an asterisk show a significant result from pairwise analyses.

Figure 5. Latency of females to mount ($\bar{x} \pm \text{SE}$) between acoustic and quality conditions. Brackets with an asterisk show a significant result from Dunn post-hoc tests, where an overall significant affect was first found.

Table 1. Output from Kruskal-Wallis tests on measures of male movement latency, female movement latency and single attempt latency (from contact), between high quality (H n), low quality (L n) and no song (N n) conditions, and pooled analysis between acoustic conditions.

777 Means and standard error of the mean are shown for populations that were tested (quality
778 conditions or acoustic conditions).

779 **Table 2.** Output from Kruskal-Wallis tests on mounting latency (s) between high quality (H
780 *n*), low quality (L *n*) and no song (N *n*) conditions, and between ambient (A *n*), traffic (T *n*)
781 and white noise (W *n*) conditions. Means and standard error of the mean are shown for
782 populations that were tested (quality conditions or acoustic conditions). Dunn's test pairwise
783 results are shown for statistically significant outcomes. Bold P-values indicate a significant (P
784 > 0.05) result.

785 **Electronic Supplementary Material S1.**

786 Procedure for wing cutting to silence males. (A) A male prior to silencing. The red line shows
787 where the file and plectrum are found on one of the wings (this area is found on both wings in
788 *Gryllus bimaculatus*), and thus the area that needs to be removed to silence the male. Dotted
789 yellow lines show where the wings were cut with fine dissecting scissors. (B) A silenced
790 male following this procedure. Red circles highlight the remaining wing stubs after silencing.
791 Males could not produce acoustic with these remaining wing segments, but they still moved
792 them as if normal courtship behaviour was occurring. This allowed us visually assess when
793 the male started their courtship display.

794 **Electronic Supplementary Material S2.**

795 Cross section of the experimental arena used for mate choice experiments. The large
796 trapezoid represents the behavioural arena with speakers on either side (circles) to broadcast
797 the acoustic condition. An additional speaker, to playback male courtship song, and camera
798 (small rectangle) and were positioned directly above the arena.

Figure 1

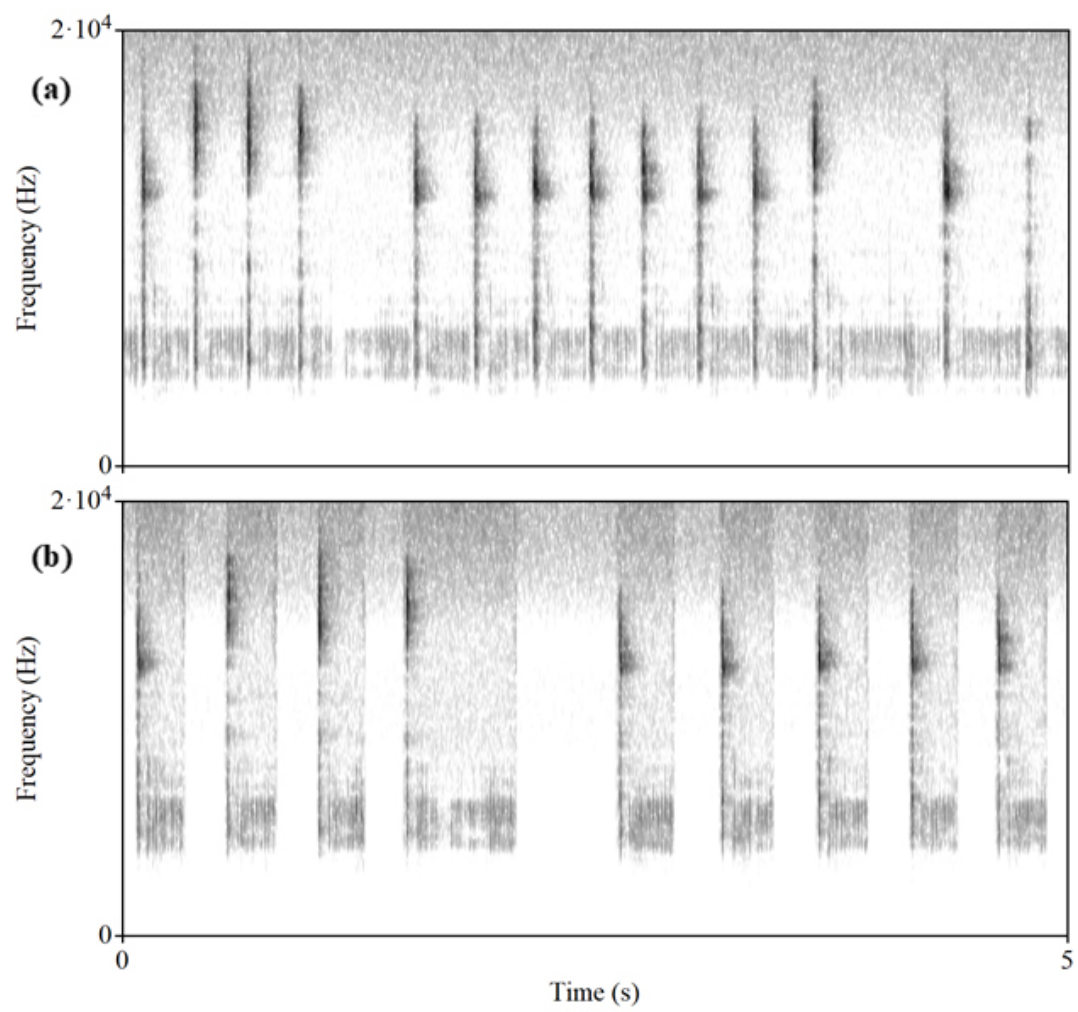


Figure 2

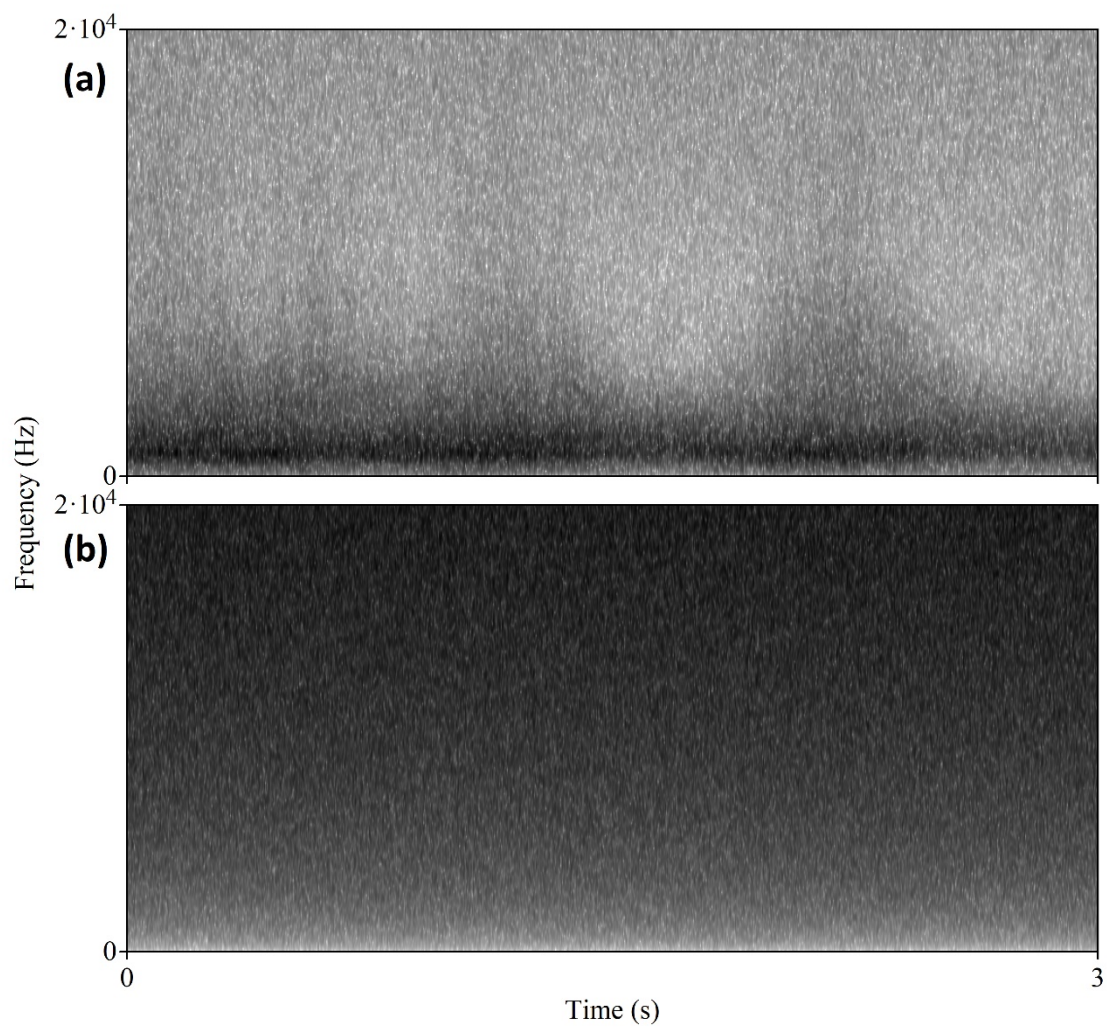


Figure 3

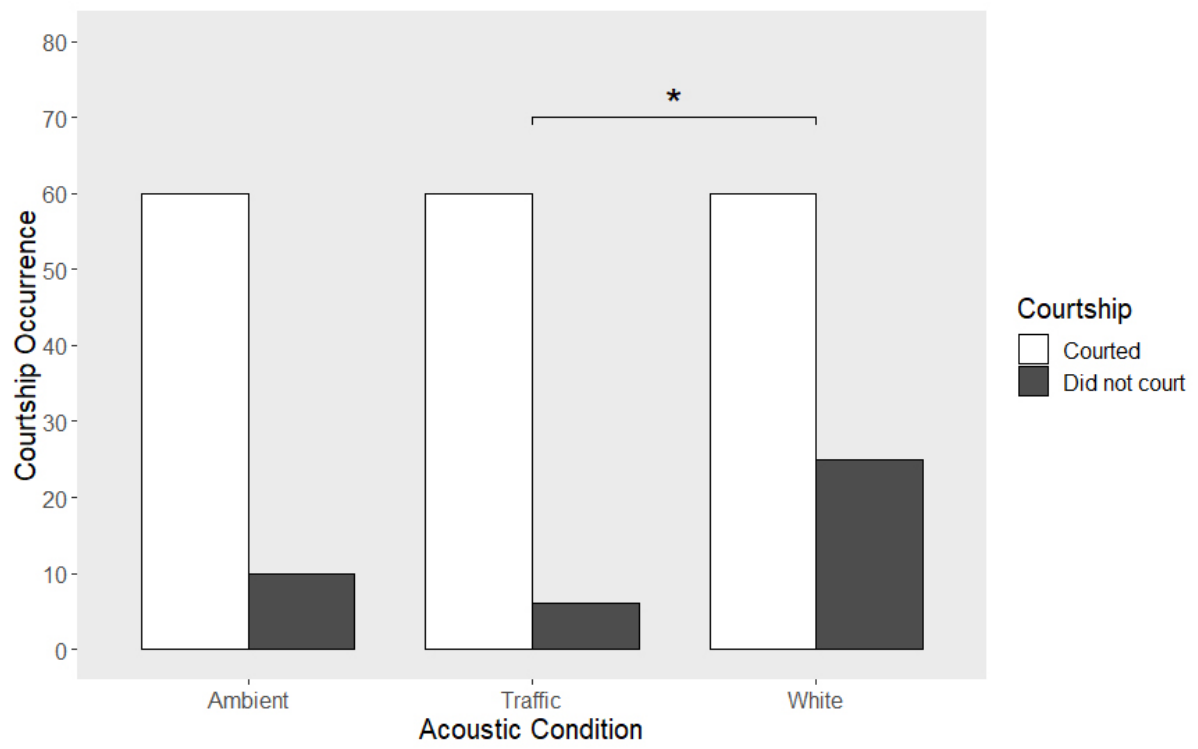


Figure 4

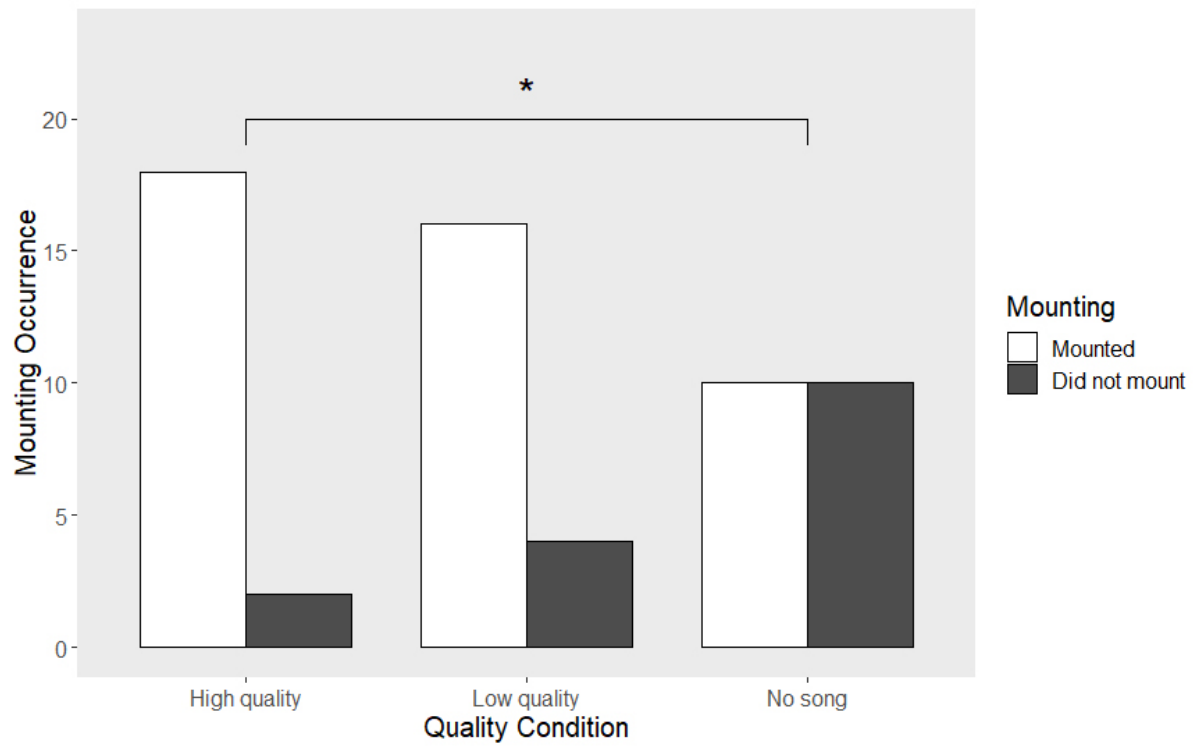
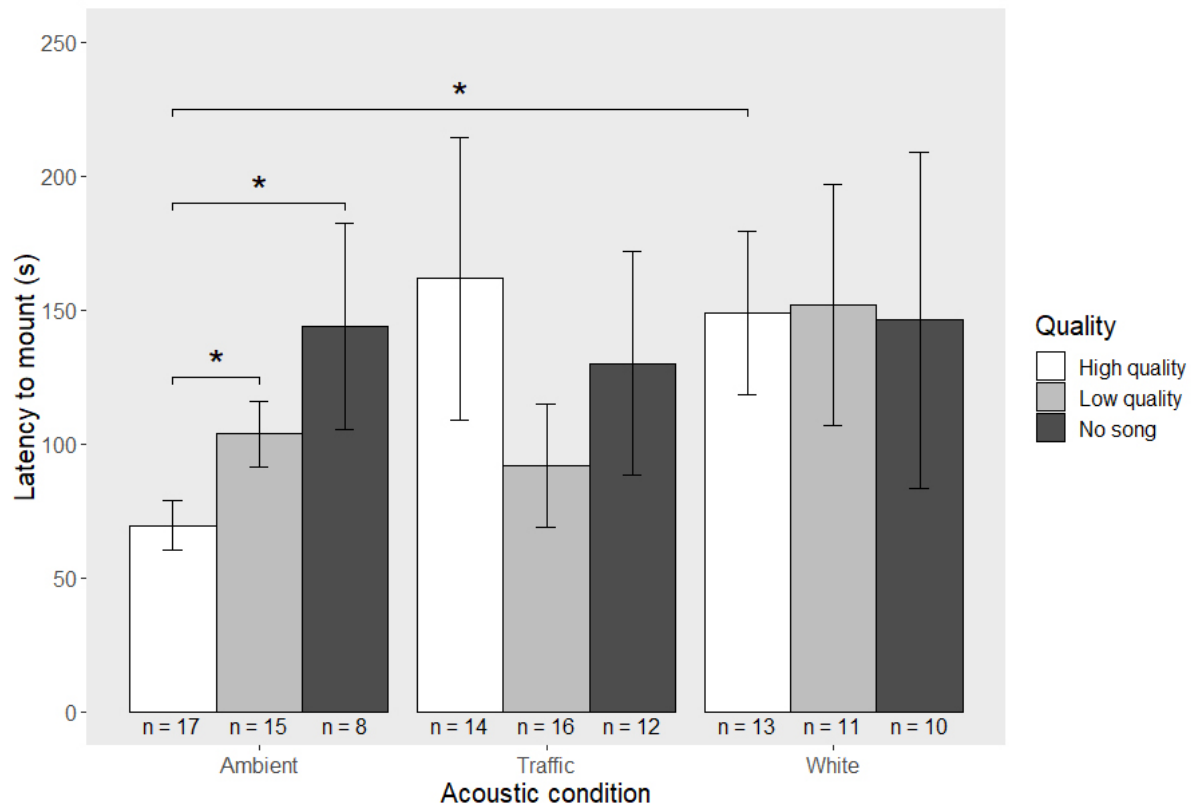


Figure 5



Supplementary Figure 1

A



B



Supplementary Figure 2

