

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

2 Running title: Mate choice under anthropogenic noise

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7 **Data Accessibility**

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

10 **Conflict of interest**

11 *We have no competing interests.*

12 **Author Contributions**

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

16 **Ethics**

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

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29 **Abstract**

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

46

47 **Keywords:** Mate Choice, Anthropogenic Noise, *Gryllus bimaculatus*, Acoustic Signals,  
48 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

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

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

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

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

121 and is only performed once a female has been detected nearby. This acoustic display is  
122 well suited for studying differences in mate choice as it is known to be used by females  
123 to make mate choice decisions (Shestakov and Vedenina 2015), has been shown to  
124 correlate with aspects of male quality (Gray and Eckhardt 2001; Rantala and Kortet  
125 2003), and is thought to be costly to the performer (Zuk and Kolluru 1998; Mowles  
126 2014). Additionally, the courtship signal is thought to be more important in detailing  
127 male quality than other acoustic produced by the male (Fitzpatrick and Gray 2001; Gray  
128 2005).

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

## 138 **Methods**

### 139 *Study Organisms*

140 Animals used in this experiment were from an existing *G. bimaculatus* colony  
141 (established in 2016) at Anglia Ruskin University (Cambridge, UK). Individuals were  
142 kept in multiple plastic terraria (30 x 17 x 20cm) in a temperature-controlled room (25 –  
143 30°C) with a 12:12h light cycle (06:00 sunrise – 18:00 sunset, local time) and at an

144 ambient noise levels (44dBa, measured through a DT-8852 IEC 61672-1 class 2  
145 compliant handheld sound level meter). All individuals were provided with fresh food  
146 (vegetables and rabbit food pellets) and water *ad libitum*. As both age and mating  
147 experience is known to affect the sexual behavior of field crickets (Cade and Wyatt  
148 1984; Lickman et al. 1998; Tanner et al. 2019), animals were separated into single sex  
149 containers prior to eclosion and individuals were only used when between 7-10 days  
150 post-eclosion. All experiments were conducted between September and December  
151 2018.

152 Male crickets were silenced by anaesthetising them at -20°C for 5 minutes, followed by  
153 cutting both their forewings anterior to the stridulatory file with fine dissecting scissors  
154 (Electronic Supplementary Material S1). Males were operated on at least 24 hours  
155 before they were used in a behavioral trial. By silencing males in this way, they were  
156 unable to produce any sounds (although it was clear when males attempted to signal  
157 through the movement of their remaining forewings) and could still exhibit other  
158 courtship behaviors. We chose this method of silencing males over others (such as using  
159 adhesives on the wings; Rillich et al. 2009) as it was necessary to see the movement of  
160 the tegmina, and amputation has been shown to work previously, with minimal impact  
161 on male sexual behavior (Balakrishnan and Pollack 1996; Shestakov and Vedenina  
162 2015).

### 163 *Acoustic Stimuli*

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

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

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

192 To test for differences in mate selection behaviors between different acoustic  
193 conditions, we also created two different types of acoustics; traffic noise and white  
194 noise (Fig. 2). The ambient noise of the observation room was also utilised as a control  
195 condition ( $44.3 \pm 3.8$  dBA). Transportation noise, including road traffic noise, is a  
196 common form of acoustic pollution (Lee et al. 2014), is known to be present in certain  
197 *G. bimaculatus* habitats (Gallego-Abenza et al. 2020), and shares acoustic traits with  
198 many other types of anthropogenic noise (i.e. low frequency, high amplitude, temporal  
199 trait fluctuations). As such we selected traffic noise as a suitable representation of  
200 anthropogenic noise. A 30-minute traffic noise recording was taken at a 5m distance  
201 from the A14 road (south Cambridgeshire) at the level of ground vegetation, and at  
202 16:00 - 18:00 local time. This traffic noise recording had an amplitude of  $79.1 (\pm 3.5)$   
203 dBA, which is similar to the amplitude faced by individuals in their natural habitat  
204 (Gallego-Abenza et al. 2020). We removed extended periods of silence from this  
205 recording (5+ seconds) and cut the file down, resulting in a 20-minute playback  
206 stimulus (Fig. 2.A). A 20-minute white noise stimulus was created using Audacity  
207 2.1.2. so that it could be played back at the same average amplitude as the traffic noise  
208 ( $79.1$  dBA; Fig. 2.B). By utilizing two different types of acoustic conditions that differ  
209 in specific spectral traits, we are able to discuss the acoustic characteristics necessary  
210 for conflicting with courtship signalling in this system.

### 211 *Courtship Interactions*

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

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

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

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

#### 245 *Behavioral Analysis*

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

#### 254 *Statistical Analyses*

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

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

264 We used Generalized linear models (GZLM) using a binary logistic function to see if  
265 amputated males were less likely to sing than those treated similarly in a previous  
266 experiment (Bent et al. 2020). Binary GZLMs were also used to check if the amount of  
267 trials males performed in affected the likelihood that they would sing and be mounted.  
268 We also used binary GZLMs to test for the effect of acoustic condition and quality  
269 condition on the number of females that mounted males, and the effect of acoustic  
270 condition on the number of males that attempted to signal. We used Kruskal-Wallis  
271 tests to analyse differences in movement latency, male signal attempt latency (from  
272 conspecific contact), and female mounting latency (from the onset of courting) among  
273 both acoustic and quality conditions. When a statistically significant result was found ( $P$   
274  $< 0.05$ ), we used suitable post-hoc tests (TukeyHSD multiple comparison analysis for  
275 binary GZLMs, Dunn's tests with Bonferroni corrections for Kruskal-Wallis tests) to  
276 conduct pairwise analyses of groups.

## 277 **Results**

### 278 *Courtship and Mounting Occurrence*

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

285 conditions (60/66,  $n = 151$ ,  $P = 0.01$ ), but no effect was found between white and  
286 ambient noise conditions (60/70,  $n = 155$ ,  $P = 0.07$ ), or ambient and traffic noise  
287 conditions ( $n = 136$ ,  $P = 0.616$ ). The amount of trials that the males had been in did not  
288 affect the likelihood that they would sing (GZLM(b): Wald  $X^2_2 = 2.116$ ,  $n = 221$ ,  $P =$   
289  $0.549$ ) or the likelihood that they would be mounted (GZLM(b): Wald  $X^2_2 = 1.245$ ,  $n =$   
290  $180$ ,  $P = 0.742$ ).

291 Females did not differ in their choice to mount males between acoustic  
292 conditions in high quality trials (GZLM(b): Wald  $X^2_2 = 4.145$ ,  $n = 60$ ,  $P = 0.126$ ). In  
293 ambient noise conditions, there was a reduction in the occurrence of mountings in no  
294 song conditions when compared to other quality conditions (GZLM(b): Wald  $X^2_2 =$   
295  $8.845$ ,  $n = 60$ ,  $P = 0.012$ , Fig. 4). Females were less likely to mount males in no song  
296 trials (50% success rate) than those in high quality trials (90% success rate,  $n = 40$ ,  $P =$   
297  $0.03$ ), but no effect was found between high quality and low quality trials (80 % success  
298 rate,  $n = 40$ ,  $P = 0.656$ ) or low quality and no song trials ( $n = 40$ ,  $P = 0.127$ ). However,  
299 this difference between quality conditions was not detected under either traffic noise  
300 (GZLM(b): Wald  $X^2_2 = 1.18$ ,  $n = 60$ ,  $P = 0.554$ ) or white noise conditions (GZLM(b):  
301 Wald  $X^2_2 = 0.959$ ,  $n = 60$ ,  $P = 0.619$ ). Under traffic noise conditions, 70% of encounters  
302 were successful in high quality trials, 80% were in low quality trials, and 65% were in  
303 no song trials. Under white noise conditions, 75% of encounters were successful in high  
304 quality trials, 55% were in low quality trials, and 50% were in no song trials.

### 305 *Behavioral Latencies*

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

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

### 318 **Discussion**

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

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

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

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

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

377 Anthropogenic noise, and the effect it has on biological systems, has been well  
378 studied throughout different taxa, with research highlighting the consequences this  
379 acoustic pollutant can have on juvenile development (Nedelec et al. 2015; Injaian et al.  
380 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

405 adjust with changing sensory conditions, sexually selected traits may need to become  
406 more exaggerated (Cunnington and Fahrig 2010), a runaway process that can lead to  
407 species extinction (Moen et al. 1999). However, as anthropogenic disturbances are an  
408 evolutionarily recent selection pressure, it is difficult to predict how species may adapt  
409 (Francis et al. 2011), meaning new evolutionary equilibriums may still be reached.  
410 Many species have adapted to signal more efficiently when non-anthropogenic abiotic  
411 factors disrupt signalling systems. For example, anole lizards have been shown to alter  
412 their visual signal when visibility is disrupted by windblown vegetation (Ord et al.  
413 2007), and tropical anurans alter the spectral characteristics of their acoustic signals  
414 when calling near noisy streams (Vargas-Salinas and Amézquita 2013). Additionally,  
415 many species adopt multi-modal signals when environmental conditions changes to  
416 mitigate any disruption (Partan 2017). Given time, many species may also be able  
417 efficiently adapt their acoustic signalling behaviour when anthropogenic noise is  
418 present.

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

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

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

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

458 Finally, this study also adds to the understanding of mate choices based on  
459 courtship songs in *G. bimaculatus*. Calling song attractiveness and preference has been  
460 studied extensively in field crickets (Simmons and Ritchie 1996; Wagner Jr and Hoback  
461 1999; Simmons et al. 2005; Meckenhäuser et al. 2011; Trobe et al. 2011; Verburgt et al.  
462 2011; Hirtenlehner et al. 2013; Samuel et al. 2013; Zhemchuzhnikov and Knyazev  
463 2015; Hedwig and Sarmiento-Ponce 2017; Zhemchuzhnikov et al. 2017), but by  
464 comparison courtship song preference has remained largely understudied (Rantala and  
465 Kortet 2003; Shestakov and Vedenina 2015). This is despite suggestions that calling  
466 songs in *Gryllus* species allow for species identification, whilst courtship songs are  
467 more likely to contain information relating to a male's quality (Fitzpatrick and Gray  
468 2001; Gray 2005). More research is necessary to understand the evolution behind  
469 courtship songs in field crickets, which will in turn better reveal the function of the song  
470 and what information females gain from attending to the signal.

## 471 **Conclusions**

472 The experiment presented here has revealed that anthropogenic noise can lead to  
473 alterations in mate choice behaviors, when mate quality is advertised through acoustic  
474 signals. In this case, both traffic noise and white noise reduced the selection preference  
475 observed in females in ambient no-noise conditions, both in terms of mounting success  
476 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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751

	$X^2_2$	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 n			H n			L n			
Ambient	8.847	8	143.914	38.596	17	69.094	9.24	15	103.72	12.341	<b>0.012</b>
High - Low					17			15			<b>0.026</b>
Low - No Song		8						15			0.825
No Song - High		8			17						<b>0.015</b>
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 n			T n			W n			
High quality	6.688	17	69.094	9.24	15	161.828	52.68	13	148.974	30.356	<b>0.035</b>
Ambient - Traffic		17			15						0.079
Traffic - White					15			13			0.957
White- Ambient		17						13			<b>0.025</b>
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*

754 **Figure Legends**

755 **Figure 1.** Frequency spectrograms showing temporal structural and frequency aspects of **(a)**  
756 the ‘high quality’ and **(b)** ‘low quality’ courtship songs created for this experiment. High  
757 quality average TP = 0.341s, average TR = 168 ticks min<sup>-1</sup>. Low quality average TP = 0.561s,  
758 average TR = 108 ticks min<sup>-1</sup>. Spectrograms were created using Praat with the following  
759 properties: window length: 0.005s, time range as shown (0 – 5s); frequency range: 0 –  
760 20000Hz

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

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

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

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

774 **Table 1.** Output from Kruskal-Wallis tests on measures of male movement latency, female  
775 movement latency and single attempt latency (from contact), between high quality (H  $n$ ), low  
776 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.

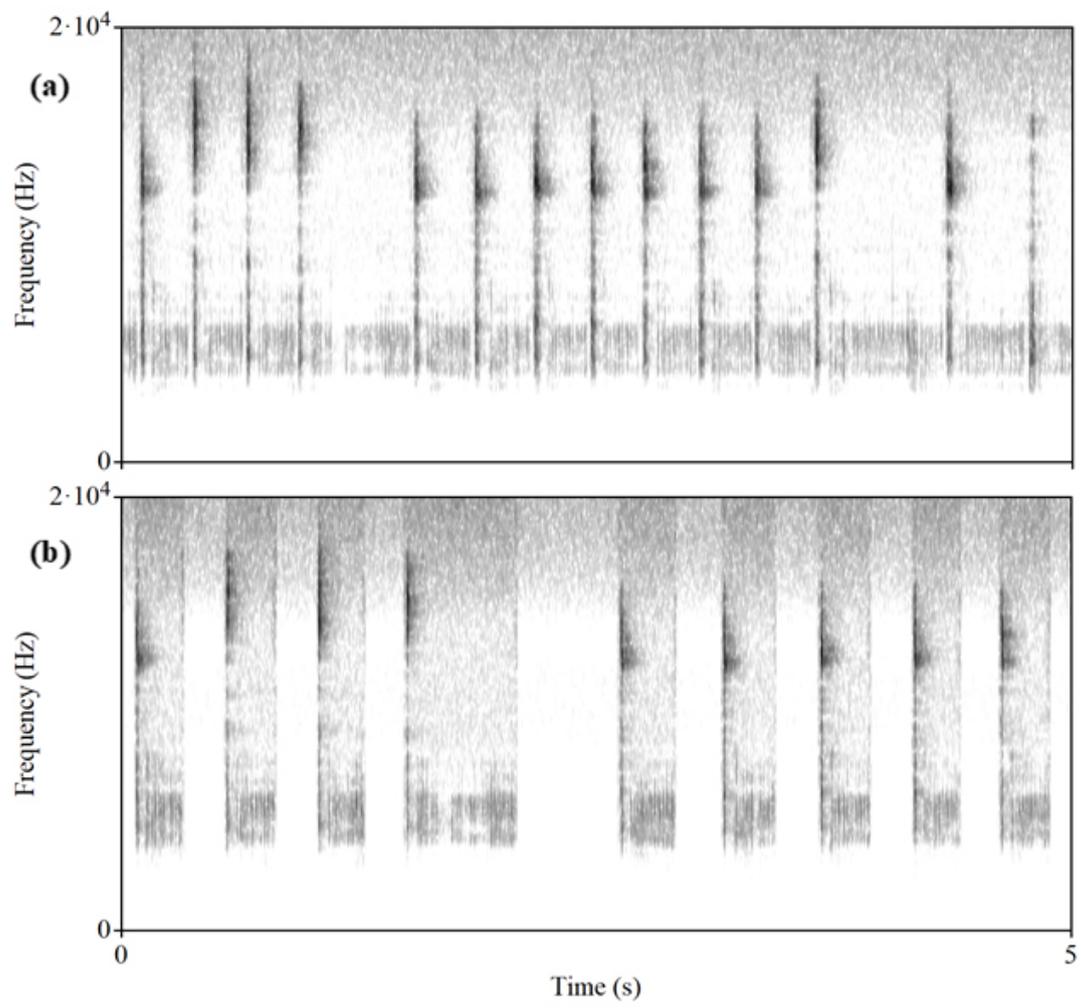
#### 785 **Electronic Supplementary Material S1.**

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.

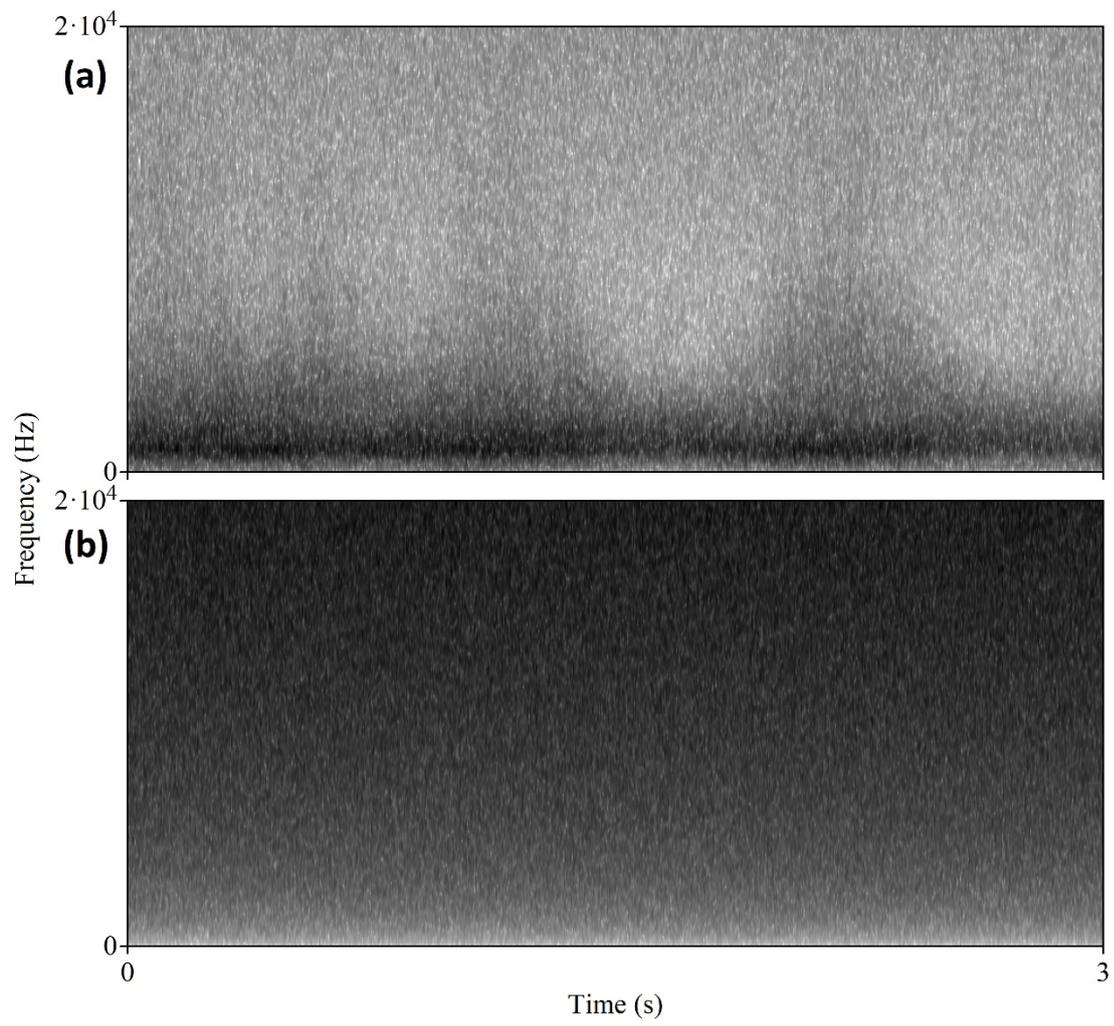
#### 794 **Electronic Supplementary Material S2.**

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.

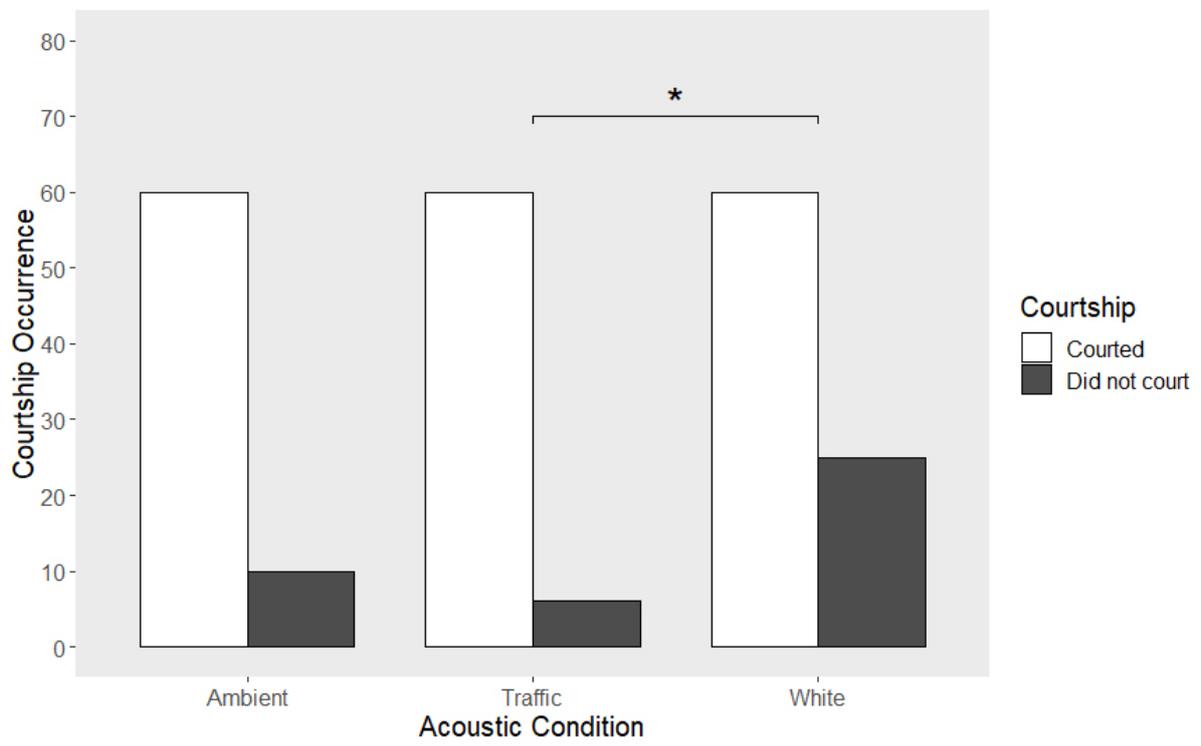
**Figure 1**



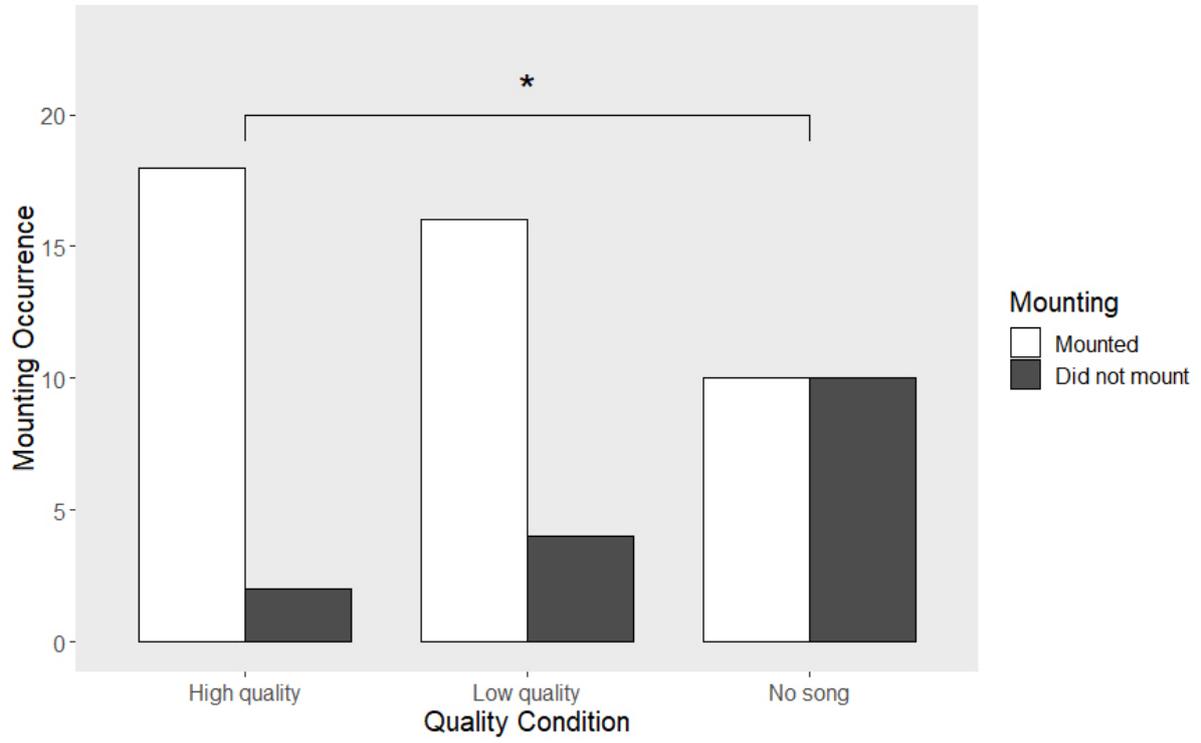
**Figure 2**



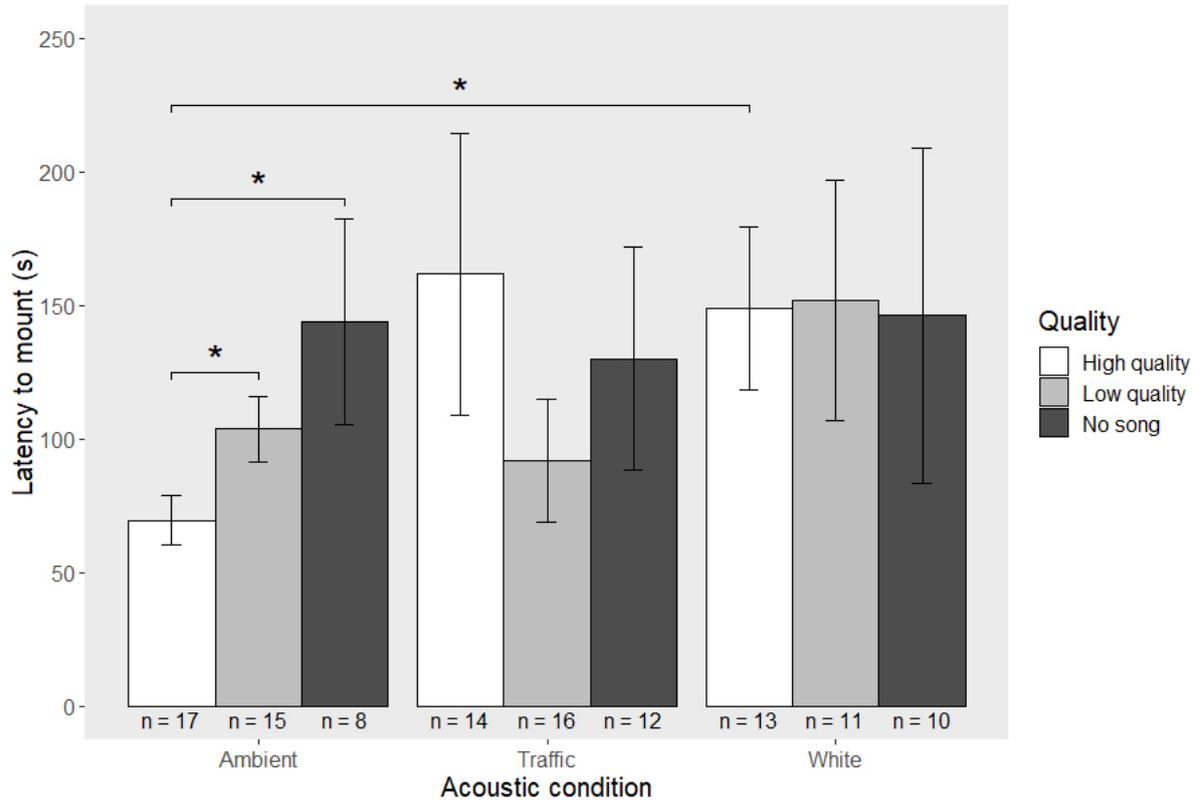
**Figure 3**



**Figure 4**



**Figure 5**



Supplementary Figure 1

A



B



Supplementary Figure 2

