

1 **Title**

2 Multiple constraints on urban bird communication: both abiotic and biotic noise shape songs
3 in cities

4

5 **Short title:** Multiple constraints on bird communication

6

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27

28 **Data Accessibility Statement**

29 Analyses reported in this article can be reproduced using the data provided by To et al. 2021.

30

31 **Lay Summary (74 words)**

32 Studies have shown that birds increase their vocalization pitch under low frequency urban
33 noise and sing at a lower pitch when exposed to high frequency insect sounds, but what if they
34 occur simultaneously? Using field recordings, we found that some urban birds no longer
35 adjusted their song in response to urban noise when insect noise is also present. This shows
36 that anthropogenic noise is not the only driver of changes in urban bird songs.

37

38 **Abstract (218 words)**

39 Ambient noise can cause birds to adjust their songs to avoid masking. Most studies investigate
40 responses to a single noise source (e.g. low-frequency traffic noise, or high-frequency insect
41 noise). Here we investigated the effects of both anthropogenic and insect noise on
42 vocalizations of four common bird species in Hong Kong. Common Tailorbirds (*Orthotomus*
43 *sutorius*) and Eurasian Tree Sparrows (*Passer montanus*) both sang at a higher frequency in
44 urban areas compared to peri-urban areas. Red-whiskered Bulbuls (*Pycnonotus jocosus*) in
45 urban areas shifted the only first note of their song upwards. Swinhoe's White-eye (*Zosterops*
46 *simplex*) vocalization changes were correlated with noise level, but did not differ between the
47 peri-urban and urban populations. Insect noise caused the Eurasian Tree Sparrow to reduce

48 both maximum, peak frequency, and overall bandwidth of vocalizations. Insect noise also led
49 to a reduction in maximum frequency in Red-whiskered bulbuls. The presence of both urban
50 noise and insect noise affected the sound of the Common Tailorbirds and Eurasian Tree
51 Sparrows; in urban areas they no longer increased their minimum song frequency when insect
52 sounds were also present. These results highlight the complexity of the soundscape in urban
53 areas. The presence of both high and low frequency ambient noise may make it difficult for
54 urban birds to avoid signal masking while still maintaining their fitness in noisy cities.

55

56 Keywords: urban noise, insect noise, bird songs, multiple noise sources

57

58 **Introduction**

59 Avian acoustic signals are important for mate attraction, territorial defense, alarm signaling
60 and other functions vital for survival and fitness (Collins 2004, Catchpole and Slater 2008,
61 Bradbury and Vehrencamp 2011). However, the efficient transmission of vocal signals is
62 affected by the presence of ambient noise from both biotic and abiotic sources, potentially
63 leading to lower fitness (Brumm and Slabbekoorn 2005, Slabbekoorn 2013, McMullen et al.
64 2014, 2016, Kleist et al. 2018). To avoid masking by noise, species may alter their
65 vocalizations through shifts in frequency, amplitude, song rate, and duration of song (Cardoso
66 and Atwell 2011, Goodwin and Podos 2013, Shannon et al. 2016, de Magalhães Tolentino et
67 al. 2018, Lee & Park 2019, Lowry et al. 2019).

68 To date many species of birds have been shown to alter their vocalizations to avoid signal
69 overlap with abiotic (e.g. rushing water, Pytte et al. 2003), or biotic factors; such as the songs
70 of other birds (Planqué and Slabbekoorn 2008), insect sounds (Slabbekoorn and Smith 2002,
71 Dingle et al. 2008, Kirschel et al. 2009, Luther 2009, Hart et al. 2015, Stanley et al. 2016) and
72 amphibian choruses (Lenske and La 2014). Anthropogenic noise is a more recent phenomenon,
73 but birds also show a wide range of acoustic responses to low-frequency anthropogenic noise
74 in particular. The most commonly documented responses of birds to low frequency

75 anthropogenic noise are to increase song frequency and/or amplitude in order to avoid signal
76 masking (e.g. Slabbekoorn and Peet 2003, Brumm 2004, Wood and Yezerinca 2006, ,
77 Slabbekoorn 2013, Guo et al. 2016, Zollinger 2017,). Increases in frequency and amplitude
78 could be directly linked, with birds singing louder also singing at higher frequencies as a
79 correlated response, rather than an independent change (Brumm and Zollinger 2011, Zollinger
80 et al. 2012, Zollinger et al. 2012); or higher frequencies may be used by birds as these
81 frequencies allow greater amplitude (Nemeth et al. 2013). However, several studies have
82 shown that birds decrease the frequency of their vocalizations in response to high-frequency
83 noise; suggesting that frequency responses can be independent of adjustments of amplitude
84 (Great Tits, *Parus major*, Halfwerk and Slabbekoorn 2009; Black capped chickadees, *Poecile*
85 *atricapillus*, Courter et al. 2020).

86 Signal masking due to high levels of anthropogenic noise has been linked to a reduction
87 in mating and reproductive success, and decreases in abundance (Habib et al. 2007, Gross et
88 al. 2010, Ríos-Chelén et al. 2013, Slabbekoorn 2013, Francis 2015, Shannon et al. 2016, Senzaki
89 et al. 2020). Although birds may avoid some of the negative impacts of signal masking by
90 changing the way in which they sing, such noise-dependent adjustments may impact mate
91 attraction under anthropogenic noise (Moiron et al. 2015, Luther et al. 2016), particularly in

92 species with lower frequency vocalizations (Francis 2015).

93 The majority of studies on the impact of noise on animal communication have focused
94 on responses to a single noise source. However, in tropical urban areas, birds are frequently
95 exposed to both low frequency anthropogenic noise and high frequency cicada choruses, often
96 simultaneously. Many cicada species generate high intensity and long-lasting acoustic signals
97 for mate attraction. While traffic noise is usually below 2 kHz, cicada calls can range from 1-25
98 kHz and can generate noise of up to 148.5 dB (Young & Bennet-Clark 1995, Fonseca et al. 2000).
99 Birds attempting to communicate under such conditions could be impacted by signal masking
100 both at the low and high limits of their song frequency range. Between increasing the
101 frequencies of their songs in response to low frequency traffic noise, and decreasing in
102 response to high frequency insect noise, birds could find that they are limited to a relatively
103 narrow bandwidth for efficient communication.

104 In this study, we recorded vocal signals of four common urban passerines in a dense semi-
105 tropical urban area, to study the effect of multiple sources of background noise on song
106 structure. We aimed to: 1) investigate whether birds alter their vocalizations in the presence
107 of anthropogenic or insect noise; 2) test for an interaction effect of anthropogenic noise and
108 insect noise on song; and 3) determine whether any response observed correlated with the

109 amplitude of anthropogenic noise or frequency of the cicada chorus. Based on previous
110 studies, we predicted that birds would sing with higher frequencies in the presence of
111 anthropogenic noise, lower frequencies in the presence of insect noise, and would produce
112 songs with narrower bandwidths under conditions where both noise sources were present.

113

114

115 **Methods**

116 *Sampling Location and Period*

117 This study was conducted in Hong Kong, a densely populated urban area with 8.8 million
118 citizens living on 1106 km² of land (Information Service Department 2018). Despite the high
119 population densities in some parts of Hong Kong, approximately 40% of land within the region
120 is designated as a country park or protected area..

121 We collected bird songs and noise measurement data from 11 urban and 11 peri-urban
122 sites across Hong Kong between 17 June and 8 September 2013 (Figure 1). Cicadas across Hong
123 Kong are mainly active from early May to late September every year and can be found in both
124 urban and rural areas, and were actively vocalizing during the whole sampling period. The
125 weather was similar across recording days: maximum temperature was in range of 30.3 – 31.1

126 °C, minimum temperature was 25.7 – 26.5°C, relative humidity was 82 – 85%, rainfall was
127 436.3 – 445.4 mm (Hong Kong Observatory 2021). These sampling locations were classified as
128 urban and peri-urban based on the nature of the surrounding buildings and habitats in the
129 area. For example, sites with high traffic highways and high-density residential buildings were
130 classified as urban areas, and we expected these urban areas to be noisier at low frequencies
131 than peri-urban sites. Urban sites included urban parks (7 sites) or roadside green spaces (4
132 sites), while peri-urban sites were in, or next to, protected areas (4 sites), traditional rural
133 villages (5 sites), or outlying islands (2 sites). Visits were made to each sampling location once
134 during the study period between 0600 to 1400 local time (UTC +8:00). At each site, songs were
135 recorded along a single transect, ranging from 1.5 to 4.2 km. Duration of recording ranged
136 from two to five hours based on the length of transects. All transects followed accessible
137 routes throughout the sampling site, such as roads, trails and footpaths. We recorded all birds
138 that sang within the sampling period. The recording started as soon as a bird was heard singing,
139 and stopped after the bird ceased singing. To avoid recording the same individual twice within
140 the same site, recordings were made at least 25m apart. If more than one individual of the
141 same species was singing at the same time, all songs in that recording were analyzed, but this
142 was counted as one sample only. Data collection occurred under fine weather conditions, i.e.

143 no rain or strong wind. For sites on outlying islands, we did not sample near the coastline to
144 limit the impact of low frequency noise produced by wave action.

145

146 *Recordings and noise measurement*

147 We recorded songs with a TASCAM DR-40 digital recorder (TASCAM, Japan) and a
148 Superlux PRA118L shotgun microphone (Superlux, Taiwan) with windscreen. Recordings were
149 set to mono channel mode at 24-bit WAV with 44.1 kHz sampling rate, no cut-off frequency
150 function was applied. All birds singing along the transect line were recorded with the same
151 settings.

152 The background noise level at each site was measured using a WESEN WS1361 (WESEN,
153 China) Type II sound level meter using C-weighting due to its sensitivity to low frequency noise.
154 The sound level meter was set on a tripod at 1.2 meters in height and at least one meter away
155 from any surface to avoid sound reflection, which would result in a higher reading
156 (Environmental Protection Department 1997). This background noise level measurement was
157 focused on the low frequency anthropogenic noise, so the noise level measurement was
158 paused when insect noise occurred in the environment. Sound measurements were taken in
159 three different directions (000°, 120°, 240°), using the $L_{eq(C)}$ (equivalent continuous sound level

160 in C-weighting) measurement for five minutes each at the start and the end of sampling. We
161 calculated overall background noise level for each site by averaging these values (Equation 1).

$$162 \quad L_{\text{eq(C)}} = 10 \log \sum_{i=t}^{i=n} \left(10^{\frac{L_i}{10}} \right) \left(\frac{1}{t_i} \right)$$

163 (Equation 1)

164

165 *Study Species*

166 Of all of the species encountered along transects, we chose those with a minimum of
167 twenty individuals recorded in both urban and peri-urban areas for further analysis. Based on
168 such criteria, only the following four species were included in the analysis: Common Tailorbird
169 (*Orthotomus sutorius*); Eurasian Tree Sparrow (*Passer montanus*); Swinhoe's White-eye
170 (*Zosterops simplex*) and Red-whiskered Bulbul (*Pycnonotus jocosus*). For these four species, a
171 total of 272 individual birds were recorded from 22 sites. No species studied was recorded in
172 all 22 sites during the sampling period. The range of sample size of each species per site was
173 from 0 to 13 (Table 1). Among all the samples, 54% of urban locations and 61% of peri-urban
174 locations had insect sounds present in the background during the recording period.

175

176 *Sound Analysis*

177 As many species sing multiple types of vocalizations, for this study we chose one specific
178 type of vocalization of each species (Figure 2) for further analysis using Avisoft SASLab Pro
179 Version 5.2.06 (Avisoft Bioacoustic, Berlin, Germany). Spectrogram settings were: FFT length
180 1024 with 100% frame size and Hamming Window, which provided a 43 Hz frequency
181 resolution and 56 Hz bandwidth resolution on the measurements. We measured the following
182 parameters using the automatic parameter measurement function: minimum frequency,
183 maximum frequency and peak frequency. Automatic parameter measurements were used to
184 reduce bias and increase consistency of the measures (Zollinger et al. 2012, Ríos-Chelén et al.
185 2017). Bandwidth (frequency difference) was calculated as the difference between the
186 maximum and minimum frequency. We analyzed at least three vocalizations for each
187 individual included in this study (range: 3 - 63). All vocalizations were measured separately and
188 then averaged for each individual.

189 For the automatic parameter measurements, a -15 dB threshold and 25 ms hold time was
190 set, with the measurement taken at the start, center and the end of the vocalization. The cut-
191 off frequency function was used on the recordings before measures were taken, based on the
192 visual inspection of the spectrogram; a high pass frequency filter removed low frequency noise,
193 and a low pass filter was applied on those recordings which contained continuous high

194 frequency noise such as insect sounds. Other noise that could potentially affect the automatic
195 measurement was cleared using the standard eraser cursor function in Avisoft using manual
196 visual judgement. We did not include any recordings where songs were so heavily masked that
197 the vocalization could not be clearly distinguished.

198 From the same recordings, the maximum frequency of the ambient noise (excluding
199 insect sounds) and the minimum frequency of insect sounds were measured with automatic
200 measurements set at the same setting for analyzing bird vocalizations. High frequency insect
201 noise is mainly produced by cicadas, and there are twenty known Cicadidae species recorded
202 in Hong Kong (Hong Kong Entomological Society 2014), but we could not identify the exact
203 species in each recording.

204

205 *Statistical Analysis*

206 All statistical analyses were run in R v3.5.1 (R Core Team 2018) and figures were
207 produced in ggplot2 (Wickham 2016).

208 We first tested the effects of the following variables; 1) species, 2) location (urban, peri-
209 urban), 3) cicada (present or not), as fixed factors; and 4) noise level as a covariate, in a linear
210 model (lm) with each of the song response parameters (minimum frequency, maximum

211 frequency, peak frequency and bandwidth. However, species were significantly different in
212 their responses, as expected, and there were three way interactions with species, location,
213 and cicada. Therefore, we ran separate linear models (using the package lme4; Bates et al.
214 2015) for the response variables for each species each including, 1) location, and 2) cicada, as
215 fixed factors, and 3) the covariate noise Level (dB).

216 As the factors in the model were not balanced we used Type III tests with contrasts and
217 used the drop1 for all models. All models included the effects of location and cicada, including
218 the interaction between the two, and noise level, as there is strong evidence that these factors
219 are likely to affect song parameters. However, we also tested whether including a
220 location*noise level interaction term significantly improved model performance (using anova
221 comparison of models), if not the term was removed. Tukey posthoc comparisons were
222 conducted using lsmeans (Lenth 2016) to identify significant differences in location/cicada.

223 We verified that final models satisfied regression model assumptions by examining
224 residual plots.

225 A second model to test for the effect of minimum cicada noise frequency was run for each
226 species and each vocal parameter including the following factors, 1) location, 2) noise level
227 and 3) cicada minimum frequency.

228 In Red-whiskered Bulbul songs, we observed two acoustic phenotypes based on
229 differences in the first note (Figure 3). In “Type A” songs, the first note is a lower frequency
230 syllable (minimum frequency, mean = 1.6 kHz, range 1.4-1.9 kHz; maximum frequency,
231 mean=3.3 kHz, range 2.9-3.7 kHz), while in “Type B” songs, the first note is a higher frequency
232 syllable (minimum frequency, mean = 1.7 kHz, range 1.5-2.3 kHz; maximum frequency,
233 mean=3.5 kHz, range 3-4.3 kHz). We tested for an effect on which note type was used in a
234 linear model with a binomial error distribution including the factors; 1) location, 2) cicada and
235 3) noise level. A second model was run as above including cicada minimum frequency instead
236 of cicada presence absence.

237 Significance of the model coefficients are given in the supplementary material Table S1.

238

239 **Results**

240 *Ambient noise levels*

241 The background noise level in urban sites (mean \pm SE = 75.23 \pm 0.82 dB(C); minimum = 70.96
242 dB(C); maximum = 80.01 dB(C); $N = 11$) was significantly higher (Independent t test: $t_{20} = 5.631$,
243 $P < 0.001$) than in peri-urban sites (mean \pm SE = 69.31 \pm 0.65 dB(C); minimum = 64.23 dB(C);
244 maximum = 71.85 dB(C); $N = 11$). The maximum frequency of anthropogenic noise in urban

245 sites (mean \pm SE = 1.425 \pm 0.038 kHz; minimum = 0.667 kHz; maximum = 2.569 kHz; N = 137)
246 was also significantly higher (independent t test: $t_{270} = -13.773$, $P = <0.001$) than in peri-urban
247 sites (mean \pm SE = 0.749 \pm 0.031 kHz; minimum = 0.200 kHz; maximum = 1.835 kHz; N = 135).

248 The minimum frequency of cicada sounds was quite variable in frequency within both
249 urban (mean \pm SE = 4.889 \pm 0.094 kHz; minimum = 2.842 kHz; maximum = 7.091 kHz; N = 74)
250 and peri-urban sites (mean \pm SE = 5.199 \pm 0.092 kHz; minimum = 2.282 kHz; maximum = 8.182
251 kHz; N = 82). The average cicada noise frequency was significantly higher in peri-urban area
252 (independent t test: $t_{154} = -2.359$, $P = 0.020$).

253

254 *Swinhoe's White-Eye*

255 There was a negative relationship between the minimum frequency of Swinhoe's White-
256 eye vocalizations and noise level ($F_{1,75} = 6.39$, $P = 0.014$, Figure 4, Table S1), when controlling
257 for location. No other vocal parameters were significantly affected by the noise conditions.
258 There was also no effect of cicada minimum frequency on any of the song parameters.

259

260 *Eurasian Tree Sparrow*

261 Minimum frequency was higher in urban areas, ($F_{1,73} = 5.82$, $P = 0.018$, , Figure 5a, Table

262 2) mainly due to the fact that birds in urban areas raised their minimum frequency when no
263 cicada were present (Tukey's $P < 0.05$).

264 Maximum and peak frequency were both significantly lower when cicadas were present
265 (Max frequency: $F_{1,73} = 14.22$, $P = 0.0003$, Figure 5b; Peak frequency: $F_{1,73} = 5.14$, $P = 0.026$,
266 Figure 5c; Table 2). The reduction in maximum and peak frequency in response to cicadas was
267 significant only in the urban areas (Tukey's $P < 0.05$). Bandwidth was also narrower in the
268 presence of cicadas ($F_{1,73} = 7.23$, $P = 0.009$, Figure 5d, Table 2).

269 There was no effect of cicada minimum frequency on any of the song parameters.

270

271 *Common Tailorbird*

272 There was a significant effect of location, and an interaction between location and
273 cicada presence, on minimum frequency (Location: $F_{1,58} = 6.04$, $P = 0.017$; Location*Cicada:
274 $F_{1,58} = 4.28$, $P = 0.043$; Figure 5a; Table 2). Posthoc tests showed that minimum frequency was
275 lower in peri-urban areas, both with and without cicada, compared to urban areas when
276 cicada were absent (Tukey's $P < 0.05$).

277 There were significant interactions between noise and location for both maximum
278 frequency and bandwidth; an increase in noise level led to an increase in both maximum

279 frequency and bandwidth in peri-urban areas, but an increase in noise level in urban areas led
280 to a decrease in both parameters (Maximum frequency: $F_{1,57}=4.33$, $P=0.042$, Figure 5b;
281 Bandwidth, $F_{1,57} = 8.26$, $P = 0.006$, Figure 5d; Table 2).

282 There was also a main effect of location on both maximum frequency and bandwidth
283 (Maximum frequency: $F_{1,57} = 4.57$, $P = 0.037$, Figure 5b; Bandwidth, $F_{1,57} = 8.26$, $P = 0.006$;
284 Figure 5d; Table 2). Maximum frequency and bandwidth both increased in urban areas.

285 Cicada frequency had no effect on any of the song parameters

286

287 *Red-whiskered Bulbul*

288 The maximum frequency was lower across both locations when there were cicadas
289 present ($F_{1,46} = 5.69$, $P = 0.021$, Figure 5b, Table 2). None of the other song frequency
290 characteristics were significantly affected by environmental variables, including cicada
291 minimum frequency.

292 Note type was affected by location ($\text{Chisq}_{1,46} = 12.93$, $P = 0.0003$) with note type B being
293 produced much more frequently in urban areas (Table 3). The difference in maximum
294 frequency between note type A and B was significant, but relatively low mean difference = 173
295 Hz, $t_{49} = 2.23$, $P = 0.03$)

296

297 **Discussion**

298 We observed, generally, that the species included in this study increased the frequency
299 of their vocalizations in urban areas with low frequency background noise, and reduced the
300 frequency in the presence of high frequency cicada noise, as found in previous studies (Dingle
301 et al. 2008, Kirschel et al. 2009, Hu and Cardoso 2010, Slabbekoorn 2013, Lenske and La 2014,
302 Roca et al. 2016). However, the presence of both low-frequency anthropogenic noise and high-
303 frequency cicada sounds affected the bird vocalizations in a complex manner, causing some
304 species to sing differently compared to when exposed to only one type of noise.

305 Common Tailorbirds and Eurasian Tree Sparrows both sang with higher minimum
306 frequencies in urban areas, but only when cicadas were not present. In urban areas, when
307 exposed to both low frequency anthropogenic noise and high frequency cicada noise,
308 vocalization frequencies did not differ from those of birds in peri-urban sites. Urban Eurasian
309 Tree Sparrows also decreased the maximum and peak frequencies of their songs, leading to
310 lower bandwidths, when cicada noise was present, in addition to the elevated levels of
311 anthropogenic noise. The narrow frequency window available to these two species for
312 avoiding signal masking thus appears to lead to reduced ability to make signal adjustments to

313 avoid masking when it is required for avoiding both high and low frequency masking noise.
314 The minimum frequency of these two species ranges from 2 - 3 kHz, so small upwards shifts in
315 minimum frequency could lead to increased masking by cicada choruses. Any benefit gained
316 from increased frequencies in response to anthropogenic noise would be counteracted by the
317 costs of signal masking by cicadas, leading to no net benefit in making vocalization adjustments.
318 There could even be an additional cost of trying to squeeze the song from the top and the
319 bottom as low bandwidth songs may be less attractive signals.

320 In Common Tailorbirds, we also found that the response to increasing noise levels differed
321 between urban and peri-urban sites; increasing noise levels in peri-urban areas led to an
322 increase in maximum frequency and bandwidth, but a decrease in these two parameters in
323 urban areas. Given that the noise was, in general, much louder in the urban sites, a potential
324 explanation for this is that when noise reaches a certain threshold, birds are constrained in
325 some way from making any further adjustments to additional increases in noise levels. In this
326 case birds may eventually appear to reach a maximum increase in frequency and then either
327 stop shifting upward or even begin to decrease in frequency (Hu and Cardoso 2010, Shiba et
328 al. 2015, Guo et al. 2016).

329 The vocalizations of Swinhoe's White-eye, which had the highest frequencies of the four

330 species studied, did not differ between urban and peri-urban populations, although we found
331 a negative relationship between background noise levels and minimum frequency, contrary to
332 predictions. The minimum frequency of this species' vocalizations is 4 kHz, much higher than
333 the peak frequency of anthropogenic noise and thus would be unlikely to suffer from signal
334 masking from anthropogenic noise, potentially explaining why they did not increase the
335 minimum frequency of their songs in response to urban noise (Parris and Schneider 2009, Hu
336 and Cardoso 2010, Parris and McCarthy 2013, Lowry et al. 2019). In fact, we found the opposite
337 response: minimum frequency of their vocalizations decreased with increasing noise levels
338 (which we discuss in more detail below). Given the frequency range of the Swinhoe's White-
339 eye vocalizations (4 - 6.5 kHz), this species would have the highest amount of overlap with,
340 and thus a high potential for masking by, the frequency of the cicada vocalizations. However,
341 we did not find any significant impact of cicada sounds in this species. Due to the extensive
342 overlap between the Swinhoe's White-eye vocalizations and the cicada noise, Swinhoe's
343 White-eyes may simply be unable to sing in a frequency that would completely avoid masking.

344 Red-whiskered Bulbul vocalizations, which have the lowest minimum frequency among
345 the four species included in this study (1.4 - 1.6 kHz), were predicted to have the strongest
346 response to noise, due to the overlap with anthropogenic noise (Parris and McCarthy 2013).

347 However, our results showed that songs of this species did not differ between urban and peri-
348 urban areas or with increasing anthropogenic noise levels. However, we did find that birds in
349 urban areas sang Type B songs more frequently – the first note in this song type had a higher
350 frequency than in Type A songs. So rather than shifting the minimum frequency of the whole
351 song, the bulbuls appear to avoid signal overlap by replacing a low frequency syllable with a
352 high frequency syllable in the introductory note, as in Great Tits and Northern Mockingbirds
353 (*Mimus polyglottos*) (Slabbekoorn 2013, Walters et al. 2019). In addition, we found an impact
354 of cicada noise on their songs; the Red-whiskered Bulbuls sang with lower maximum
355 frequencies in the presence of cicadas. These results mirror results from other species that
356 sing with lower maximum frequencies in the presence cicada noises (Gray-breasted Wood-
357 wrens, *Henicorhina leucophrys*, Dingle et al. 2008; Green Hylia *Hylia prasina*, Kirschel et al.
358 2009). White-crowned Sparrow (*Zonotrichia albicollis*) reduced song bandwidth during frog
359 chorusing (Lenske and La 2014).

360 It has been argued that observed increases in frequency in noisy areas is simply an
361 involuntary byproduct of birds singing at a higher amplitude (the “Lombard effect”, Nemeth et
362 al. 2013). While we did not measure song amplitude in this study, we found evidence in all four
363 species that frequencies decreased in the presence of background noise, which would seem

364 to contradict this hypothesis. In Swinhoe's White-eyes, minimum frequencies decreased in
365 areas with higher levels of anthropogenic noise. For urban Eurasian Sparrows, maximum and
366 peak frequencies, along with bandwidth, declined in the presence of cicadas. Finally, for the
367 Common Tailorbirds, increased noise levels led to a decrease in maximum frequency and
368 bandwidth in urban areas (in contrast to the impact of increasing anthropogenic noise levels
369 in peri-urban areas which led to an increase in these parameters). These results provide a
370 counterpoint to the argument that birds simply respond to noise by increasing the amplitude
371 of their songs, leading to an involuntary increase in frequencies and suggest that birds can
372 control the frequency and amplitude of their vocalizations independently.

373 Frequency characteristics of biotic noises in our study were more variable than low
374 frequency anthropogenic noise, likely due to the diversity of species of cicada; at least twenty
375 species of cicada have been recorded in Hong Kong (Hong Kong Entomological Society 2014).
376 Although there is limited information available on the frequency range of each of these species,
377 it is likely that there is significant variation in the frequency, amplitude, and timing of calls
378 between these species. In our study, the minimum frequency of cicada choruses ranged from
379 2 kHz up to 8 kHz, with a mean value of 5 kHz. This variation could imply that the impact of
380 bird songs will be highly variable, depending on which cicada species is present and on the

381 frequency range of the birds.

382 Overall our results together imply that a more complex soundscape, including both urban
383 and biotic noise, may limit potential song adaptation as well as our ability to predict how birds
384 respond in such complex situation. Response to noise/urbanization appeared to be reversed
385 in urban areas when cicadas were also present. Previous investigations into the impact of
386 different types of noise has indicated responses differ. When Silvereyes (*Zosterops lateralis*)
387 were experimentally exposed to both low and high frequency noise, they lowered the
388 minimum frequency of their vocalizations when exposed to high frequency noise, but showed
389 only a small effect in response to low frequency noise (Potvin and Mulder 2013). White-
390 throated Sparrows had different vocalization adjustments in response to biotic and abiotic
391 noise separately (Lenske and La 2014). However, unlike in our study, neither study compared
392 the effect of both sources simultaneously on bird vocalizations. LaZerte et al. (2016) showed
393 the response of Black-capped Chickadees (*Poecile atricapillus*) to a noise varies between quiet
394 and noisy areas; males in noisy environments shifted their frequency upward in response to
395 increasing levels of background noise, but shifted downwards in quiet areas. These examples,
396 in addition to our results, suggest that there may be potential trade-offs in responding to high
397 and low frequency noise-sources simultaneously. It is also possible that under scenarios when

398 it becomes impossible to adjust frequency parameters to avoid song masking, birds might
399 adjust other parameters to compensate.

400 Temporal song characteristics, amplitude, and the timing of vocalizations have all been
401 shown to play a role in a species' response to urban noise (Luther 2009, Slabbekoorn 2013,
402 Lenske and La 2014, Hart et al. 2015, Stanley et al. 2016, Zollinger 2017). As we did not
403 measure these aspects of the vocalizations in this study, it is possible that changes in these
404 features may explain some of the absence of frequency response observed in our study.

405 It is notable that insects could be affected by anthropogenic noise themselves, and have
406 a similar response to birds such as altering their acoustic signals (Costello and Symes 2014,
407 Morley et al. 2014). As a result, there could be a cumulative effect as insects adjust their
408 acoustic signal in response to urban noise, and then birds respond to both noises (Kirschel et
409 al. 2009). The nature of both anthropogenic noise and insect sounds could vary from time to
410 time, and place to place. Studies showed birds change their song based on the noise profile at
411 the time they sing, rather than the overall noise level (Shannon et al. 2016, Gentry et al. 2017).
412 Hence, birds may need to flexibly adjust their vocalizations based on the real-time situation.
413 However, these adjustments cannot always maintain the original communication function fully
414 under anthropogenic noise (Moiron et al. 2015, Luther et al. 2016). With the addition of

415 intense insect noise, communication effectiveness may be further reduced. Birds that live in
416 cities with intensive biotic noise, such as tropical and subtropical cities, are facing heavier
417 pressure on communication than was previously known. These birds might struggle to
418 communicate under the influence of both noises.

419

420 *Conclusions*

421 The results of our study complement the growing body of evidence that birds adjust the
422 frequencies of their vocalizations in response to anthropogenic noise, but highlight that this
423 response is not straightforward when multiple noise sources are present. The response of the
424 four species included in this study to background noise differed depending on the frequency
425 of the noise source, and differed when in the presence of both high and low frequency noise
426 sources simultaneously. The presence of two different noise sources may therefore present a
427 trade-off between increasing and decreasing frequency characteristics in order to avoid signal
428 masking.

429 As low frequency anthropogenic noise has already been shown to reproductive success
430 and fitness in urban birds (Brumm and Slabbekoorn 2005, Slabbekoorn 2013, McMullen et al.
431 2014, Moiron et al. 2015, Shannon et al. 2016, Kleist et al. 2018), it seems likely that birds in

432 cities simultaneously exposed to high and low frequency noise will face additional challenges.
433 As these areas are very likely to be in tropical cities where little attention has been paid to the
434 impact of noise on birds, we strongly encourage more studies to understand how birds adjust
435 their songs when exposed to multiple noise source types and the impact on reproduction and
436 survival.

437

438

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588

589 **Figure Legends**

590 **Figure 1.** Map shown the sampling location of urban sites (red) and peri-urban sites (blue) across
591 Hong Kong.

592 **Figure 2.** Spectrograms showed the vocalizations of the four species studied under different ambient
593 noise situations.

594 **Figure 3.** Two different types of Red-whiskered Bulbul song, grouped based on the structure of the
595 first note (highlighted in red).

596 **Figure 4.** Scatterplot showing the relationship between the minimum frequency of the Swinhoe's
597 White-eye and noise level, including the 95% confidence intervals for the fitted line

598 **Figure 5.** Boxplots showed the mean value of the four acoustic parameters a) minimum frequency,
599 b) maximum frequency, c) peak frequency and d) bandwidth, in the four different situations peri-
600 urban, no insect noise; peri-urban, with insect noise; urban, no insect noise; urban, with insect noise
601 of all four species studied.

602 **Figure 6.** Scatterplot showing the relationship between the a) maximum and b) bandwidth frequency,
603 of the Common Tailorbird and noise level separately for urban and peri-urban areas, including the
604 95% confidence intervals for the fitted lines

Table 1. Sample size, individuals, (*N*) of the four studied species. The number of sites (*N* of site) indicates how many sites the samples were collected from.

Species	<i>N</i>	
	Peri-urban (<i>N</i> of site)	Urban (<i>N</i> of site)
Common Tailorbird (<i>Orthotomus sutorius</i>)	41 (9)	22 (8)
Eurasian Tree Sparrow (<i>Passer montanus</i>)	26 (6)	52 (11)
Swinhoe's White-eye (<i>Zosterops japonicus</i>)	39 (9)	41 (10)
Red-whiskered Bulbul (<i>Pycnonotus jocosus</i>)	29 (7)	22 (9)

Table 2. Mean value of acoustic parameters of the four bird species studied in the four different ambient noise environments (peri-urban, no insect noise; peri-urban, with insect noise; urban, no insect noise; urban, with insect noise).

	Average Frequency \pm SE (Hz)			
	Peri-urban		Urban	
	No insect noise	With insect noise	No insect noise	With insect noise
<i>Swinhoe's White-eye (Zosterops simplex)</i>				
<i>N</i>	19	20	22	19
Minimum Frequency	4120 \pm 45	4084 \pm 44	4152 \pm 42	4041 \pm 45
Maximum Frequency	6229 \pm 89	6300 \pm 87	6457 \pm 83	6270 \pm 89
Peak Frequency	4838 \pm 70	4885 \pm 68	4976 \pm 65	4814 \pm 70
Bandwidth	2109 \pm 80	2216 \pm 79	2305 \pm 74	2229 \pm 80
<i>Eurasian Tree Sparrow (Passer montanus)</i>				
<i>N</i>	16	10	22	30
Minimum Frequency	3020 \pm 81	2989 \pm 102	3273 \pm 69	2999 \pm 59

Maximum Frequency	6064 ± 130	5616 ± 164	6220 ± 110	5703 ± 94
Peak Frequency	4237 ± 90	4135 ± 114	4525 ± 77	4222 ± 66
Bandwidth	3044 ± 126	2627 ± 160	2947 ± 108	2704 ± 92

Common Tailorbird (*Orthotomus sutorius*)

<i>N</i>	9	32	10	12
Minimum Frequency	2320 ± 109	2392 ± 58	2863 ± 103	2553 ± 95
Maximum Frequency	5022 ± 262	4900 ± 139	5680 ± 249	5196 ± 227
Peak Frequency	3509 ± 141	3486 ± 75	3897 ± 134	3646 ± 122
Bandwidth	2702 ± 269	2508 ± 142	2818 ± 255	2642 ± 233

Red-whiskered Bulbul (*Pycnonotus jocosus*)

<i>N</i>	9	20	9	13
Minimum Frequency	1758 ± 60	1625 ± 40	1705 ± 60	1680 ± 50
Maximum Frequency	3639 ± 88	3362 ± 59	3477 ± 88	3382 ± 73
Peak Frequency	2649 ± 65	2445 ± 44	2526 ± 65	2551 ± 54

Bandwidth

1881 ± 64

1736 ± 43

1773 ± 64

1701 ± 53

Table 3. Observed frequency of Red-whiskered Bulbul song type in different noisy conditions.

Song Type	Peri-urban	Urban
Type A	16	2
Type B	13	20

Table S1. Estimates for coefficients of linear models for significant results.

Song trait	Species	Variable	Estimate	t (df)	P
Minimum Frequency	Swinhoe's White eye	Noise Level	30.05±11.89	2.53 (75)	0.014
	Eurasian Tree Sparrow	Location	183.38±76.01	2.41 (73)	0.018
	Common Tailorbird	Location	156.95±63.86	2.46 (58)	0.017
		Location*Cicada	99.82±48.26	2.06 (58)	0.043
Maximum Frequency	Eurasian Tree sparrow	Cicada	241.28±63.99	3.77 (73)	0.0003
	Common Tailorbird	Location	5830.66±2727.40	12.14 (57)	0.037
		Location*Noise evel	78.77±37.84	2.08 (57)	0.042
	Red-whiskered Bulbul	Cicada	94.07±39.45	2.38 (46)	0.021
Peak Frequency	Eurasian Tree Sparrow	Cicada	100.83±44.47	2.267 (73)	0.026

Bandwidth	Eurasian Tree Sparrow	Cicada	166.7±61.98	2.69 (73)	0.009
	Common Tailorbird	Location	-7794.86±2715.08	2.87 (57)	0.006
		Location*Noise Level	108.241±37.67	2.87 (57)	0.006
