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Galápagos yellow warblers in low- and high-traffic environments differ in song plasticity in response to noise.

Leon Hohl [*,1], Alper Yelimlieş [*,1,2], Çağlar Akçay [3], Sonia Kleindorfer [1,2,4]

[*]co-first authors

[1] Konrad Lorenz Research Center for Behavior and Cognition, University of Vienna, Grünau im Almtal, 4645 Upper Austria, Austria

[2] Department of Behavioral and Cognitive Biology, University of Vienna, 1030 Vienna, Austria

[3] School of Life Sciences, Anglia Ruskin University, Cambridge, UK

[4] College of Science and Engineering, Flinders University, Adelaide 5001, Australia

ORCID:

LH: 0009-0001-4485-7685

AY: 0000-0003-2319-6141

CA: 0000-0003-0635-9586

SK: 0000-0001-5130-3122

Corresponding Author:

Çağlar Akçay

School of Life Sciences, Anglia Ruskin University, East Road, Cambridge, UK CB1 1PT

Email: caglar.akcay@aru.ac.uk

Phone: +44 (0)1245 493131

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Galápagos yellow warblers in low- and high-traffic environments differ in song plasticity in response to noise.

Abstract

Acoustic communication between animals is increasingly disrupted by noise in human-altered environments. This can make signals less effective and create selection pressure to increase their detectability. A good example of this is bird song. Song is a signal used in agonistic interactions between territorial rivals, and birds may modify their song and singing behaviour in response to noise. However, if these modifications are still ineffective, this can lead to increased conflict between rivals. Here, we asked whether experimental traffic noise induces immediate changes in acoustic characteristics of song and aggressive behaviour in populations that differ greatly in traffic noise exposure. We conducted simulated territorial intrusions on Galápagos yellow warblers (*Setophaga petechia aureola*) living on Santa Cruz (high traffic) and Floreana (low traffic) islands. We assessed the focal birds' physical response levels and recorded their vocalisations in response to playback of conspecific song (control) and conspecific song coupled with traffic noise (noise treatment). Although we found that physical response levels did not differ between islands or treatment types, birds on each island adjusted their songs differently in response to noise. In particular, birds increased the minimum frequency of their songs in response to the noise treatment, and the increase was more pronounced on Floreana Island (low traffic) than on Santa Cruz (high traffic). In contrast, song duration increased in response to noise treatment only on the high-traffic island. Our results suggest behavioural flexibility in birdsong in response to traffic noise, which appears to depend at least in part on prior experience with traffic noise.

Keywords: Anthropogenic noise, Birdsong, Aggression, Galápagos, Yellow Warbler

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Highlights

- Galápagos yellow warblers on different islands modified their song structure in an experience-dependent way when exposed to a simulated intruder and traffic noise.
- In Santa Cruz (higher traffic) birds increased the duration of their songs in response to experimental noise while in Floreana (lower traffic) they did not.
- Santa Cruz birds sang with higher minimum frequencies in general, but Floreana birds increased minimum frequencies in response to noise more than the Santa Cruz birds
- Birds on neither island changed their physical aggression towards the simulated intruder dependent on experimental noise.

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Interference from noise during signal transmission has important implications for animal communication (Bradbury & Vehrencamp, 2011). Given the rapidly increasing scale of human activities, animals need to produce and perceive signals in an ever-noisier world (Brumm, 2013; Halfwerk & Slabbekoorn, 2015). This is especially prevalent in the acoustic domain, where loud noises caused by traffic, construction, or other activities may mask animal vocalisations with implications for lower reproductive success and/or survival (Bowen et al., 2020; De Jong et al., 2018; Grade & Sieving, 2016).

The effect of anthropogenic noise on vocalisations has been most widely studied in songbirds (Brumm & Zollinger, 2013; Duquette et al., 2021). Bird song is a culturally transmitted acoustic signal that functions in both inter-sexual communication such as mate attraction as well as communication within each sex, such as territory defence against same-sex competitors (Catchpole & Slater, 2008). signaling in territorial disputes can be mutually beneficial because both parties can assess their rivals without the cost of fighting, which may result in injury or death (Logue et al., 2010; Maynard Smith & Harper, 1988). When anthropogenic noise masks animal signals, however, assessment of rivals via signals is impaired, increasing the probability that the opponent will need to resort to a conflict involving physical aggression. In support of this scenario, there is evidence from several species of songbirds that high levels of acoustic noise are correlated with increased physical aggression (Akçay et al., 2020; Davies & Sewall, 2016; Phillips & Derryberry, 2018). Of course, increased aggressiveness in urban environments could also be explained by other variables such as resource availability (Foltz et al., 2015), but experimental studies have shown that at least in some cases noise can lead to increased territorial aggression in birds (Grabarczyk & Gill, 2019; Önsal et al., 2022; but see Kleist et al., 2016).

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To improve transmission of signals in noise, songbirds may use one of several adjustments to their signals and signaling behaviours (Brumm & Zollinger, 2013; Kunc & Schmidt, 2021). One common adjustment is to increase the amplitude of the signal, known as the Lombard effect (Brumm & Zollinger, 2011; Hage et al., 2013; Kunc et al., 2022). Alternatively, they can increase the redundancy of the signal through repetition or by singing for longer (Brumm & Slater, 2006; Ríos-Chelén et al., 2013). Increasing redundancy would be potentially more effective in response to intermittent noise (as opposed to constant noise) as there would be a possibility of at least partially avoiding masking during signal production.

Because anthropogenic noise generally occupies lower frequency bandwidths, another possibility to reduce the effects of masking is to change the spectral parameters of the vocalisations (Roca et al., 2016; Slabbekoorn & Peet, 2003). One possibility is to raise the frequency of vocalisations so that there is less overlap with low-frequency noise. Such changes in frequency may come about through either ontogenic effects (Moseley et al., 2018; but see Liu et al., 2021), or through immediate and short-term plasticity (Bermúdez-Cuamatzin et al., 2011; Verzijden et al., 2010). It is worth noting that the changes in frequency may partly come about as a by-product of the Lombard effect as singing louder can lead to higher frequencies (Nemeth & Brumm, 2010).

Whether animals can cope with noise by changing their signaling may depend on previous experience with noise (Courter et al., 2020; Gallego-Abenza et al., 2020; LaZerte et al., 2016). There are only a few studies that have directly examined whether previous experience with noise affects short-term changes in song in response to acute noise. In one study, LaZerte and colleagues (2016) found that black-capped chickadees (*Poecile atricapillus*) shifted their song frequencies to higher frequencies in response to noise only if they already lived in noisier territories. By contrast, birds in quieter territories shifted their song frequencies downward. In a

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similar vein, urban, but not rural, white-crowned sparrows (*Zonotrichia leucophyrs*) produced songs with lower maximum frequencies and bandwidths when exposed to experimental noise (Gentry et al., 2017). Given that urban birds live in noisier territories this again suggests a role for experience. In contrast to these results, other studies found that in at least some species, individuals living in rural or quieter areas can also respond to experimental noise with immediate plasticity in vocalisations (Bermúdez-Cuamatzin et al., 2011; Potvin & Mulder, 2013; Verzijden et al., 2010). Here we test whether immediate acoustic and behavioural plasticity in the face of experimental noise is dependent on previous experience with chronic noise in two island populations of a common songbird in the Galápagos archipelago, the Galápagos yellow warbler (*Setophaga petechia aureola*).

While long considered a natural laboratory to study natural selection (B. R. Grant & Grant, 1979; P. R. Grant & Grant, 2014), the Galápagos Archipelago is not spared the human footprint and is now increasingly also a source of research into urban ecological frameworks that explore human-wildlife conflict and the evolutionary dynamics of endemic island systems. In Darwin's finches, there is a growing body of research into the effects of urbanisation on altered foraging behaviour (De León et al., 2019; Rivkin et al., 2021), nesting success (Harvey et al., 2021), boldness (Gotanda, 2020), song phenotypes and aggressive behaviour (Colombelli-Négrel et al., 2023). Given the large growth in the human population on the Galápagos Islands (Kricher & Loughlin, 2022; INEC 2016), there is a concomitant increase in the number of trucks and cars, and as a result higher levels of noise as well as more roadkill (García-Carrasco et al., 2020). Between 1980 and 2013, there was a 57-fold increase in traffic on Santa Cruz Island (23 vehicles in 1980 and 1326 vehicles in 2013; Márquez 2000; García-Carrasco et al. 2020). The endemic Galápagos yellow warbler is widely distributed across the archipelago, both on inhabited and uninhabited islands, and is the most commonly killed bird (> 70%) among roadkill on the

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heavily trafficked main road of Santa Cruz Island (García-Carrasco et al., 2020). Being commonly found across the human disturbance gradient, the Galápagos yellow warbler has the potential to be an important study system for investigating the impacts of human activities on island birds.

Here we aim to measure behavioural and song structure plasticity in response to a simulated intruder under traffic noise in the Galápagos yellow warblers and to compare the magnitude of response between two islands (Santa Cruz and Floreana) that differ in human population size and traffic. If the birds show behavioural and song structure plasticity in their response to an intruder song in a noisy environment, we expect that individuals will modify their songs and singing behaviours to increase detectability (increase in frequency, longer duration, more songs). Crucially, if the ability to modify song structure depends on experience with noise (Gentry et al., 2017; LaZerte et al., 2016), more modifications will be made on the island with more traffic exposure (Santa Cruz). Finally, we also expected that if birds do not modify their song or modifications are ineffective in dealing with experimentally increased noise, they will increase physical aggression by more closely approaching the simulated intruders and engaging in more search behaviours for the intruder.

Methods

Study species and sites

The Galápagos yellow warbler is an abundant songbird that occurs on all vegetated islands and across all habitats of the Galápagos Archipelago, Ecuador (Chaves et al., 2012; Snow, 1966). It is a small (~12 g) songbird that is year around territorial (Snow, 1966). Both sexes sing distinctive songs (Yelimlies et al. unpublished data) to defend the territory. Males have a reddish-brown crown and a chest streaking of the same colour against a yellow plumage,

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whereas females are uniform yellow (Salgado-Ortiz et al., 2008), making the sexes distinguishable using binoculars in the field.

We conducted the experiment in the lowlands and highlands on Santa Cruz Island and Floreana Island. All study areas have previously been part of long-term annual nesting monitoring of Darwin's finch breeding biology monitored by our research group since 2000 (Common et al., 2022; Kleindorfer et al., 2021). Santa Cruz highland study plots were located in the Los Gemelos area (-0.625982, -90.384829), and the lowland study plots in the El Barranco area (0.739068, -90.301467). The Floreana Island highland study plots were located in the Cerro Pajas (-1.299829, -90.455674) and Asilo de la Paz (-1.313595, -90.454935) areas, and the lowland sites near the Loberia area (-1.282974, -90.49208). Both islands have permanent human settlements but differ in human population size and number of vehicles (INEC 2016; Galápagos Conservancy 2023). Santa Cruz has a population of over 15,000 humans (INEC 2016) and over 1,000 vehicles (Ovidio et al. 2009; García-Carrasco et al. 2020) compared with Floreana which has a human population of ~100 (Galápagos Conservancy 2023) and ~10 vehicles (Hohl et al. personal observation).

Number of vehicles

To estimate relative traffic density between the two islands, we placed passive sound recorders developed by the New Zealand Department of Conservation along the main road in the highlands at Los Gemelos on Santa Cruz (28 and 29 Jan) and Cerro Pajas on Floreana Island (4 and 5 Feb). The number of passing vehicles was counted by listening to the recordings at x8 speed on headphones of recordings made between 0600 and 0800 and 1000 and 1200. We measured dB (A-weighted) in the field with a sound level meter (Voltcraft SL-100) standing

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roadside to measure the maximum dBA at 3 m from passing vehicles on 28 Jan on Santa Cruz Island.

Active territories

We carried out playback trials at 38 (22 Floreana, 16 Santa Cruz) active yellow warbler territories in January and March 2022 on Santa Cruz, and February 2022 on Floreana. Focal birds were identified from 3-7 visits to the nest territories across three weeks. Sites were systematically searched every two days for the presence of male and female pairs and active nests (Lawson et al., 2023). A male or a male and female pair was considered resident if they were active on the territory over a three-day period as evidenced by nest building and/or males showing mate-guarding (following a female) and/or females exhibiting nest defence behaviours (alarm-calling, perch-switching, wing-flicking, circle-flight), which have been used to assign partulid nesting status in similar studies (Hobson & Sealy, 1989; Mitra, 1999). As in Lawson et al. (2023), if these conditions were met, the territory was included in the experiment.

Playback stimuli

We prepared 15 song and 10 traffic noise stimulus files with our field recordings using the software Audacity. The 15 yellow warbler song stimuli (9 from Floreana, 6 from Santa Cruz) were created from recordings made in January and February 2022 using a Zoom H5 (Zoom Corporation, Japan) recorder with a Sennheiser MKE 600 (Sennheiser electronic GmbH & Co. KG, Germany) directional microphone. Recordings were made at a 48 kHz sampling rate and 24-bit resolution. We selected songs with a high signal-to-noise ratio from the recordings, filtered frequencies below 1500 Hz, and normalised the peak amplitude of each song. The song stimuli consisted of 2 or 3 renditions of a single song type presented at a rate of 6 songs per minute.

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We generated traffic noise stimulus files using recordings of vehicles passing on the main road through Santa Cruz Island during January 2022 by standing on the roadside and pointing the microphone towards the road (using the same equipment and settings above). We edited the traffic noise recordings to first clip unique traffic events (a vehicle passing). Then, we merged on average 5.5 (SD = 1.2) events per minute to create a stimulus tape of traffic noise. In other words, our noise stimulus simulated a traffic of 5.5 vehicles per minute passing by. This noise stimulus simulates the typical intermittent and varying levels of noise (which increases and decreases as a vehicle passes) that birds experience. Figure 1 shows the average frequency spectra for the noise and the song stimuli. Each playback stimulus consisted of 4 minutes: 1-minute baseline (silence), 1-minute song (song or song coupled with traffic noise, depending on treatment), 1-minute silence or traffic noise, and 1-minute song (song or song coupled with traffic noise). The song stimulus for each subject was a local song from a non-neighbouring conspecific recorded at least 200 m away from the same island. Both stimuli (song and noise) were broadcast at 85 dBA max (measured at 1 m from the speaker with Voltcraft SL-100, Conrad Electronic, Germany).

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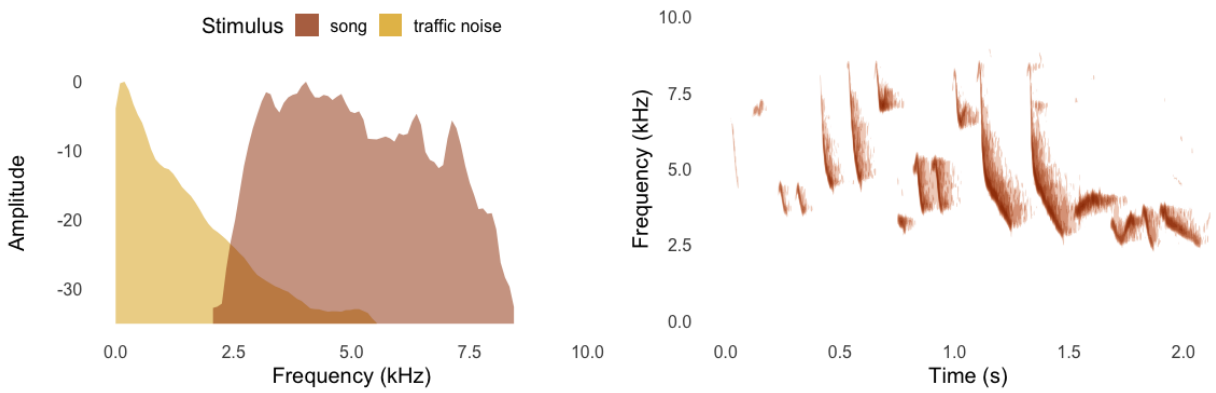

Figure 1. On the left: mean frequency spectra for one example traffic noise stimulus file and one song stimulus file, showing the overlap between the two. On the right: spectrogram of the same yellow warbler song stimulus.

Procedure and response measures

Each male was tested twice, once with song only and once with song plus traffic noise, the two trials being 1 day apart except for the 3 birds that were tested 2 to 5 days apart due to rainy weather. 17 of the subjects received the noise treatment before the song treatment and for the rest the order was reversed. For each trial, we placed two JBL Clip 4 Bluetooth speakers (JBL Inc., USA) at 0.5 m and 1.5 m above ground (usually clipped on a branch). The lower speaker was used to broadcast the traffic noise on the traffic noise trials and the upper one was used for the song. After placing the speakers, two observers were positioned 10 m apart and waited until the male was seen within 20 m to start the playback. Once the male was located within range, one observer started recording with a Zoom H5 recorder with a Sennheiser MKE 600 microphone and started the 4-minute playback stimulus via a smartphone. During the trials, starting with the 1-minute baseline period, both observers narrated the movements of the focal bird and recorded songs. From the narrations, we extracted the closest approach distance, number

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of flights, and crosses to the speaker (defined as flights within a 1m radius of the conspecific playback speaker). These measures are commonly used to quantify physical aggressive responses in songbirds (Falls & Brooks, 1975; Searcy et al., 2006).

Song analysis

From the trial recordings, we extracted high-quality songs that did not overlap with other vocalisations using the software Raven Pro 1.6.4 (K. Lisa Yang Center for Conservation Bioacoustics, 2022). The resulting dataset consisted of 490 songs (Santa Cruz = 225, Floreana = 265) from 36 birds (Santa Cruz = 15, Floreana = 21). We then measured the minimum and maximum frequency of the selected songs -20 dB relative to the peak frequency (i.e. the threshold method, Brumm et al., 2017); and calculated bandwidth as their difference using the freq_detec function from the warbleR package in R (Araya-Salas & Vidaurre, 2017; R Core Team, 2023). We also measured the peak frequency and the duration of the songs within Raven Pro (for the latter we used the duration 90% function which measures the duration in each selection that is between the times dividing the selection to the 5th and 95th percentile of the energy in the selection).

Statistical analysis

All data were analysed using R. Because our measures of physical approach were all correlated with each other (minimum distance, crosses, flights) we used a principal component analysis (PCA) using the package psych in R (Revelle, 2017) for these variables (see Table 1 for the correlation matrix and coefficients). The first component (PC1) of the PCA (unrotated, correlation matrix) explained 76% of the variance and had high factor loadings for behavioural

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response associated with physical aggression so it was taken as the aggression scores. A high aggression score (PC1) indicates a close approach and many flights and crosses over the speaker.

To test our hypothesis that behavioural response differs when exposed to an intruder in a noisy environment, we created a linear mixed model (LMM) using the aggression scores (PC1) as the dependent variable, island and treatment as the fixed factors, and the individual ID as the random factor. We also analysed the number of songs and the song structure parameters using separate LMMs with the same fixed and random factors. All models were created in R using package nlme (Pinheiro et al., 2022).

Ethical note

This study complies with all current Austrian laws and regulations and was supported by Animal Experiment License Number 66.006/0026-WF/V/3b/2014 issued by the Austrian Federal Ministry for Science and Research (EU Standard, equivalent to the Animal Ethics Board). The subjects were not captured during the experiment and each trial only took 3 minutes. The birds returned to their normal activities within a few minutes.

Results

Traffic conditions between islands

There was significantly more vehicle traffic on Santa Cruz Island (62 ± 7.8 per hr) than on Floreana Island (1.8 ± 0.3 per hr) ($t = 7.679$, $df = 6$, $P < 0.001$). The average amplitude of 27 vehicles recorded at 3 m was 84.7 ± 0.7 dBA max, with a minimum of 79.9 and a maximum of 95.4.

Behavioural responses to playback

Although all yellow warblers approached the speaker and displayed territorial defence behaviours during the playbacks (Table S2), there were no significant differences in the

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aggression scores (PC1) depending on the treatment, island, or their interaction effect (Table 1, Figure 2). Number of songs in response to playback also did not differ between the birds depending on treatment, island, or their interaction (Table 1, Figure 2).

Variable	*Predictors*	*Estimates*	*CI*	*Statistic*	*p*
Aggression score	**(Intercept)**	-0.04	-0.48 – 0.40	-0.18	0.858
	treatment [Song+Noise]	0.13	-0.35 – 0.60	0.54	0.591
	island [Santa Cruz]	0.12	-0.56 – 0.80	0.36	0.719
	treatment [Song+Noise] × island [Santa Cruz]	-0.36	-1.08 – 0.37	-0.99	0.327
Number of songs	**(Intercept)**	8.64	6.19 – 11.08	7.16	**<0.001**
	treatment [Song+Noise]	-0.18	-3.64 – 3.28	-0.11	0.916
	island [Santa Cruz]	2.49	-1.28 – 6.26	1.34	0.189
	treatment [Song+Noise] × island [Santa Cruz]	0.31	-5.03 – 5.64	0.12	0.908

Table 1. Effects of island type and treatment on Aggression Scores (PC1) and number of songs. Results are shown for linear mixed effect models with island and treatment as fixed effects, and territory ID as a random effect.

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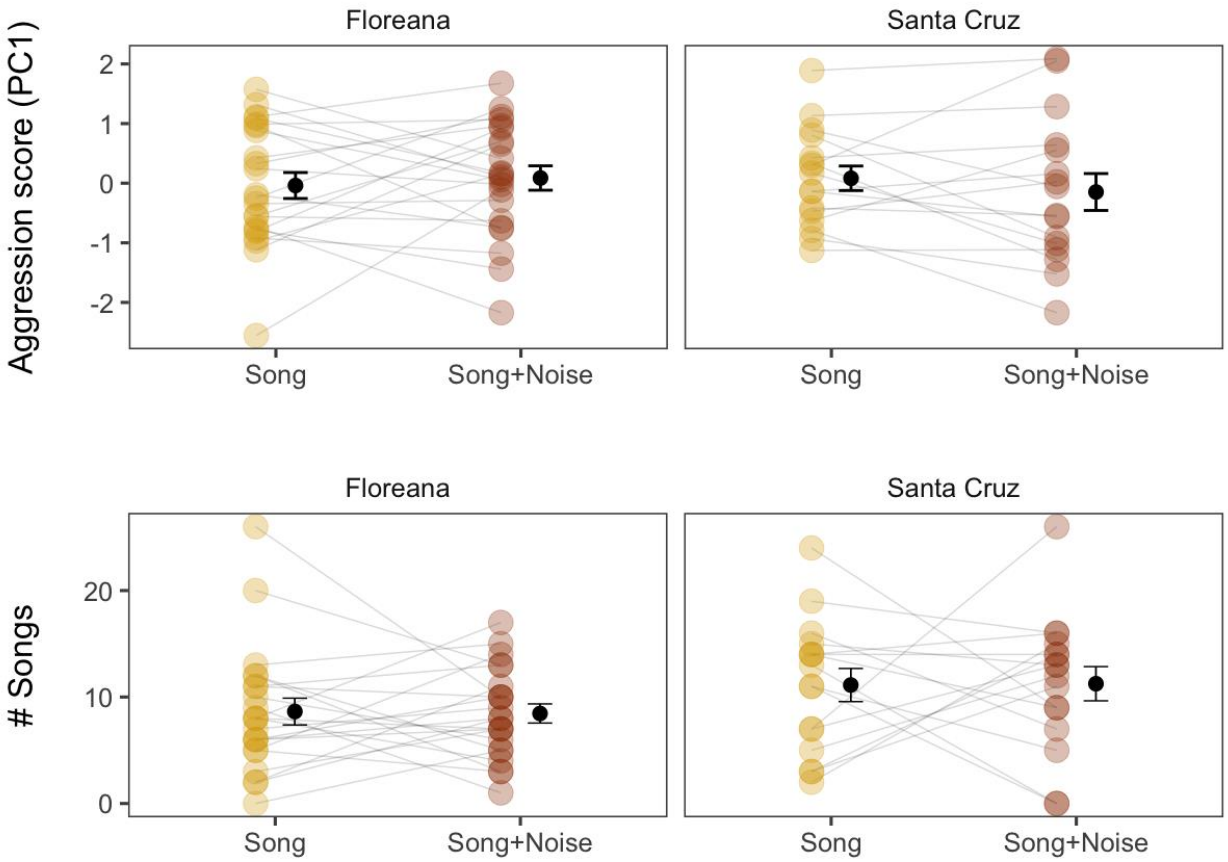

Figure 2. Behavioural response showing mean ± SE for a) Aggression Scores (PC1), and b) the number of songs. No significant differences across the islands or treatment groups existed in any of the variables (see results).

Song structure

Male yellow warblers changed their song structure when exposed to a simulated intruder with noise compared to without noise (see Table 2, Figure 2). In particular, males on Santa Cruz sang songs with higher minimum frequencies than on Floreana Island and birds on both islands increased the minimum frequency of their songs under experimental traffic noise. The latter effect, however, was more pronounced on Floreana as evidenced by a significant interaction

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effect (Table 2, Figure 3). The maximum frequency and the bandwidth (the difference between maximum and minimum frequency) did not differ between islands or treatments. The duration of songs was similar across the two islands but yellow warblers on Santa Cruz sang longer songs in song + noise treatment compared to the song-only treatment while birds on Floreana did not adjust the duration of their song, as evidenced by a significant interaction effect of treatment and island (Table 2, Figure 3). Finally, the peak frequency did not differ between islands and was slightly but significantly increased in noise treatment.

Variable	*Predictors*	*Estimates*	*CI*	*Statistic*	*p*
Minimum frequency	**(Intercept)**	2468.62	2398.14 – 2539.11	68.83	**<0.001**
	treatment [Song + Noise]	100.49	48.70 – 152.29	3.81	**<0.001**
	island [Santa Cruz]	125.92	13.69 – 238.15	2.28	**0.029**
	treatment [Song + Noise] × island [Santa Cruz]	-82.44	-158.64 – -6.24	-2.13	**0.034**
Maximum frequency	**(Intercept)**	7151.61	6933.72 – 7369.49	64.51	**<0.001**
	treatment [Song + Noise]	65.37	-96.06 – 226.80	0.80	0.427
	island [Santa Cruz]	-121.03	-467.92 – 225.85	-0.71	0.483
	treatment [Song + Noise] × island [Santa Cruz]	111.43	-126.07 – 348.94	0.92	0.357
Bandwidth	**(Intercept)**	4684.76	4464.13 – 4905.40	41.73	**<0.001**
	treatment [Song + Noise]	-36.70	-192.48 – 119.08	-0.46	0.644

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	island [Santa Cruz]	-246.46	-598.04 – 105.11	-1.42	0.163
	treatment [Song + Noise] × island [Santa Cruz]	193.92	-35.28 – 423.11	1.66	0.097
Duration	**(Intercept)**	0.95	0.87 – 1.03	23.75	**<0.001**
	treatment [Song + Noise]	-0.09	-0.15 – -0.03	-3.05	**0.002**
	island [Santa Cruz]	-0.09	-0.21 – 0.04	-1.40	0.170
	treatment [Song + Noise] × island [Santa Cruz]	0.16	0.08 – 0.25	3.68	**<0.001**
Peak frequency	**(Intercept)**	4493.20	4317.17 – 4669.23	50.16	**<0.001**
	treatment [Song + Noise]	186.74	5.06 – 368.43	2.02	**0.044**
	island [Santa Cruz]	65.72	-212.16 – 343.60	0.48	0.634
	treatment [Song + Noise] × island [Santa Cruz]	6.44	-260.89 – 273.78	0.05	0.962

Table 2. Effects of island type and treatment on song structure parameters. Results are shown for LMMs with island and treatment as fixed effects, and territory ID as a random effect.

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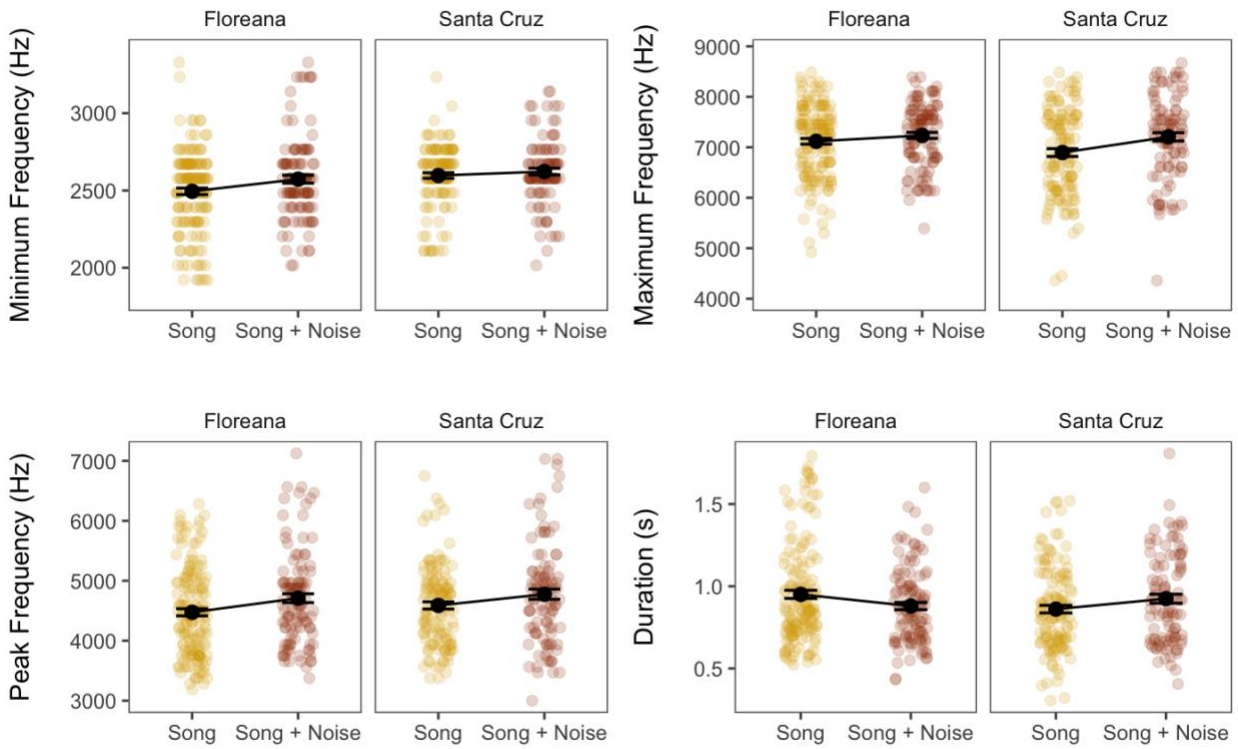

Figure 3. Song structure variables depending on treatment and island a) Minimum frequency (Hz), b) Maximum Frequency (Hz), c) Peak Frequency, and d) Duration. Each coloured circle represents a song, black dots in the middle and intervals show group means ± SE.

Discussion

In the present study, we asked whether Galápagos yellow warblers show increased aggression and flexibility in their song when faced with a simulated intruder in experimentally increased noise. Unlike some of the previous studies showing an increase in aggression levels with increasing ambient noise (Akçay et al., 2020; Davies & Sewall, 2016; Phillips & Derryberry, 2018), or experimental noise (Grabarczyk EE & Gill, 2019; Önsal et al., 2022) we found no effect of experimental traffic noise on physical aggression. Instead, we found that birds on Santa Cruz sang songs with significantly higher minimum frequency than birds on Floreana

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and, when experimentally exposed to traffic noise, birds in both islands increased the minimum frequency of their songs. The latter effect was more pronounced on Floreana. We also found an increase in song duration with experimental noise but only on Santa Cruz. The peak frequency of songs was also slightly increased with experimental noise compared to the song-only treatment. The other acoustic parameters (maximum frequency and bandwidth) did not differ between the islands or treatments. We discuss these results below.

The difference in the baseline (under no experimental noise) minimum frequencies between the islands may be due to long-term selection for higher frequency songs on Santa Cruz Island as traffic levels have increased over the past few decades. A similar explanation was proposed for the changes in frequency parameters in the white-crowned sparrow songs in the San Francisco Bay Area in the USA (Luther & Baptista, 2010; Luther & Derryberry, 2012). Whether such changes may have come about through preferential cultural learning of songs with higher frequencies is still debated (Moseley et al., 2018; Zollinger et al., 2017). Interestingly, the white-crowned sparrows in the San Francisco Bay returned to singing lower minimum frequency songs in response to the dramatic reduction in human activities and anthropogenic noise during COVID-19 shutdowns of Spring 2020 (Derryberry et al., 2020). This latter finding suggests that irrespective of whether the long-term changes in song frequencies in bird songs are due to developmental or other proximate mechanisms, the birds retain adaptive plasticity to adjust song features as adults.

An alternative hypothesis for why the birds on Santa Cruz may be singing songs with higher minimum frequencies may be due to differences in body size. Larger birds may produce songs with lower minimum frequency (Friis et al., 2022; Ryan & Brenowitz, 1985). Therefore, if there are differences in yellow warbler body size between the islands, such morphological

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differences could explain differences in song frequency (Potvin, 2013). While we did not measure yellow warbler morphology across the islands, two previous studies did. Browne and colleagues (2010) found that there were some size differences between yellow warblers on different islands with Santa Cruz birds being smallest, although their sample sizes were relatively small (including for Floreana; n = 4). Another study by Chaves and colleagues (2012), with a larger sample size did not find any morphological differences between yellow warblers from four Galápagos islands, but their dataset did not include birds from Floreana. Therefore, this eco-morphological hypothesis needs further data to test it fully.

Interestingly, birds on Floreana adjusted their minimum frequency more than the birds on Santa Cruz but birds on Santa Cruz increased the duration of their song more than the birds on Floreana. These findings point to a potentially complex effect of previous experience on adaptive adjustments on song. On one hand, the finding that birds in Santa Cruz increase the duration of their songs more than birds in Floreana is consistent with the hypothesis that long-term selection due to-- or previous individual experience with noise leads to the ability to rapidly and adaptively adjust songs features (Gentry et al., 2017; LaZerte et al., 2016). On the other hand, the minimum frequency patterns that show stronger adjustments on Floreana than in Santa Cruz appear to run against this hypothesis. A potential reason behind these seemingly contradictory findings may be that there is a limit to plasticity in minimum frequencies; perhaps birds on Santa Cruz are already singing at higher minimum frequencies (and/or higher amplitudes as we discuss below) and may not be able to further increase their minimum frequency. Instead, they may attempt to cope with the increased noise levels by increasing the duration of their songs.

It is worth noting that the changes in frequency, while potentially adaptive, may also come about by increases in song amplitude. Nemeth and colleagues (2013) showed that

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amplitude and frequency covary and that birds with greater song amplitude also have higher frequencies. In our study, we did not measure the amplitude of the songs, and therefore can't rule out an effect of the amplitude changes on song frequencies. Nemeth and Brumm, (2010) found that increasing amplitude is more effective in overcoming masking effects in noisy environments, which they measured experimentally in the great tit (Parus major) and blackbird (Turdus merula) populations. Specifically, higher amplitude songs travelled further whereas there was greater attenuation of higher pitched songs. In regard to the argument of efficacy, in urban habitats, the upward shift in song frequency increased the communication distance to a significantly lesser degree than amplitude (Nemeth & Brumm, 2010). Measuring amplitude in the field with freely moving animals is difficult but future studies should endeavour to disentangle these effects. Another fruitful avenue would be to measure the transmission of the songs from the two islands under different noise treatments to see if the frequency as well as duration adjustments do in fact lead to better transmission.

If song produced during territorial defence is an aggressive signal that is used to resolve agonistic interactions instead of physical aggression, then acoustic noise that interferes with effective signaling may result in increased aggression. Several studies across taxa have found increased levels of aggression in noisy environments (in humans: Alimohammadi et al., 2018; Moudon, 2009; in songbirds: Akçay et al., 2020; Phillips & Derryberry, 2018). In contrast, we found that despite the pronounced difference in traffic levels between islands, yellow warblers did not show island-level differences in their physical response behaviour to the simulated intruder, nor did they increase aggression when they were faced with intermittent traffic noise. It is possible that short-term and long-term adjustments in song frequency (and possibly amplitude) are sufficient to deal with interference from the traffic noise. It is also possible that yellow

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warblers use signals in other modalities (such as visual modality, e.g. wing-out and tail-spread display) more effectively in increased noise (Akçay & Beecher, 2019; Önsal et al., 2022).

In addition to posing a problem for sound transmission (Parris & Schneider, 2009; Schmidt & Balakrishnan, 2015), traffic also poses a risk to survival (García-Carrasco et al., 2020; Grilo et al., 2021; Parris & Schneider, 2009). Globally, roadkill is becoming an increasing threat to the survival of animals (García-Carrasco et al. 2020; Grilo et al. 2021). In the Galápagos, yellow warblers make up by far the largest portion (up to >70% on the main road) of roadkill on Santa Cruz, despite having similar abundances to other songbirds (García-Carrasco et al. 2020). Why yellow warblers make such a high proportion of roadkill on Galápagos is unclear, although some ecological and morphological traits are likely to be important. A study in Portugal found that foliage-gleaning birds as well as to a lesser extent birds with high flight manoeuvrability had higher mortality risk due to vehicle collision (Santos et al. 2016). The combination of these risk factors in the Galapagos yellow warblers may be leading disproportionate mortality of this species on roads but this hypothesis needs further testing. A way that humans can help reduce roadkill and acoustic competition is to regulate speed limits – given vehicle velocity is positively correlated with noise (Cowan, 1993) and areas with lower speed limits have less roadkill (Farmer & Brooks, 2012; Hobday, 2010).

In summary, we found that traffic noise affects the song structure of the Galápagos yellow warblers, with some differences between islands with different traffic levels. On both islands, when exposed to traffic noise, birds increased the minimum frequency of their songs. And on the island with high traffic, resident birds also sang longer songs when exposed to a simulated intruder song with traffic noise. While the Galápagos Islands are a natural living laboratory that inspired Charles Darwin's formulation of the theory of evolution by natural

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selection (Darwin, 1859; P. R. Grant & Grant, 2014), our study adds to the growing body of knowledge that the archipelago is affected by anthropogenic impacts that are expanding globally. The Galápagos yellow warbler, common in all the habitats of vegetated islands, is a good model system for studying the human impacts on this unique archipelago.

Data availability

Data and R-code to reproduce analyses can be found in the supplementary materials.

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