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Consequences of anthropogenic noise to song elaboration, signal value and reproductive success: field and captive studies in neotropical Bananaquits and Bengalese finches

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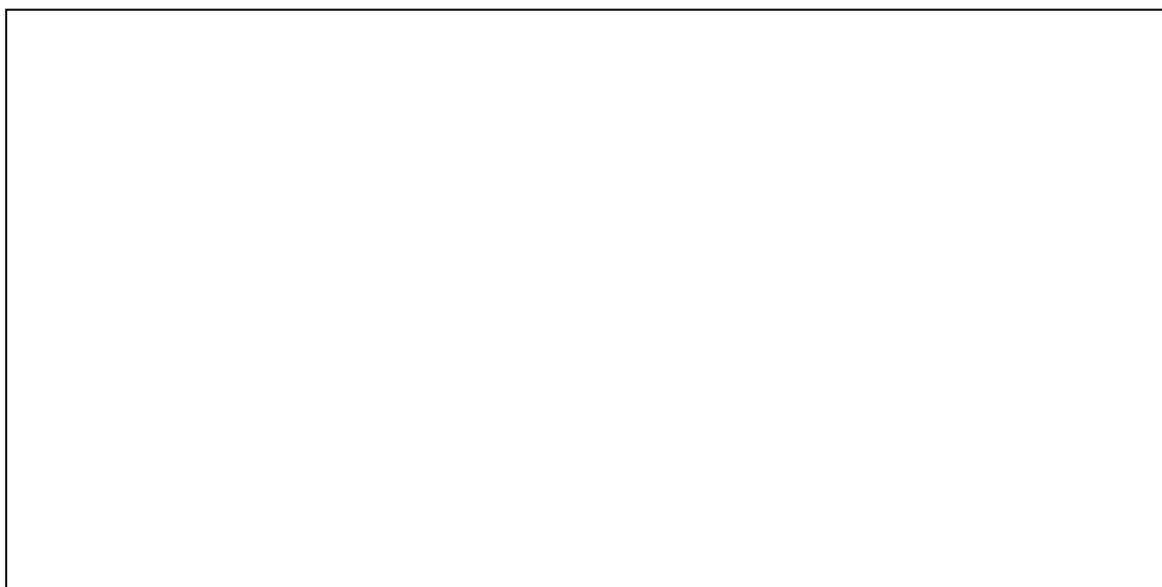
**Consequences of anthropogenic noise to song elaboration, signal value and reproductive success: field and captive studies in neotropical Bananaquits and Bengalese finches**

Ph. D. Thesis presented to the Graduate Program in Experimental Psychology at the Instituto de Psicologia, Universidade de São Paulo, Brazil, to obtain the degree of Doctor of Experimental Psychology. Concentration area: Experimental Psychology. Advisors: Patrícia Izar Mauro, Hilton Ferreira Japyassú, Hans Slabbekoorn.

São Paulo  
2019

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## RESUMO

Winandy, Gabrielle Sá Melo. **Consequências do ruído antropogênico sobre a elaboração do canto, valor do sinal e do sucesso reprodutivo: estudos de campo e de cativeiro em cambacicas neotropicais e manons**. 2019. 96f. Tese (Doutorado em Psicologia Experimental) – Instituto de Psicologia, Universidade de São Paulo, São Paulo, 2019.

O ruído antropogênico pode interferir no comportamento animal, como na comunicação acústica, defesa de território, atração de parceiros e sucesso reprodutivo. Como o ruído pode mascarar os sinais acústicos, os animais podem mudá-los como uma aparente resposta de adequação, entretanto, isso pode acarretar desvantagens à qualidade do sinal. Mudanças na frequência e duração, por exemplo, podem evitar o mascaramento do ruído, mas também podem levar à restrição de outros parâmetros acústicos que carregam informação sobre a qualidade do emissor. Com tais efeitos de mascaramento e mudanças nos cantos e na qualidade do sinal, diferentes parâmetros da vida dos organismos (e.g., defesa de território, escolha de parceiro, reprodução, cuidado parental) podem também ser negativamente afetados. No presente estudo, investigamos se reduções na faixa de frequência ou duração dos cantos restringiram a diversidade silábica ou elaboração do canto em um pássaro neotropical, a cambacica (*Coereba flaveola*) (capítulo 1). Em seguida, utilizando playbacks de cantos elaborados versus simples, nós investigamos se os ajustes vocais ao ruído levam a um potencial compromisso funcional da elaboração dos cantos (capítulo 2). Por fim, em exposição de longo prazo ao ruído, nós testamos se três diferentes fases do processo reprodutivo são afetadas em manons (*Lonchura striata var. domestica*) (capítulo 2). Nós demonstramos que as cambacicas cantam em frequências mais altas e de largura de bandas mais estreitas em territórios mais ruidosos, independente da variação da densidade do território, sem mudanças significativas na duração. Tais mudanças espectrais do canto estão correlacionadas a um número menor de tipos de sílabas diferentes. Ademais, as cambacicas respondem acusticamente diferentes à variação na elaboração do canto, o que sugere que as restrições vocais dependentes do ruído comprometem o valor do sinal. Essa restrição acústica pode refletir um comprometimento funcional entre audibilidade e valor do sinal: as frequências mais altas e mais restritas podem ser mais audíveis, mas cantos menos elaborados podem enfraquecer a mensagem da qualidade do sinal. Em relação aos experimentos de cativeiro, os manons expostos ao ruído atrasam a postura dos ovos, entretanto fases subsequentes de incubação e cuidado parental não foram afetadas. O mascaramento auditivo dos cantos dos machos pode melhor explicar o atraso na postura dos ovos, uma vez que comportamentos que precedem tais fases e que são dependentes de vocalizações, como corte e escolha de parceiros, podem ser afetados. Cantos menos elaborados, de banda de frequência e qualidade restritos podem trazer consequências para a aptidão individual, uma vez que pode representar menor qualidade do emissor. A longo prazo, as mudanças comportamentais observadas podem moldar a diversidade acústica, o processo de evolução da comunicação e a seleção sexual.

**Palavras-chave:** qualidade do sinal; complexidade do canto; ajustes dependentes do ruído; plasticidade comportamental; elaboração do canto.

## ABSTRACT

Winandy, Gabrielle Sá Melo Winandy. **Consequences of anthropogenic noise to song elaboration, signal value and reproductive success: field and captive studies in neotropical Bananaquits and Bengalese finches.** 2019. 96f. Tese (Doutorado em Psicologia Experimental) – Instituto de Psicologia, Universidade de São Paulo, São Paulo, 2019.

Anthropogenic noise can interfere with animal behaviour, from acoustic communication, to territorial defense, mate attraction and reproductive success. In response to a masking effect, animals may change their acoustic signals as an apparent adjustment strategy, but with a drawback on signal quality. Songs and calls may show noise-dependent changes associated in frequency and duration, which reduce masking, but may constrain other acoustic parameters that might carry information about the sender. With such masking effects and changes in male songs and signal quality, the female preference, fertility, incubation and parental care can also be negatively affected. In the present study, we study a Neotropical songbird, the bananaquit (*Coereba flaveola*) to investigate whether noise-dependent reduction in frequency bandwidth or song duration restricted syllable diversity or song elaboration (chapter 1). Using playback stimuli of elaborate versus simple songs, we also investigated the potential for a functional compromise of the song elaboration in this songbird (chapter 2). Using long-term noise experiment in captivity, we tested whether the noise affected three different phases of reproduction in the Bengalese finch (*Lonchura striata var. domestica*) (chapter 3). We show that in noisier territories, bananaquits sing higher frequency songs, of narrower bandwidth, without significant variation in song duration. These results were not affected by the density of conspecifics. The spectral adjustment was correlated to a lower number of different syllable types present in songs. Moreover, the acoustical responses of bananaquits differed in song elaboration, suggesting the noise-dependent vocal restrictions may compromise the signal value and communicative function. This finding is in line with an acoustic restriction and may reflect a functional trade-off between audibility and signal value whereas higher frequencies may be more audible but reduced song elaboration may weaken the message quality of the sender. Our results from the captive experiment show that the Bengalese finches exposed to noise delayed laying eggs, however subsequent phases of hatching and parental care were not affected. Auditory masking of male songs may explain the delay in egg-laying, as some behaviours that precedes such phase and which depend on vocalizations (e.g., mating, female choice) can be affected. The variation in song elaboration and its quality, and the reproductive consequences of noise exposure could yield to consequences for individual fitness in cities. Over the long-term this could also shape the acoustic diversity and processes of sexual selection in urban environments.

**Key words:** song quality; song complexity; noise-dependent adjustments; behavioural plasticity; song elaboration.

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## INTRODUCTION

Anthropogenic activities convert natural habitats into urban and rural areas in a process called urbanization (Schmid et al. 2018, Marzluff and Ewing, 2001, Marzluff 2001). A worldwide increase of urbanization in the past decades has yielded to a homogenization of communities (Devictor et al. 2007, McKinney 2006). This homogenization is due mainly to the destruction and removal of vegetation (McKinney 2005). Consequently, in large cities the biodiversity and the structure and function of ecosystems are reduced (Devictor et al. 2007). Biodiversity reduction in urban areas typically occurs by replacement of several native and specialist species by a few non-native and generalist urban adapters (McKinney et al. 2006, Morelli et al. 2016, Devictor et al. 2007). Such functional homogenization has been shown in bird communities (Devictor et al. 2007). Several urban bird species exemplify well the adaptability of non-native species: the rock dove (*Columba livia*), European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*) (Buijs and Van Wijnen 2001, Marzluff 2001). However, besides deforestation, other aspects of human activities can negatively affect the presence, attraction, and disappearance of species.

The anthropogenic noise also negatively affects animal communities in urban ecosystems (Buxton et al. 2017, Mennitt et al. 2015), for example, by playing a role on the decline of density and diversity of populations (e.g. Saha and Padhy 2011, Bunkley et al. 2017). At the individual level, anthropogenic noise can negatively affect the behavior and physiology of animals (e.g., Rheindt 2003), impairing their activities and chasing them out of urban areas (Kight and Swaddle 2011, Popper and Hawkins 2016). The reasons for negative effects and the severity of impact can vary per species and population. In noisy places, animals may not hear signals of conspecifics, potential predators or prey. Consequently, they may be hampered in finding mates, raise offspring, or bothered in some other important social interactions. They might also be less likely to survive as they can starve from not finding prey or be suddenly killed by a predator. Long exposures to noisy conditions can also raise physiological stress levels and debilitating their health (e.g., Halfwerk et al. 2011b, Meillère et al. 2015, Purser and Radford 2011, Wale et al. 2013, Davies et al. 2017). In summary, anthropogenic noise may negatively affect animal survival and

reproduction, as it can distract them from important activities, mask their auditory signals, damage their hearing and increase their physiological stress.

The masking effect of anthropogenic noise has been shown to negatively affect vocally communicating animals. The noise can decrease the detectability and recognisability of the vocal signals as it can overlap signals in both time and frequency (Barber et al. 2010, Parris and McCarthy, 2013; Templeton et al. 2016). Consequently, anthropogenic noise may negatively affect vocal function in several behavioural contexts, such as courtship, territory defence and predation avoidance (Quinn et al. 2006, Fernandez-Juricic and Telleria, 2000, Halfwerk et al. 2011b, Kleist et al. 2016). For example, female great tits (*Parus major*) and female canaries (*Serinus canaria*), respond less to male songs under elevated noise (Halfwerk et al. 2011b, Huet des Aunay et al. 2014). Savannah sparrows (*Passerculus sandwichensis*) respond less promptly to conspecific alarm calls or predators when under noise (Antze and Koper 2018). Despite all the masking effects and negative noise-related threats to survival and reproduction (Halfwerk et al. 2011a, Kleist et al. 2018, Potvin et al. 2015), some species thrive in urban environments and this might be due to some vocal adjustments.

Several types of noise-dependent vocal adjustments occur likely to increase audibility of signals in noisy conditions. For example, in some bird species, individuals may raise the minimum frequency, change the duration, raise amplitude and syllable rate of their songs, and alter timing of vocal activity to more quiet times of day (Brumm, 2004, Potvin et al. 2013, Gil et al. 2014). City birds sing higher-pitched, longer and more intense vocalisations than conspecific rural birds (Slabbekoorn and Den Boer-Visser, 2006, Ríos-Chelén et al. 2013, Brumm and Zollinger, 2011). These adjustments may decrease the masking effect of the typical low-frequencies of anthropogenic noise and increase song audibility (Brumm and Slabbekoorn 2005, Pohl et al. 2012).

Noise-dependent signal changes may not only be beneficial but can also have some negative consequences. Although high-frequency songs may increase detectability and female responsiveness relative to low-frequency songs in noisy situations (Halfwerk et al. 2011a), they can be less attractive to females choosing a partner to mate (Huet des Aunay et al. 2014, Halfwerk et al. 2011a). Spectral and temporal adjustments could also lead to vocal restrictions on signal efficiency, as they may prevent the use of specific syllables with potentially high signal value

(Halfwerk et al. 2011a, Huet des Aunay et al. 2014) or reduce available repertoire size or song elaboration, which may also signal some sender quality (Buchanan and Catchpole 2000, Kipper et al. 2006). Reduction in frequency band use may restrict the use of particular syllables and limit possible syllable variation, and consequently limit song repertoire size of an individual (Montague et al. 2012; Fouda et al. 2018). As a physiological stressor, anthropogenic noise can increase the level of stress-related hormones, such as corticosterone, which can interfere with female fertility and reproduction (Davies et al. 2017, Wright et al. 2007, Blickley et al. 2012, Angelier et al., 2009). Finally, noisy conditions may also affect vigilance behaviour during feeding (Fernandez-Juricic and Telleria, 2000; Quinn et al., 2006), which could reduce food intake and offspring provisional rate. Summarizing, through masking of communication and inducing stress, anthropogenic noise can, in the short-term, negatively affect survival chances, mate attraction and reproduction; in the long-term it can modify patterns of sexual selection and acoustic communication in urban ecosystems, potentially resulting in population decline or local extinction of some species.

Neotropical and captive bird species can be good model systems to study the negative impacts of anthropogenic noise on behaviour and reproduction. There are still few studies of urban birds of Latin America as most investigations have focused on temperate species of Europe and North America (MacGregor-Fors, I. and Escobar-Ibáñez 2017, Ortega-Álvarez and MacGregor-Fors, 2011, Delgado-V and Correa-H, 2013). Neotropical bird species can be excellent models to study noise-induced vocal and behavioural changes due to their wide range of vocal variability, complexity and flexibility to noise (e.g. Bermúdez-Cuamatzin et al. 2009, 2011), specially the song learners oscines (Ríos-Chelén et al. 2012). Bananaquits (*Coereba flaveola*), for example, have songs that widely vary in complexity and in frequency use as I will show along this thesis (also see Hilty and Christie 2018). The syllable diversity per song and elaboration of the repertoire can vary among birds of the same geographical area or city, making it potentially a noise-dependent vocal flexibility. Captive bird species may also contribute to experimental studies that investigate the mechanisms underlying noise-dependent vocal and behavioural changes. There are still few studies experimentally investigating the long-term negative impacts of noise on song and behavioural development and reproductive success in captivity, such as in zebra finches (*Taeniopygia guttata*) (Potvin et al. 2015, 2016).

In the present thesis, I studied the influence of noise on signal elaboration and quality in free living wild birds and I tested whether long-term noise exposure can cause alterations in reproductive success using captive birds in an aviary. I explored patterns of noise dependent variation in a descriptive study on song elaboration in wild neotropical bananaquits. I also tested experimentally whether noise-dependent song elaboration in this species plays a role in territorial signalling in the city of Salvador. For the captive experiments, I used Bengalese finches (*Lonchura striata* var. *domestica*) as a model species in breeding aviaries under low or experimentally elevated noise levels. In my first data chapter (Winandy et al. submitted a), I explored how noise-dependent song adjustments yield a trade-off between audibility and signal quality, which could affect mate choice and sexual selection. In my second data chapter (Winandy et al. submitted b), I explored how noise-dependent song elaboration might affect territorial defence in city populations and I addressed how it could affect mate choice and sexual selection in urban populations. Finally, in my third chapter (Winandy et al. in preparation), I explored how experimental, long-term, noise exposure may negatively impact reproductive success. In the general discussion after my data chapters, I integrated my findings and addressed how noise-impaired signal value, territorial signalling and reduced reproductive success can contribute to species extermination and homogenization of urban bird communities.

## 2. URBAN NOISE LEVELS SHIFT THE TRADE-OFF BETWEEN SONG FREQUENCY BANDWIDTH AND SYLLABLE DIVERSITY<sup>1</sup>

Increasing the chances of being heard under noise may involve reduced quality of acoustic signal. While many songbirds, through elaborate songs, may inform conspecifics of their quality, this ability may be impaired when noise abound. We show that city bananaquits, a Neotropical songbird, sing less elaborate songs when signaling at higher and narrower frequency ranges, those typically used under noisy situations. Such song restriction may alter the course of sexual selection in urban environments.

### 2.1 Abstract

Anthropogenic noise can interfere with animal behaviour through masking of acoustic communication. In response to masking, animals may change their acoustic signals as an apparent adjustment strategy, but this may have a drawback on signal quality. Songs and calls may show noise-dependent changes in frequency and duration, which may yield some masking avoidance, but may also constrain other acoustic parameters that might carry information about the sender. In the present study, we investigated whether noise-dependent reduction in frequency bandwidth or song duration restricted syllable diversity or song elaboration in a Neotropical songbird, the bananaquit (*Coereba flaveola*). We show that bananaquits sing higher frequency songs, of narrower bandwidth, in noisier territories, independent of variation in territory density, without significant variation in song duration. The spectral adjustment was correlated to a lower number of different syllable types present in songs. This finding is in line with an acoustic restriction and may reflect a functional trade-off between audibility and signal value: higher frequencies may be more audible but less elaborate songs may weaken the message of sender quality. Consequently, noise pollution may not only alter avian communities, but also shape acoustic diversity and processes of sexual selection in urban environments.

### 2.2 Introduction

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<sup>1</sup> Félix, RP<sup>2</sup>; Sacramento, RA<sup>2</sup>; Mascarenhas, R<sup>2</sup>; Batalha-Filho, H<sup>2,3</sup>; Japyassú, HF<sup>2,3</sup>; Izar, P<sup>1</sup>; Slabbekoorn, H<sup>4</sup>.

Anthropogenic noise has increased in natural and human-altered habitats (Buxton et al. 2017, Mennitt et al. 2015), where it might negatively affect vocally communicating animals. The elevated noise levels can cause acoustic interference by masking the functional variation in frequency and amplitude of vocal signals, for example in frogs, birds, and mammals (Wollerman and Wiley, 2002, Lohr et al. 2003; Erbe et al. 2016). Acoustic overlap in time and frequency with anthropogenic noise may decrease the detectability and recognisability of animal vocalizations and thereby disrupt or alter communication (Barber et al. 2010, Parris and McCarthy, 2013; Templeton et al. 2016). As a consequence, anthropogenic noise may negatively impact vocal function and undermine survival and reproductive success (Halfwerk et al. 2011a, Kleist et al. 2018, Potvin et al. 2015), for example through reduced foraging efficiency while avoiding predation, and less success in territory defence and mate attraction (Quinn et al. 2006, Halfwerk et al. 2011b, Kleist et al. 2016).

Evidence for a detrimental impact of anthropogenic noise via interference of acoustic communication has been reported in a variety of studies. Ambient noise levels may for example affect courtship: female great tits (*Parus major*) and female canaries (*Serinus canaria*) were found to respond less to the low-frequency songs of males in experimentally elevated noisy conditions than in ambient control conditions (Halfwerk et al. 2011b, Huet des Aunay et al. 2014). Ambient noise levels may also undermine communication about predation risk. Savannah sparrows (*Passerculus sandwichensis*) delay feeding visits to their nestlings when hearing conspecific alarm calls or predators. This behaviour is likely reducing predation for both parents and nestlings, but feeding latency declined under noisy conditions despite the presence of alarm calls or predator songs (Antze and Koper 2018). A solution, at least to some extent, would be to adjust acoustic signals in such a way that masking by anthropogenic noise would be less.

Several types of noise-dependent vocal adjustments could make signals more audible under noisy conditions. For bird songs, the vocal adjustments include modifications of the minimum frequency, changes in duration, amplitude and syllable rate, and altered timing of vocal activity (Brumm, 2004, Potvin et al. 2013, Gil et al. 2014). Specifically, city birds have been reported to sing higher-pitched, longer and more intense vocalisations than birds of the same species from more quiet territories

in rural areas (Slabbekoorn and Den Boer-Visser, 2006, Ríos-Chelén et al. 2013, Brumm and Zollinger, 2011). Such song adjustments may decrease the masking effect of the typical low-frequencies of anthropogenic noise and increase song audibility (Brumm and Slabbekoorn 2005, Pohl et al. 2012). Spectral and temporal adjustments, however, could also lead to vocal restrictions on signal efficiency, as they may prevent the use of specific syllables with potentially high signal value (Halfwerk et al. 2011b, Huet des Aunay et al. 2014) or reduce available repertoire size, which may also signal some sender quality (Buchanan and Catchpole 2000, Kipper et al. 2006).

There is indeed some evidence that spectral and temporal shifts under noisy conditions may impose inherent restrictions on syllable diversity. Montague et al. (2012) found that European robins (*Erithacus rubecula*) increased the minimum frequency of their songs in response to an elevation in ambient noise levels, which was associated with a synchronous decrease in frequency bandwidth, song duration and syllable length, as well as a decrease in the number of different syllable types. Montague et al. (2012) argued that birds may respond with adjustments in song structure to masking noise, but that acoustic plasticity may be restricted by mechanistic correlations among different song parameters. If such restrictions to acoustic variety affect song function, birds face a trade-off between audibility and signal value (Slabbekoorn and Ripmeester 2008, Gross et al. 2010, Slabbekoorn 2013; Luther et al. 2016), regardless of whether acoustic changes are a direct response to elevated noise levels, or an indirect consequence of a noise-dependent change in another parameter.

Song frequency use or syllable diversity may also be affected by factors other than ambient noise. For some bird species, population density can increase in human-altered habitats due to the increased availability of food resources and decreased presence of predators (Chace and Walsh 2006; Ciach and Fröhlich 2017, Tomialojć 1998). High territory density may induce competitiveness among males and change their singing behaviour (Dabelsteen and Pedersen 1990; Ripmeester et al. 2010, Narango and Rodewald 2015). Territory density may therefore be correlated to motivational variation in temporal components of song, such as syllable length, syllable rate, number of syllables and song length (Hamao et al. 2011, Narango and Rodewald 2015). However, it has also been shown that having more audible territorial neighbours was associated with spectral components of song, such as the

minimum frequency in urban great tits (*Parus major*) (Hamao et al. 2011), and the peak frequency in urban blackbirds (*Turdus merula*) (Ripmeester et al. 2010). Population density may therefore be an alternative explanation, and thereby a confounding variable, for noise-dependent song variation.

The Neotropical songbird bananaquit (*Coereba flaveola*) is an excellent model system to study noise-dependent song variation and to test for potential signalling trade-offs due to inherent acoustic correlations among song parameters. Bananaquits exhibit quite complex vocalisations, including a variety of high- and low-pitched syllable types, while song diversity and elaboration has been reported to vary among birds from different areas (Hilty & Christie, 2018). Furthermore, they are relatively abundant and have urban and rural distribution where they breed and sing in a variety of microhabitats with variable ambient noise levels. The bananaquit is typically also used to human presence and thereby very suitable to approach for song recordings and analyses of geographic variation and correlation to environmental variables.

In this study, we recorded bananaquit songs in urban territories with variable ambient noise levels to correlate song variation to noise, taking territory density into account. We aimed to answer the following questions: 1) are bananaquit songs higher, narrower and shorter in noisier territories? 2) is territory density a confounding variable and also correlated to song frequency or duration? and 3) does any noise-dependent song structure determine a restriction on song elaboration yielding less and lower diversity in syllables? If so, we would provide more insight into how noise pollution may alter conditions for sexual selection and evolutionary change in urban bird species, as well as shape species and song diversity of urban bird communities.

## **2.3 Material and Methods**

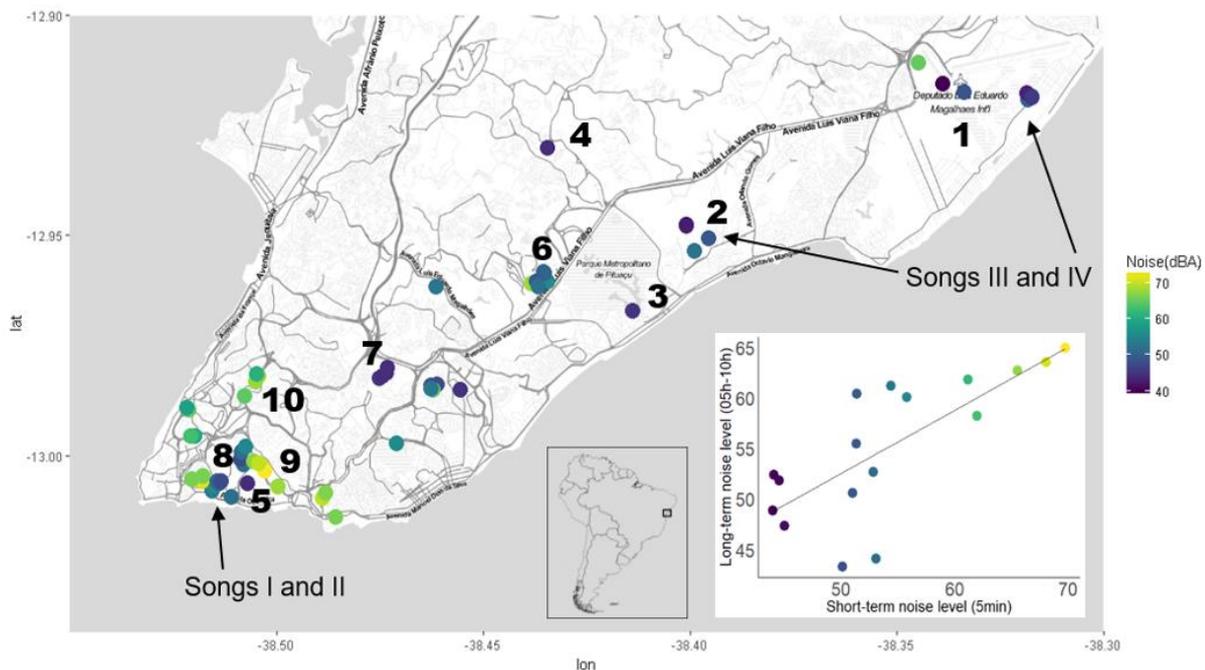
### *2.3.1 Subject species and study area*

Bananaquits (*Coereba flaveola*) are small nectivorous birds with a downward curved bill that occur across the Neotropics, from Southern Mexico to Northern Argentina and the Caribbean islands. They breed in a wide variety of habitats, predominantly at low elevation, including city gardens, urban parks, disturbed areas, and forest borders (Hilty & Christie, 2018). They are largely monomorphic and

appear in the study area with a grey back, black crown and cheek, white eyebrow, light-grey throat, and a bright yellow belly (this plumage varies geographically). Bananaquits are persistent singers and breed throughout the year (Hilty & Christie, 2018). The song is a relatively short series of high-pitched syllables, with more or less repeated sound elements that are often repeated in stereotypic fashion. They can sing during all parts of the day, including the rush hours (personal observation).

We conducted our study in urban parks and built-up areas (commercial and residential) in the city of Salvador, Bahia, Brazil, between January of 2016 and March of 2018. We visited localities 400m to 25km apart (figure 1). Our sampling covered a range of environmental noise levels from 40 to 73 decibels (dBA). We recorded bananaquits, for example, in the relatively quiet areas of the tropical sand dunes ('restinga') of 'Parque das Dunas', at the borders of the Atlantic forest fragments of 'Jardim Botânico', 'Parque de Pituaçu', 'Vale Encantado' and 'Parque Zoobotânico Getúlio Vargas', and in urban gardens of 'Jardim da Saudade' and the Federal University of Bahia (figure 1). Noisier bananaquit territories were recorded in more busy, urban areas – i.e., main avenues with high concentration of concrete buildings and low quantities of natural trees and gardens, which occur alongside Atlantic forest fragments, such as 'Coelba', at the borders of the sand dunes, and in urban gardens such as at the 'Garibaldi' and 'Dique' localities (figure 1). We consider our sampling design as a contiguous population in urbanized area with high heterogeneity in terms of vegetation, buildings and noise level. All territories, localities and respective ambient noise levels are represented in the map (figure 1), which was generated using 'ggmap' package in R™ software (Kahle and Wickham 2013).

Figure 1 – Map representing the distribution of the recorded birds in the city of Salvador, Bahia, Brazil, and the variation of noise levels along sampled territories and in the correlational graph (long-term and short-term noise recordings). The colour of the dots represents the intensity of the environmental noise (in decibels, A-curve), measured during the recording of bananaquit songs. In a continuous coloured scale, purple dots represent quieter territories and yellow dots the noisier territories. Indicated by numbers are example of quieter and noisier localities and their respective habitat types: Tropical sand dunes of 1) ‘Parque da Dunas’; Atlantic forest fragments of 2) ‘Vale Encantado’, 3) ‘Parque de Pituacu’, 4) ‘Jardim Botânico’, 5) ‘Parque Zoobotânico Getúlio Vargas’ and 6) ‘Coelba’; Urban Gardens of 7) ‘Jardim da Saudade’ and 8) Federal University of Bahia; Main avenues in 6) ‘Coelba’, 9) ‘Garibaldi’ and 10) ‘Dique’. Spectrograms of more and less elaborated songs indicated in different localities (songs I to IV) are represented in figure 2.



Source: designed by the author

### 2.3.2 Song recordings and noise measurements

We recorded bananaquit songs with a Sennheiser™ (Wedemark, Germany) shotgun directional microphone (ME67 + K6) connected to a Sony™ (Tokyo, Japan) PCM-D50 digital recorder. Song recordings were taken in WAV format, at a sampling rate of 44kHz. We recorded the birds mostly between 05h00 and 10h00 AM, from a distance of 5 to 10 meters to the focal bird. To improve signal to noise ratio and the quality of the recordings in noisy locations, we positioned the microphone as close as possible to the bird and as far as possible from the noise source. We positioned the

direction of the microphone in parallel to the direction of the noise source as the sides of the microphone are the least sensitive. Additionally, we positioned a barrier, like a car or a wall, between the noise source and the microphone when possible (c.f. Slabbekoorn 2012).

We measured the ambient noise level in each territory where we were able to record sufficient bananaquit songs. We assessed the sound levels of ambient noise for 5 minutes, starting at the beginning of song recording, using a sound pressure level meter with data logger Skill-Tec™ (São Paulo, São Paulo, Brazil) SKDEC-02 (A-weight, slow response, range 30-130 dB, 1s interval). We positioned the equipment upwards, kept vertically on a tripod at 150 cm from the ground. The 5 minutes sample was integrated into a single, average sound level (dBA) as the ambient noise measure per territory.

The variation in sound levels within territories across the time period of sampling may undermine comparisons across territories (Slabbekoorn & Peet 2003; Arroyo-Solis et al. 2013). This potential problem was checked with repeated sound level measurements over the morning in a subset of bananaquit territories. We averaged ten five-minute samples per locality recorded between 05.00h and 10.00h, sampled at the start of each half hour. The long-term average was compared to the nearest short-term territory samples.

### 2.3.3 *Song processing and measurements*

We filtered song recordings using the software Audacity™ v. 2.1.2 (Carnegie Mellon University, Pittsburgh, Pennsylvania, USA), cutting-off the noise for each recording at different limits between 0.5-2.5kHz. The omission of low-frequency background noise renders a distinct presence of the target songs in the amplitude wave, which supported the measurement of song duration on the spectrogram. We generated song spectrograms and waveforms with Raven™ PRO software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) version 1.5. The chosen parameters for the calculation of spectrograms were Hann windows, DFT size 512 samples, and an overlap of 50%.

We measured the following spectral and temporal song parameters: minimum, maximum and peak frequency (Hz), frequency bandwidth (Hz) and song duration (s). We assessed these measures by cursor placement and the automated writing to file

procedure of the Raven™ PRO software. There are advantages and disadvantages to the method of cursor placement compared to a fully objective automated measurement technique by amplitude cut-off points relative to the peak amplitude in the song (Zollinger et al. 2012; Ríos-Chelén et al. 2016). However, our method is valid for our targets and potential issues about effect and artefact size are addressed in the discussion (c.f. Verzijden et al. 2010).

Observer bias is possible to some extent for some of the measurements (e.g. minimum and maximum frequency), as we had clear directional expectations about noise-dependent song variation (c.f. Brumm et al. 2017). However, our recording strategy in the field, to optimize signal-to-noise ratio, reduced direct audibility of current noise level during song measurements. Furthermore, noise level fluctuations in time also reduced the link between noise conditions at specific recording times and average noise levels. Consequently, song analyses were largely blind to variation in the relative noise level among territories, which should prevent problems of observer bias.

We also quantified four measures of song elaboration related to the number of repeated song units and the acoustic variety among these units (Garamszegi and Moller 2004; Soma and Garamszegi, 2011; Hill et al. 2017): 1) number of syllables (sound units per song), 2) number of syllable types (different sound units per song), syllable rate (number of syllables sung per second); 4) number of phrases (number of sequential syllable repetitions). These measures of song elaboration have been shown to play a role in communication related to sexual and territorial advertisement (Hoi-Leitner et al. 1995; Catchpole and Leisler 1996; Soma and Garamszegi, 2011; Hill et al. 2017).

#### 2.3.4 *Territory density assessment*

We assessed the number of singing bananaquits within a range of 100m of a target territory. Within this distance, birds are expected to properly hear each other and face relevant territorial interactions (Ripmeester et al. 2010). Bananaquits may hear more neighbours than human observers, due to a perceptual focus on conspecific song features and advantageous perch heights. However, it is important to have a measure of territory density that is sampled in a standardized way and which adequately reflects variation among territories in density related competition.

### 2.3.5 Statistical Analysis

We correlated spectral and temporal song measurements (minimum, maximum, peak frequency, frequency bandwidth and song duration) to the noise level measures associated with individual song recordings. We also tested for a confounding impact by social competition by correlating these measurements to territory density. We used linear models throughout by selecting the 'lm' function of R™ software (R Core Team, 2017).

We performed a principal component analysis (PCA) with the four song elaboration measurements to evaluate which variables contributed most to the variation among individuals. We performed the PCA using the 'factoextra' package in the R™ software (Kassambara and Mundt 2017). From the first and second components extracted (PC1 and PC2), we used the most prominent variables in PC1 and PC2 to test for the possible constraint of noise-dependent song adjustments on song elaboration. We also used these variables for testing for a possible interplay between song elaboration and territory density. Using the same software and 'lm' function, we performed linear models to investigate whether there was a correlation between the spectral variables minimum frequency and frequency bandwidth, the number of neighbours, and the two most prominent song elaboration measurements.

## 2.4 Results

We recorded songs and assessed ambient noise levels in 65 bananaquit territories and measured the territory density of 37 of these in terms of the number of singing male neighbours audible to the human observer. Three out of four of the spectral song measurements correlated significantly to noise level. Song minimum and maximum frequencies were higher in noisier territories (table 1, figures 2 and 3). Despite the fact that both the minimum and maximum frequencies were positively correlated, we also found the frequency bandwidth was significantly correlated to noise level, with a narrower frequency range in noisier conditions (table 1). Peak frequency and song duration were not correlated to noise level (table 1). The spectral variables that were correlated to noise level were not correlated to territory density (table 3).

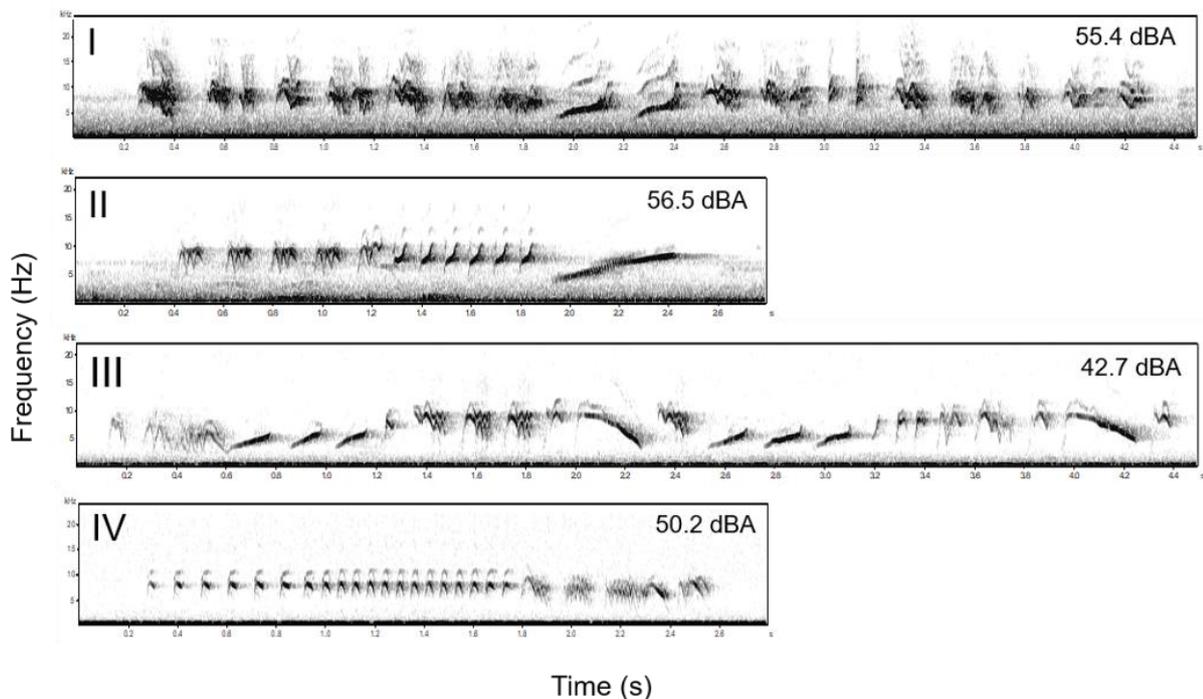
Table 1 – Fitted values of linear models between noise level and song components (i.e., syllable sequence length and syllable diversity) and between the noise level and the spectral and temporal song measurements. Values indicate respectively the significance values and the Regression coefficient value. N=65.

**Fitted linear models for comparisons between noise and song variables**

Song variables	R squared	t value	Significance value
Low frequency (Hz)	0.356	5.897	<b>&lt;0.001</b>
High frequency (Hz)	0.114	2.847	<b>0.006</b>
Frequency bandwidth (Hz)	0.097	-2.608	<b>0.01</b>
Peak frequency (Hz)	0.002	0.384	0.7
Song duration (Hz)	0.004	-0.517	0.607

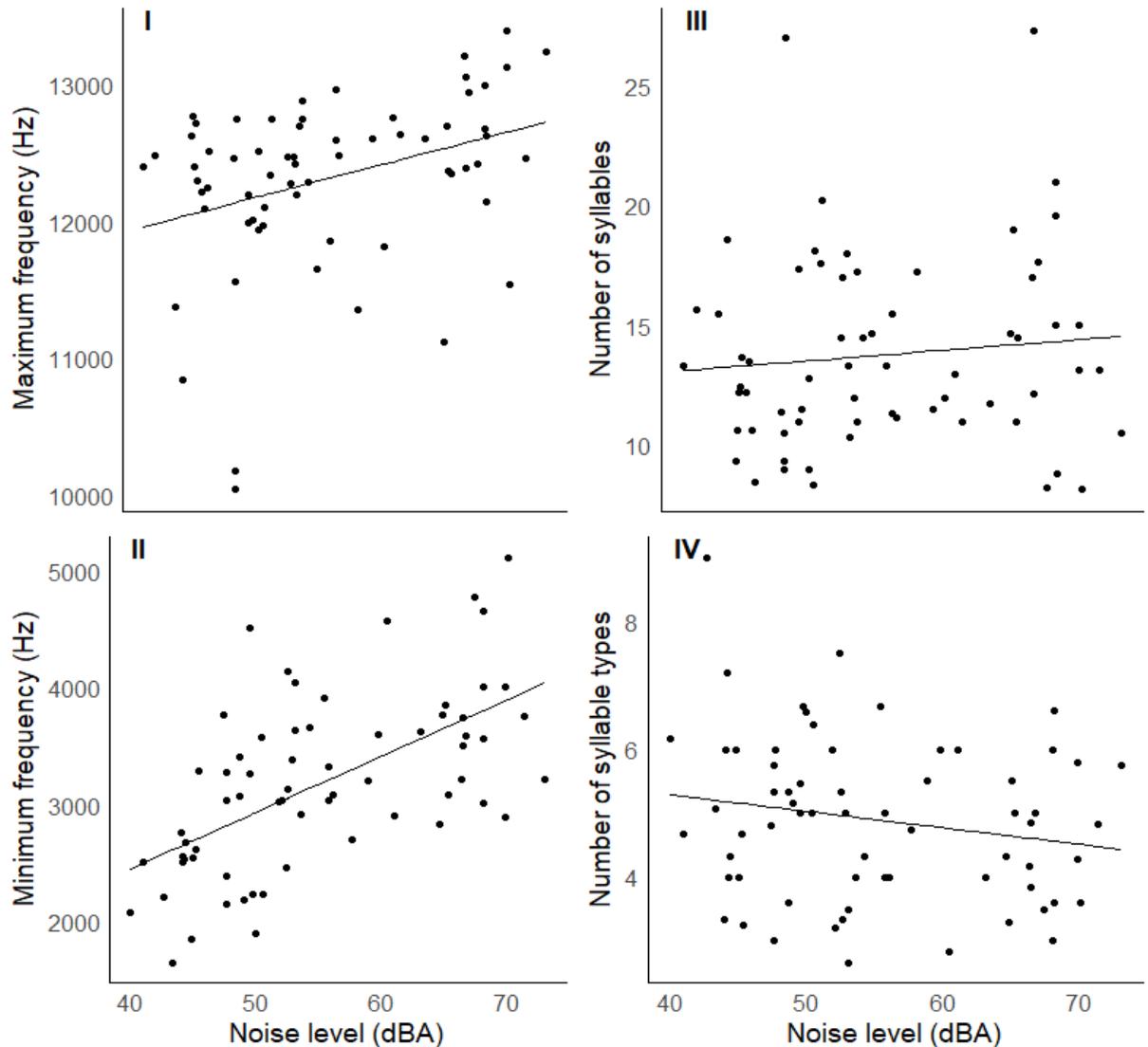
Source: designed by the author

Figure 2 – Spectrograms of four bananaquit songs differing in levels of elaboration, i.e., in the number of different syllable types. Both more (I,III) and less (II, IV) elaborate songs occur in the most geographic distant locations accessed. This indicates that the observed pattern of variation in song elaboration may not be a consequence of possible diverging dialects. The more elaborate songs (I and III) were recorded both in ‘Federal University of Bahia’ (8) and ‘Vale Encantado’ (2) localities and the less elaborate songs (II and IV) in ‘Federal University of Bahia’ (8) and Parque das Dunas (1) localities.



Source: designed by the author

Figure 3 – Relationship between noise level and bananaquit song variables. In noisier situations, bananaquits sing higher songs (A and B), do not change the song syllable length (C) and tend to decrease the song syllable diversity, but not significantly (D). The black lines represent the fitted linear model.



Source: designed by the author

The short-term noise measurements, sampled in a 5-minute period during song recordings, correlated well with the long-term noise assessments, based on ten 5-minute periods spread out over the 5-hour period of song recordings between 5.00 and 10.00h in the morning ( $r^2 = 0.5598$ ,  $t = 4.367$ ,  $p = 0.0005$ ,  $N = 17$ , figure 1).

The principal component analysis derived two components that reflected different aspects of song elaboration (table 2). The first principal component (PC1), which explained 47% of the variation, most prominently represented the number of syllables. The second principal component (PC2), which explained 29% of the

variation, most prominently represented the number of different syllable types. The number of syllables and the number of different syllables were not correlated with noise level or territory density (table 3).

Finally, the noise-dependent frequency variation in minimum frequency and the frequency bandwidth were significantly related to one of the two measures of song elaboration. There was no correlation to the number of number of syllables (minimum freq.:  $r^2=, 0.008$ ,  $t=-0.726$ ,  $p=0.471$ ; freq. bandwidth:  $r^2= 0.037$ ,  $t=1.576$ ,  $p=0.12$ ), but minimum song frequency was negatively correlated to the number of different syllable types, which means that a higher minimum frequency yields less diversity in syllable types ( $r^2=0.1946$ ,  $t=-3.902$ ,  $p<0.0$ , figure 4). Also, song frequency bandwidth was positively correlated to the number of different syllables, which means that a narrower frequency range yields less diversity in syllable types ( $r^2=0.124$ ,  $t=2.989$ ,  $p=0.003$ ; figure 4).

Table 2 – Principal component loadings, eigen values and percentage of variation explained for song elaboration measurements. In bold are the loadings of the variables that most represent each component (PC1 and PC2). N=65.

Variables	PC1	PC2
Song features		
Number of syllables	<b>.939</b>	.175
Number of syllable types	.088	<b>.909</b>
Number of phrases	<b>.791</b>	.110
Syllable rate	.604	-.550
Summary		
Variance explained	47%	29.25%
Eigen values	1.880	1.170

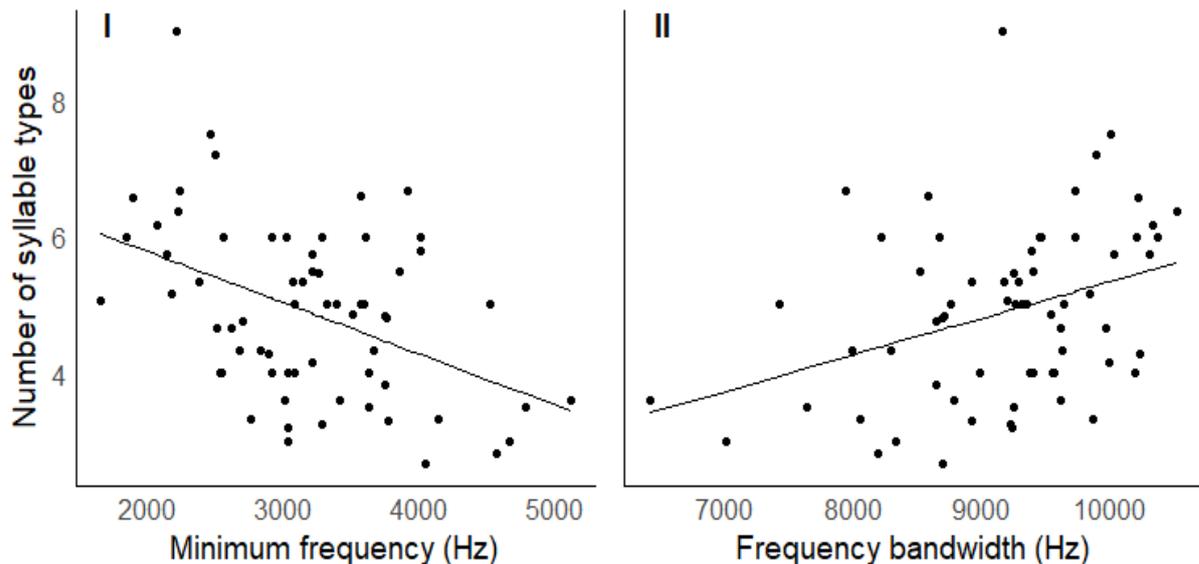
Source: designed by the author

Table 3 – Fitted values of linear models comparing the relationship between the number of singing neighbours and the song variables. N=37.

Fitted linear models for comparisons between male density and song variables			
Song variables	Mean square	F statistics	Significance value
Low frequency (Hz)	328.350.191	1.001	.405
High frequency (Hz)	247.368.578	.969	.419
Frequency bandwidth (Hz)	33.480.268	.066	.978
Song duration (s)	.453	.216	.885
Peak frequency (Hz)	1.004.700.216	.973	.417
Number of syllables	15.106	.300	.825
Number of syllable types	2.724	.581	.632

Source: designed by the author

Figure 4 – Phenotypic restriction of the song syllable diversity (i.e., number of syllable types) by the increase of the minimum song frequency (A) and the decrease of the frequency bandwidth (B). The black lines represent the fitted linear model.



Source: designed by the author

## 2.5 Discussion

We investigated whether bananaquit songs exhibited noise-dependent variation in spectral and temporal parameters and, if so, whether higher or shorter

songs would determine a restriction on song elaboration. Bananaquit songs varied spectrally with noise levels, but song duration was not correlated. We found significantly higher minimum and maximum frequencies and significantly narrower frequency bandwidth in noisier territories. These noise-dependent spectral changes were not affected by territory density, but appear to restrict song elaboration, as higher and narrower songs were more repetitive and showed less diversity in the number of different syllable types in a song.

### 2.5.1 Trade-off between audibility and signal value

The noise-dependent changes in frequencies can make bananaquit songs more audible under noisy conditions, but at the same time make them of lower signal value to the singer (Slabbekoorn 2013; Luther et al. 2016). Bananaquits sang higher frequencies songs of narrower bandwidth in noisier territories. Spectral masking avoidance of low-frequency traffic noise (c.f. Slabbekoorn & Peet 2003) may increase song audibility, as, for example, great tit females responded more, and male black-capped chickadees (*Poecile atricapillus*) responded faster, to high than to low frequency songs in noisy situations (Halfwerk et al. 2011b, Lazerte et al. 2017). However, a higher song frequency may also affect signal value, as low-frequency songs may signal male size and may play a critical role in mate attraction and competitor deterrence (Brown et al. 1996, Linhart et al. 2012, Luther and Magnotti 2014).

A decrease in frequency bandwidth may also benefit audibility through improvement of signal-to-noise ratio by spectral concentration of signal energy (c.f. Hanna et al. 2011), but at the same time may limit performance features related to combinations of bandwidth and trill rate (c.f. Podos 1997). Such songs with restricted bandwidth feature have been shown to elicit lower response levels from territorial rivals than songs with broader frequency bandwidth (Luther et al. 2016).

On top of the direct consequences of noise-dependent spectral changes, we found a correlated reduction of song elaboration that may also affect signal value. In European robins, a noise-dependent increase in minimum song frequency also correlated to a decrease in the number of different syllable types in a song (Montague et al. 2012). This association of spectral restriction and decline in syllable diversity, now found in multiple species, may be related to an elimination of low-

frequency syllables and result from inherent acoustic restrictions due to physical constraints. Alternatively, lower syllable diversity may also emerge through the production of more repetitive trills of the same syllables (as illustrated by the example in figure 2). This could be a counter strategy to cope with noisy conditions in itself (Shannon and Weaver 1949, Wiley 1994) as suggested for noise-dependent repetition rates in chaffinches (*Fringilla coelebs*) of Europe (Brumm and Slater 2006) and urban silvereyes (*Zosterops lateralis*) of Australia (Potvin et al. 2012).

The noise-dependent song variation found for the bananaquits can therefore represent a functional trade-off in several ways. The spectral adjustments and increased redundancy may make their songs more audible in the noisy territories. However, the reduced syllable diversity may also affect perceived repertoire size, which may undermine the abilities of singing bananaquits to signal sender quality (Buchanan and Catchpole 2000, Kipper et al. 2006). Kagawa and Soma (2013) found for example that larger and heavier Java sparrows (*Lonchura oryzibora*) sang more elaborate songs, i.e., with more note types. Whether a detrimental effect on signal content due to reduced syllable diversity applies to bananaquits remains to be tested and could be done by playback experiments.

### 2.5.2 Methodological validation

Accurate measurements of noise-dependent song frequency variation may be hindered by the fading song amplitude at spectral extremes and the presence of noise around these same frequencies (Verzijden et al. 2010; Zollinger et al. 2012; Ríos-Chelén et al. 2016). Fully objective automated measurements using amplitude cut-off points, relative to the peak amplitude in the song, avoid this problem but compromise on the assessment of the actual song frequency range. Measurements by cursor placement are the most precise determination of spectral song extremes, but may suffer from observer bias (c.f. Brumm et al. 2017) and may introduce a noise-dependent artefact (Verzijden et al. 2010).

For the current study, we selected spectral measurements by cursor placement and we believe that observer bias is not a problem (see methods) while any possible artefact is of a much smaller scale than the effect size in our results. Bananaquit songs show, for example, variation over a range of 3467 Hz in the minimum frequency between the highest and lowest songs and the noise-dependent

shift in this parameter concerns about 1500 Hz over the sampled range of noise levels, from 40-70 dB(A). This is a large effect size compared to other descriptive studies on noise-dependent song variation (Slabbekoorn et al. 2012; Nemeth et al. 2012) and far beyond the artefact size in studies that determined this experimentally for measurements on song playbacks with variable noise levels for chiffchaffs (*Phylloscopus collybita*): 49 Hz (Verzijden et al. 2010), and red-winged blackbirds (*Agelaius phoeniceus*): 615 Hz (Ríos-Chelén et al. 2017).

As a final methodological issue, we here briefly address territory density as a potentially confounding variable. Bird densities may increase in cities, for example, by an increase in the availability of nesting sites and food resources (Shochat 2004, Roob et al. 2008). Higher densities may affect urban song variation through altered levels of interaction and agitation that potentially covary with variation in noise levels (Ripmeester et al. 2010, Hamao et al. 2011; Narango and Rodewald 2015). It is possible that bananaquits densities can be higher in more urbanized (and consequently noisier) areas as the species relies on nectar, a very common resource especially in urban parks with a selection of ornamental flowers (Kaluza et al. 2016). However, high densities of bananaquits can indeed be seen in foraging clusters at particular localities, but this seems less so during singing behaviour and territorial defence (personal observations). In our study, we also did not find any relationship between our territory density measure and spectral or elaboration features of bananaquit songs. We therefore consider the noise-dependent song variation to be independent of this potentially confounding factor for our species.

### 2.5.3 Conclusions

Neotropical bananaquits present another case of noise-dependent variation in song frequency use, correlated with a decline in song elaboration. We found that bananaquits sang higher and within a narrower frequency range in noisier situations, which results in masking avoidance of low-frequency traffic noise at least to some extent. Songs in more noisy territories were also more repetitive and less diverse in terms of the number of different syllable types, which may reduce signal value for mate attraction and territorial defence. Consequently, these findings are congruent with an auditory trade-off and suggest that urban birds face a functional compromise between audibility and signal quality. We believe our findings indicate that

anthropogenic noise may not only alter avian communities and the song features they sing in urban areas, but that these changes can also affect fundamental processes of sexual selection that may undermine individual fitness and the fate of populations.



### 3. NOISE-RELATED SONG VARIATION AFFECTS COMMUNICATION: BANANAQUITS ADJUST VOCALLY TO PLAYBACK OF ELABORATE OR SIMPLE SONGS<sup>2</sup>

#### 3.1 Abstract

Birds communicate through acoustic variation in their songs for territorial defense and mate attraction. Noisy urban conditions often induce vocal changes that can alleviate masking problems, but that may also affect signal value. We investigated this potential for a functional compromise in a neotropical songbird: the bananaquit (*Coereba flaveola*). This species occurs in urban environments with variable traffic noise levels and was previously found to reduce song elaboration in concert with a noise-dependent reduction in song frequency bandwidth. Singing higher and in a narrower bandwidth may make their songs better audible in noisy conditions of low-frequency traffic. However, it was unknown whether the associated decrease in syllable diversity, affected their communication. Here we show that bananaquits responded differently to experimental playback of elaborate versus simple songs. The variation in syllable diversity did not affect general response strength, but the tested birds gave acoustically distinct song replies. This result suggests that noise-dependent vocal restrictions may change the signal value of songs and compromise communicative function. It remains to be investigated whether there are consequences for individual fitness and how such effects may alter the diversity and density of the avian community in noisy cities.

#### 3.2 Introduction

In the last decades, the noise levels in human-altered and natural habitats have substantially increased and affected the way birds sing (Rabin and Greene 2002, Mennitt et al. 2015, Buxton et al. 2017). Anthropogenic noise can interfere with communication among birds because it can mask their songs through overlap in frequency and time (Brumm and Slabbekoorn 2005, Barber et al. 2010, Parris and McCarthy 2013). Several noise-dependent vocal changes have been reported in city birds (Brumm 2004, Potvin and Mulder 2013, Gil et al. 2014), which typically yield an

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<sup>2</sup> Em parceria com os coautores Japyassú, HF, Izar, P, Slabbekoorn, H.

increase in song detectability and improved efficiency of communication (Brumm and Slabbekoorn 2005, Pohl et al. 2012). However, vocal changes may not only affect signal detectability but also signal value (Slabbekoorn and Ripmeester 2008, Gross et al. 2010) and noise-dependent song variation may thereby concern a functional compromise (Slabbekoorn 2013, Luther et al. 2016). Although reports on noise-dependent song variation are widespread, tests of the potential for functional consequences for communication are still rare (see e.g. Mockford and Marshall 2009, Ripmeester et al. 2010, Luther and Derryberry 2012).

There are several ways birds change their songs by which they could counteract masking by urban noise. Several species have been found to sing higher frequencies and/or narrower-banded songs in noisier environments (Slabbekoorn and Peet 2003, Verzijden et al. 2010, Bermúdez-Cuamatzin et al. 2011, Montague et al. 2012, Lazerte et al. 2016). As anthropogenic noise is typically biased to low-frequency bands, higher-frequency songs are better audible than lower-frequency songs (Brumm and Slabbekoorn 2005; Nemeth and Brumm 2010; Halfwerk et al. 2011) and concentrating all acoustic energy in a narrower band can also raise signal-to-noise ratio (Hanna et al. 2011). Birds are also reported to sing at higher amplitudes if noise levels rise and they can sing shorter or in alternating time periods when noise levels are fluctuating (Brumm 2004, Gil et al. 2014, Gentry et al. 2017).

Although such noise-dependent changes may be successful in masking avoidance, they may also restrict the potential for communication by undermining the signaling function of the songs (Slabbekoorn and Ripmeester 2008, Gross et al. 2010, Slabbekoorn 2013, Luther et al. 2016). Reduction in frequency band use, for example, may restrict the use of particular syllables and limit possible syllable variation, and consequently limit song repertoire size of an individual (Montague et al. 2012, Fouda et al. 2018, Winandy et al. 2019a). Song elaboration in birds may signal male size or other parental qualities (e.g. Kipper et al. 2006, Botero et al. 2009; Kagawa and Soma 2013) and can be a good predictor of potential offspring survival and thus affect female preference (Hasselquist et al. 1996, Buchanan and Catchpole 1997, Buchanan and Catchpole 2000). Therefore, noise-dependent reduction in song elaboration may negatively affect the signal value of a song and undermine information transfer about sender qualities.

Potential signal value or communicative function of a song can be explored by controlled exposure to playbacks of recorded songs and by experimental

manipulation of specific acoustic variation (e.g. Nelson 1988; Slabbekoorn and ten Cate 1998; Linhart et al. 2012). Playback of urban and rural song variation has, for example, revealed recognition of urban acoustic features in natural territories of great tits (*Parus major*) and European blackbirds (*Turdus merula*). Individual birds approach more closely, stay longer or respond vocally more quickly to playback of songs dependent on whether they are from birds from the same habitat type or similar background noise levels (Mockford and Marshall 2009, Ripmeester et al. 2010). The potential impact of noise-dependent variation in spectral range has been tested in few studies in both male-female (Halfwerk et al. 2011, Huet des Aunay et al. 2014) and male-male communication (Luther and Magnotti 2014, Lazerte et al. 2017).

The bananaquit (*Coereba flavoela*), an abundant bird species of neotropical cities, is a good model system to study the potential signal value of song elaboration. We previously showed bananaquits exhibit noise-dependent variation in song elaboration: they sing elaborate songs, rich in syllable types and syllable transitions in quiet territories, but also simple and repetitive songs that are poor in syllable diversity in more noisy territories (Winandy et al. 2019a). They are relatively abundant across city habitats, used to human presence, and can be highly territorial to conspecific intruders (Hilty and Christie 2018; personal observations). Consequently, bananaquits are very suitable for playback studies that demand close approach of researchers for behavioral observations and recordings.

In this study, we performed a playback exposure experiment and tested whether bananaquits responded differently to elaborate versus simple songs. More elaborate songs were characterized by higher syllable diversity (i.e., more syllable types per song), but also by lower minimum and higher maximum frequencies. We aimed at answering the following questions: 1) do simpler songs trigger stronger responses than the more elaborate songs? 2) do individuals match song elaboration? 3) do elaborate songs trigger wider frequency range songs from territory owners? If so, this would provide evidence for a signaling value of song elaboration, a trait affected by urban noise level in bananaquits (Winandy et al. 2019a). Therefore, this study could provide new insight into how noise pollution, through the simplification of urban songs, can alter the evolution of sexually selected signals.

### **3.3 Material and methods**

### 3.3.1 *Study Site and Species*

We conducted our playback experiment in 20 bananaquit (*Coereba flaveola*) territories in the city of Salvador, Bahia, Brazil (12°57'50.9"S, 38°30'21.0"W). We tested the birds during the Brazilian summer, between February and March of 2018. This species can sing and breed throughout the year (Hilty and Christie 2018) and territorial responsiveness does not fade during summer. The territories were located in different habitat types and traffic noise regimes: in Atlantic Forest urban parks, urban gardens and areas close or next to main avenues, with variable stands of concrete buildings and trees. We performed the experiment only during relatively quiet moments of the day for each territory, between 05H00 and 07H00 in the morning. In this way, we avoided possible interference of noise levels on song playback detection by the territory owners, which was not our target in this study.

Bananaquits are nectarivorous songbirds that occur across the Neotropics from Mexico to Argentina and the Caribbean islands. They can be easily observed in several types of human-altered habitats, from highly urban to rural areas. They are territorial birds that sing and defend their territories throughout the day and year (Hilty and Christie 2018). The songs are composed of series of high-pitched syllables, which vary from complex sequences of diverse element types with high transition rates to highly repetitive series of less variable syllable types (Winandy et al. 2019).

### 3.3.2 *Sound Recording and Analyses*

Before exposing the birds to the playback, we recorded their pre-playback songs for one minute. Usually, in one minute of recording the bananaquits sang about 10 songs, but for some individuals we obtained less than 5 songs. We recorded the birds from a distance of 2 to 14 meters, using a Tascam DR-44WL recorder connected to a Sennheiser™ (Wedemark, Germany) shotgun directional microphone (ME67 + K6). In total, we performed acoustic analyses on  $11.08 \pm 5.24$  (mean  $\pm$  SD) songs per individual. We used Raven™ PRO software, version 1.5 (Cornell Laboratory of Ornithology, Ithaca, NY, USA) for all processing of recordings and song measurements. Spectrograms settings were kept constant as: FFT length: 512, window: hann, overlap: 75%.

All song recordings, pre-playback and response songs, were first cut in shorter song sequences, separated from recorded playback stimuli, before the analyses. In this way, the observer was always blind to the origin and nature of songs in the stimuli used for the playback experiment. We used cursor placement to extract three spectral song variables (c.f. Verzijden et al. 2010, Winandy et al. 2019a): minimum frequency, maximum frequency and frequency bandwidth. The low-noise conditions during playback and the observer being blind to the stimulus type reduced the chance for observer bias or artefact effects in our spectral measurements (Verzijden et al. 2010, Brumm et al. 2017). Additionally, we counted the number of syllable types per song as a measure of song elaboration.

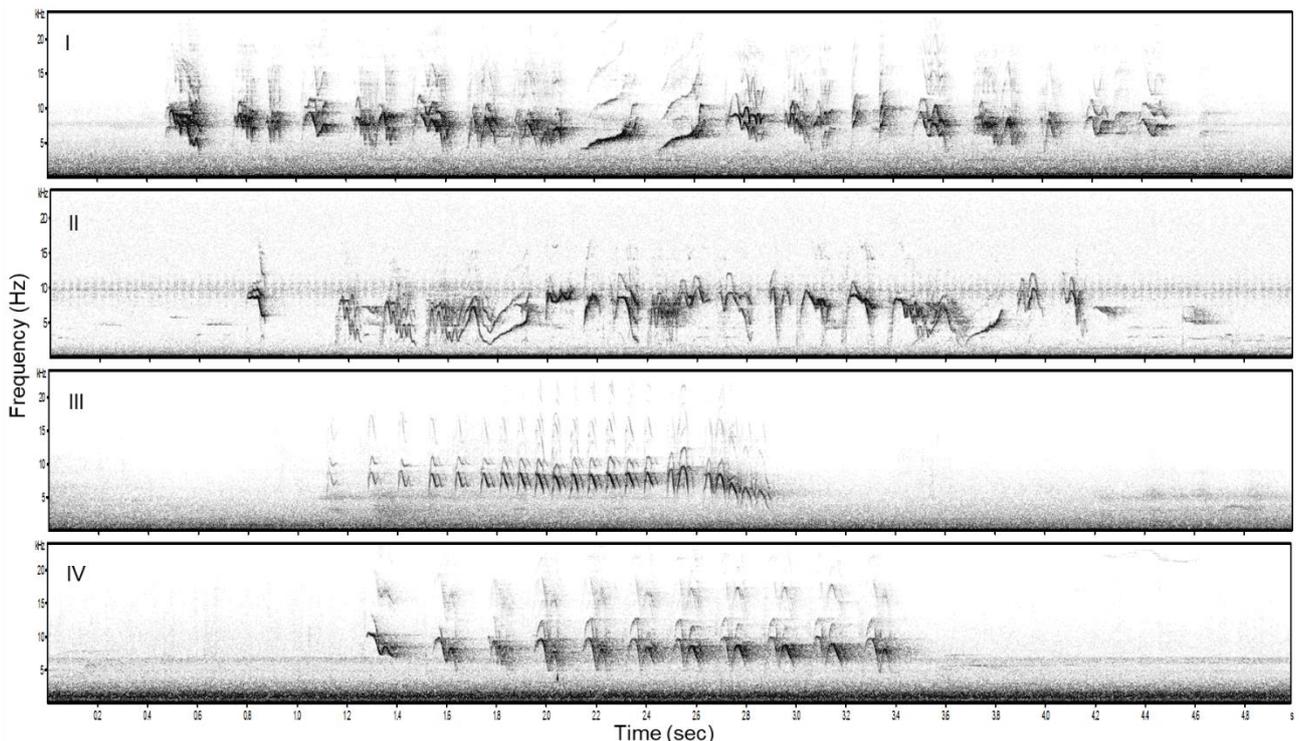
### 3.3.3 Playback Stimuli

We used songs of 20 bananaquits recorded at our study site in 2016 and 2017. We chose 20 song recordings varying in levels of song elaboration, from 10 individuals that sang relatively elaborate and 10 relatively simple songs, reflected in the number of different syllable types per song ( $X_{\text{simple}} \pm SD = 3.1$  and  $0.77$ ,  $X_{\text{elaborate}} \pm SD = 5.7$  and  $1.6$ , two-way ANOVA:  $F_{1,19} = 21.46$ ,  $N = 20$ ,  $P < 0.001$ , figure 5) and in the minimum and maximum song frequencies (two-way ANOVA for minimum frequency:  $F_{1,19} = 35.55$ ,  $N = 20$ ,  $P < 0.001$ , maximum frequency:  $F_{1,19} = 6.14$ ,  $N = 20$ ,  $P = 0.02$ , figure 1). We made sure that there were no significant differences in the length (measured in total number of syllables per song and song duration), peak frequency, syllable rate and frequency bandwidth between the two song categories (two-way ANOVA for number of syllables:  $F_{1,19} = 0.07$ ,  $N = 20$ ,  $P = 0.7$ , song duration:  $F_{1,19} = 3.37$ ,  $N = 20$ ,  $P = 0.08$ , peak frequency:  $F_{1,19} = 1.21$ ,  $P = 0.2$ ,  $N = 20$ , syllable rate:  $F_{1,19} = 2.444$ ,  $N = 20$ ,  $P = 0.135$ , frequency bandwidth:  $F_{1,19} = 2.928$ ,  $N = 20$ ,  $P = 0.1$ ), as they may also convey information and, for example, be indicative of motivational states on birds (Langemann et al. 2000, Ripmeester et al. 2007, Lattin and Ritchison 2009, Linhart et al. 2012, Luther et al. 2016, Linhart et al. 2013). All the songs were high-pass filtered to remove the low-frequency background noise and normalized to an equal peak amplitude in Audacity™ v. 2.1.2 (Carnegie Mellon University, Pittsburgh, Pennsylvania, USA).

Each playback stimulus consisted of 3 different songs of the same individual and song category (simple or elaborate). Songs from the same individual were only

used for one stimulus and thus not in different song categories. The 3 songs were played back twice in the same sequence with a silent interval of 3 seconds between each of them (c.f. Ripmeester et al. 2010). We created in this way 10 unique exemplars of each playback stimulus: 10 simple and 10 elaborate playback stimuli.

Figure 5 – Examples of two elaborate (I and II) and two simple (III, IV) song stimuli used in the playback experiment.



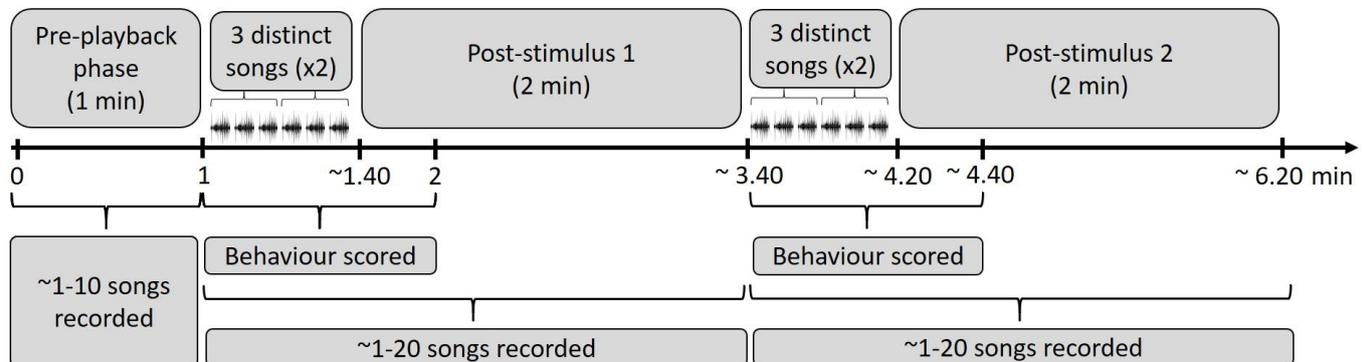
Source: designed by the author

### 3.3.4 Playback Design

We played back the stimulus songs in bananaquit territories of actively singing birds without nearby competitors that could be agonistically interacting at the time of the experiment. These procedures were meant to reduce variation in behavioral responses related to different motivational states. We placed the JBL clip 2 loudspeaker at about 5 to 10 meters from the focal male and the observer was positioned 5 to 10 meters further away. We measured the amplitude of the playback with a Skill-Tec™, SKDEC-02 (São Paulo, São Paulo, Brazil) sound pressure level meter (A-weighted, fast response, range 30-130 dB, 1s interval) and adjusted playback levels to a volume of 70dB(A) at a distance of 1 meter from the speaker.

After the start of the playback of the first song stimulus series, simple or elaborate, we scored the behavior of the focal individual for one minute. During the playback and for two minutes after it was ended, we also recorded the songs. After the two minutes interval, we played back a song stimulus from the opposite category and recorded songs and scored response behaviors for the same periods as before (figure 6). We avoided testing direct neighbors that could have been exposed to previous playbacks. The following behaviors and song measurements were scored: number of flights over the loudspeaker, shortest distance of the focal male to the loudspeaker, number of songs, number of calls and song and call rate.

Figure 6 – Time periods overview of the playback procedure in the field. A stimulus of three distinct elaborate or simple songs of the same individual was played twice after one minute of pre-playback recording phase. After the start of the playback of the first song stimulus series, we scored behavior for one minute. During the playback and for two minutes after it ended, we recorded the songs. Following that, the second stimulus was played back to the same focal individual: three distinct songs twice of the opposite stimulus category (simple or elaborate songs, depending on the order of exposure).



Source: designed by the author

### 3.3.5 Statistical Analysis

We conducted all statistical analyses in R studio software (R Core Team), using the packages lme4 (Bates et al. 2015) and MuMIn (Barton 2016). We performed generalized linear models (GLM) and Akaike's information criterion (AIC) model selection to find out whether the song variables and behavioral responses were affected by the stimulus type (simple vs elaborate song playbacks) and/or by the order of the stimuli. All song measurements and behavioral responses were

entered as response variables in the models. The stimulus category and playback order were entered as fixed factors in the full model and individual as random factor. We computed the statistics for all possible models, which included: 1) single predictors (stimulus category, order), 2) their additive combinations (category + order) and 3) the null models (without effect of any predictor). The response variables: number of syllable types, total number of syllables and number of flights were entered as interval variables in Poisson generalized models with log-link function.

We selected the best models based on the AICc values, considering  $\Delta\text{AICc} > 2$  a criterion for substantial difference between models. The model selection was made using the function dredge model selection (package MuMIn). We calculated the marginal ( $R^2m$ ) and conditional ( $R^2c$ )  $R^2$  values to evaluate how much the fixed effects ( $R^2m$ ) or the entire model ( $R^2c$ ) explained the variance of the response variables (Nakagawa and Schielzeth 2013). Finally, we performed post-hoc Tukey's tests for each response variable for which we obtained a minimal model selection. This analysis informed which pairs of playback conditions were significantly different in song or behavioral responses.

As we did in our previous correlational study, we investigated again the possible trade-off between the signal frequency reduction and the restriction in song elaboration. Therefore, we fitted linear models to test the relationship between the spectral and elaboration variables with two different datasets: one that included only the spontaneous songs sung before the start of the playback experiment and another with all songs, both the spontaneous and playback triggered songs.

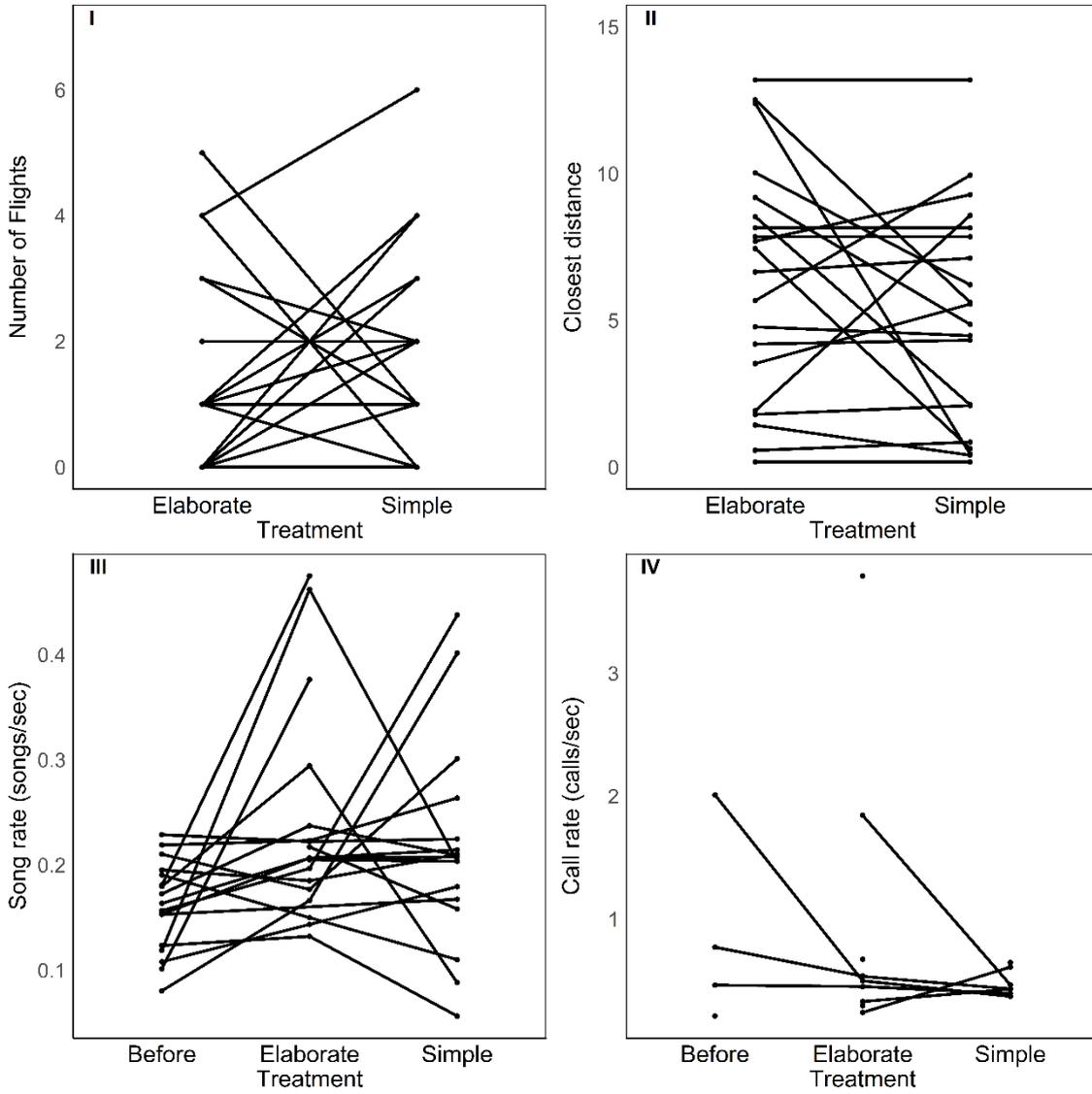
### 3.4 Results

There was no effect of the stimulus type (elaborate vs simple) on behavioral response strength and vocalization rate. The number of flights, the approach to the speaker, the song and call rates were all not affected by stimulus category or by the playback order (figure 7, table 4). However, individuals responded in acoustically distinct ways to each playback type. Their songs had fewer syllables and were lower and wider in frequency when they responded to the elaborate song stimuli compared to when they responded to the simple song stimuli (figure 8).

The model selection for song variables showed that the number of syllables per song was significantly affected by the playback stimulus (table 5). The birds sang less syllables per song after being exposed to the elaborate song stimulus than before the playback experiment (table 6). The spectral variables: minimum frequency, maximum frequency and frequency bandwidth (Hz) were explained by both the song stimulus category and the order of the stimulus playback (table 5). Regarding the order, when the elaborate stimulus was played first, the differences in the spectral responses between treatments were more pronounced (figure 8). Birds significantly lowered the minimum frequency of their songs after being exposed to the elaborate playback (figure 8, table 6). Moreover, they sang significantly wider frequency songs when responding to the elaborate stimulus, followed by a bandwidth decrease when exposed to the simple playback as the second stimulus (figure 8, table 6).

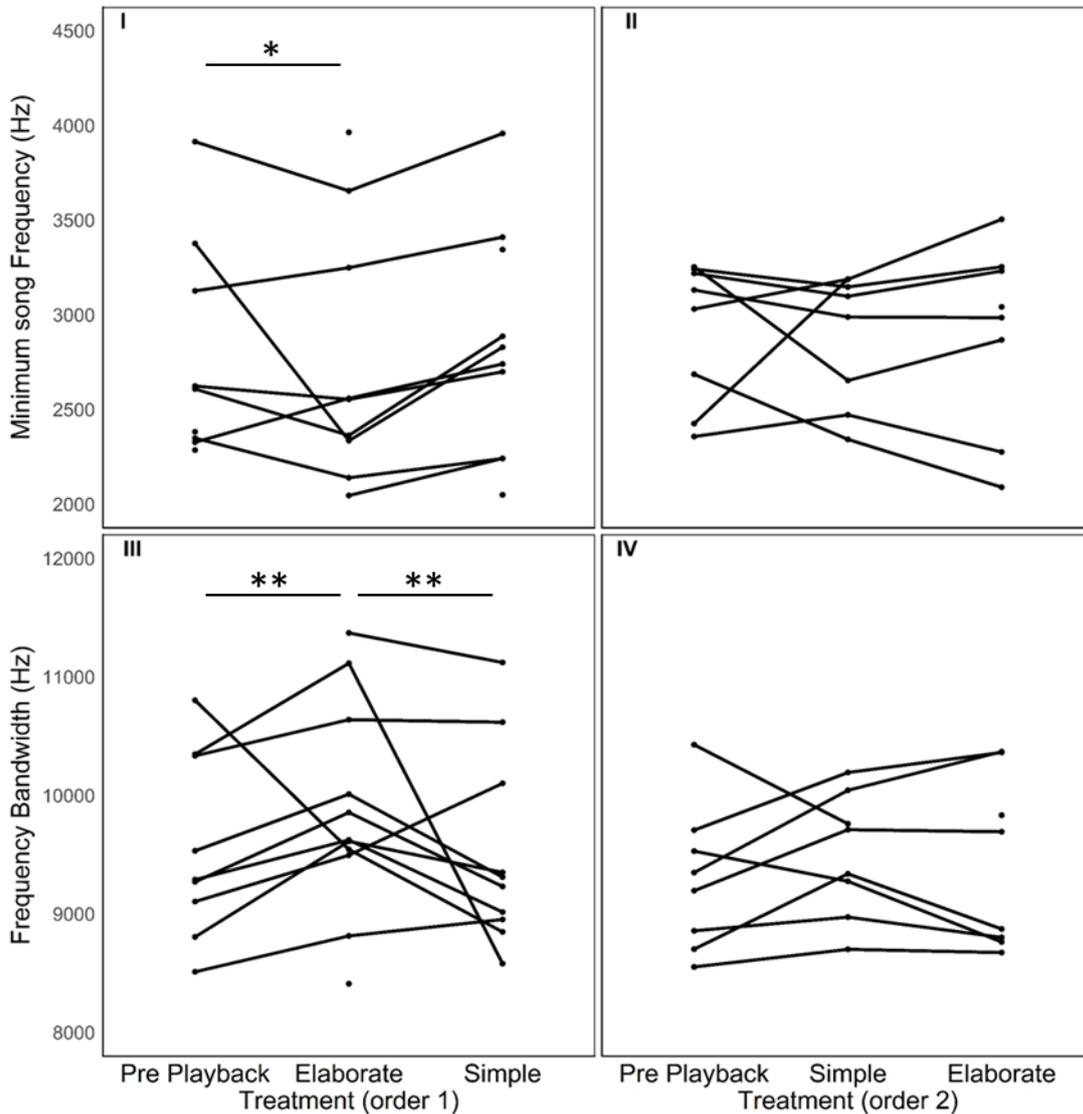
Finally, the correlation between song elaboration and song frequency previously found for bananaquit songs was not found for the songs from the playback experiment in the current study. The number of syllable types per song and the spectral variables, minimum song frequency and frequency bandwidth, were not correlated. The correlation did not occur when all songs from the playback experiment were included, i.e., for both spontaneous and playback triggered songs (Linear model for low frequency:  $R^2 = -0.01$ ,  $F_{1, 53} = 0.05$ ,  $N = 20$ ,  $P = 0.81$ ; Linear model for frequency bandwidth:  $R^2 = -0.01$ ,  $F_{1, 53} = 0.36$ ,  $N = 20$ ,  $P = 0.54$ ), or when we only included the spontaneous songs from the pre-playback phase (Linear model for low frequency:  $R^2 = -0.06$ ,  $F_{1, 16} = 0.01$ ,  $N = 17$ ,  $P = 0.89$ ; Linear model for frequency bandwidth:  $R^2 = -0.06$ ,  $F_{1, 16} = 0.03$ ,  $N = 17$ ,  $P = 0.86$ ).

Figure 7 – Strength of behavioral responses to each song playback (top) and vocalization rate (bottom) before and during each song playback. There were no significant changes between scoring periods (see text).



Source: designed by the author

Figure 8 – Spectral variation in the songs sung in response to each stimulus category. Each line connects song measures of one individual in three different periods of the playback procedure. As playback order had an effect, we provide the data in two separate sets of graphs. Individuals that were exposed first to the elaborate songs followed by the simple songs are depicted in the graphs on the left. The responses of individuals that were exposed first to the simple songs followed by the elaborate songs are depicted in the graphs on the right. \* indicates statistically significant differences between the measures in two of the playback periods. (\*:  $P < 0.05$  and \*\*:  $P < 0.01$ ).



Source: designed by the author

Table 4 – Results of the model selection for all behavioral response variables (indicated in bold).  $\Delta AICc > 2$  indicates a significant difference between two models.  $R^2m$  indicates the proportion of variance of the response variable explained by the fixed factor and  $R^2c$  indicates the proportion of variance explained by the entire model.

Model	df	logLik	AICc	$\Delta AICc$	weight	$R^2m$	$R^2c$
<b>Number of flights ~ ...</b>							
1* 1 + (1 ID)	2	-70.4	145.1	0	0.38	0	0.08
2 Order + (1 ID)	3	-69.3	145.3	0.15	0.35	0.04	0.16
3 Playback + (1 ID)	3	-70.12	146.9	1.8	0.15	0.01	0.1
4 Playback + Order + (1 ID)	4	-69.13	147.4	2.28	0.12	0.05	0.16
<b>Closest distance ~ ...</b>							
1 * 1 + (1 ID)	3	-109.28	225.2	0	0.41	0	0.35
2 Playback + (1 ID)	4	-108.37	225.9	0.65	0.29	0.03	0.41
3 Order + (1 ID)	4	-108.88	226.9	1.67	0.18	0.01	0.38
4 Playback + Order + (1 ID)	5	-107.93	227.6	2.39	0.12	0.04	0.44
<b>Song rate ~...</b>							
1 * Order + (1 ID)	4	53.99	-99.4	0	0.34	0.04	0.04
2 Playback + (1 ID)	5	55.1	-99.3	0.11	0.32	0.07	0.07
3 1 + (1 ID)	3	52.54	-98.7	0.66	0.24	0	0
4 Playback + Order + (1 ID)	6	55.1	-96.9	2.49	0.1	0.07	0.07
<b>Call rate ~ ...</b>							
1* 1 + (1 ID)	3	-54.52	115.5	0	0.38	0	0.74
2 Playback + (1 ID)	5	-52.42	116.1	0.58	0.29	0.03	0.77
3 Playback + Order + (1 ID)	6	-51.48	116.7	1.23	0.21	0.03	0.79
4 Order + (1 ID)	4	-54.46	117.7	2.23	0.13	0	0.74

\* indicates the best model. N = 20 individuals.

Source: designed by the author

Table 5 – Results of the model selection for all song response variables (indicated in bold).  $\Delta AICc > 2$  indicates a significant difference between two models.  $R^2m$  indicates the proportion of variance of the response variable explained by the fixed factor and  $R^2c$  indicates the proportion of variance explained by the entire model.

Model	df	logLik	AICc	$\Delta AICc$	weight	$R^2m$	$R^2c$
<b>Number of syllable types ~ ...</b>							
1* 1 + (1 ID)	2	-795.41	1594.9	0	0.45	0	0.08
2 Playback + (1 ID)	4	-793.89	1595.9	1.02	0.27	0.01	0.08
3 Order + (1 ID)	3	-795.34	1596.7	1.88	0.18	0	0.08
4 Playback + Order + (1 ID)	5	-793.88	1597.9	3.05	0.1	0.01	0.08
<b>Number of syllables ~ ...</b>							
1* Playback + (1 ID)	4	-1186.91	2381.9	0	0.57	0.01	0.28
2 Playback + Order + (1 ID)	5	-1186.5	2383.1	1.22	0.31	0.01	0.28
3 1 + (1 ID)	2	-1190.89	2385.8	3.89	0.08	0	0.28
4 Order + (1 ID)	3	-1190.65	2387.3	5.43	0.04	0	0.28
<b>Minimum song frequency ~ ...</b>							
1* Playback + Order + (1 ID)	6	-3243.93	6500.1	0	0.58	0.01	0.63
2 Playback + (1 ID)	5	-3245.51	6501.2	1.1	0.33	0.01	0.63
3 1 + (1 ID)	3	-3249.14	6504.3	4.28	0.07	0	0.62
4 Order + (1 ID)	4	-3249.06	6506.2	6.15	0.03	0	0.62
<b>Maximum song frequency ~ ...</b>							
1* Playback + Order + (1 ID)	6	-3352.68	6717.6	0	0.58	0.01	0.47
2 Playback + (1 ID)	5	-3354.86	6719.9	2.3	0.18	0.01	0.46
3 Order + (1 ID)	4	-3356.32	6720.7	3.18	0.12	0	0.46
4 1 + (1 ID)	3	-3357.34	6720.7	3.19	0.12	0	0.45
<b>Frequency bandwidth ~ ...</b>							
1* Playback + Order + (1 ID)	6	-3443.05	6898.3	0	0.95	0.02	0.56
2 Playback + (1 ID)	5	-3447.13	6904.4	6.11	0.05	0.01	0.56
3 Order + (1 ID)	4	-3451.92	6911.9	13.64	0	0	0.55
4 1 + (1 ID)	3	-3452.98	6912	13.73	0	0	0.54

\* indicates the best model. N = 20 individuals.

Source: designed by the author

Table 6 – Post-hoc tests for the song response variables where a best model with at least one fixed factor was selected.

<b>Best Model</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>z value</b>	<b>Pr(&gt; z )</b>
<b>Number of syllables ~ Playback + (1 D)</b>				
Elaborate – Pre playback *	-0.1	0.04	-2.54	0.029
Simple – Pre playback	-0.03	0.04	-0.81	0.69
Simple – Elaborate	0.07	0.03	2.14	0.08
<b>Minimum frequency (Hz) ~ Playback + Order + (1 D)</b>				
Elaborate - Pre playback *	-220.53	81.35	-2.71	0.017
Simple - Pre playback	-122.03	85.95	-1.42	0.32
Simple - Elaborate	98.5	45.14	2.18	0.07
<b>Frequency bandwidth (Hz) ~ Playback + Order + (1 D)</b>				
Elaborate - Pre playback *	451.4	129.46	3.49	<0.01
Simple - Pre playback	235.47	136.76	1.72	0.19
Simple - Elaborate *	-215.93	71.82	-3.01	<0.01
<b>Maximum frequency (Hz) ~ Playback + Order + (1 D)</b>				
Elaborate - Pre playback	228.9	105.9	2.16	0.07
Simple - Pre playback	112.5	111.8	1.01	0.56
Simple - Elaborate	-116.5	58.7	-1.98	0.11

\* indicates which pairs of playback periods were statistically distinct in song or behavioral responses. N = 20 individuals.

Source: designed by the author

### 3.5 Discussion

We performed a playback exposure experiment to test whether bananaquits responded differently to elaborate versus simple songs. We found the following answers to our questions: 1) playback of simpler songs did not trigger stronger (or weaker) behavioral responses than playback of more elaborate songs; 2) individuals did not match song elaboration to the stimulus categories, and even decreased syllable numbers in their song in response to more elaborate songs; however 3) songs triggered by elaborate song playback had a lower minimum frequency and wider frequency range compared to songs sung before the playback. The frequency

of songs sung after the elaborate playback were also significantly wider than songs sung after simple song playback.

### 3.5.1 *Song Elaboration Is Meaningful*

Our current results reveal that noise-induced changes in song elaboration concern meaningful changes to territorial birds in neotropical bananaquits. Variation in responsiveness related to variation in song elaboration is in line with other studies in the literature. In simulated territorial intrusions, for example, dark-eyed juncos (*Junco hyemalis*) responded stronger to structurally more elaborate songs, spending longer periods closer to the playback speaker (Reichard et al. 2011). In chaffinches (*Fringilla coelebs*), both males and females responded stronger to more elaborate songs, i.e., signals with a higher number of different trill phrases (Leitão et al. 2006), suggesting this song parameter plays a role in both male-male competition and mate attraction. As we found an impact of song elaboration on response song variation and not on response strength, a signaling function of this song feature may be widespread but vary in content among species.

The impact of song elaboration on response song variation in our study on bananaquits concerned syllable number and spectral variation. We found no matching in elaboration, as more elaborate stimuli led to less elaborate response songs. We did not expect this, but less elaborate and more stereotypic songs can be associated with male-male interactions, while more elaborate and diverse can be more important for female choice (Hasselquist et al. 1996, Searcy and Beecher 2009, Kagawa and Soma 2013). However, we did find spectral matching in the minimum song frequency and in an increase in the frequency bandwidth when individuals responded to the elaborate playback. Similar changes in song frequency use have been found to be meaningful in other species in various ways. Frequency song matching, for example, can be an aggressive signal between rival birds during dispute (Searcy and Beecher 2009) as reported for Kentucky warblers (*Oporornis formosus*, Morton and Young 1986) and black-capped chickadees (*Poecile atricapillus*, Horn et al. 1992, Otter et al. 2002). But also, a relative frequency variation among communicating birds (i.e., frequency mismatch) may be important, as shown for willow warblers (*Phylloscopus trochilus*, Linhart and Fuchs 2015). Wider frequency bandwidths can also indicate higher aggressiveness, as white-crowned

sparrows (*Zonotrichia leucophrys nuttalli*) respond less strongly to songs of restricted bandwidth (Luther et al. 2016). Although we still have limited insight into the content of the message, we suggest that, conform the literature, the spectral variation and matching in bananaquit songs may also be meaningful.

The modified spectral response, in the absence of a strength in other behavioral responses, could also reflect that song elaboration plays a role in moderating territorial disputes (Slabbekoorn and ten Cate 1996, Searcy and Nowicki 2000, Otter et al. 2002). Graded variation in agonistic signals can convey increasing and decreasing levels of threats, before this becomes actually apparent in more overt changes in behavioral displays or approach tendencies (Searcy and Beecher 2009). The fact that the order in which the stimuli were played influenced the escalation behavior of bananaquits in our study confirms such a possibility and warrants further exploration through playback experiments simulating dynamic changes in song elaboration (c.f. Hof and Podos 2013).

### 3.5.2 *Elaboration Versus Bandwidth as a Signal*

There was an interesting discrepancy between the correlational analyses of the spectral and elaboration parameters in our previous (Winandy et al. 2019a) and the current study. In the previous observational study, we found frequency bandwidth and minimum frequency to be determined by noise level, and a lower and narrower frequency range was correlated with less elaborate song. In the current experimental study, however, we found a change in bandwidth dependent on song elaboration, but song frequency range was not correlated with song elaboration. We believe that this discrepancy requires further exploration of the potential role for ambient noise in signaling bananaquits.

There are two contextual differences in the recording sets that could explain the inconsistency of the correlation: noise level during recordings and whether song was sung in response to playback. In the previous study, we recorded the birds in quiet and also in noisy conditions, while in the current study, we only recorded the birds in relatively quiet moments of the day. As we found the correlation among the song parameters only in the first study, in which noisy conditions were present, we believe that the traffic noise could be causally linked to the presence of that significant correlation. This is another indication that noisy conditions may play a role

in song syllable use restriction through noise-dependent bandwidth availability. In the previous study we also only recorded spontaneous songs, while in the current study, we recorded both spontaneous and playback induced songs. However, in the current study, we found no correlation before or after the playback. We therefore argue that motivational state is not a likely explanation for the lack of correlation between song elaboration and frequency bandwidth in the current study.

### 3.5.3 *The Audibility-Signal Efficiency Trade-Off*

The combination of results of the previous observational study (Winandy et al. 2019a) and the current playback study allows a new perspective on the signal audibility/efficiency trade-off (Slabbekoorn and Ripmeester 2008, Gross et al. 2010, Slabbekoorn 2013). On the one hand, noise-dependent changes in frequency use may improve signal audibility as 1) avoiding low frequencies leaves a larger part of the song unaffected by masking low-frequency traffic noise (Nemeth and Brumm 2010; Halfwerk et al. 2011); and 2) concentrating sound energy in a spectrally more narrow bandwidth will also improve the signal-to-noise ratio (Hanna et al. 2011). On the other hand, as song elaboration is meaningful to the birds themselves (current study), the correlation between frequency bandwidth and song elaboration under noisy conditions (previous study, Winandy et al. 2019a) can be interpreted as evidence for a restriction on signal efficiency by noise-dependent song bandwidth contraction. When this signal audibility/efficiency trade-off is relaxed under relatively quiet conditions, the correlation between song frequency bandwidth and song elaboration apparently also fades.

Few studies have addressed the consequences of this trade-off between signal audibility and signal efficiency under noisy conditions. We here show for bananaquits that the noise-dependent variation in frequency use concerns biologically relevant signal variation, but for general conclusions the trade-off remains to be tested in more species. We especially need to gain insight into whether vocal changes that improve audibility actually yield any benefit to the signaler. We do know for example from a few earlier playback studies that spectral changes potentially driven by masking traffic noise affect response levels and are therefore proven to be biologically relevant (Mockford and Marshall 2009, Ripmeester et al. 2010, Luther and Derryberry 2012). However, we just started to find out about the potential for

reduced responsiveness to urban song features, as modified by anthropogenic noise conditions, in a mate choice context (Halfwerk et al. 2011, Huet des Aunay et al. 2014) as well as in a territorial context of male-male communication (Luther and Magnotti 2014, Lazerte et al. 2017).

### **3.6 Conclusions**

In the present study, we showed that bananaquits recognize the variation in song elaboration (in terms of syllable diversity and spectral extremities) as they respond with syllable adjustment and spectrally distinct songs to the variation in song elaboration in our playback stimuli. As song elaboration was shown in an earlier study to be restricted by noise-dependent song frequency bandwidth, the current results confirm that song adjustments could increase audibility through masking avoidance, but at the same time affect the signaling function. This provides another example of how the rise in anthropogenic noise levels in avian habitat may not only affect what birds sing, but also what they communicate. We still have little insight into fitness consequences of masking avoidance and changes of noise-induced adjustments in signaling content. We therefore believe that more studies are warranted into human impact on the ecology and evolution of singing birds in their acoustically altered environments due to noisy human activities worldwide.

## 4. THE IMPACT OF EXPERIMENTAL NOISE EXPOSURE ON REPRODUCTIVE SUCCESS IN CAPTIVE BENGALESE FINCHES<sup>3</sup>

### 4.1 Abstract

Anthropogenic noises can disrupt animal reproductive success through three different possible mechanisms: auditory masking, physiological stress or auditory distraction. Through such mechanisms, the noise can affect communication during mating, as the songs of males and female preferences are changed, the fertility, the incubation and, at last, the parental care of offspring. Some of these negative impacts have been shown by field studies. However, long-term noise exposures in captivity would be more useful to clearly understand whether the noise impacts different phases of reproductions and the underlying mechanisms of disturbances. In the present study we tested whether an experimental noise exposure affected three different phases of reproduction in Bengalese finches: 1) the pre-nesting phase, 2) the egg-laying phase, and 3) hatchling and nestling phase. Our results show that the birds exposed to noise delayed the egg-laying, therefore, the pre-nesting phase, when compared to birds reproducing in quiet conditions. The subsequent phases were not affected: egg-laying, hatchling and nestling phases did not change. The number and weight of the laid egg, hatchlings and nestlings were similar among quiet and noisy treatments. An auditory masking of male songs is the most likely explanation for our results, as behaviors that rely on such vocalizations and that precede egg laying, as mating, can be affected. Physiological stress could also be an underlying mechanism. However, it is less likely to occur as other reproductive phases affected by stress-related hormones do not change.

### 4.2 Introduction

The growing level of urbanization worldwide has affected populations and communities of birds (Vitousek et al. 1997; Clergeau et al. 2006; McKinney 2006; Lepczyk et al. 2008). The spread of urban surfaces in the landscape or the industrial development has been negatively correlated to the density of populations and to the diversity of species, leading to a homogenization of bird communities (Clergeau et al.

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<sup>3</sup> Em parceria com os coautores Japyassú, HF, Slabbekoorn, H, Izar, P.

2006; Lepczyk et al. 2008, Kunc et al. 2013). Such simplification can be partially attributed to a reduction of the reproductive success of birds inhabiting such highly urban environments (Kuitunen et al. 2003, Rodewald et al. 2013, Mennechez and Clergeau 2006, Seress et al. 2012, Dietz et al. 2013, Bailly et al. 2015). However, it is not only, or not necessarily just, the expansion of urban surfaces by itself that is the causal factor to impact the survival of the remaining species.

Several urban factors modify the environmental conditions in and around cities in such a way that they may negatively affect animals (e.g. Flousek 1989; Swiergosz et al. 1998; Warren et al. 2006; Saha and Padhy 2011; Ciach and Frohlich 2017; Sanderfoot and Holloway 2017). Pollutants as street lightning, industrial and traffic noise and particulate matter can all negatively influence the presence and well-being of humans and animals alike (Saha and Padhy 2011, Bernath-Plaisted and Koper 2016, Gaston et al. 2013, Ciach and Frohlich 2017, Sanderfoot and Holloway 2017). From these factors, anthropogenic noise has recently received much attention (Wright et al. 2007, Rabin et al. 2003, Kight and Swaddle 2011, Slabbekoorn et al. 2010, Francis and Barber 2013), and it has been shown that this pollutant may play a role on the density and diversity deterrence of populations (e.g. Saha and Padhy 2011, Bunkley et al. 2017).

Anthropogenic noise can negatively impact bird populations through interference on behavior and physiology of individuals (e.g. Rheindt 2003). It can disrupt the acoustic communication, foraging, antipredator and mating behaviors (Halfwerk et al. 2011b, Meillère et al. 2015, Purser and Radford 2011, Wale et al. 2013, Davies et al. 2017), which can frequently occur through acoustic masking of signals (Barber et al. 2009). For example, the noise can reduce the responsiveness of female birds to sexually preferred songs of males it usually masks (Halfwerk et al. 2011b, des Aunay et al. 2014), suggesting consequent disruptions on mating. Moreover, the noise can be a potential physiological stressor and distractor. It can increase the level of stress-related hormones as corticosterone that interfere on female fertility and reproduction (Davies et al. 2017, Wright et al. 2007, Blickley et al. 2012, Angelier et al., 2009) but also the vigilant against feeding behavior (Fernandez-Juricic and Telleria, 2000; Quinn et al., 2006), which could affect nestling provisioning rates. Therefore, as vocal communication and stress-related hormones play a role on reproduction (Bradbury and Vehrencamp 1998, Thierry et al. 2013,

Angelier et al., 2009), the anthropogenic noise can also have a negative impact on the reproductive success of birds.

The negative impact of noise on the reproductive success have been recently investigated by few field studies. In a population of great tits (*Parus major*) breeding alongside a motorway, females laid smaller clutches in noisier than in quieter territories and had fewer successful fledglings independently of the clutch size (Halfwerk et a. 2011). Also, Eastern bluebirds (*Sialia sialis*) produced less fledglings in noisier than in quieter territories (Kight et al. 2012). Such findings suggest that the noise may play a negative effect on the egg-laying phase and on the efficiency of the incubating behavior. The noise may also affect the parental provisioning rate to the nestlings. House sparrows (*Passer domesticus*) breeding in a natural area affected by industrial noise had lighter fledglings than birds breeding in quiet areas (Schroeder et al. 2012). Although it is known that anthropogenic noise is negatively related to the reproductive success of birds in different phases, whether there is a causal relationship driving this correlation is still not clearly understood. Some cofounding effects present in the urban or natural habitats other than noise may also play a role on the reproductive success.

Experiments of long-term noise exposure in captivity are useful to clearly understand the impact of noise on the reproductive success of birds, as some cofounder effects can be controlled (e.g. predation risk and territory quality; see Angkaew et al. 2019, Halfwerk et al. 2011, Reijnen and Foppen 1994, Habib, Bayne and Boutin 2007). In a recent experimental long-term noise exposure, captive zebra finches breeding in noise did not delay the egg-laying, but needed higher nesting attempts, possibly due to an observed higher embryo mortality (Potvin et al. 2015a). Additionally, they bred fewer successful fledglings that had a delayed growth rate on the first two weeks of development (Potvin et al. 2015a). The authors suggested these breeding failures may occur due to a decreased parental incubation and provisioning rate, however these possibilities remain to be experimentally tested. Despite this single study performed in captivity, there is still a lack of direct evidences whether the noise may play a role on the initial stages of reproduction (pre-egg laying or egg-laying phases), as suggested for some field studies showing correlations between noise and delay of egg-laying (Halfwerk et al. 2011, Kight et al. 2012).

Bengalese finches (*Lonchura striata var. domestica*) are suitable birds for captive experiments. They are easy to maintain and reproduce in cages and their

reproductive behavior has been already described (Eisner 1960, 1961, 1969). They can breed throughout all year long, instead of having specific breeding seasons (Eisner 1960). Clutch sizes range from 2 to 9 eggs, with 5-6 the most common quantity, and both females and males alternate incubation that usually last 16 days (Eisner 1960, 1961). The influence of several factors on their reproductive behavior have been studied. For example, the level of song complexity from which females are exposed and the quantity of available nest material can influence the readiness of individuals to start nest building and egg-laying (Slater 1970, Okanoya 2004). Moreover, it has been shown that clutch size and hormone treatments, as estrogen, play a role on the incubation time and costs (Coleman and Whittall 1988, Eisner 1969). However, the impact of anthropogenic noise on the reproductive success of Bengalese finches has not yet been studied.

In the present study, we tested whether experimental noise exposure affected three different phases of reproduction in Bengalese finches: 1) the pre-nesting phase, 2) the egg-laying phase, and 3) hatchling and nestling phase. We predicted a negative impact of noise on each phase with 1) less or delayed nesting attempts in newly formed couples, as the communication between mates and the female readiness to reproduce can be disrupted by the noise, 2) smaller clutch sizes, with smaller, lighter and less fertilized eggs, as noise can be affect female hormone levels related to ovulation and fertility, and 3) fewer hatchlings/nestlings of lower weights and less fledgling success, as noise can be a stressor factor decreasing incubation efficiency and parental provisioning rate.

### **4.3 Material and methods**

#### *4.3.1 Species subject and Housing*

We used 48 individuals of adult Bengalese finches donated by a local breeder. We chose Bengalese finches as a model species due to their propensity to breed in captivity and to the existence of knowledge about their reproduction cycle and their song characteristics and flexibility. It is known the time period of the song crystallization and the species ability to short-term adjust the frequency and amplitude of songs under noisy regimes (Shiba et al. 2016). These song adjustments yield to the suggestion that the noise may interfere on the vocal communication.

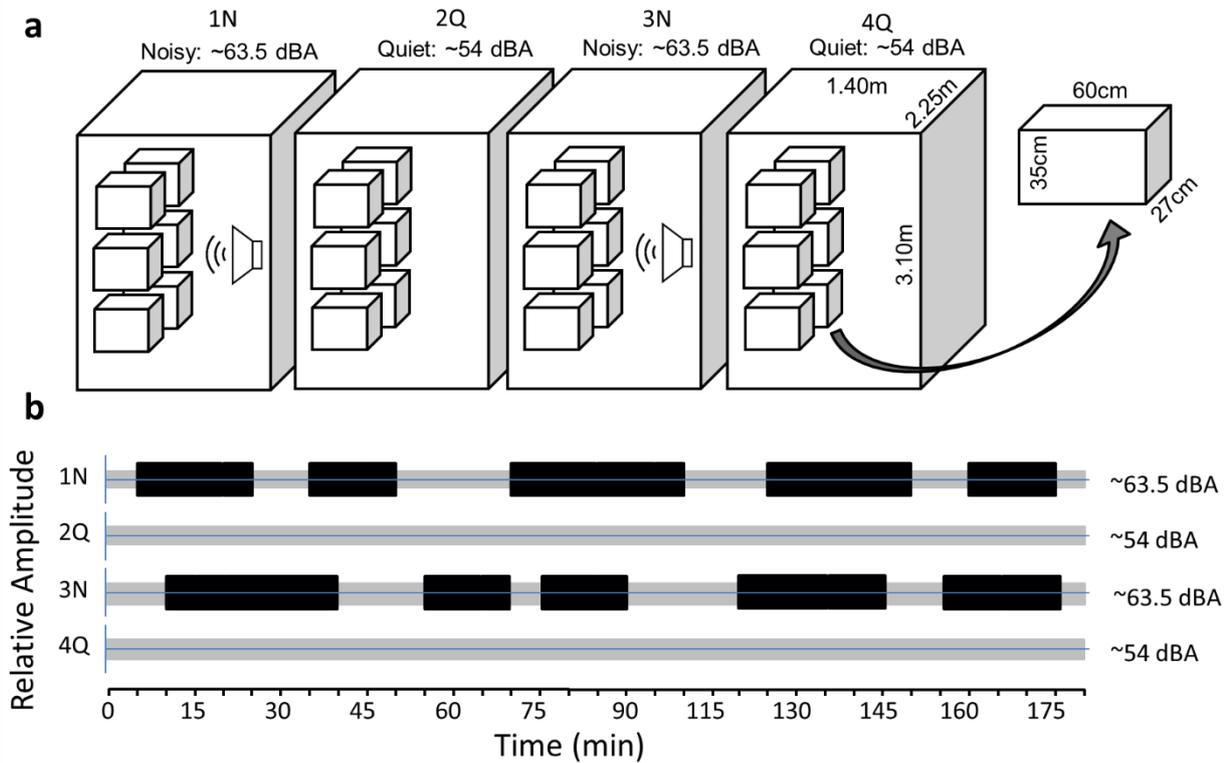
Whether there is a possible interference on the communication during courtship or parental care, the reproductive success of the species might also be affected.

Prior to the formation of the breeding pairs, we housed the individuals in a common room during 2 months for acclimatization to the laboratory conditions. In this room, we kept the birds in groups of 3 or 4 individuals of the same sex in separate cages (L:60 H:35 D:27 cm) on a 12:12 h light/dark cycle. All birds were given daily fresh water, finch seed mixture, cuttlebone and eggshell *ad libitum*, along with a portion of escalore leaves and *MegaZoo* vitaminized cornmeal finch mixture twice per week.

#### 4.3.2 *Experimental Protocol*

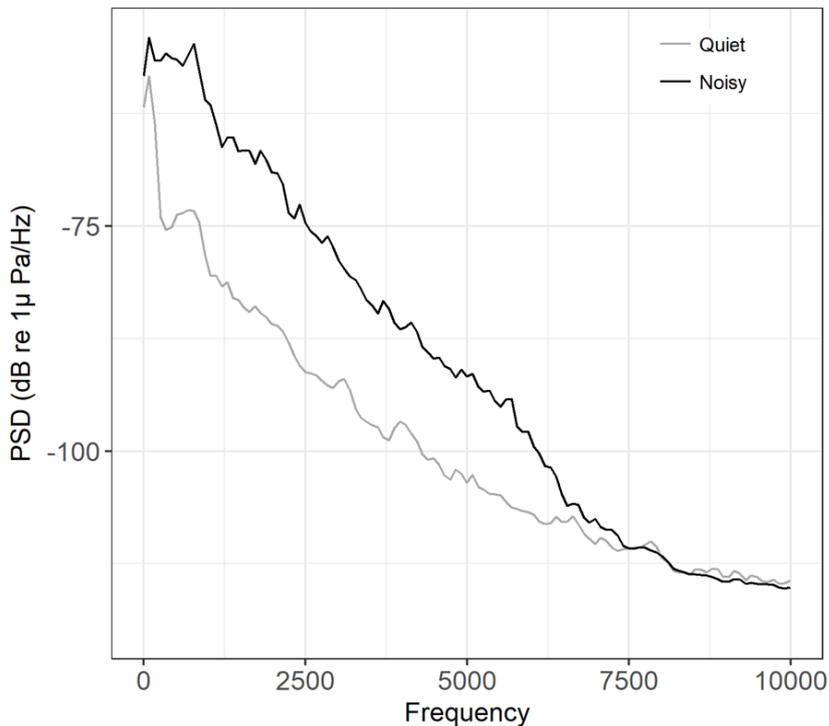
We formed 24 breeding pairs of bengalese finches and randomly distributed them in four treatment rooms in individual cages (L:60 H:35 D:27 cm), with 6 pair per room. The birds were kept in a light/dark cycle of 14h:10h and boiled eggs were added to the vitaminized cornmeal portions to increase the proportion of proteins in the diet during reproduction. Birds from two rooms (12 pairs) were exposed intermittently to an artificial experimental noise at the level of 65 dBA SPL during all day and night and the birds from the other two rooms were not exposed to noise (Figure 9a, Figure 10). The intermittence of the noise was created by playing it back in four different durations intercalated with four different durations of quietness (no noise playback), all in random orders. The durations were 15, 20, 25 and 30 minutes of noise played back between 5, 10, 15 and 20 minutes of quietness (figure 9b). Each noise was playback back with a fade in and fade out in 20 seconds. For the noise playback we used ipods connected to Dell speakers. The sound levels inside the rooms where regularly checked and measured using a sound pressure level meter with data logger Skill-Tec TM (São Paulo, São Paulo, Brazil) SKDEC-02 (A-weight, slow response, range 30-130 dB, 1s interval).

Figure 9 – Experimental setup of treatments and background noises: a. Two rooms were designated to the noisy treatment and two other rooms to the control/quiet treatment. Each room contained 6 cages/breeding pairs. b. Relative amplitude of the background noise (dBA) per treatment in time. Experimental artificial noises of different durations were played back intermittently and randomly in the noisy treatments.



Source: designed by the author

Figure 10 – Spectral and Amplitude average characteristics of the background noise measured in each room by the sound pressure level meter.



Source: designed by the author

We synthesized the noise imitating the frequency spectrum of a traffic noise (Figure 10). We created this noise with Audacity™ v. 2.1.2 (Carnegie Mellon University, Pittsburgh, Pennsylvania, USA), where, using the equalization option, we attenuated a white noise by 3dB every 500 Hz until the upper limit of 10kHz (Halfwerk and Slabbekoorn 2009, Lazerte et al. 2016). The background noise of the treatment rooms without the birds ranged between 45 and 68 dBA SPL and between 48 (birds quiet) and 74 dBA SPL (birds chattering loudly). The total LAeq average sound level was 63.48 dBA SPL with and 62.48 dBA SPL without birds. The other 12 bengalese finch pairs divided between the other two rooms were not exposed to noisy regimes and were used as control groups. The background noise of the control rooms ranged from 49 (birds quiet) to 78 dBA SPL (birds chattering loudly) and between 50 and 56 dBA SPL without birds. The total LAeq average sound level for the control room was 52.31 dBA SPL without and 54.18 dBA SPL with the birds inside.

#### 4.3.3. Measurements of the Reproductive Success

For each breeding pair we measured eight variables related to the reproductive success at three different phases: 1) pre-nesting phase: latency to the first egg laying, 2) nesting phase: number of laid eggs, number failed eggs (that were broken during egg laying and incubation period or that never hatched despite having or not a visible embryo), number of dead embryos (by visual inspection of the content of the broken eggs or eggs that never hatched after the offspring fledged), 3) post-hatching phase: number of dead offspring (hatchling and nestling that died), number of fledglings, fledgling survival rate (i.e., the number of fledglings/(number of dead embryos + dead nestlings + fledglings)) and nestling growth (each 3 days from 5 to 23th post-hatching days and in the 25<sup>th</sup> day, in grams). Additionally, we measured the mass (in grams) of the nestlings and the size (length and width) and mass (grams).

#### *4.3.4. Statistical Analysis*

We performed all the analysis in R studio software (R Core Team), using the packages lme4 (Bates et al. 2015), glmmTMB (Brooks et al. 2017) and coin (Hothorn et al. 2008). We performed generalized linear models (GLM, poisson and gaussian distributions) but also zero-inflated Poisson models (ZIP) and GLM with negative binomial distributions, as mostly of the response variables were zero-inflated. All the measured variables of reproductive success entered as response variables. The response variables number of laid eggs, number of failed eggs, number of dead embryos, number of dead nestlings, number of fledglings, fledgling survival rate and egg-laying latency entered the models as intervals in Poisson for GLM and ZIP, and in negative binomial distributions for GLM. Therefore, we computed statistics for six different models, which included a single predictor (the noise treatment) and the null models (without the effect of any predictor): GLM with Poisson distribution, GLM with negative binomial distribution, ZIP with Poisson distribution and null models for each type of model and distribution. The mass and size measurements of the eggs and nestlings entered the models as numeric in Gaussian distributions in the generalized models only, as they were not zero-inflated. Therefore, for the egg measurements, we computed two models, the GLM including noise as a factor and the respective null model. Finally, to find out whether the noise and not only the age of the nestlings was playing a role on their mass gain, we computed four models: two GLMs including

separately the single predictors (noise or age), a GLM with their additive combination (noise + age) and the null model (without predictors).

To find out whether the noise exposure affected the reproductive success, we selected the best models based on the Akaike's information criterion (AIC). The model with lowest AICc value were considered the best model and two units of difference between them was considered as substantial to select only one best model. We performed non-parametric randomization tests for each response variable we obtained a best model that included the noise treatment. This analysis informed us whether there were significant differences between the treatments in the reproductive success variables. As an addition to the results interpretation, we performed non-parametric stratified bootstrapped 95% confidence intervals with respect to the treatment and applied the zero-inflated Poisson models (ZIP) to it. The mean effect size and the 95% confident intervals were taken from 10000 samples. Through this method, we could evaluate the importance of the effect of the noise on the response variables by visually inspecting the level of skewness between the 95% CIs.

#### *4.3.5. Ethical Note*

The present study was approved by the local Ethical Committee in the Use of Animals for Research (CEUA) of the Institute of Biology of the Universidade Federal da Bahia, Salvador, Brasil (reference number: 01/2018), place where the experiment was executed.

## **4.4. Results**

Our results show an effect of the noise exposure on the first phase of reproduction, the pre-nesting phase. The latency to egg laying was higher for the birds breeding in the noisy condition (Tables 7 and 8; Fig. 11a). However, after started the egg-laying phase, the number of laid eggs did not differ between the treatments noisy and quiet (Table 7; Fig. 11b). The number of eggs that failed (broken during oviposition or that never hatched) tended to be higher for the birds breeding in the noisy condition, when compared to the quiet regime. For this response variable, the best model included the treatment as a factor, however, the

differences between noise regimes were not statistically significant (Tables 7 and 8; Fig. 11c). The failed eggs were not represented by the embryo mortality, as the number of dead embryos did not differ between noise treatments (Table 7). This indicates that the number of egg failures must be represented mainly by the non-fertilization and discard of the eggs instead of lost during embryo development. Furthermore, the noise did not affect the mass of the eggs but tended to influence their shape. The eggs from the noisy condition tended to be narrower than the eggs from the quiet (Tables 7 and 8; Fig. 12a) but not longer or lighter (Table 7, Fig. 12b-c).

Finally, our study does not show an effect of the noise on hatchling/nestling phase, as the nestling and fledgling death, survival and growth rate were not affected. Neither the number of nestlings and fledglings per couple that died (Table 7, Fig. 11d) nor the fledgling survival rate differ between the noisy and quiet treatments. Regarding the nestling development, the noise did not influence the gain of mass of the nestlings between their 5<sup>th</sup> and 25<sup>th</sup> post-hatching days (Table 7, Fig. 12d).

Table 7 – Results of the model selection for all song response variables (indicated in bold).  $\Delta AICc > 2$  indicates a significant difference between two models.

<b>Results of model selection and AIC values</b>				
<b>Response variables</b>	<b>Distribution</b>	<b>df</b>	<b>AIC</b>	<b><math>\Delta AIC</math></b>
<b>Egg laying latency</b>				
<b>GLM</b>	<b>Negative binomial</b>	<b>3</b>	<b>146.966</b>	<b>0</b>
GLM null	Negative binomial	2	154.8479	7.8819
GLM	Poisson	2	155.0309	8.0649
ZIP model	Poisson	4	159.0309	12.0649
GLM null	Poisson	1	184.2471	37.2811
ZIP null model	Poisson	2	186.2471	39.2811
<b># Laid eggs</b>				
ZIP Null model	Poisson	2	118.9907	0
ZIP model	Poisson	4	122.3304	3.3397
GLM Null	Negative binomial	2	124.9652	5.9745
GLM	Negative binomial	3	126.4762	7.4855
GLM Null	Poisson	1	129.7962	10.8055
GLM	Poisson	2	130.7424	11.7517
<b># Failed eggs</b>				
<b>GLM</b>	<b>Negative binomial</b>	<b>3</b>	<b>119.032</b>	<b>0</b>
GLM null	Negative binomial	2	119.6016	0.5696
ZIP model	Poisson	4	124.1958	5.1638
ZIP null model	Poisson	2	124.967	5.935
GLM	Poisson	2	126.2835	126.2835
GLM null	Poisson	1	130.4795	130.4795
<b># Dead embryos</b>				
ZIP null model	Poisson	2	23.18288	0
ZIP model	Poisson	4	NA	NA
GLM null	Negative binomial	2	25.38955	2.20667
GLM	Poisson	2	27.7245	4.54162
GLM	Negative binomial	3	29.72442	6.54154
GLM null	Poisson	1	32.65597	9.47309

(continua)

<b>Results of model selection and AIC values</b>				
<b>Response variables</b>	<b>Distribution</b>	<b>df</b>	<b>AIC</b>	<b>ΔAIC</b>
<b># Dead nestlings</b>				
GLM null	Poisson	1	25.72037	0
ZIP null model	Poisson	2	26.38254	0.66217
GLM	Poisson	2	26.67387	0.9535
GLM null	Negative binomial	2	26.67884	0.95847
GLM	Negative binomial	3	27.93107	2.2107
ZIP model	Poisson	4	29.31344	3.59307
<b># Fledglings</b>				
ZIP null model	Poisson	2	53.05051	0
ZIP model	Poisson	4	53.83661	0.7861
GLM	Negative binomial	3	55.09559	2.04508
GLM null	Negative binomial	2	55.86038	2.80987
GLM	Poisson	2	58.53239	5.48188
GLM null	Poisson	1	63.27062	10.22011
<b>Fledglings survival rate</b>				
ZIP null model	Poisson	2	19.80562	0
GLM null	Negative binomial	2	19.80571	9E-05
GLM	Negative binomial	3	21.79158	1.98596
ZIP model	Poisson	4	23.79149	3.98587
GLM	Poisson	2	inf	
GLM null	Poisson	1	inf	
<b>Nestling mass (g)</b>				
GLM (~Age)	Gaussian	8	867.1088	0
GLM (~Treatment + Age)	Gaussian	9	869.1077	1.9989
GLM Null model	Gaussian	2	1397.8389	530.7301
GLM (~Treatment)	Gaussian	3	1399.8187	532.7099
<b>Egg mass (g)</b>				
GLM Null model	Gaussian	2	-25.80432	0
GLM (~Treatment)	Gaussian	3	-25.60657	0.19775

(continua)

Continuação

**Results of model selection and AIC values**

Response variables	Distribution	df	AIC	$\Delta$ AIC
<b>Egg width (mm)</b>				
<b>GLM (~Treatment)</b>	<b>Gaussian</b>	<b>3</b>	<b>128.2343</b>	<b>0</b>
GLM Null model	Gaussian	2	130.2123	1.978
<b>Egg length (mm)</b>				
GLM Null model	Gaussian	2	194.3958	0
GLM (~Treatment)	Gaussian	3	196.2597	196.2597

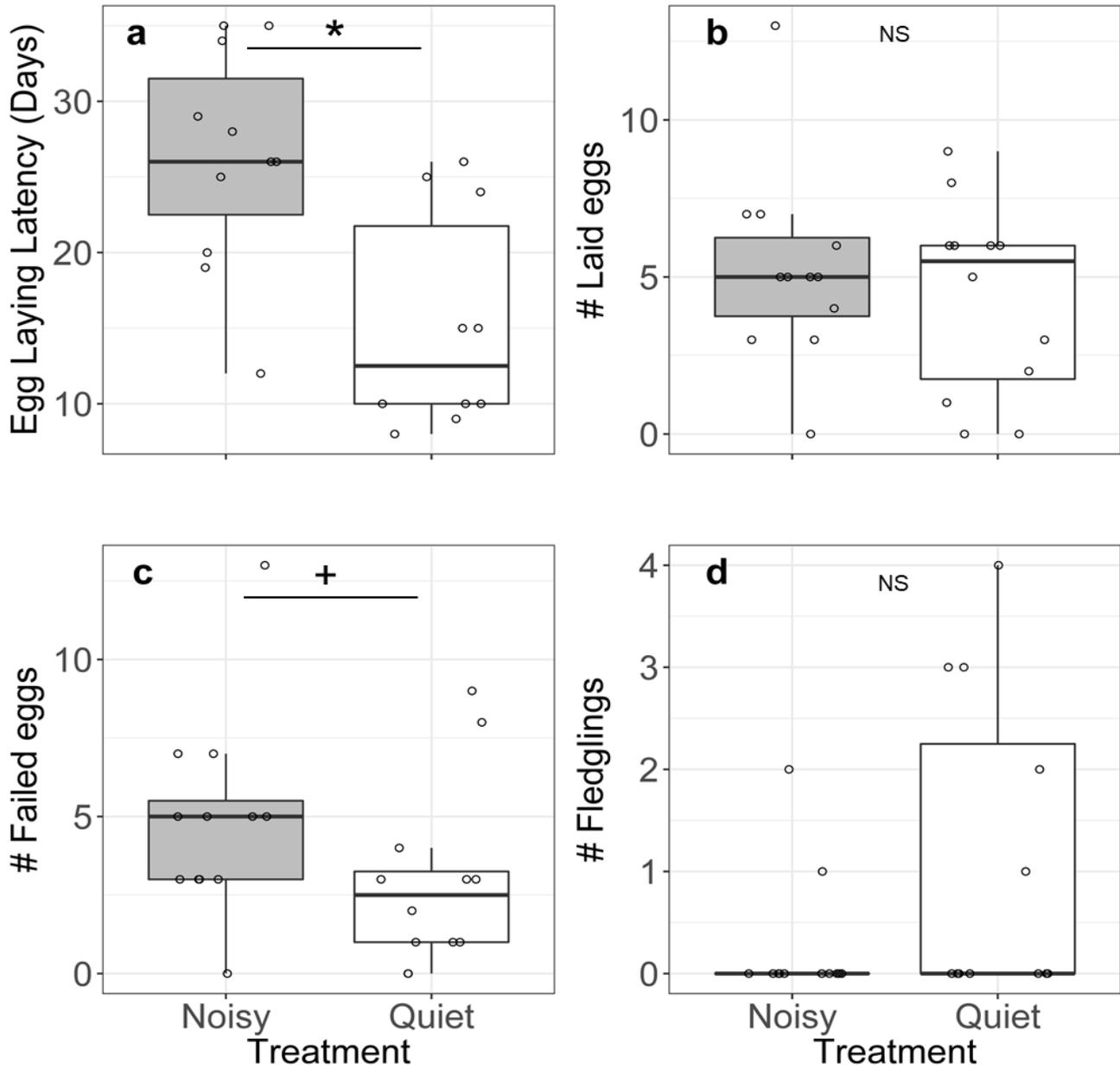
Source: designed by the author

Table 8 – Results of the best models in which treatment significantly impacted the response variables

Summary of best models with treatment					Randomization test	
Response variable	Estimate value	Std. Error	z value	p-value	z value	p-value
Egg laying latency	-0.54724	0.15328	-3.57	<0.001	2.8123	0.003
# Failed eggs	-0.5222	0.3163	-1.651	<0.01	1.5498	0.14
Egg width (mm)	0.29501	0.14795	1.994	0.05	-1.9512	0.05

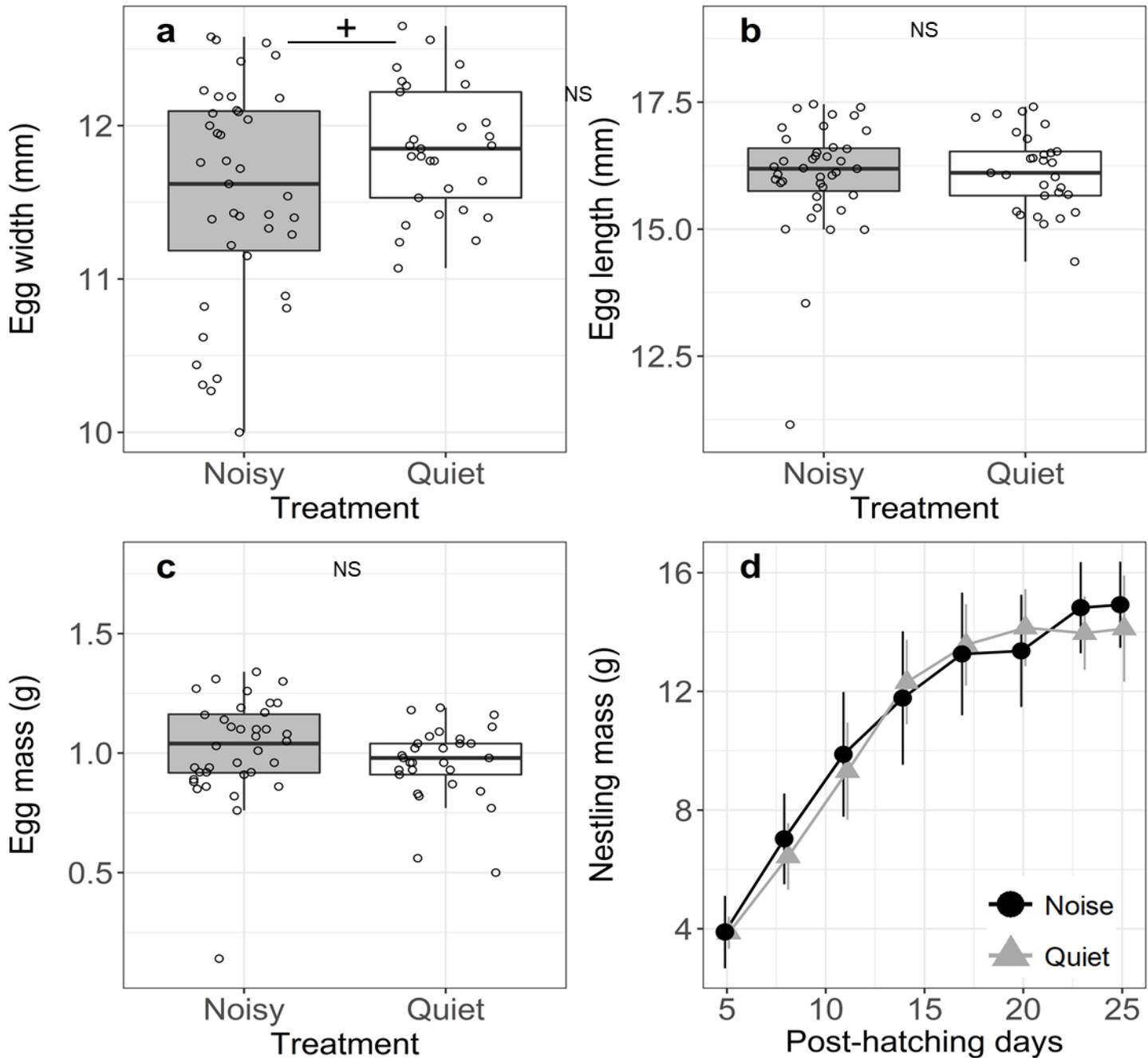
Source: designed by the author

Figure 11 – Efficiency of the reproductive phases per noise treatment: a. latency to egg laying; b. number of laid eggs; c. number of failed eggs and number of fledglings between noisy and quiet treatments.



Source: designed by the author

**Figure 12** – Egg measurements for noisy and quiet treatments and nestling growth rate between 5<sup>th</sup> to 25<sup>th</sup> post-hatching days: a. egg width (mm); b. egg length (mm); c. egg mass (g) and nestling mass (g).



Source: designed by the author

#### 4.5. Discussion

In the present study, we found evidence for the effect of experimental noise exposure on the 1) pre-egg laying phase, as we found a negative effect of noise on the delay of egg-laying. However, we do not show an effect of noise on the 2) egg-

laying and 3) hatchling and nestling phases, as clutch sizes, embryo deaths, hatchling and nestling survival and growth rate were not affected. Despite the lack of impact on the embryonic deaths, the number of eggs that failed, either during oviposition or after incubation, tended to be higher in the noisy condition, indicating that might be a lost due to non-fertilization. The shape of the eggs also tended to be different between treatments, being slightly narrower for birds exposed to the noise.

#### *4.5.1. Effect of chronic experimental noise exposure on the delay of egg laying*

Three different mechanisms can explain the delay of egg laying of birds chronically exposed to noise: acoustic masking, auditory distraction and auditory stress. As an acoustic masker, the noise can negatively impact behaviors that rely on acoustic signals that precedes egg laying, as mating. As an auditory distractor, the noise can disturb the attention of the birds during foraging attempts, decreasing their food intake through the increase of their vigilant state. As a chronic stressor, the noise can negatively affect the female hormone balance, increasing their stress hormone levels. Therefore, lessened communication and nutrition and increased stress and vigilance can debilitate the general health of individuals and their readiness to reproduce and to lay eggs.

For the Bengalese finches, the acoustic masking may be one of the most reasonable explanation for the delay in egg-laying. It is well known that the noise masks low-frequency song sequences, bandwidths that usually attracts female preferences in courtship contexts (Pasteau et al. 2007, Halfwerk et al. 2011b, des Aunay et al. 2014). On the current study, at least half of the lowest frequency bandwidth of Bengalese songs was overlapped by the noise, despite their wide singing ranges (usually from 0.5 to 7-10 kHz, Winandy et al. in prep.). From such overlaps, we suggest the noise masks and decrease the detectability of the most preferable song sequences, bringing negative impacts to the courtship, which would delay the readiness to reproduce and the egg-laying. Moreover, despite the ability of Bengalese finches to increase the amplitude, the fundamental frequency and the syllable gaps of their songs as a possible masking avoidance (Shiba et al. 2016, Sober and Brainard 2009, Tachibana et al. 2017), these vocal adjustments may possibly decrease their attractiveness (Halfwerk et al. 2011b, des Aunay et al. 2014) and delay the reproduction. Therefore, the noise may have masked and reduced the

detectability of the most attractive low-frequency song bandwidths, yielding the birds to sing higher detectable but less attractive high song frequencies. Such possible auditory masking and noise-induced vocal changes could yield to a delay in the egg laying.

An auditory stress, but unlikely an auditory distraction, could also explain why Bengalese finches delayed the egg-laying. Chronic exposures to noise are known to increase birds stress related hormones, as corticosterone (Wright et al. 2007, Blickley et al. 2012). Such hormonal imbalance can negatively affect birds health and behavior (Davies et al. 2017, Wright et al. 2007, Blickley et al. 2012, Angelier et al., 2009), which could yield to a delay and/or reduction of reproductive efforts. In the meantime, it is less likely that the noise can distract the attention of the birds during foraging activities which could reduce their food intake (Fernandez-Juricic and Telleria, 2000; Quinn et al., 2006). Although food intake was not measured directly, feeding did not appear to differ among treatments. We based this on the fact that food replenishment was comparable in all studied rooms. Therefore, from the three possible mechanisms by which noise could affect the animals readiness to reproduce and to lay eggs, the auditory masker and stressor are the most likely causes.

Differently from Bengalese finches, the delay of egg-laying did not occur for other species also chronically exposed to noise (Potvin et al. 2015, Kuitunen et al. 2003, Halfwerk et al. 2011a, Schroeder et al. 2012). Different methodological setups in captivity and/or seasonality of wild breeding conditions could explain opposite results. Potvin et al. (2015) chronically exposed captive zebra finches to a continuous recorded highway noise with machinery sounds during daylight. Differently, our Bengalese finches were exposed to different and intermittent durations of an artificial low-frequency noise along with variable intervals of quietness, during all day and night. Both studies, despite having unpredictable fluctuating noise decibel levels, differed in the amplitude of the decibel variation. Bengalese finches, in opposite of zebra finches, were possibly exposed to higher decibel variation regarding the quiet intervals added to the noise exposures. Such higher variability might have prevented or delayed the birds to habituate to the stressor and start reproducing. Moreover, 24h hours of intermittent noise exposure, rather than a daylight continuous noise, might have intensified the stress effects for Bengalese finches.

Differences in seasonality may explain why wild breeding species do not delay the egg-laying (e.g., Kuitunen et al. 2003, Halfwerk et al. 2011a, Schroeder et al.

2012) and Bengalese finches do. Season is a key factor for the reproductive success of some species. During specific seasons birds can find the necessary resources to reproduce successfully as food, nests, lack of competitors and favorable temperature (Harriman et al. 2017, Kwon et al. 2108, Samplonius and Both 2019). Whether some delay occur in the start, i.e., in the egg laying, or during the reproductive efforts, breeding can fail (e.g., Samplonius and Both 2019 report an overlap of competition for nests). Bengalese finch does not have a specific breeding season (Eisner 1960) and could be more sensitive to the impact of noise than species that are strongly triggered by other biotic and abiotic factors to start the breeding behavior. However, more comparative studies are needed to understand the role of breeding seasonality on the susceptibility to a negative effect of noise.

#### 4.5.2. *No impact on eggs weight, number and fertilization*

Maybe the chronic noise was not such a strong or prolonged auditory stressor as it did not affect the egg-laying phase. A significant impact of the noise as an auditory stressor, increasing birds stress hormones as corticosterone (Davies et al. 2017, Wright et al. 2007, Blickley et al. 2012, Angelier et al., 2009), would reduce their reproductive efforts on nesting or incubation, yielding to a higher egg failure or embryo mortality (Potvin et al. 2015). As Bengalese finches, other species under noise also did not engage less in incubation, excepting two (see Halfwerk et al. 2011, Potvin et al. 2015). Wild populations of house sparrows (*Passer domesticus*), flycatchers (*Ficedula hypoleuca*) and eastern bluebirds (*Sialia sialis*) did not have reduced clutch sizes when reproducing under noisy conditions (Schoroeder et al. 2012, Kuitunen et al. 2003, Halfwerk et al. 2011). Therefore, it is unlikely that the noise acted as a physiological stressor, as investment on the egg-laying and incubation (with exception of great tits, Halfwerk et al. 2011, and captive zebra finches, Potvin et al. 2015) did not appear to have been negatively impacted. Instead, it may possibly have acted as a masking sound on earlier phases of reproduction, affecting egg-laying latency (Bengalese finches, current study) or nesting attempts (zebra finches, Potvin et al. 2015).

Nevertheless, it is still possible that the noise may play a role on the egg-laying phase, as it can still possibly affect fertilization and incubation. Bengalese finches of our study tended to have a higher number of failed eggs that were possibly

not fertilized, as the dead embryos of these eggs did not differ between treatments. Zebra finches from noisy conditions, had a higher embryo mortality, yielding to more nesting attempts till they produced hatchlings, which suggest less incubation investments (Potvin et al. 2015). It is possible that a reduction in fertilization be explained by a masking effect of noise on male songs, impairing communication between mates (Halfwerk et al. 2011, des Aunay et al. 2014), or by decreasing the investment and receptivity of females to noise-dependent adjusted male songs (Halfwerk and Slabbekoorn 2014). Therefore, both explanations could lead to less copulation success, higher abandonments of eggs or nest, or less investments in incubation.

#### 4.5.3. No effect on hatchling/nestling stage

The noise might not play a role on the parental care of hatchlings and nestlings of captive Bengalese finches as it does for wild living species. Our Bengalese finches do not produce different number of hatchlings and fledglings, with no weight differences, among noise and quiet conditions. The number of nestling deaths, successful fledglings and nestlings growth rate are similar among treatments. Meanwhile, wild Eastern bluebirds (*Sialia sialis*) and great tits (*Parus major*) breeding in noisy territories produce fewer number of fledglings with lower body masses than pairs from quieter sites (Kight et al. 2012, Halfwerk et al. 2011). An increased vigilance or predator scanning against a decreased offspring provisional rate (Fernandez-Juricic and Telleria, 2000; Quinn et al., 2006), as reported for house sparrows (Schroeder et al. 2012), or an auditory masking of the parent-offspring communication (Lucass et al. 2016) could explain why such species decline the parental care. However, as it is not the case for Bengalese finches, both explanations would not play a role on their reproductive success once they start hatchling/nestling care.

One explanation for the lack of negative impact of the noise on the hatchling and nestling success is the captive breeding condition. The negative influence of the noise on the offspring provisional rate and survival is reported for natural living bird populations (Kight et al. 2012, Halfwerk et al. 2011, Schroeder et al. 2012) but not for captive and domestic species - Bengalese finches of our study and zebra finches (Potvin et al. 2015). Foraging and parental care in natural habitats is more

challenging than in captivity. Finding food in natural and non-predictable environments has to be counterbalanced with vigilance against predator (Fernandez-Juricic and Telleria, 2000; Quinn et al., 2006). In captivity, the breeding and feeding condition are more predictable, where food and nesting material are offered in *ad libitum* and in predictable containers and nests. Moreover, the maintenance environment of aviaries and cages is much more restricted than in natural habitats and reduce or eliminate predation risks. Bengalese finches are captive and domesticated birds which counts for a possible loss of antipredator behaviors (Carrete and Tella 2015). Therefore, the natural, unpredictable and more risky breeding conditions - rather than the captive habitat and domesticated species – would pronounce the negative effects of noise on parental care and provisioning.

#### 4.5.4. Conclusion

Our study provides insights of noise-induced impact on the delay of egg-laying and, therefore, on the breeding cycle of captive Bengalese finches. However, our study does not show a negative impact of the noise on successive breeding phases, such the hatching, hatchling and nestling phases of reproduction. It is more likely that an auditory masking effect is the mechanism explaining our result. The noise can negatively affect behaviors that rely on acoustic signals and that precedes egg laying, as mating. Other possible negative effects as physiological stressor and auditory distraction could also occur, although they are less likely for our captive Bengalese finches. As a possible strong physiological stressor, the noise would impair other reproductive phases highly influenced by stress related hormones, as fertility and embryo and nestling mortality. As an auditory distractor, the noise would reduce the feeding attempts or offspring provisional rate due to increased vigilant behaviors. However, these two last mechanisms are less likely to occur as Bengalese finches do not show significant decreased fertility, hatchling survival and nestling mass in the noisy treatment. Therefore, only a masking of the male songs or a reduced attractiveness of noise-related adjusted vocalizations could yield to a delayed mating and, consequently, nesting attempts and egg-laying.

## 5. DISCUSSION

Urbanization, habitat homogenization, deforestation and anthropogenic noise have altogether negatively impacted animals, adding to them more challenges in surviving and reproducing in recently human altered habitats (Buxton et al. 2017, Devictor et al. 2007, McKinney 2006). To thrive in acoustically loud and stressing environments, animals have been found to change their communicating vocal signals in ways that might increase audibility (Brumm and Slabbekoorn 2005, Pohl et al. 2012). They also may change other behaviors, as time of activity or singing, territory defense and predation avoidance against feeding attempts (Gil et al. 2014, Quinn et al. 2006, Halfwerk et al. 2011b, Kleist et al. 2016). There are other species, however, that change their home range and avoid sound impacted areas (Saha and Padhy 2011, Bunkley et al. 2017), contributing to the homogenization of animal communities in cities. For example, city birds sing songs higher in frequency and with more intensity than their forest conspecifics (Slabbekoorn and Den Boer-Visser, 2006, Ríos-Chelén et al. 2013, Brumm and Zollinger, 2011). Savannah sparrows usually delays feeding visits to their nestlings under alarm calls, however, decrease this latency to feed when an interfering noise is present (Antze and Koper 2018). Although, it is known anthropogenic noise can negatively impact and induce behavioral changes on animals, it is far an entire understanding the consequences such alterations cause to the animal populations.

The overall address of the thesis is the negative impact of the anthropogenic noise on bird communication and its consequences on territory defense and reproductive success. For such study we used field observations to look for patterns and further experiments conducted on the field and on captivity. The thesis explores the patterns of noise-dependent vocal adjustments in a neotropical bird species and focus on patterns of alteration of song elaboration, based on the combined song use of frequency bandwidth, duration and syllable types. In continuity, we explore some of the consequences of the observed pattern of noise-related variation in song elaboration. We then addressed how such pattern of song elaboration could play a role on territorial signaling in the noisy city environment. Such investigation could lead insights into whether noise-dependent song restrictions cause consequences on the value of signals and compromise communicative function. Finally, yet looking forward the consequences of noise-induced vocal changes, we investigated how

noise could negatively interfere on the success of reproduction, a highly vocally dependent behaviour in bird species. We used as model a captive bird species, the Bengalese finch, due to its facility to maintain and reproduce in laboratory.

### **5.1 Noise-related changes in song elaboration**

The noise can overlap songs in both frequency and time. Such overlap can mask several song components or syllables. Such overlap, in turn, may yield animals to remove elements, simplifying and/or shortening the song. When singing in noise, for example, birds may remove the low-frequency elements of their songs, and, sometimes, replace them by more repeatable high-frequency components. When noise also overlap the singing behavior in time, may be more difficult to sing longer bouts of songs. As a mechanism to counteract long or constant noise exposures, birds can shorten their songs, which may increase the probability of being completely heard without auditory interferences. Our study species, the bananaquit, provides a great example of noise-dependent changes in the frequency of songs that might restrict song elaboration (Chapter 1). We found that bananaquits significantly sings in higher minimum and maximum frequencies and in narrower frequency bandwidths in noisier territories. These noise-dependent restrictions in frequency use appear to restrict song elaboration, as the higher and narrower songs tends to be more repetitive and with less different syllable types. Shorter, simpler and higher songs may increase audibility in noise as they are less likely to be overlapped by low-frequency anthropogenic noises and to have spectrally concentrated signal energy (Hanna et al. 2011).

### **5.2 Behavioral consequences of singing simpler songs in noise**

Simpler, higher, narrower and shorter songs may probably contribute to the signal detection in noisy environments. However, they can come at the cost of a reduced signal value or attractiveness (Slabbekoorn 2013; Luther et al. 2016). Higher-frequency songs can avoid the masking effect of low-frequency anthropogenic noise (Slabbekoorn and Peet 2003). However, they can be less attractive and have reduced signal value, as the low-frequency songs usually signal bigger and healthier males, playing a role on mate attraction and territorial

deterrence (Brown et al. 1996, Linhart et al. 2012, Luther and Magnotti 2014, Leitão et al. 2006). Despite the minimum frequency, narrower songs in frequency bandwidth can also play a role on signal value, as they limit the performance features of combinations of bandwidth and trill rates (Podos 1997). Our studied bananaquits sing both higher and narrower frequency songs in noisier than in quieter territories (Chapter 1), which suggests that the noise may play a role on reducing signal value in this species, probably also negatively affecting mate attraction and territorial deterrence.

As signaling less elaborate and narrower songs can change the value of the signal, it may also play a role on mating and territorial defense. Birds that sing songs with narrow frequency bandwidths elicit lower response levels from territorial rivals than songs with broader frequency bandwidths (Luther et al. 2016). Java sparrows (*Lonchura oryzibora*) that sing more elaborate songs. i.e., with more note types, are also the larger and heavier individual, which suggests that song elaboration may play a role on mate attractions. In the present thesis, we investigated whether the noise induced reduction in song elaboration played a role on detrimental effects to territorial deterrence for the city bananaquits. The birds gave acoustically different responses to experimental playbacks of elaborate versus simple songs (Chapter 2). Such finding suggests the noise-dependent vocal restrictions bananaquits exhibits may change the value of the signals and compromise the territorial defense in noisy territories of the city. The consequences the noise may play on territoriality can bring long-term consequences for individual fitness and the diversity of birds communities in cities.

### **5.3 Reproductive consequences of noise-induced stress**

Noise can negatively affect the reproductive success of birds which home ranges rely in cities. Different mechanisms could yield to such negative effect: noise can disturb the animals through shifting their attentions from feeding or mating to vigilance; it can also mask vocal signals and difficult the communication between mating couples; or noise can act as a chronic stressor and it can increase the physiological stress responses of animals, which would decrease their readiness to reproduce (Quinn et al. 2006, Halfwerk et al. 2011b, Kleist et al. 2016, Huet des Aunay et al. 2014, Kleist et al. 2018, Potvin et al. 2015). We studied the negative

impacts long exposures to anthropogenic noise could have on the avian reproductive success in a captive population of Bengalese finches (*Lonchura striata var. domestica*). We monitored different reproductive phases of the birds that were both exposed to intermittent and continuous fragments of an artificial low-frequency noise and birds that were not exposed to any additional noise. We found a significant effect of the noise on the first reproductive phase, the pre-nesting phase (Chapter 3). The birds exposed to the noise delayed the egg-laying when compared to birds reproducing in quiet conditions. The subsequent phases, egg-laying, hatchling and nestling, were not affected. The number and weight of the laid eggs and the number of hatchlings and nestlings were similar among couples reproducing in quiet and noisy treatments.

The negative effect of noise on the delay of egg-laying is most likely explained by an auditory masking of the vocalizations. Behaviors that depend on acoustic signals to be expressed, as mating, could be interrupted or delayed by the noise. A decreased communication efficiency due to noise masking or reduced female attractiveness to noise-induced altered male songs would delay the mating and the readiness of birds to nest and to start laying eggs. Signal masking or noise induced vocal changes would not affect subsequent phases of reproduction as the birds in our experimental captive setup would not rely that much on vocal signals to incubate and to feed and take care of the offspring. Life restricted to captivity and cages would not impose foraging challenges and predator risks. Another possible explanation would be the physiological stress the noise may cause. Birds exposed chronically to the noise could have higher levels of stress hormones, as corticosterone, and reduced limiar of fear and vigilant responses against feeding attempts (Davies et al. 2017, Wright et al. 2007, Blickley et al. 2012, Angelier et al., 2009, Fernandez-Juricic and Telleria, 2000; Quinn et al., 2006). Therefore, higher stress levels could impair several phases of reproduction: the couple readiness to nest, the female fertility and readiness to lay eggs and the offspring provisional rate could all be affected. However, such causal mechanism is less likely to occur as several reproductive phases that could be affected by high levels of stress are not reduced with noise exposure. The hatching and nestling success could be impaired in possibly stressed and debilitated parents, however, they do differ between treatments.

## 5.4 Implications for other avian species

The bananaquit is one of the most abundant species throughout the Latin America and is easily found in human altered habitats as cities and rural areas. Due to its abundance in anthropogenic areas, the species appear to not be under threat of extinction through the negative impacts of the urbanization and noise. The same is comparable for Bengalese finches, as the species is bred in captivity and is used to the presence and activities of humans. However, the facility of approaching an abundant and naturally occurring species in cities and the facility to maintain and breed in captivity another made viable the execution of the issues addressed in the present thesis. The questions studied in this thesis were valuable to understand and predict the possible impacts of the noise to other species. Furthermore, we show that, even for the abundant city bananaquits, there are noise-related vocal changes in song elaboration (chapter 1) and, such vocal changes in the context of territoriality elicit different song replies from conspecifics (chapter 2). Such differences in the vocal responses suggest at least an impact of the noise on the value and contempt of the signal. Additionally, we show that the noise affects and delay the reproductive efforts of the captive Bengalese finches (chapter 3). Therefore, even highly adaptable species to human altered and noisy habitats are prone to the negative impacts of the noise and somehow may not cope with them efficiently.

To evaluate the possible impacts of the anthropogenic noise on other taxonomic groups we should be cautious to some indicators other than abundance and home range in cities. Spectral and temporal characteristics of the vocal signals, possible modifications of their content or value of the signals and the efficiency of the reproduction and survival attempts may all give clues about whether and how the noise is negatively impacting a species. Therefore, taking the example of the bananaquits, whether a species lack the ability to change the frequency of their songs, the frequency bandwidth and the song elaboration according the noise level, it should not avoid the masking effects of the noise. Whether noise avoidance does not occur, the communication may fail. Meantime, even when spectral and elaboration changes occur in a song, it can bring a cost to the value of the signal. Therefore, some behaviors may still be negatively impacted by the noise, as territorial defense and female choice during courtship. When such costs occur to the contempt of the

signals, the noise may also negatively affect the reproductive success of the population living in urban habitats.

Therefore, species that sing in low-frequency songs and that does not show song flexibility in the frequency or syllable content domain are prone to suffer the negative consequences of the noise. However, species that counteract the masking effects of the noise by changing the frequency use, and the song elaboration, through the reduction of the syllable diversity or the increase of the repetition rate of their songs, can still possibly suffer further negative consequences on mate choice, territorial defense and reproductive efficiency. Red-winged blackbirds (*Agelaius phoeniceus*) reduce the frequency bandwidth of their songs in noise (Hanna et al. 2011). European robins (Montague et al. 2012) show a noise-dependent increase in song frequency and decrease in the number of syllable types. Chaffinches (*Fringilla coelebs*) of Europe (Brumm and Slater 2006) and urban silvereyes (*Zosterops lateralis*) of Australia (Potvin et al. 2012) were found to have noise-dependent repetition rates of songs. Would be valuable to investigate, for example, whether such species also suffer further negative impacts of their noise-dependent vocal changes on signal content and related behaviors as territoriality, mating and reproductive efficiency (e.g., Luther et al. 2016).

## 5.5 Future directions

The urbanization of habitats into rural and urban areas is widely widespread around the world and increases at higher rates as time pass. The homogenization of the natural areas together with increasing intensities of anthropogenic noises are forcing species to move from their natural occurring home ranges to more silent but unknown places or to adjust themselves to the new sounds and other challenges of the cities. The present thesis shows how a neotropical bird may adjust themselves to the urban and noisy habitat and some negative consequences they may suffer. It shows some patterns of noise-related song changes in frequency and elaboration in a species that signs highly complex and flexible songs. Also, it shows one of the consequences the noise-related changes in song elaboration may play on the value of the signals. Finally, it presents how long-term exposures to noise may affect the success of reproduction of a captive species. Besides the herein and several other findings showing and suggesting the negative impacts of the noise on birds behavior

and reproduction, more knowledge and evidences are still required. More research is needed, for example, to understand the mechanisms underlying the noise-related decreases in song elaboration. Furthermore, studies investigating how noise-induced changes in signal value may play a role on the evolution of the signals and communication in cities are still necessary. More work on the long-term consequences of such vocal changes on the evolution of mating and sexual selection is also needed.

Besides our study investigating the consequences of the variability of song elaboration on signal value (chapter 2), other consequences of such noise-related signal adjustments on social interactions could be explored. The role of song elaboration on territorial dispute, on female preferences and on reproductive success of bananaquits or other neotropical bird species from cities could be studied. For the further investigations, playback experiments using high and low elaborate songs could be done in the context of male-male song matching, imitating song alternation or overlap among song types, either in quiet and noisy territories of temporarily removed owners. Additionally, another playback experiment using high and low elaborate songs could be done in the courtship context to evaluate their differences of attractiveness to females, during the reproductive period. Investigating the female preferences to the high and low elaborate songs in urban and forest populations would give us a clue of the consequences of such noise-related vocal changes on the course of sexual selection in cities. Finally, further studies could look for males that have an average singing behavior that differ in song elaboration and correlate with rates of extra-pair paternity. Doing so, we could gain insights about the course and evolution of the noise-dependent signal changes in cities and the consequences to the reproductive success.

## **5.6 Concluding remark**

The urbanization of natural areas and the global increase of anthropogenic noises have rapidly changed the habitats and the function of ecosystems. Such environmental changes challenge the animals ability to survive and reproduce. Abundant and naturally occurring species in cities can be good models to study the behavioral strategies animals can use as a way to counteract the negative impacts of the noise or other urban stressors. Experimental manipulation in the field is highly

valuable and still needed to understand the mechanisms underlying the behavioral changes to the noise and the consequences such stressor may cause on the evolution of the noise-induced changes. However, captive species and indoor experimental setups still provide great facility in evaluating several long-term impacts of the noise, such as in the processes of vocal learning, mate choice and reproduction. Finally, given the rapid and widespread increase of urban habitats, the number of studies on the negative consequences of urban stressors to animals fitness and behavioral evolution has increased but still many questions remains to be answered.

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