

Spatiotemporal vocal interactions in a ground-dwelling Neotropical  
passerine

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A Research-Based Master's Thesis  
for the  
Degree of Magister Scientiae in Biodiversity and Ecology

May 11, 2022

## ABSTRACT

In many species, communication involves an exchange of information between the sender and the receiver, by using different types of signals. Among the different types of communication, acoustic communication has been studied for many decades and much of that work has been on bird song. Birds can communicate information across vast distances through song, which is mostly used for mate attraction and territory defence. The majority of studies on bird song focuses on oscine passerines, with suboscines receiving little attention, and little is known regarding song interactions within and among the sexes in suboscines. There is also an important gap in knowledge regarding the possible presence of a domino effect in singing behaviour between individuals during the dawn chorus and about differences in the singing activity of both sexes during the day. In this study, I investigated spatiotemporal interactions in the Mexican antthrush (*Formicarius moniliger*), a suboscine passerine in which both sexes sing and defend year-round territories. More specifically, I investigated the effect of sex, testosterone levels and time of day on singing activity of males and females and whether duetting occurs in this species. I also investigated the effect of presumed territory intruders on singing activity of territory holders through playback experiments. The effect of vocalizing individuals on the singing activity of neighbouring birds during the dawn chorus was also examined. For the purposes of the study, I analysed recordings of songs of the Mexican antthrush obtained from eight Song Meter autonomous recording devices, which were placed in territories within the study population. In Mexican antthrushes duetting does not occur and males sing more on average than females, with testosterone found to have a negative effect on female but not male singing activity. Also, singing activity is higher at dawn and decreases through the day, with song levels greater when neighbours are also singing, suggesting that domino effect occurs. Also, the playback experiments, resulted in significantly more responses from ringed individuals to female song over no playback. The pattern of singing activity in relation to time of day, indicates possible territory defense and mate attraction then and increased foraging activity at later times. The importance of territory defense and mate attraction is also highlighted from the results of the playback experiments. The positive effect of singing neighbours in the singing activity of the individuals indicates a strong social effect in the Mexican antthrush, as has been

found in other species. Thus, in the Mexican antthrush, combined with a pattern of a domino effect, singing activity increases in the presence of conspecific vocal activity from neighbours and presumed intruders. Furthermore, stronger responses were found to female song playback overall, and females sang significantly more when neighbouring males were singing, suggesting the possibility that neighbouring individuals of the opposite sex may be interacting.

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## **ACKNOWLEDGEMENTS**

I would like to thank my thesis supervisor, Associate Professor Dr. Alexander N. G. Kirschel, for accepting me to his Behavioural Ecology and Evolution Lab and giving me the opportunity to work on this project, for his important assistance and contribution in the classification and statistical analysis of the data and comments on the thesis.

I would also like to thank Ph. D candidate Matteo Sebastianelli for introducing me in the program Raven Pro, for his assistance in processing the recordings and contribution in the statistical analysis.

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## SEMINAR ANNOUNCEMENT



University of Cyprus  
Department of Biological  
Sciences

*Master Research Dissertation in Biodiversity and  
Ecology (BIO 831/601)*

### *Student Presentation*

Wednesday, 11 May 2022 at 10:00

Building XΩΔ01, Room 003, Panepistimioupoli Campus

*This seminar is open to the public*

**Stavroula Elia**

*Thesis Supervisor:* Assoc. Prof. Alexander Kirschel

**“Spatiotemporal vocal interactions in a ground-dwelling  
Neotropical passerine”**

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studied for many decades and much of that work has been on bird song. Birds can communicate information across vast distances through song, which is mostly used for mate attraction and territory defence. The majority of studies on bird song focuses on oscine passerines, with suboscines receiving little attention, and little is known regarding song interactions within and among the sexes in suboscines. There is also an important gap in knowledge regarding the possible presence of a domino effect in singing behaviour between individuals during the dawn chorus and about differences in the singing activity of both sexes during the day. In this study, I investigated spatiotemporal interactions in the Mexican antthrush (*Formicarius moniliger*), a suboscine passerine in which both sexes sing and defend year-round territories. More specifically, I investigated the effect of sex, testosterone levels and time of day on singing activity of males and females and whether duetting occurs in this species. I also investigated the effect of presumed territory intruders on singing activity of territory holders through playback experiments. The effect of vocalizing individuals on the singing activity of neighbouring birds during the dawn chorus was also examined. For the purposes of the study, I analysed recordings of songs of the Mexican antthrush obtained from eight Song Meter autonomous recording devices, which were placed in territories within the study population. In Mexican antthrushes duetting does not occur and males sing more on average than females, with testosterone found to have a negative effect on female but not male singing activity. Also, singing activity is higher at dawn and decreases through the day, with song levels greater when neighbours are also singing, suggesting that domino effect occurs. Also, the playback experiments, resulted in significantly more responses from ringed individuals to female song over no playback. The pattern of singing activity in relation to time of day, indicates possible territory defense and mate attraction then and increased foraging activity at later times. The importance of territory defense and mate attraction is also highlighted from the results of the playback experiments. The positive effect of singing neighbours in the singing activity of the individuals indicates a strong social effect in the Mexican antthrush, as has been found in other species. Thus, in the Mexican antthrush, combined with a pattern of a domino effect, singing activity increases in the presence of conspecific vocal activity from neighbours and presumed intruders. Furthermore, stronger responses were found to female song playback overall, and females sang significantly more when neighbouring males were singing, suggesting the possibility that neighbouring individuals of the opposite sex may be interacting.

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## INTRODUCTION

### Animal communication

Communication has been observed in numerous organisms. It is important for their social life, survival, and fitness (Catchpole, Slater 2003, Naguib 2006). Communication involves an exchange of information between the sender and the receiver through the use of different types of signals (Catchpole, Slater 2003). Communication can occur between conspecific or heterospecific individuals, and its function ranges from synchronization of breeding behavior, territory defense, warning, or search for food (Bradbury, Vehrencamp 1998, Naguib 2006). There are different type of cues which are used in communication. These can be acoustic, visual, chemical, electrical and tactile signals. The type of signal they will use depends on the type of information and its biological significance, and the sound transmission properties of the environment (Penar, Magiera et al. 2020). Moreover, communication is affected by factors such as the species social system, for example the ranking of an individual in a group, the behavioral context (e.g. expressing aggression), and the ability of senders and receivers to produce, receive and process the given information (Naguib 2006, Penar, Magiera & Klocek, 2020).

Among the different communication signals, chemical cues are characterized as the least complex, least energy and time consuming (Naguib 2006). In this case, information is provided using pheromones and other chemicals found in urine and body glands (Naguib 2006). In this way, information is available even in the absence of the sender and it can cover long distances. Unlike chemical cues, visual signals are directional since the information is conveyed to specific individuals, but the transmission distance and direction are affected by the structure of the habitat. Visual signals in a dense vegetated habitat can be used only in proximity of the receiver, whereas they can cover longer distances in open habitats. Examples of visual signals are body coloration and visual displays, and they can provide information on the nutrition state of an individual, the age and developmental history (Naguib 2006). Thus, they can be used in mate choice, as they are often honest indicators of male quality. Electric signals are mainly used by aquatic organisms, and

they help predator and prey to locate each other in conditions of low visibility (Naguib 2006). Electric signals travel fast in the media and are not directional. The spatial localization of the signal is difficult and depends on the comparison of signal amplitudes on different parts of the body or on comparisons of the amplitudes at different locations made while swimming. The transmission range varies between several cm and m, and depends on different factors, such as the presence of noise and the sensitivity of the receiver's receptors (Hopkins 1977). Another type of communication cue is tactile signals and are usually used to strengthen bonds between pairs or between individuals of a social group and when youngsters beg for food from their parents (Naguib 2006). The production of tactile signals is not energy demanding. They are used for short range communication. Thus, it is easy to locate them and can be used day or night (Catchpole, Slater 2003). Moreover, acoustic signals are a communication cue used by different groups of organisms, including fish, insects, amphibians, crustaceans, reptiles, arachnids, birds and mammals (Penar, Magiera et al. 2020).

Acoustic signals serve in mate attraction and guarding, feeding, defending territories, escaping from predators and can be used at any time of the day, and cover long distances (Keen, Ross et al. 2014, Farina, James 2016, Gasc, Francomano et al. 2017). Because they are mostly used in long range communication, however, they are affected by distance and vegetation structure. In dense vegetated areas, acoustic signals tend to have lower frequencies and slower repetition rates whereas in open habitats they tend to have higher frequencies (Morton 1975, Slabbekoorn, Eilers et al. 2002, Naguib 2006). Acoustic signals sometimes carry species-specific and individual-specific information and can be a way of assessing an individual's health (Penar, Magiera et al. 2020). For example, a female bird can choose between a male who often sings a complex song and a male which is less active (Catchpole, Slater 2003). The use of acoustic signals is common and therefore acoustic communication has been widely used to address questions in the fields of ecology (Buxton, Brown et al. 2016, Penar, Magiera et al. 2020).

## **About bioacoustics**

The growing interest in acoustic communication and biological sounds, has led to new fields of study such as soundscape ecology, ecoacoustics and bioacoustics (Buxton et al., 2016, Farina, James, 2016, Mcloughlin, Stewart & McElligott, 2019). Soundscape ecology resulted from the

combination of landscape ecology, bioacoustics, community ecology and engineering. It involves the combined study of biological, geophysical and anthropic sounds and their temporal and spatial variation across the landscape. It also examines the changes in the spectral, temporal and spatial structure of biological sounds in relation to large-scale environmental changes, like habitat alteration, climate change and spread of invasive species (Buxton, Brown et al. 2016, Gasc, Francomano et al. 2017). Like soundscape ecology, ecoacoustics examines the ecological role of natural and anthropogenic sounds at the level of population and community, in a landscape scale (Farina, James 2016, Eldridge, Guyot et al. 2018). Bioacoustics is a combination of biological and acoustic sciences, ecology, signal processing, mathematics and machine learning (Mcloughlin, Stewart et al. 2019, Penar, Magiera et al. 2020) and investigates the production, transmission and reception of animal-produced sounds (Mcloughlin, Stewart et al. 2019). Also, it studies the dependence of sound signal properties on the type of medium the sound propagates through, thus helping to understand the evolution of acoustic mechanisms as well as the evolution of animals that developed them (Penar, Magiera et al. 2020).

Bioacoustics is becoming increasingly popular in the last years because of its important contribution in different environmental aspects, such as monitoring populations or even in understanding how vocalizations are affected by climate change (Penar, Magiera et al. 2020) One example of its importance is the application of bioacoustics to monitor biodiversity, and especially to collect data for rare and elusive species (Frommolt, Tauchert 2014, Mcloughlin, Stewart et al. 2019, Penar, Magiera et al. 2020). Applications of bioacoustics range from detecting presence and absence of a species of interest, numbers and density of occurrence, seasonal concentrations and movements during migrations and provides insight to unknown behavioral aspects (Frommolt, Tauchert 2014, Stowell, Wood et al. 2016, Mcloughlin, Stewart et al. 2019, Penar, Magiera et al. 2020). An example of these is the use of passive acoustic monitoring to investigate the cryptic behavior and mechanisms of communication of forest elephants, to estimate population size, to quantify the threats and assess the efficacy of conservation strategies. The use of passive acoustic monitoring provided access to the diel activity patterns of the elephants without human disturbance and more specifically it revealed increased nocturnal behavior and higher activity at the forest clearings at night (Wrege, Rowland et al. 2017). Moreover, with bioacoustic monitoring we can study the effects of climate change in environmental acoustics (Penar, Magiera et al. 2020). With the increasing temperatures and landscape changes caused by climate change, there is an impact

on the communication between animals and changes in soundscapes (Penar, Magiera et al. 2020). For example, in the oceans, sound absorption depends on temperature, pressure, salinity and acidity (Penar, Magiera et al. 2020). Changes in these factors can reduce the absorption of sound and natural and anthropogenic noise will travel further, affecting the acoustic behavior of marine organisms (Mcloughlin, Stewart et al. 2019, Penar, Magiera et al. 2020). Another field where bioacoustics is used is monitoring the health and welfare of farm animals based on their vocalizations. This allows their monitoring at any time of the day, in the absence of humans and reduces the time humans must spend in conditions that may be harmful for their health (Mcloughlin, Stewart et al. 2019).

The collection of data for avian research was historically done with point counts and line transects, where human observers visit different parts of a site, for a specific time and identify species from their vocalizations and with visual detection (Frommolt, Tauchert 2014, Shonfield, Bayne 2017). The increasing development of bioacoustics equipment has led to an increase in the use of recordings and, in some cases, they have replaced human observers (Shonfield, Bayne 2017, Penar, Magiera et al. 2020). Based on the objective and species of interest, acoustic recordings can be used by their own or in combination with human observers. However, both methods have their advantages and disadvantages. In the case of field surveys, sometimes the observers can detect birds from larger distances than those of the acoustic recorders and behavioral aspects of the individuals can be observed (Shonfield, Bayne 2017). Unlike acoustic recorders that are placed in a fixed location, observers can move around the survey area when more observations are needed and they can discern between signals from different directions, which is impossible when a single-channel recorder is used. The human presence in the field can also be a disadvantage because of the possible disturbance and this can affect the vocal activity of the target species and also the data collected are affected from observer bias (Catchpole, Slater 2003, Digby, Towsey et al. 2013).

With the use of acoustic recorders studies on species occurrence, abundance and richness studies become easier to conduct, thus providing an important contribution in conservation and monitoring (Blumstein, Mennill et al. 2011, Shonfield, Bayne 2017). Also, by using several recording devices together with localization algorithms we can map territories, since we can accurately locate the origin of the sound (Blumstein, Mennill et al. 2011). Recorders can be synchronised to record simultaneously for several days or weeks, covering a large area at low cost. This allows scientists

to obtain large datasets of recordings of long duration that will be analysed at any time by human personnel. In this way, surveys can be conducted in remote areas, or in areas with low visibility (Bardeli, Wolff et al. 2010, Zwart, Baker et al. 2014, Ulloa, Gasc et al. 2016). With this approach, human presence is very limited as well as the disturbance to the study species. The only human interference is during the placement and retrieving the recorder, or when replacing the batteries (Zwart, Baker et al. 2014).

The performance of the two approaches varies. In some cases, fewer species or individuals have been detected when acoustic recorders were used than with point counts (Frommolt, Tauchert 2014, Shonfield, Bayne 2017). Some important factors affecting the performance when processing the recordings are the variability among observers and the ability to detect faint and low calls (Digby, Towsey et al. 2013). Factors affecting the performance of both methods are background noise, accessibility to the location, experience, the study design, with weather conditions also affecting point counts (Digby, Towsey et al. 2013, Zwart, Baker et al. 2014). Moreover, during periods of high vocal activity, humans may not be able to distinguish between different calls during point counts, but they are easily detected when inspecting a spectrogram, despite overlapping calls (Digby, Towsey et al. 2013).

The interest in acoustic surveys is growing even more with improvements in the equipment that allow registration of vocal activity for long time periods, and the more affordable recording devices (Shonfield, Bayne 2017, Penar, Magiera et al. 2020). The most important development in the field of bioacoustics was the invention of the spectrograph recorder. When first invented, its cost was a restricting factor for use, but it was an important tool in the detailed analysis of the sound, resulting in a growing number of studies. Nowadays, the spectrograph recorder has been replaced from software programs which also produce sonograms and thus have an important contribution not only in the field of animal communication but also in other scientific fields such as environmental monitoring (Catchpole, Slater 2003, Penar, Magiera et al. 2020). The simplest device used is a microphone which can be directional or omnidirectional and a recorder (Frommolt, Tauchert 2014, Penar, Magiera et al. 2020). Autonomous recording units are also commonly used and under continuous development, with new models powered by solar energy, can operate in a variety of weather conditions and have available wireless sensors for data transmission and large storage capacity (Potamitis, Ntalampiras et al. 2014). Moreover, microphone arrays give the opportunity

to estimate the location of vocalizing animals (Trifa, Kirschel et al. 2008). This is possible with the time delay from the arrival of the signal at different directional microphones, which record the sound independently from the direction of the source (Frommolt, Tauchert 2014). In a study on the acoustic localization of antbirds in a Mexican rainforest, an acoustic localization system was developed with self-contained nodes and each one consisted of a subarray of four microphones and a wireless network among others, which allowed the remote monitoring of the system. The use of the sub-array helped in the estimation of the direction and time of arrival of the sound (Collier, Kirschel et al. 2010).

There are some factors to consider when choosing the equipment. One of them is the position of the recording device, since not all places are ideal and this plays a role in the outcome (Blumstein, Mennill et al. 2011). An example is the use of microphone arrays that were placed in dry parts of a restored wetland site for the monitoring of Eurasian bitterns, but when the bird was crossing the area among the recorders could not be located (Frommolt, Tauchert 2014). In such cases, omnidirectional microphones which record the sounds from all directions are preferred. Other factors are the target organisms and the objectives of the study. The species of interest will determine the equipment used because of the sensitivity in the frequency range they show and the ability to provide high quality signals at the end. Also, the sampling rate required for the study determines the size of the acoustic files and, as a result, the storage requirements. Protection from different environmental conditions may be required for the microphone and the recording units because they can reduce the quality of the recordings. In cases where the equipment could be disturbed by animals, it should be camouflaged or animal deterrents can be used (Blumstein, Mennill et al. 2011).

## **Processing recordings**

Since the field of bioacoustics is under continuous development, it is now possible to send data to a server in real time for processing (Shonfield, Bayne 2017). The technological improvement of the equipment used to acquire recordings, has led to large collections of audio and, as a consequence, the need for an improved storage capacity and organization (Gasc, Francomano et al. 2017). Generally, there are three approaches to study vocalizations and the choice of the approach is based on the research question (Garcia, Favaro 2017). The first one includes extensive

research for the classification, description, and subsequent analysis of the signals under various contexts and various model species. The second one emphasizes in the investigation of the connection between the structure of the signals and their function, leading to the development of various theoretical frameworks. The last approach is to investigate the sound production mechanisms involved in the vocalization process, by connecting anatomical structures to acoustic characteristics (Garcia, Favaro 2017). For the first two approaches, the processing of large audio datasets is needed. The methods used for this purpose are under continuous development (Garcia, Favaro 2017, Shonfield, Bayne 2017). These methods include listening and visually scanning spectrograms, machine-based detection measurements and classification algorithms (Blumstein, Mennill et al. 2011). More specifically, the methods are originated from speech recognition technology and include Hidden Markov Models based on spectrograms, mel-frequency cepstral coefficients, spectrogram cross correlation, binary template matching of spectrograms and band limited energy detection in spectrograms among others (Katz, Hafner et al. 2016, Shonfield, Bayne 2017). Some software programs used from researchers are based on these methods, like for example Raven Pro (Bioacoustics 2019), Kaleidoscope Pro (Wildlife Acoustics) and R packages (Shonfield, Bayne 2017), such as *warbleR* (Araya-Salas, Smith-Vidaurre 2017).

The detailed analysis of the audio includes signal detection, classification, and feature extraction. Before starting the detailed analysis, the filtering of the recording may be needed (Stowell, Wood et al. 2016). With the use of band-pass filters, the interference of background noise can be effectively reduced, and this helps to get better results at the processing phase (Potamitis, Ntalampiras et al. 2014). After filtering, the signal recognition process ensues. This includes signal detection and classification (Blumstein, Mennill et al. 2011). Signal detection involves the selection and extraction of the signals of interest from a recording, and signal characterization or classification is the identification and grouping of the extracted vocalizations to the right species or individual (Acevedo, Corrada-Bravo et al. 2009, Blumstein, Mennill et al. 2011). This can be done by listening to the recording and simultaneously having a visual image of the sound provided by the spectrogram. This requires expert listeners and good equipment quality. Sometimes, the spectrogram can be used without listening to the recording when the signal is simple (Chen, Maher 2006, Blumstein, Mennill et al. 2011, Shonfield, Bayne 2017). Except from the manual inspection of spectrograms, in recent years there is an increase in the development and use of automated methods (Blumstein, Mennill et al. 2011, Ulloa, Gasc et al. 2016). Some of these methods are

Hidden Markov Models (HMMs), Gaussian mixture models, artificial neural networks, and spectrogram cross-correlation (Stowell, Wood et al. 2016, Ulloa, Gasc et al. 2016). Software programs for bioacoustics analysis such as Raven Pro (Bioacoustics 2019) and Kaleidoscope Pro (Wildlife Acoustics) are based on these methods (Stowell, Wood et al. 2016). Audio feature extraction involves the extraction of acoustic measurements from the signal and is an important step in the process of species recognition (Trifa, Kirschel et al. 2008, Blumstein, Mennill et al. 2011, Mcloughlin, Stewart et al. 2019). In some cases, the extracted acoustic features have some variability between them when the recordings are originated from different populations or individuals (Trifa, Kirschel et al. 2008). Acoustic measurements include time and frequency variables such as duration and peak frequency, among others, which are taken automatically from waveforms, spectrograms, and power spectra (Blumstein, Mennill et al. 2011).

Signal classification methods can be supervised or unsupervised (Blumstein, Mennill et al. 2011, Mcloughlin, Stewart et al. 2019). In the first case, annotated data are used to train the system and in the case of unsupervised methods the structure of the data guides the system for the classification (Blumstein, Mennill et al. 2011). The recognizers are trained and tested on manually pre-segmented data (e.g. song cuts) that contain the target signal (Potamitis, Ntalampiras et al. 2014). Supervised methods for signal classification include machine learning algorithms such as linear discriminant function analysis, decision trees, artificial neural networks, and support vector machines (Acevedo, Corrada-Bravo et al. 2009, Bardeli, Wolff et al. 2010, Jančovič, Kökür 2011, Ulloa, Gasc et al. 2016). Improvements in this domain include better recognition algorithms to discriminate neighbouring and simultaneously singing individuals (Bardeli, Wolff et al. 2010). Automatic methods include mel-frequency cepstral coefficients, detecting features based on signal energy or entropy distribution, hill-climbing algorithms, and Hidden Markov Models (Trifa, Kirschel et al. 2008, Blumstein, Mennill et al. 2011). The choice of the method for species detection, recognition or feature extraction depends on the study species and the signal characteristics of the species, since some of these methodological approaches have been designed on specific types of signals increasing the need for more generic approaches (Blumstein, Mennill et al. 2011, Stowell, Wood et al. 2016). For better results, the use of species – specific algorithms is suggested (Potamitis, Ntalampiras et al. 2014, Ulloa, Gasc et al. 2016).

Energy thresholding is a simple method for signal detection and is applied in specific frequency regions of interest. Signals are detected when the value of energy is higher than a threshold, whereas they are ignored when it is below the threshold (Stowell, Wood et al. 2016). Spectrogram cross - correlation is another method for signal detection and recognition. In this case, examples of species – specific signals, known as templates, are used in spectrogram screening to find signals with a profile highly similar to the templates (Stowell, Wood et al. 2016, Ulloa, Gasc et al. 2016). Hidden Markov Models are frequently used in human speech recognition but can also be used in bird detection and recognition and is suitable for temporal and frequency data. In this method, pre-processing steps are done completely automatically (Trifa, Kirschel et al. 2008, Potamitis, Ntalampiras et al. 2014, Stowell, Wood et al. 2016). Mel-frequency cepstral coefficients are originated from human speech recognition. Calculating the parameters is an easy process and involves the segmentation of a signal. Then the power spectrum of each frame is converted into a mel – frequency spectrum using filters (Fagerlund 2007, Blumstein, Mennill et al. 2011). Another method called Support Vector Machine (SVM) is characterized by high accuracy compared to other methods and is based on statistical learning theory and structural risk minimization (Fagerlund 2007, Acevedo, Corrada-Bravo et al. 2009). The R software (R Core Team 2021) has some packages available for sound analysis. The package *Seewave* (Sueur, Aubin et al. 2008) has functions for time, amplitude, and frequency analyses (Shonfield, Bayne 2017). The *monitoR* (Katz, Hafner et al. 2016) package can be used for acoustic detection and identification. It has functions for renaming recordings, detecting vocalizations, and classifying them to species and is characterized by easy creation, storing and manipulation of templates, efficient manual verification of identifications, visual and aural spectrogram browsing and manual annotation of song events in spectrograms (Shonfield, Bayne 2017). The *warbleR* (Araya-Salas, Smith-Vidaurre 2017) package is based on the functions of the *Seewave* and *monitoR* packages and provides analysis of the acoustic signal by measuring signal parameters, such as frequency, time and amplitude and performs pairwise spectrogram cross – correlation (Shonfield, Bayne 2017). Another software available is the ARBIMON (Aide, Corrada-Bravo et al. 2013) – acoustic software. With its use, recording analysis is less time consuming, and this is achieved by inspecting a subset of the recordings and preparing examples of species-specific vocalizations. These will be used as training data to train the model (Aide, Corrada-Bravo et al. 2013). Spectrograms are frequently used, especially when manually detecting species vocalizations. Spectrograms are a visual

representation of sound. They show how the spectrum of a sound varies over time in three – dimensional plot. The horizontal axis represents the time in seconds (s), the vertical axis the frequency in kilohertz (kHz) and the amplitude is represented by colour intensity (Anderson, Dave et al. 1996, Katz, Hafner et al. 2016).

Each method has different performance, and the choice of the method is usually done based on the research question (Shonfield, Bayne 2017). With the use of support vector machines, (Andreassen, Surlykke et al. 2014) were able to classify bat calls with an accuracy of 96%. The survey has revealed two bat species not previously seen in the survey area and an abundance of social calls. In another study they used the spectrogram cross – correlation method to detect the calls of the screaming Piha (*Lipaugus vociferans*). They consider the method as fast, especially for loud and stereotype songs such as the Piha´s. (Ulloa, Gasc et al. 2016). In another study, the use of species – specific pattern recognition algorithms and more specifically for the Savi´s warbler (*Locustella luscinoides*), yielded a 92% detection rate of the songs of Savi´s warbler (Bardeli, Wolff et al. 2010). In a study for four species of woodpeckers, the authors used manual and automatic methods to detect calls in 24 h recordings. The processing time was shorter for the automated scanning method but reviewing the annotation lists generated by the automated method was more time consuming than the manual method. The scanning accuracy was much less in the automated method as it gave more false positives as well as the scanning comprehensiveness because it identified fewer target sounds (Swiston, Mennill 2009). Neal et al. (2011) used a supervised time frequency audio segmentation method for the extraction of syllables of bird call from noisy environment and have managed to extract 93,6% of the acoustic energy of bird song and the false – positive rate was 8,6% (Neal, Briggs et al. 2011). Hidden Markov models have been used for the automatic recognition of five species of antbirds in a Mexican rainforest. In that study, species recognition was very high reaching 99,5% when recordings with low background noise levels were used, and recognition rates were still high even in recordings with high background noise levels, reaching 90% (Trifa, Kirschel et al. 2008). The use of time Dynamic Time warping method in a study (Anderson, Dave et al. 1996) for the automatic recognition of indigo bunting (*Passerina cyanea*) and zebra finch (*Taeniopygia guttata*) songs gave 97% accuracy for recording, in a low – noise environment and 84% accuracy when the songs had low amplitude (Anderson, Dave et al. 1996). One problem in the comparison of recognition methods is the non-existence of detection

threshold. A common framework for the development and assessment of automatic recognition methods is needed (Blumstein, Mennill et al. 2011, Shonfield, Bayne 2017).

The manual inspection of spectrograms for species detection and recognition, is a very common method and one of the very first methods that have been used for this purpose (Swiston, Mennill 2009). Nowadays, automatic methods are becoming more widely used (Chesmore 2004). Manual inspection is a laborious, time consuming process (Kogan, Margoliash 1998, Chesmore 2004, Swiston, Mennill 2009, Blumstein, Mennill et al. 2011, Buxton, Brown et al. 2016). Factors affecting the time needed are the number of target sounds and the degree of experience of the observer (Kogan, Margoliash 1998, Swiston, Mennill 2009, Blumstein, Mennill et al. 2011). Using both automated and manual approaches is suggested (Kogan, Margoliash 1998, Shonfield, Bayne 2017, Abrahams, Geary 2020). Scanning accuracy and comprehensiveness also varies, because humans can better discriminate the different signals and more importantly those with great similarity. A disadvantage of the automated methods is that sometimes, they return many false positive detections and classifications (Swiston, Mennill 2009, Abrahams, Geary 2020).

The use of detectors for the annotation of songs and calls is sometimes considered efficient and effective, as they may be able to detect rare and elusive species (Shonfield, Bayne 2017). However, two important issues are the overlapping calls in time and frequency between species and increased background noise. Both led to high numbers of false positives and false negatives (Blumstein, Mennill et al. 2011, Potamitis, Ntalampiras et al. 2014, Shonfield, Bayne 2017). Improvements to reduce these issues and increase recognition and detectability start from the field. One example is the use of many recording units for long periods of time (Wood, Popescu et al. 2019). The position of the recorders is also important, and it has to cover the territory size of the target species, but with caution to avoid overlap. A radius of 50 m is suggested for the recording of many bird species but this depends on microphone model and condition (Abrahams, Geary 2020). Considering both factors and having a correct balance between them will improve the sound pressure on the microphone and help in the high frequency attenuation and as a result the accuracy and precision in detectability estimates (Aide, Corrada-Bravo et al. 2013, Potamitis, Ntalampiras et al. 2014, Abrahams, Geary 2020). Also, environmental conditions, and habitat characteristics affect the quality of the recordings (Potamitis, Ntalampiras et al. 2014, Gasc, Francomano et al. 2017). At the processing stage of the recordings, the complexity and variability of the signals affect the

detection as well as the signal – to – noise ratio (SNR) (Blumstein, Mennill et al. 2011, Aide, Corrada-Bravo et al. 2013, Shonfield, Bayne 2017). Moreover, in a spectrogram there are some parameters that should be considered. The main parameters are the Fast Fourier Transform (FFT), window length, the overlap between successive FFT windows, the zero padding of the FFT and the shape of the window used. These parameters change the time frequency resolution of the spectrogram and as a result affect the detection performance. Performance can also be increased by using separate band pass filters for each training and test signal or by using specific feature analyses (Trifa, Kirschel et al. 2008). In methods that require training and testing datasets attention should be given in the preparation and choice of the signals and in the number of samples (Trifa, Kirschel et al. 2008). In general, comparing the performance of recognizers is difficult because there are not established standard assessment metrics or detection thresholds. The need for a common framework in the development and assessment of recognizers for automated species recognition has been recognized. This framework can include standard performance metrics and visualization tools, techniques for parameter tuning, facilities for running detectors and classifiers and generating feature sets and tools to determine the extent of under or over fitting of a system to training data. A standard corpus of data sets is needed, that can be analysed by a variety of recognition systems to determine the relative strengths and weaknesses of each system. For the development of common standards and new technology a strong collaboration between researchers to exchange experiences is crucial (Blumstein, Mennill et al. 2011, Shonfield, Bayne 2017).

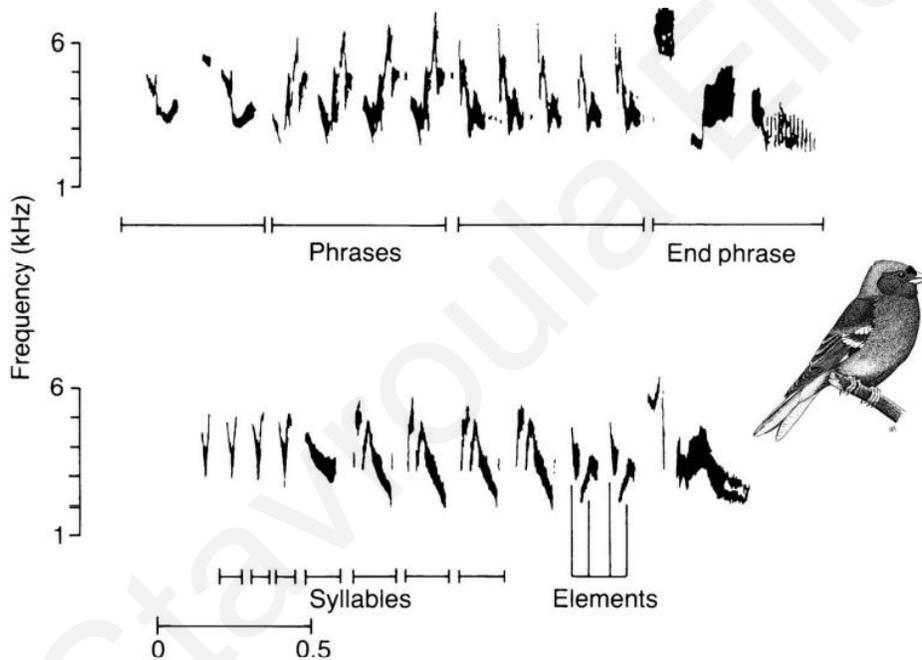
A major problem when processing recordings is noise. Noise has a negative impact on the quality of the recording and the detection and recognition of the target signal (Bardeli, Wolff et al. 2010, Ulloa, Gasc et al. 2016). Noise is characterized as background noise and interference (Ulloa, Gasc et al. 2016). Background noise can be geophonic sounds such as wind and rain (Ulloa, Gasc et al. 2016) or it can be anthropogenic (e.g. traffic noise) (Bardeli, Wolff et al. 2010, Ulloa, Gasc et al. 2016). This type of noise has a strong impact because it affects a big part of the frequency range (Bardeli, Wolff et al. 2010). Interference (or biogenic noise) refers to sounds produced by other vocalizing species such as amphibians or birds and interfere with the target signal (Bardeli, Wolff et al. 2010, Ulloa, Gasc et al. 2016). The level of this type of noise fluctuates through the day (Bardeli, Wolff et al. 2010). Also, there is noise produced from the recording equipment. This can be electromagnetic and thermal noise from the electromagnetic circuit and distortion by the conversion of the signal from analogue to digital (Ulloa, Gasc et al. 2016). Noise is also caused by

micromovements when handling the equipment or from cable swinging. The effect of background noise is that it affects the signal-to-noise ratio and as for interference noise, decreases the accuracy by increasing the false – positive detection rate (Ulloa, Gasc et al. 2016).

## About bird song

Among the different types of communication, acoustic communication has been the study subject for many researchers especially in recent years and also there is a growing interest in studying bird song (Catchpole, Slater 2003). Bird song has been characterized as one of the most beautiful and complex sounds in nature, among others (Catchpole, Slater 2003). Through song, birds can share information across long distances (Kumar 2003, Potamitis, Ntalampiras et al. 2014). In other instances, acoustic communication is coupled with visual displays (Kumar 2003). Acoustic vocalizations are classified as calls, songs, and mechanical sounds (Potamitis, Ntalampiras et al. 2014, Abrahams, Geary 2020). Calls are termed as short monosyllabic sounds, whereas songs are larger and more complex, as they consist of many syllables (Fagerlund 2007, Jančovič, Kökür 2011, Potamitis, Ntalampiras et al. 2014). Mechanical sounds are the sounds produced by mechanical movements of parts of the body, like for example from wing and beak clapping (Potamitis, Ntalampiras et al. 2014). The classification of sound into songs and calls is based on four criteria. One of them is the physical characteristics of the vocalization which refers to the structure of the signal. A call is made up from a single element followed by a silent gap, whereas songs consist of groups of elements. Another criterion is the biological function, in which songs are used mainly for mate attraction and territory defence and calls for warnings and other types in intraspecific communication (Kumar 2003, Fagerlund 2007). Moreover, on the basis of structural differences of the syrinx, songbirds produce complex songs due to the presence of acromyodian syrinx, whereas non-songbirds have a mesomyodian syrinx (Warner 1972). Also, songbirds have up to seven pairs of extrinsic muscles and non-songbirds have only two (Kumar 2003). The last criterion is neurobiology. Songbirds and parrots have developed a special telencephalic vocal control system for the production of learned vocalizations, which is absent in non-songbirds (Wild 1997, Kumar 2003). Bird song can be divided into three hierarchical levels; phrases, syllables and elements (Figure 1) (Fagerlund 2007, Potamitis, Ntalampiras et al. 2014). A phrase is made from a series of units which occur together in a particular pattern. Those units are called syllables and often within a phrase one syllable can be different from an other. Syllables may have simple or

complex structure. When the structure of a syllable is complex, they are made from smaller building blocks, called elements or notes (Catchpole, Slater 2003, Kumar 2003, Fagerlund 2007). In calls, phrases are absent, and the signal is made of one syllable or a series of syllables (Fagerlund 2007). Songs and calls are characterized by frequency and amplitude. The frequency is the number of waves /cycles per second and is measured in Hertz (Hz), and the amplitude refers to the loudness of the sound and is measured in decibels (dB) (Kumar 2003). The amplitude can be represented using waveforms and the frequency with spectrograms, in relation to time (Catchpole, Slater 2003, Charif, Waack et al. 2010). Recordings with high amplitude may indicate that the source of the sound is close to the recorder and those with low amplitude indicate the opposite (Farina, James 2016).



**Figure 1:** A sonogram showing the three hierarchical levels of the song of a male chaffinch (Catchpole, Slater, 2003)

Birdsong has several functions. Because it covers long distances, one of its most important roles is territory establishment and defence (Kumar 2003, Fagerlund 2007, Tobias, Gamarra-Toledo et al. 2011). In many species, singing rates are increasing during the breeding season to attract mates and for territory establishment (Kumar 2003, Fagerlund 2007, Tobias, Gamarra-Toledo et al. 2011). Bird song varies in the individual and species level and thus, it helps in the recognition, identification and discrimination between individuals and between species. Thus, singing

individuals can recognize and communicate with their neighbours, mates and young. In this way, they avoid unnecessary aggression and as a result they save energy and time (Seddon, Tobias 2010) and, in some cases, hybridization is prevented (Kumar 2003, Seddon 2005, Seddon, Merrill et al. 2008, Tobias, Gamarra-Toledo et al. 2011). During the day, singing activity is more common at dawn and dusk (Kumar 2003, Farina, James 2016). Dawn and dusk are the times of the day where singing activity reaches a maximum and because of the simultaneous singing of different species, they are mentioned as dawn and dusk choruses, respectively (Catchpole, Slater 2003, Kumar 2003, Penteriani, Delgado et al. 2014, Farina, James 2016). However, at dawn there are species which start singing earlier than others and especially those with bigger eyes because they can see better at low light intensities and as a result, they start their day earlier (Catchpole, Slater 2003). Singing activity at dawn and dusk is high for various reasons (Catchpole, Slater 2003). First, dawn is the best time of the day to sing because sound transmission is favoured by the microclimatic conditions occurring at that time, such as reduced wind and air turbulence. Second, foraging at that time is difficult, due to the low light conditions, and prey is less available due to the low temperatures (Catchpole, Slater 2003). Birds with high energy levels can spend more time singing at both times, whereas those who have not fed well will spend less time signing in the morning and in the afternoon (Cuthill, Macdonald 1990, Hardouin, Robert et al. 2008, Penteriani, Delgado et al. 2014). Another reason is that dawn is the ideal time of the day for territory holders to defend their territories because at that time invasions are more likely to happen from other individuals to take over any vacant territories (Catchpole, Slater 2003). After foraging during the day, the afternoon is a good time to show to other individuals that they are in good shape to defend their territories and attract mates (Cuthill, Macdonald 1990, Hardouin, Robert et al. 2008, Penteriani, Delgado et al. 2014). In both choruses, the duration of the singing activity among individuals varies and depends on food supplementation and their physical state (Cuthill, Macdonald 1990, Catchpole, Slater 2003, Hardouin, Robert et al. 2008, Penteriani, Delgado et al. 2014). They also reveal inter-individual differences in competitive ability and habitat quality (Cuthill, Macdonald 1990, Hardouin, Robert et al. 2008, Penteriani, Delgado et al. 2014).

Singing activity is affected by the seasons, reaching a maximum during the breeding season and a minimum in winter, whereas in the tropics there is very little variation give the relatively more stable climate (Farina, James 2016). Other factors affecting the sound are temperature, humidity, atmospheric pressure, vegetation cover and land structure (Kirschel, Blumstein et al. 2009, Farina,

James 2016, Gasc, Francomano et al. 2017). In open habitats high-frequency sounds are more widespread, whereas in densely vegetated areas low-frequency sounds are more favourable. This is because high frequencies are easily absorbed and low-frequency sounds can travel further in forested areas (Gasc, Francomano et al. 2017) and also by singing low frequency songs, the attenuation and degradation of the sound is minimized (Seddon 2005, Kirschel, Blumstein et al. 2009). Moreover, sound attenuation and degradation at the different forest levels vary and thus the height from which a bird sings, is important for the sound transmission (Seddon 2005). As a result, birds living in forests produce lower-pitched songs, with slower rates of syllable repetition compared to those living in open habitats (Seddon 2005). Other factors affecting vocal activity are the age, sex, breeding status and the presence of other vocalizing individuals (Bruni, Mennill et al. 2014, Abrahams, Geary 2020). Song traits like song pitch, duration, note number and note diversity require energy and good physical state, to increase (Catchpole, Slater 2003). It has been found that there is a correlation between song pitch and body size, and hence with body mass. The peak frequency of the song is negatively correlated with body size in interspecies and intraspecies scale (Seddon, Merrill et al. 2008, Kirschel, Blumstein et al. 2009, Kirschel, Zanti et al. 2020, Uribarri, Rodríguez-Cajarville et al. 2020). Song production is also affected by noise (Kirschel, Blumstein et al. 2009). Biotic and abiotic noise cover the sound spectrum, driving birds to adapt their song in restricted frequency range. Usually, abiotic noise, like wind and running water cover the low frequencies of the spectrum, and biotic noise like vocalizing insects cover the high frequencies of the spectrum (Kirschel, Blumstein et al. 2009).

For many years, it was assumed that song is mainly a male trait because of its role in female attraction and territory defence (Geberzahn, Goymann et al. 2009, Cain, Langmore 2015). Thus, female song remained poorly studied. This is evident in the literature and biological sound collections which are richer in male song specimens (Odom, Benedict 2018). The song of females does not have many differences in the structure and function of the males and has been suggested to be ancestral (Odom, Hall et al. 2014, Odom, Benedict 2018, Kirschel, Zanti et al. 2020). Song is more common in female individuals of tropical species, where they can sing in association with the male or in coordinated duets (Odom, Hall et al. 2014, Odom, Benedict 2018, Kirschel, Zanti et al. 2020). In temperate regions, song is absent from female individuals of many species, because of their migratory behaviour and the fact that they do not form year-round territories and long-lasting bonds (Price 2009, Kirschel, Zanti et al. 2020). Female song has several functions such as

advertising fertility, attracting mates, coordinating breeding behaviour and common care of the offspring (Price 2009, Tobias, Gamarra-Toledo et al. 2011, Cain, Langmore 2015). Also, it is important in intrasexual competition for resources, like nesting sites, or in determining dominance (Price 2009, Tobias, Gamarra-Toledo et al. 2011, Cain, Langmore 2015). A study on 323 species from different areas of the world, indicated that female song was present in 229 species and a study on European passerine species showed that female song was present in 43% of the species (Odom, Hall et al. 2014) and since research on female song is increasing, more cases are being found of females singing in temperate species, for example in Cyprus wheatear (*Oenanthe cypriaca*) (Patchett, Kirschel et al. 2021). Further study is required to investigate the selection pressures that have caused females to stop singing in some species while song has been maintained in both sexes in other species and to identify the mechanisms that shape the brain function and behaviour in female song (Odom, Hall et al. 2014, Odom, Benedict 2018).

A great majority of avian species belongs to the order Passeriformes, with about 5700 species (Lovell, Lein 2004). The order Passeriformes includes two suborders, the oscines and the suboscines (Lovell, Lein 2004). Suboscine passerines account for the 10% of the avian fauna and are mostly found in South America, with few species also found in North America, Africa, Asia and Australia (Slater, Mann 2004, Tobias, Brawn et al. 2012, Touchton, Seddon et al. 2014). Neotropical suboscine passerines are classified into non-tracheophone or brochophone and tracheophone suboscines (Touchton, Seddon et al. 2014). Non-tracheophone suboscines are the species of the infraorder Tyrannides, such as tyrant flycatchers (Tyrannidae), manakins (Pipridae) and cotingas (Cotingidae). Tracheophone suboscines are the species of the infraorder Furnariides, such as antbirds (Thamnophilidae) and ovenbirds (Furnariidae) (Tobias, Brawn et al. 2012). A difference between the oscines and the suboscines, among others, is song development. In oscine passerines song is developed by learning through auditory feedback during development, whereas in suboscines song is shaped by genetic differences and developmental constraints (Isler et al. 1998, Lovell, Lein 2004, Slater, Mann 2004). In suboscines, individuals of both genders sing and in some cases, they form coordinated and synchronised duets (Slater, Mann 2004). The differences in the mechanisms of song development between the two suborders occurred after they diverged 85-90 million years ago (Lovell, Lein 2004). During the process, suboscines lost the vocal control areas in the forebrain which are associated with song learning but have evolved mechanisms of sound production in their courtship displays (Kirschel, Zanti et al. 2020). In oscines, a motor pathway in

the anterior brain is responsible for song learning (Isler et al. 1998). Also, natural or sexual selection on suboscine vocalizations has caused greater diversity on the syrinx morphology compared to oscines (Tobias, Brawn et al. 2012). The fact that song learning is absent in most suboscines is supported by captive-rearing experiments, in which chicks were raised in silence or where exposed to the song of related species but in the end, when they were fully grown, they produced normal adult song (Tobias, Brawn et al. 2012). In addition, there are cases of high genetic variation in which vocal differences are present, even when the species are similar in morphology (Isler et al. 1998, Tobias, Brawn et al. 2012, Touchton, Seddon et al. 2014). Having a stereotyped song helps in the detection and interpretation of signals in the noisy environment of the tropical regions in which they live and favours conspecific mate recognition (Seddon, Tobias 2010, Tobias, Brawn et al. 2012).

The two passerine suborders do not differ only in song development but also in other traits such as body mass, survival rate, stress responses, reproductive effort and immune function (Tobias, Brawn et al. 2012). Species in tropical regions have a slow pace of life and longer lifespan and as a result they have smaller clutch size and invest more in post-fledging parental care (Tobias, Brawn et al. 2012). Even though suboscines have a big percentage in the global avian diversity, oscines received the most attention in research in the last years and when suboscines were studied most attention was paid to non-tracheophone suboscines (Lovell, Lein 2004, Tobias, Brawn et al. 2012). Now this has changed and research on tracheophone suboscines is growing. Tracheophone suboscines can be found in a variety of ecosystems and they are socially diverse, exhibiting social monogamy and cooperative breeding, as well as year-round territoriality (Tobias, Brawn et al. 2012). Also, they are easy study systems as they are highly responsive to song playbacks. Their ability to live in human modified environments and their conspicuous behaviour make them easy to find and follow for observational studies and to record their vocalizations (Tobias, Brawn et al. 2012). Due to their stereotyped song, they are ideal for acoustic analyses used in tests of evolutionary theory and speciation and for studying geographic variation (Tobias, Brawn et al. 2012).

## Study species

One example of a tracheophone suboscine is the Mexican or Mayan antthrush (*Formicarius moniliger*) (Figure 2). The Mexican antthrush is a ground antbird and belongs to the family Formicariidae (Collier, Kirschel et al. 2010). As a ground antbird it spends most of its time on the ground, foraging on invertebrates in the leaf litter (Kirschel, Earl et al. 2009, Kirschel, Cody et al. 2011). It inhabits humid evergreen and semi-deciduous forests, from sea level until 1800 m elevation (Howell 1994). Its range extends from the southern Mexican states of Veracruz and Oaxaca eastwards to Central Honduras (Howell 1994, Collier, Kirschel et al. 2010). In Honduras, the species is mostly found in humid forests from 400 to 1200 m elevation (Howell 1994). They are sexually monomorphic and socially monogamous and they occupy territories of approximately 2 ha, which both sexes defend year-round (Kirschel, Earl et al. 2009). Breeding season has been recorded between April and June. The female lays two white eggs in a cavity and both parents incubate them and provide parental care to the offspring (Kirschel, Zanti et al. 2020). Both males and females sing but their song is indistinguishable to the human ear making identification of the sex in the field challenging (Kirschel, Earl et al. 2009). Throughout the year they usually sing at dawn and dusk, but in the breeding season singing activity increases during the day as well (Kirschel, Earl et al. 2009). Its song consists of an introductory note which is longer than the others, followed by a short pause and then a sequence of eight to fifteen similar notes and all of them have similar length (Figure 2) (Howell 1994, Kirschel, Cody et al. 2011). The last notes tend to be sharply down slurred and the whole song is transmitted within a narrow frequency band of 1500-2500 Hz (Howell 1994, Kirschel, Earl et al. 2009). The Mexican antthrush is an ideal study system for tracking movements in two dimensions because it spends most of the day on the ground foraging and singing, making it easy to follow for vocalization recording and observations (Collier, Kirschel et al. 2010, Kirschel, Cody et al. 2011).

Previous work on Mexican antthrushes examined the ability of Hidden Markov Models to classify songs of five species of antbirds, including the Mexican antthrush, that share the same territory in a rainforest and examined different factors affecting the performance (Trifa, Kirschel et al. 2008). Other work involved the evaluation of the performance of an acoustic localization system to localize birds singing from the forest undergrowth (Collier, Kirschel et al. 2010). Moreover, with the use of supervised and unsupervised methods (Kirschel, Earl et al. 2009) could classify

antthrush's songs to individuals. Then, this work was extended at a point where scientists could track individuals over time by using recordings obtained in different years and they could study the interactions of individuals of the same and opposite sex and their interactions with the environment (Kirschel, Cody et al. 2011). The most recent study on the Mexican antthrushes identified how males and females responded to presumed intruders of either sex in the process of playback experiments, by assessing their likely size based on the frequency of their song (Kirschel, Zanti et al. 2020).

In this thesis, I will investigate the singing activity of the Mexican antthrush. More specifically, I aim to investigate the response of colour-ringed individuals to intruders represented by male and female conspecific playback and the singing activity during the day. I will investigate how sex and testosterone levels affect singing activity across the ringed population and the singing activity in the first two morning hours and in the whole day. Also, I will investigate if the presence of vocalizing individuals during the first two hours after dawn, is a motivational factor for other individuals of nearby territories to start singing as well and thus if there is domino effect. Moreover, I will investigate if duetting is present in the Mexican antthrush. I expect male individuals to sing more than female individuals and testosterone levels to be an important factor in this. I also expect increased singing activity in the morning hours due to the dawn chorus and it will be decreased at the other times of the day and increased singing activity in the presence of an intruder, represented by the playback. For the purposes of the study, I used recordings obtained from eight Song Meter devices (Wildlife Acoustics) that were placed in the territories of the colour-ringed individuals and also songs from directional recordings of colour-ringed individuals obtained in the process of playback experiments. The study will provide some more information on the spatiotemporal vocal interactions of this tracheophone suboscine passerine and also will shed some more light in the recently studied field of female song.



**Figure 2:** The Mexican antthrush (*Formicarius moniliger*) (Photo by Holger Teichmann, eBird) and its song

## MATERIALS AND METHODS

### Fieldwork

Fieldwork took place from late April (28/4) until mid-May (15/5) of 2016, at the Chajul Tropical Biology Station of the Montes Azules Biosphere Reserve, in Chiapas, Mexico (Collier, Kirschel et al. 2010). The reserve covers an area of 331 000 ha of lowland tropical rainforest. The study area covered 50 ha of mature tropical evergreen forest, although there were places where the forest was of lower stature, and at an elevation of 150-165 m on the northern bank of Lacantun River (Kirschel, Cody et al. 2011). Fieldwork included playback experiments from the early morning hours of the day until midday (6:55 am-13:35 pm) and then in the afternoon (17:15 pm-18:15 pm). Playbacks were recordings of the songs of ringed individuals that were high-pass filtered at 400 Hz to remove high amplitude noise, and each recording included two songs of an individual, with a silent gap of 22 seconds between them. Those individuals were colour ringed and recorded using a directional microphone during previous field seasons in the same area and had also been genetically sexed. The recordings were played in a looped for the whole duration of the experiment and the output volume was at the same level for all the experiments. The playback equipment consisted of an Apple iPod MP3 player and a TivoliAudio PAL loudspeaker (Kirschel, Zanti et al. 2020). The experiments took place during the breeding season of the Mexican antthrush and the presence of the playback made the territory holders more alerted and more active, thus they were attracted close to the source of the playback songs and they were vocalizing more. This allowed the researchers to record their songs using a Sennheiser ME67/K6 directional microphone and a Marantz PMD 670 digital recorder directly onto compact flash cards at a sample rate of 44.1 kHz and a resolution of 16 bits per sample (Kirschel, Earl et al. 2009), while walking along trails. Mist nets were placed to catch the singing individuals for biometrical measurements and colour-ringing, to aid in the identification of individuals in the field. Feather and blood samples (50-100µL) were also obtained via venipuncture of the brachial vein for genetic sex identification and to determine the concentration levels of testosterone (Taves, Schmidt et al. 2010, Kirschel, Zanti et al. 2020).

All individuals had radiofrequency identification tags fitted on their backs which aided in locating individuals and identifying them when rings could not be seen, thus allowing for identification of individuals using radiotelemetry. At the end of each recording the date, time, location of the territory, and playback stimulus used were announced, along with the colour ring combination or telemetry number of the individual singing and at times, information about the behaviour of the bird and if there were other anthruses vocalizing nearby. The duration of the directional recordings ranged from 5 to 40 minutes, depending on the activity of the individual and its approach, and 15 individuals were recorded. In the area around the station, 8 Song Meter devices (Wildlife Acoustics) were placed in a way to cover all the territories within the study plot and the distance between them was ~200 m, except between SM7 and SM8 where the distance between them was 80 meters since the area was inaccessible, due to the dense vegetation. GPS devices designed by Wildlife Acoustics for use with song meters to provide a time stamp for synchronization were also attached. The song meters were set to record from 30 minutes before dawn (close to 06:00) to 30 minutes after dusk (approaching 20:00) and WAV files were saved in hour-long segments.

### **Song feature extraction and Discriminant Function Analysis**

All the recordings obtained from the field were processed using Raven Pro 1.6. Once in Raven Pro, the waveform, selection spectrum and spectrogram view were used. For a high-resolution spectrogram, the 3 dB Filter Bandwidth was set to 50 Hz and the DFT size to 16384 samples. The brightness and contrast of the spectrogram were adjusted to aid in visual signal detection. Signals of high amplitude have a dark colour and those with low amplitude a faint colour, affecting the detection. Due to the high quality of the signals and the low background noise, the note selection of the songs from the directional recordings was done manually. In recordings with more than one singing individuals, I labelled them based on information about their colour-ring combination provided from announcements during or at the end of the recordings. This was also possible by observing the differences of the signals in spectrograms. When the ring coloration or transmitter number was not mentioned, the bird was labelled as unknown. Some of the announcements during the recording mentioned the presence of the playback, making it easier to recognise when it was a singing individual or playback, and avoid selecting the notes of it. The waveform was used as a reference for the precise selection of the signal, to match the actual begin and end times.

From the recordings obtained from the song meters, I used from three song meters the recordings for all the hours of the day for six days, and from five song meters I used the recordings of the first two hours of the day for six days. These recordings were processed differently from the directional recordings due to the high levels of background noise which impact detectability. First, those songs of the antthrushes which had Avg Power Density lower than -80.00dB were selected manually and then all the selected songs were saved as separate WAV files. Then, those WAV files were filtered using the Batch Band Filter that Raven provides, to filter the background noise around the targeted signal, with frequencies lower than 1 kHz and higher than 3 kHz. The filtered files were saved as new WAV files. Afterwards, the Band Limited Energy Detector was used for the note detection and the target signal parameters were set as follows: minimum frequency 1600 Hz, maximum frequency 2100 Hz, minimum duration 0.02875 s, maximum duration 3.99625 s, minimum separation 0.01438 s and SNR Threshold 5.0 dB. If the detector failed to detect a note, it was selected manually, using the waveform as a reference. For both directional and song meter recordings, when selecting a note, the values for temporal and spectral features were recorded automatically which are begin and end time (s), low and high frequency (Hz), delta time (s), duration 90% (s), frequency contour 5% (Hz), frequency contour 50 % (Hz), frequency contour 95% (Hz), peak frequency (Hz), peak frequency contour (Hz), PFC Average Slope (Hz/ms), PFC Num Inf Pts, PFC Slope (Hz/ms), peak time relative, occupancy and max frequency (Hz). The number of the notes was documented manually. All the measurements obtained from the feature extraction process of the directional experiments were transferred into Microsoft Excel (Microsoft Corporation, 2018), where I prepared spreadsheets for the calculation of additional features using formulas (Table 1). I prepared two spreadsheets, one for the recordings of the song meters and one for the directional recordings. Then, I used STATA 11.2 (StataCorp, College Station, TX, U.S.A.) to run Discriminant Function Analysis (DFA) with leave-one-out classification, using the songs from the directional recordings in which the ID of the bird singing was known, to test how well I could classify each song to ringed and tagged individuals. Based on the correct classification percentage, the songs of the directional recordings were used as a training dataset for the classification of the songs to individuals recorded on the song meters. To classify the songs to individuals in the case of the song meters, I again ran a linear Discriminant Function Analysis in STATA 11.2. This time I used two approaches, first, classifying the songs based on the training set from directional recordings alone (hereafter SM), and second, to improve the classification

results, I added information about the geographic coordinates of the song meters and geographic coordinates of the territories of the ringed and unringed individuals and calculated the distance between the two (SMdist). Bouts of song recorded on the song meters were assigned to the individual classified by the DFA if the percentage of correct classification of the songs within the bout was >50% for the SM and >70% for the SMdist. In the case where the SM was <50% and the SMdist >70%, the songs were classified as unidentified – in some cases likely unringed individuals that were present at the study site and seen vocalising.

**Table 1:** Song features calculated with the use of formulas into Microsoft Excel (Microsoft Corporation, 2018)

1	Duration from the start of the first note to the start of the second note
2	Duration of the main song
3	Duration of the first half of the song
4	Duration of the second half of the song
5	Duration of the last note
6	Duration of the second half of the song
7	Duration of the last note of the second half of the song
8	Peak Frequency of the first note
9	Peak Frequency of the second note
10	Peak Frequency of the last note
11	Peak Frequency of the first half of the song
12	Peak Frequency of the second half of the song
13	Average Peak Frequency of the main part of the song
14	Rate of the main part of the song
15	Rate of the first half of the song
16	Rate of the second half of the song
17	Total number of notes

### Genetic sex identification and Testosterone analysis

As mentioned above, for the genetic sex identification, DNA was extracted from blood or feather samples obtained from the fifteen individuals. To determine the sex, PCR amplification was performed, and P2 (TCTGCATCGCTAAATCCTTT) and P8 (CTCCCAAGGATGAGRAAYTG) primers were used (Griffiths, Double et al. 2002). Then, the PCR products were run in 1% agarose gel for 40 minutes (135 Volts), revealing one or two bands for males and females, respectively.

Also, to determine the concentration levels of testosterone in the plasma, >100  $\mu$ L of blood was centrifuged for 10 min to separate the plasma, which was then extracted from tubes using a Hamilton syringe and then placed in  $-20^{\circ}\text{C}$  (Taves, Schmidt et al. 2010). Samples were then sent to Max Planck institute for Ornithology in Seewiesen Germany to identify testosterone levels.

## **Analyses of song rates**

Based on the total number of songs of individuals recorded on the song meters, I was interested to determine if factors such as sex, testosterone, time of day and the presence of playback have an effect on the singing activity of the species. Also, I was interested to determine if there is a domino effect taking place during the morning hours of the day and how the singing activity of female individuals is affected by the presence of their singing partner and neighbour individuals of both sexes. For these reasons, I ran generalized linear mixed models (GLMM) fitted with a negative binomial distribution, in the glmmTMB R package (Brooks, Kristensen et al. 2017). In order to test for the effect of sex, testosterone, time of day and presence of playback in the singing activity, I used a candidate model approach to compare nine models, using the entire dataset of songs from 30 minutes before dawn to 30 minutes after dusk from three song meters and the first two hours and fifteen minutes from 30 minutes before dawn from the remaining five song meters. These candidate models had total songs per individual within 15-minute windows as response variable and individual as random factor. Fixed factors tested in these models were combinations of sex, time, presence / sex of playback, testosterone level and the interaction of sex and testosterone, sex and time, and sex and time as a quadratic term. In order to test if there was a domino effect of song among neighbours, I compared candidate models focusing on the songs recorded in the two hours and fifteen minutes starting 30 minutes before dawn. Again, I used total songs per individual within 15-minute windows as response variable and individual as random factor. Fixed factors were combinations of time, neighbour singing (yes/no), sex of playback and playback ID. For neighbour singing, I considered the partner and any individuals singing in adjacent territories picked up on the same or adjacent song meters. In order to determine if female singing activity is affected by song of its partner or singing activity of the neighbours of both sexes, I compared candidate models on a dataset of the female songs from the first two hours and fifteen minutes from 30 minutes before dawn. Again, the response variable was total songs per individual withing 15-minute windows and the random factor was individual. Fixed factors were combinations of testosterone,

time, sex of the playback, partner singing, neighbouring male singing and the interaction of partner singing with neighbouring male singing (there were no neighbouring females singing in the data set).

In all cases, each model was developed based on the level of significance of the fixed factors to the response variable and non-significant fixed factors were excluded from the models. Model selection was based on lowest corrected Akaike information criterion (AICc) score calculated using the package AICcmodavg (Mazerolle 2019). Model validation was performed in DHARMA (Harting 2019) and back-transformed GLMM estimates were produced using the effects package (Fox, Weisberg 2018). Graphic representations of the singing activity of the ringed and unringed/unknown individuals in the first two hours and fifteen minutes of the day for a period of six days, and also of the results of the models were produced using the package ggplot (Wickham 2016) in R and singing levels of individuals from the same territory combined in figures using Adobe Illustrator (Adobe Inc., 2019).

## RESULTS

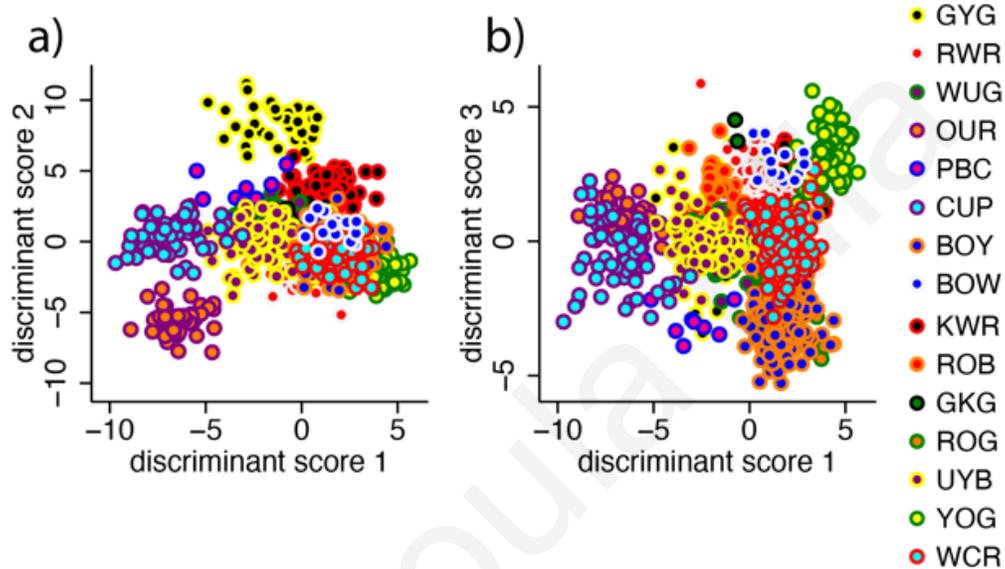
From the 15 individuals recorded singing during the playback experiments, 12 were males and 3 were females (Table 2).

**Table 2:** The colour ring combination of the study population of Mexican antthrushes and their sex.

	<b>Individual</b>	<b>Sex</b>
<b>1</b>	WCR	Male
<b>2</b>	CUP	Male
<b>3</b>	YOG	Male
<b>4</b>	BOY	Male
<b>5</b>	GKG	Male
<b>6</b>	BOW	Male
<b>7</b>	KWR	Male
<b>8</b>	RWR	Male
<b>9</b>	ROB	Male
<b>10</b>	WUG	Male
<b>11</b>	ROG	Male
<b>12</b>	UYB	Male
<b>13</b>	GYG	Female
<b>14</b>	OUR	Female
<b>15</b>	PBC	Female

Ringed individuals were identified on 44 directional recordings. More specifically, PBC, GYG, GKG and BOW were recorded once, CUP and ROG twice, KWR, ROB, WUG and OUR three times, UYB, YOG and WCR four times, BOY five times and RWR seven times. Using these recordings, the correct classification percentage of the Discriminant Function Analysis with the leave-one-out classification method is equal to 90%. Discriminant scores were calculated for the songs obtained from the sample of 44 recordings and the results are illustrated in the following scatter plot (Figure 3). With the use of three discriminant scores, the clustering of the songs into

the different groups of individuals becomes more distinguishable (Figure 3a,b). The total number of 15-min segments that were processed from the song meters were 1260 15-min segments, of which 990 were from three song meters (from 30 min before dawn to 30 min after dusk) and 270 recordings from the remaining five song meters (135 min starting from 30 min before dawn) (Table 3). Also, the classification process yielded a total of eight uringed/unknown individuals that were recorded in a total of 99 15-min segments.



**Figure 3:** Scatter plots of discriminant scores 1 and 2 (a) and of discriminant scores 1 and 3 (b) illustrating the separation of songs per individual.

**Table 3:** Total number of 15-min segments in which each ringed individual was classified based on the Discriminant Function Analysis.

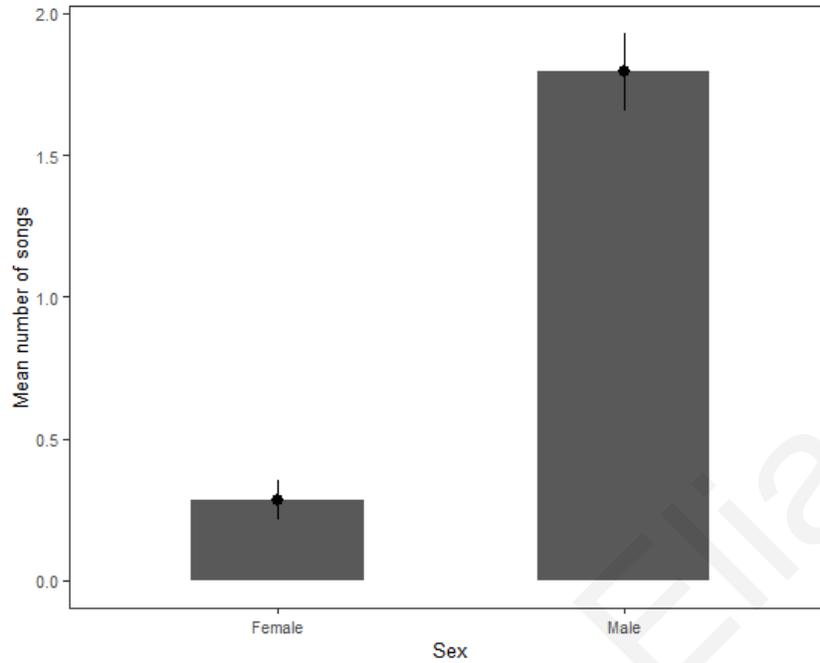
	<b>Individual</b>	<b>Total number</b>
<b>1</b>	WCR	69
<b>2</b>	YOG	22
<b>3</b>	BOY	10
<b>4</b>	GKG	34
<b>5</b>	BOW	1
<b>6</b>	KWR	18
<b>7</b>	RWR	22
<b>8</b>	ROB	47
<b>9</b>	WUG	5

<b>10</b>	UYB	11
<b>11</b>	GYG	13
<b>12</b>	OUR	9
<b>13</b>	PBC	8

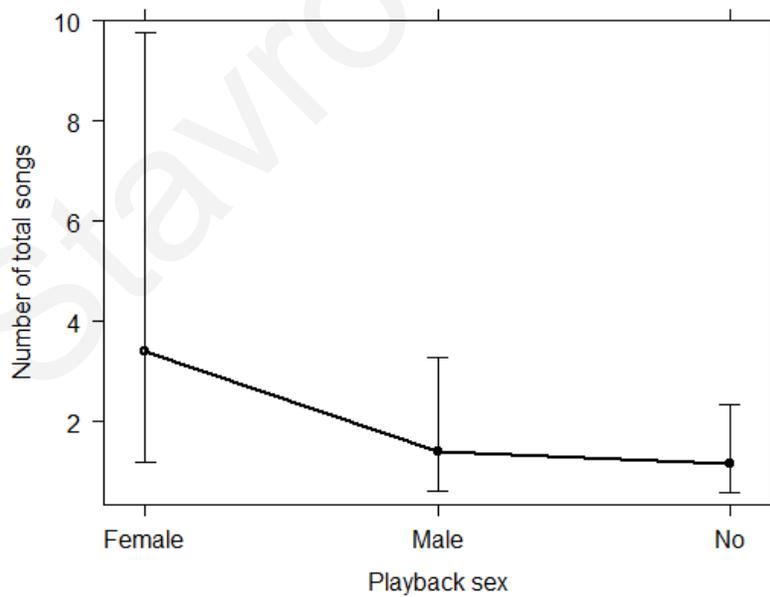
From the entire dataset of 2447 15-min segments from 14 ringed individuals, I found that time of day had a significant effect with birds singing more earlier than later in the day, and a lack of interaction with sex suggests there is no difference in males and females in this behaviour (Figure 6, Table 4). There was also a significant effect of playback, in that fewer songs were sung without playback than the reference condition of female playback. Interestingly, birds sang less in response to male playback than female playback, but this result was not significant (Figure 5, Table 4). In addition, males sang substantially more than females (Figure 4, Table 4), but perhaps surprisingly, not significantly so. Finally, testosterone had no effect on singing activity and was not included in the final model (Table 4).

**Table 4:** The effect of playback sex, sex, time and their interaction on total number of songs

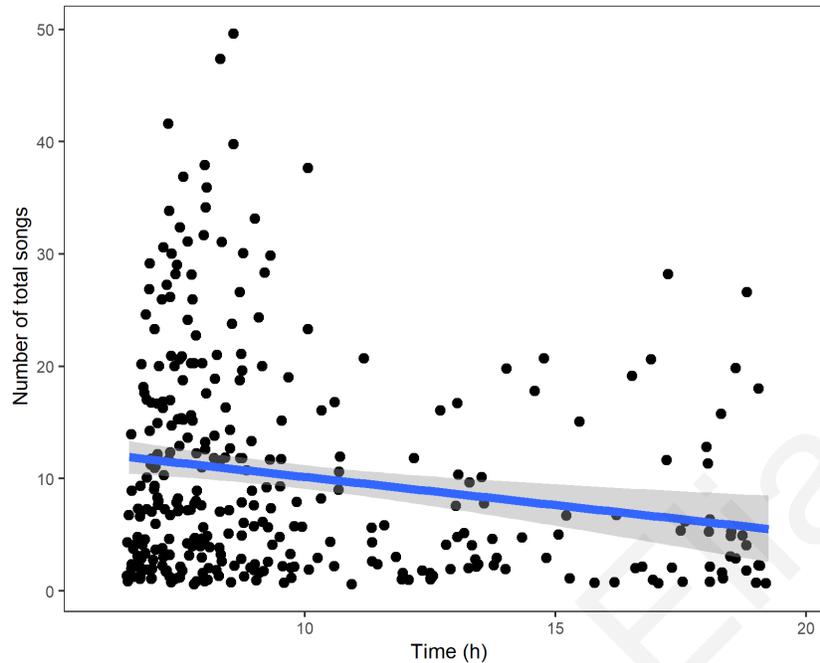
<b>Response: Total songs</b>	<b>Estimate</b>	<b>Std. error</b>	<b>z</b>	<b>p</b>
Intercept	0.2586	0.83	0.312	0.7553
Sex: male	1.3782	0.8198	1.681	0.0927
Time	-1.2297	0.3049	-4.033	5.52E-05
Playback sex: male	-0.8842	0.487	-1.816	0.0694
Playback sex: no	-1.071	0.4225	-2.535	0.0112
Male x time	0.5494	0.3191	1.722	0.0851



**Figure 4:** Bar plot with standard error illustrating the mean number of songs sung by male and female anthruses.



**Figure 5:** Line plot illustrating the effect of the sex of the playback in the singing activity of the ringed individuals and in the absence of playback.

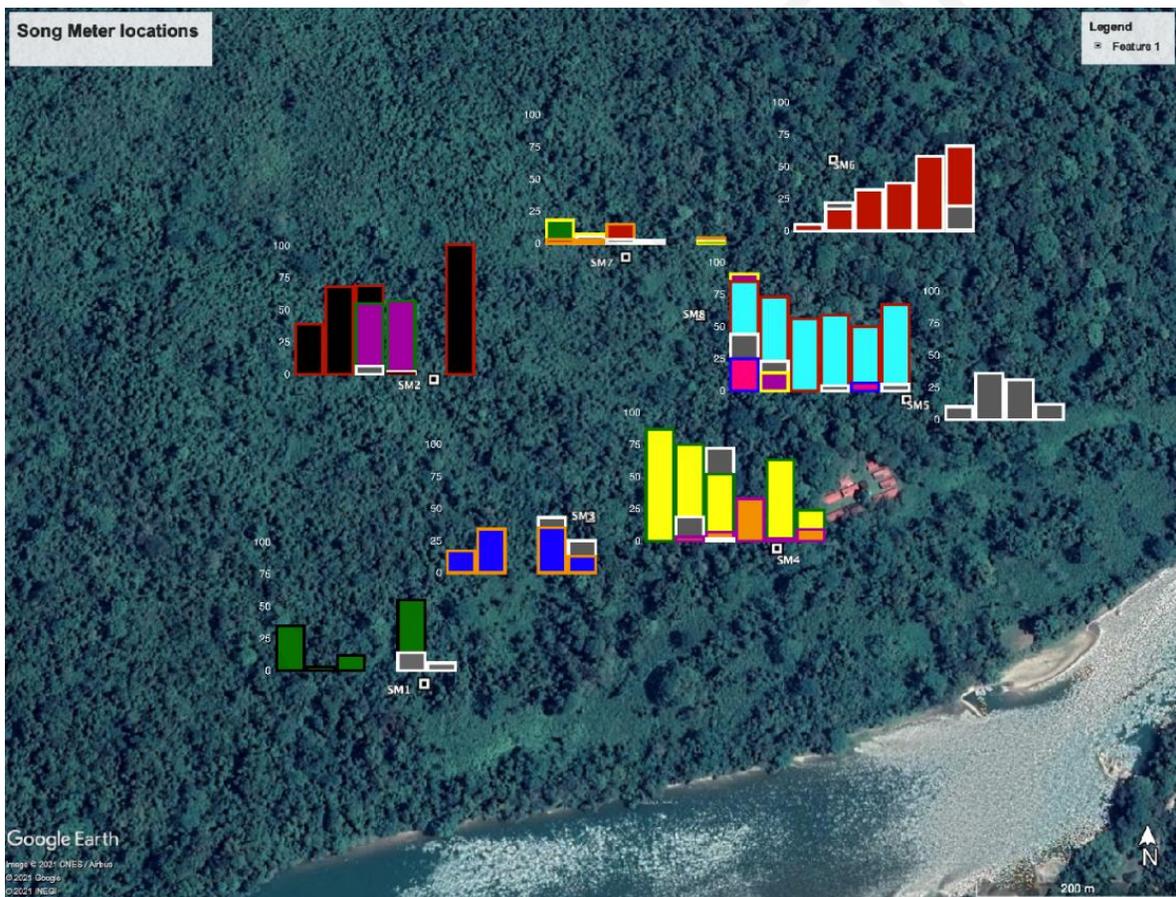


**Figure 6:** Line plot illustrating the change in the singing activity of the ringed individuals during the day, from 30 min pre-dawn to 30 min post dusk.

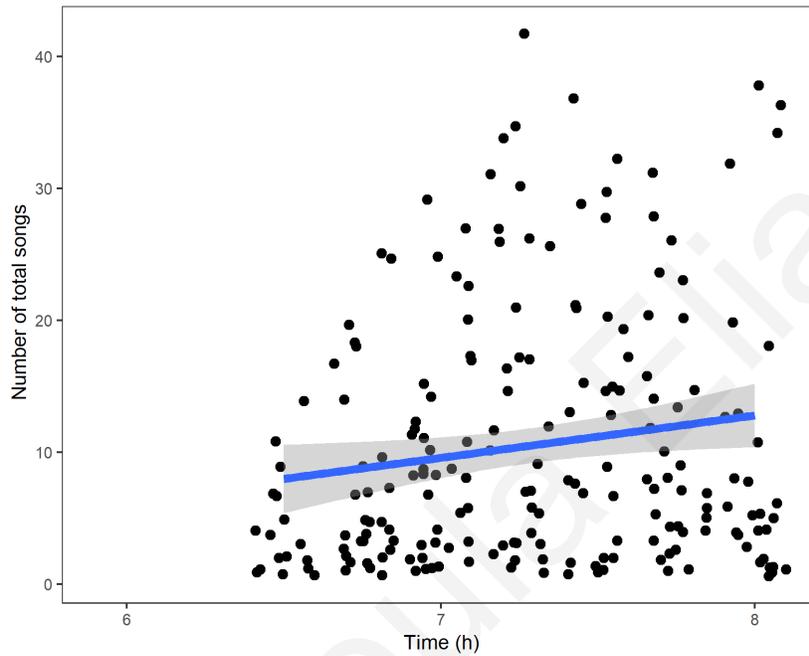
From the dataset of 1188 15-min segments from 22 color-ringed and unringed or unknown individuals, I found that time of day had a strong positive effect in the singing activity of the individuals. More specifically, there is increased singing activity during the first 135 minutes of the day from 30 min before dawn, but they avoid singing at the first 30 minutes (Figures 7 and 8, Table 5). Also, there was a significant effect to the singing activity of individuals when there were other vocalizing individuals within or adjacent to their territory, in that more songs were sung (Figures 7 and 9, Table 5). Moreover, birds sang less when there was no playback compared to the presence of male playback, but this result was not significant (Table 5).

**Table 5:** The effect of time, playback sex and neighbour singing on the total number of songs for the first 135 minutes from 30 min before dawn.

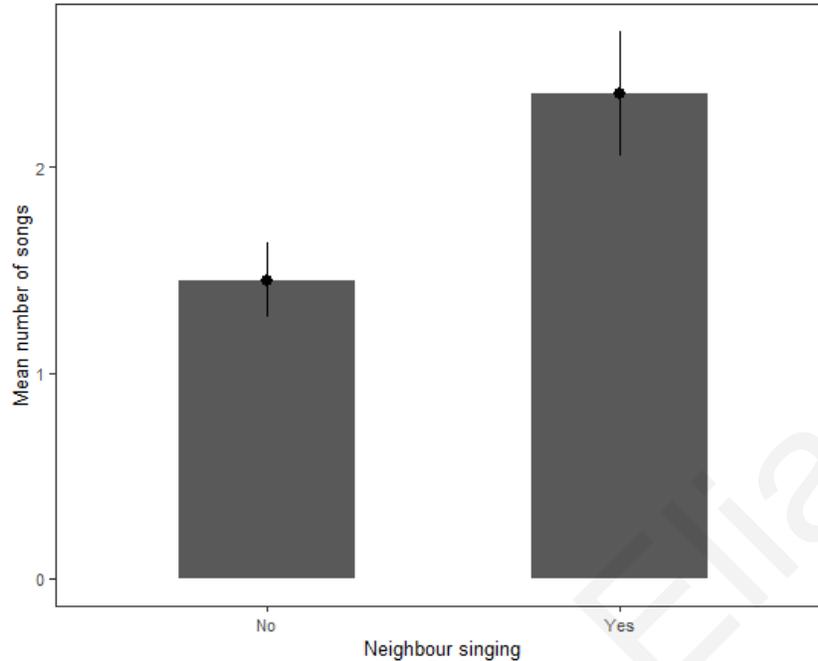
Response: Total songs	Estimate	Std. error	z	p
Intercept	0.51976	0.39898	1.303	0.1927
Time	0.51814	0.08052	6.435	1.24E-10
Playback sex: no	-0.5067	0.29367	-1.725	0.0845
Neighbour singing: Yes	0.40531	0.16099	2.518	0.0118



**Figure 7:** Satellite image of the study area at Estacion Chajul just north of the Rio Lacantun. Bar plots illustrating the total number of songs of ringed and unringed or unknown individuals recorded for the first 135 min from 30 min before dawn, for a period of six days. Each plot is positioned in the territory or between territory boundaries of recorded individuals.



**Figure 8:** Line plot illustrating singing activity during the first 135 min from 30 min before dawn.

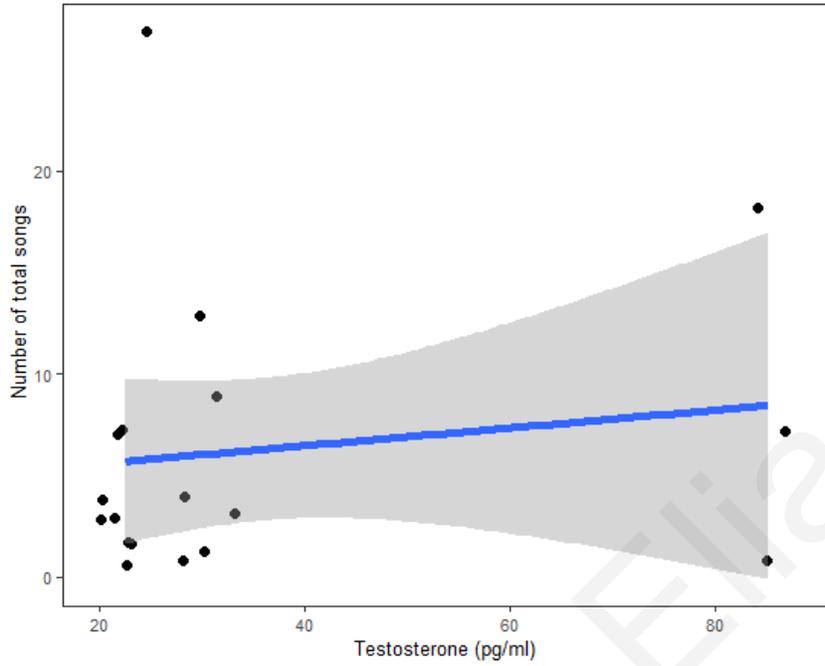


**Figure 9:** Bar plot with standard error illustrating the effect of the presence and absence of neighbours singing during morning singing activity.

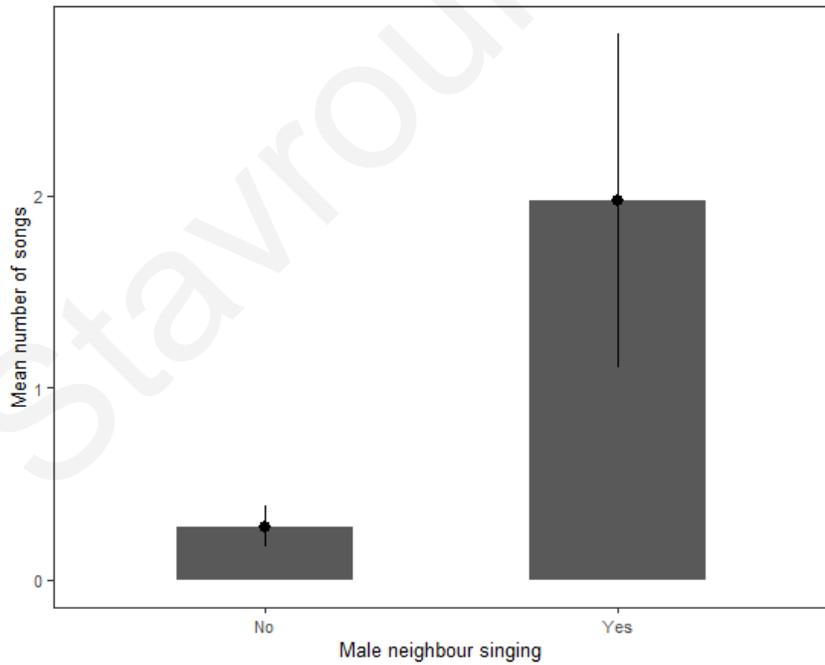
From the dataset of 162 15-min segments from three female individuals, I found that testosterone had a negative effect in the singing activity of the females (Figure 10, Table 6). Also, interestingly, females sang substantially more songs when their neighbouring male birds were singing (Figure 11, Table 6). In addition, there was no effect on singing activity of females when their partner was singing and thus partner singing was not included in the final model (Table 6).

**Table 6:** The effect of testosterone and neighbour male singing on the total number of songs of the three female Mexican antthrushes for the first 135 min from 30 min before dawn.

<b>Response: Total songs</b>	<b>Estimate</b>	<b>Std. error</b>	<b>z</b>	<b>p</b>
Intercept	-0.3613	0.6605	-0.547	0.58436
Testosterone	-0.7426	0.3469	-2.141	3.23E-02
Neighbour male: Yes	1.7028	0.565	3.014	0.00258



**Figure 10:** Line plot illustrating the effect of testosterone on singing activity of females.



**Figure 11:** Bar plot with standard error illustrating the effect of singing male neighbours on the singing activity of females.

## DISCUSSION

### Testosterone and singing rates

In this study, testosterone was found to have no effect on singing activity of males but a negative effect on females. The hormone testosterone can influence behaviour by binding to androgen receptors found in the nuclei of the song control system or by being converted in the brain into 17- $\beta$  estradiol ( $E_2$ ) by the enzyme aromatase (Hau, Wikelski et al. 2000, Ritschard, Laucht et al. 2011, Quispe, Sèbe et al. 2016). In temperate birds, testosterone levels in the plasma increase from baseline levels in early spring and remain elevated during the reproductive period, resulting in increased singing rates (Hau, Wikelski et al. 2000, Apfelbeck, Mortega et al. 2013, York, Radford et al. 2016). Also, in a number of species which maintain territories year-round including the majority of tropical birds, testosterone remains in baseline or in lower levels in the plasma throughout the year (Hau, Wikelski et al. 2000, Apfelbeck, Mortega et al. 2013, York, Radford et al. 2016). In those species in which testosterone levels are low, their brain may have higher sensitivity to low levels of the hormone (Apfelbeck, Mortega et al. 2013). In addition, in species where females have been observed to sing, it happens when testosterone levels are above the threshold level for song production (Catchpole, Slater 2003). Studies on tropical species in which male and female individuals were exposed to testosterone implants, males' and females' singing activity increased and also females produced male song (Hau, Wikelski et al. 2000, Chiver, Schlinger 2019). Thus, as a tropical species, Mexican antthrush may maintain low levels of testosterone, which may explain the findings in this study. Also, females had lower levels of testosterone than males, but it is important to consider that one of the females had very high levels of testosterone (85.25 pg/ml) than the others. Although in the case of the Mexican antthrush there was no apparent effect of testosterone on song, the results must be taken with caution because testosterone levels can vary a lot from day to day and when plasma was extracted may have been some days before the birds were recorded and any changes in testosterone levels during that time

are possible. Also, the small sample size of the females should be considered. Further investigation of the role of testosterone in the singing activity of both sexes is required.

### **Singing activity between the two sexes and responses to playback**

In the tropics, long-term territoriality and pair-bonds are common in many bird species (Price 2009). Song has been observed in both sexes of those species, it can be sexually monomorphic or dimorphic, but serves a common purpose (Price 2009, Cain, Langmore 2015). In the dense vegetated environment of the tropics, individuals of both sexes sing to maintain contact between them within their territory or to coordinate their breeding activity and offspring care from year to year (Slater, Mann 2004, Price 2009, Cain, Langmore 2015). They also sing to attract mates and to defend territories and mates from same or opposite sex (Slater, Mann 2004, Price 2009, Tobias, Gamarra-Toledo et al. 2011, Cain, Langmore 2015). Although song is present in both sexes and has common functionality, singing rates between the sexes of a species may vary (Price 2009, Cain, Langmore 2015), as well as to the level of coordination (Price 2009). There are species in which both sexes sing solo songs, species where the sexes occasionally coordinate their song and species where perfectly synchronized duets have been observed (Slater, Mann 2004, Price 2009, Kirschel, Zanti et al. 2020). Duetting can be the song of a male followed by an immediate response from the female and the opposite, even before the individual which started singing first finishes its song, or a cycle in which male and female sing in turns (Slater, Mann 2004).

Duetting does not occur in Mexican antthrush, because based on the statistical analysis there is no relation between the total number of songs sung by females when their partner was singing. This agrees with observations from previous work which state that female antthrushes have been observed to sing solo and do not coordinate their song with the male (Kirschel, Zanti et al. 2020). In the case of a duetting species, female warbling antbirds rarely sing solo but they reply to the song of their partner (Seddon, Tobias 2006). In the warbling antbird, duets have been observed during interactions with neighbors and playback experiments indicating common territory defense (Seddon, Tobias 2006). Also, females have been observed to interrupt their partner's song when they were singing to other females, indicating mate guarding (Seddon, Tobias 2009). As previously mentioned, male Mexican antthrushes sang more songs than females, though in the analyses the result was not significant. This might be because of unbalanced recordings in the sample, with most male songs during the dawn chorus when singing rates overall were higher. I expect with a

larger sample males would be shown to sing significantly more. Males singing more than females is of course expected even in tropical species where both sexes sing, and has been found in other tropical species, including *Hypocnemis peruviana*, *Hypocnemis subflava* (Tobias, Gamarra-Toledo et al. 2011) and Spotted antbird (*Hylophylax n. naevioides*) (Bard, Hau et al. 2002). In general, male birds sing most when critical reproductive resources are most valuable or threatened, or when mate attraction is most important (Cain, Langmore 2015). This may be possible in the case of the Mexican antthrush. Male antthrushes possibly sing to warn other males from entering an occupied territory or to attract females. This is supported from previous work in which males have been recorded in the same territory for several years, but female partners have been replaced from others within three years and also found more than one female within some territory boundaries (Kirschel, Cody et al. 2011).

During playback experiments performed in the present study, total responses from males and females were higher for female playback than male playback, and also I found that females increased their singing activity when neighboring males were vocalizing. A possible explanation, is that females will mostly defend their territory against other females and they choose to which males they will respond to. In a previous experiment, it has been found that females respond to those males with higher frequency songs, hence to smaller males than their size in order to avoid physical cost when taking part in territory defense (Kirschel, Zanti et al. 2020). In that study, they will not have known the presumed male intruders they were responding to in playback experiments, though here it is expected they know the neighboring males they are responding to. Considering all the above, it seems that the main reason male and female antthrushes sing, is for territory defense. A territory will provide vital resources to adult individuals and their offspring, including nesting sites and food even at the times of the year where food availability is low (Cain, Langmore 2015).

### **The effect of time and neighbors' singing activity on song rates**

Another finding in this study, is the high singing rates during the first two hours after dawn and in the following hours the singing activity is reduced. This finding has also been observed in other study systems, including Rufous-and-white wrens (Mennill, Vehrencamp 2005), Venezuelan troupials (Odom, Omland et al. 2016) and *Hypocnemis* antbirds (Tobias, Gamarra-Toledo et al.

2011). There are several potential reasons behind the high singing activity in the morning hours and its reduction for the rest of the day in the Mexican antthrushes. It has been suggested that singing at dawn is associated with territory defence (Mace 1989, Tobias, Gamarra-Toledo et al. 2011). At dawn, energy levels are low and song requires less energy than approaching an intruder (Mace 1989, Berg, Brumfield et al. 2006) and by singing in the morning is a way to advertise survival overnight and as a result the territory is still occupied (Dalziell, Cockburn 2008, Stanley, Walter et al. 2016). Other reasons for increased singing at dawn include defence of fertile females, advertise fitness to their mate and attract mates (Dalziell, Cockburn 2008, Tobias, Gamarra-Toledo et al. 2011). Another possible reason is foraging activity (Mace 1989). Individuals start singing early in the morning where light levels are low making foraging difficult (Mace 1989, Berg, Brumfield et al. 2006, Dalziell, Cockburn 2008, Bruni, Mennill et al. 2014, Stanley, Walter et al. 2016). As the time passes and light levels increase, they spend more time foraging rather than singing. Moreover, reduced predation risk at the early morning hours of the day where predators are not active compared to the other hours of the day is a possible reason (Dalziell, Cockburn 2008, Bruni, Mennill et al. 2014). Resting could also be a reason of reduced singing activity later in the day (Mace 1989). In addition, based on the acoustic transmission hypothesis, atmospheric conditions at dawn, such as temperature, humidity and wind, are ideal for sound transmission (Berg, Brumfield et al. 2006, Dalziell, Cockburn 2008, Bruni, Mennill et al. 2014, Stanley, Walter et al. 2016). Low air turbulence at dawn maximizes sound propagation (Stanley, Walter et al. 2016). Weather conditions affect singing activity during the day (Bruni, Mennill et al. 2014). The masking effect of the sound of rain decrease communication efficiency (Bruni, Mennill et al. 2014). Reduction in the singing activity during the day under rainy conditions has been found in rufus-scrub birds (O’Leary, Stuart 2021). In the case of the Mexican antthrush, rainy weather is dominant in the habitat they live and in many of the recordings that have been analysed, the sound of rain covered the frequency spectrum and the birds were not vocalizing. Social factors such as the number of neighbours affect singing activity during the morning hours (Bruni, Mennill et al. 2014). This relation between increase neighbour density and higher song rates has been observed in studies of different species, including the *Hypocnemis* antbirds, the rufus-scrub birds and the rufus-and-white wrens (Mennill, Vehrencamp 2005, Tobias, Gamarra-Toledo et al. 2011, O’Leary, Stuart 2021). In the case of the Mexican antthrush, I found that singing individuals influence individuals of nearby territories to start singing as well. In the study area, each individual’s territory

was adjacent to one or more territories. I also found that the birds started singing after 6:30 am. Inspecting recordings, there was spectral overlap between the song of the Mexican antthrush and a loud din created by nocturnal cicadas. By starting to sing after 6:30 am, Mexican antthrushes avoid the masking of their song by cicadas and achieve better transmission of their song to intended receivers. This finding agrees with observations from cicada playback experiments, in which species with songs that overlap in frequency with the insects' call, reduced their singing activity during playback. Thus, in order to avoid masking by insects, birds may shift the time they start singing, as has been observed in spotted antbird, dot-winged antwren, black-cheeked woodpecker and red-capped manakin (Stanley, Walter et al. 2016).

## **Conclusion**

This study on Mexican antthrush revealed that they sing more earlier than later in the day, that singing activity commences after ambient noise caused by singing nocturnal cicadas subsides, and that males sing more on average than females. It also revealed that testosterone found to have no effect on singing activity of males but a negative effect on females though, these results require further investigation because of a low sample size and difficulty of associating the time when blood samples were collected with the days individuals were recorded. In order to better understand the effect of testosterone in the singing activity of the Mexican antthrushes, the densities of androgen receptors of the nuclei responsible for song production in male and female birds and the size of the high-level control nucleus (HVC) could be examined. Experiments under captive conditions in which birds will be exposed to substances which block testosterone action and comparison of the singing rates under these conditions with the singing rates of the wild population could also be done. Another finding is that there is no effect of partner singing activity on singing activity of the female, indicating the absence of duetting. Female singing activity increased when neighboring males were singing, but it is not known whether it was the female responding to neighboring males or vice versa, either to defend their territory or to extra pair copulations. At this point, it should be mentioned that in the study site there were more than three ringed females. However, those females did not sing in the process of acquiring directional recordings. It is possible that some of the unringed/unknown individuals recorded singing by the song meters were not unringed birds, but the female individual of the pair which I could not classify due to the lack of its song in the training dataset. Also, the playback experiments, resulted in more responses from ringed individuals to

female playback than to male playback, possibly because of female-female competition over territory combined with males seeking to attract potential mates. In the absence of territory intruders, the singing activity is reduced. Combined with a pattern of a domino effect, the study demonstrates both correlatively and experimentally that singing activity is heightened by conspecific vocal activity nearby, and especially from neighbors and presumed intruders.

Stavroula Elia

## ABBREVIATIONS

ABBREVIATION	MEANING
e.g.	for example
cm	centimeter
m	meter
ha	hectare
s	seconds
min	minutes
h	hours
Hz	Hertz
WCR	White Cyan Red
YOG	Yellow Orange Green
BOY	Blue Orange Yellow
GKG	Green Black Green
BOW	Blue Orange White
KWR	Black White Red
RWR	Red White Red
ROB	Red Orange Blue
WUG	White Purple Green
UYB	Purple Yellow Blue

GYG	Green Yellow Green
OUR	Orange Purple Red
PBC	Pink Blue Cyan
CUP	Cyan Purple Pink
ROG	Red Orange Green

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## BIBLIOGRAPHY

ABRAHAMS, C. and GEARY, M., 2020. Combining bioacoustics and occupancy modelling for improved monitoring of rare breeding bird populations. *Ecological Indicators*, **112**, pp. 106131.

ACEVEDO, M.A., CORRADA-BRAVO, C.J., CORRADA-BRAVO, H., VILLANUEVA-RIVERA, L.J. and AIDE, T.M., 2009. Automated classification of bird and amphibian calls using machine learning: A comparison of methods. *Ecological Informatics*, **4**(4), pp. 206-214.

AIDE, T.M., CORRADA-BRAVO, C., CAMPOS-CERQUEIRA, M., MILAN, C., VEGA, G. and ALVAREZ, R., 2013. Real-time bioacoustics monitoring and automated species identification. *PeerJ*, **1**, pp. e103.

ANDERSON, S.E., DAVE, A.S. and MARGOLIASH, D., 1996. Template-based automatic recognition of birdsong syllables from continuous recordings. *The Journal of the Acoustical Society of America*, **100**(2), pp. 1209-1219.

ANDREASSEN, T., SURLYKKE, A. and HALLAM, J., 2014. Semi-automatic long-term acoustic surveying: A case study with bats. *Ecological Informatics*, **21**, pp. 13-24.

APFELBECK, B., MORTEGA, K., KIEFER, S., KIPPER, S., VELLEMA, M., VILLAVICENCIO, C.P., GAHR, M. and GOYMANN, W., 2013. Associated and disassociated patterns in hormones, song, behavior and brain receptor expression between life-cycle stages in male black redstarts, *Phoenicurus ochruros*. *General and comparative endocrinology*, **184**, pp. 93-102.

ARAYA-SALAS, M. and SMITH-VIDAURRE, G., 2017. warbleR: an R package to streamline analysis of animal acoustic signals. *Methods in Ecology and Evolution*, **8**(2), pp. 184-191.

BARD, S.C., HAU, M., WIKELSKI, M. and WINGFIELD, J.C., 2002. Vocal distinctiveness and response to conspecific playback in the spotted antbird, a Neotropical suboscine. *The Condor*, **104**(2), pp. 387-394.

BARDELI, R., WOLFF, D., KURTH, F., KOCH, M., TAUCHERT, K.-. and FROMMOLT, K.-., 2010. Detecting bird sounds in a complex acoustic environment and application to bioacoustic monitoring. *Pattern Recognition Letters*, **31**(12), pp. 1524-1534.

BERG, K.S., BRUMFIELD, R.T. and APANIUS, V., 2006. Phylogenetic and ecological determinants of the neotropical dawn chorus. *Proceedings of the Royal Society B: Biological Sciences*, **273**(1589), pp. 999-1005.

BIOACOUSTICS, C.R.P., 2019. Interactive Sound Analysis Software. *The Cornell Lab of Ornithology: Ithaca, NY, USA*,

BLUMSTEIN, D.T., MENNILL, D.J., CLEMINS, P., GIROD, L., YAO, K., PATRICELLI, G., DEPPE, J.L., KRAKAUER, A.H., CLARK, C. and CORTOPASSI, K.A., 2011. Acoustic monitoring in terrestrial environments using microphone arrays: applications, technological considerations and prospectus. *Journal of Applied Ecology*, **48**(3), pp. 758-767.

BRADBURY, J.W. and VEHCAMP, S.L., 1998. Principles of animal communication.

BROOKS, M.E., KRISTENSEN, K., VAN BENTHEM, K.J., MAGNUSSON, A., BERG, C.W., NIELSEN, A., SKAUG, H.J., MACHLER, M. and BOLKER, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal*, **9**(2), pp. 378-400.

BRUNI, A., MENNILL, D.J. and FOOTE, J.R., 2014. Dawn chorus start time variation in a temperate bird community: relationships with seasonality, weather, and ambient light. *Journal of Ornithology*, **155**(4), pp. 877-890.

BUXTON, R.T., BROWN, E., SHARMAN, L., GABRIELE, C.M. and MCKENNA, M.F., 2016. Using bioacoustics to examine shifts in songbird phenology. *Ecology and evolution*, **6**(14), pp. 4697-4710.

- CAIN, K.E. and LANGMORE, N.E., 2015. Female and male song rates across breeding stage: testing for sexual and nonsexual functions of female song. *Animal Behaviour*, **109**, pp. 65-71.
- CATCHPOLE, C.K. and SLATER, P.J., 2003. *Bird song: biological themes and variations*. Cambridge university press.
- CHARIF, R., WAACK, A. and STRICKMAN, L., 2010. Raven Pro 1.4 user's manual. *Cornell Lab of Ornithology, Ithaca, NY*, **25506974**.
- CHEN, Z. and MAHER, R.C., 2006. Semi-automatic classification of bird vocalizations using spectral peak tracks. *The Journal of the Acoustical Society of America*, **120**(5), pp. 2974-2984.
- CHESMORE, D., 2004. Automated bioacoustic identification of species. *Anais da Academia Brasileira de Ciências*, **76**, pp. 436-440.
- CHIVER, I. and SCHLINGER, B.A., 2019. Sex-specific effects of testosterone on vocal output in a tropical suboscine bird. *Animal Behaviour*, **148**, pp. 105-112.
- COLLIER, T.C., KIRSCHER, A.N. and TAYLOR, C.E., 2010. Acoustic localization of antbirds in a Mexican rainforest using a wireless sensor network. *The Journal of the Acoustical Society of America*, **128**(1), pp. 182-189.
- CUTHILL, I.C. and MACDONALD, W.A., 1990. Experimental manipulation of the dawn and dusk chorus in the blackbird *Turdus merula*. *Behavioral Ecology and Sociobiology*, **26**(3), pp. 209-216.
- DALZIELL, A.H. and COCKBURN, A., 2008. Dawn song in superb fairy-wrens: a bird that seeks extrapair copulations during the dawn chorus. *Animal Behaviour*, **75**(2), pp. 489-500.
- DIGBY, A., TOWSEY, M., BELL, B.D. and TEAL, P.D., 2013. A practical comparison of manual and autonomous methods for acoustic monitoring. *Methods in Ecology and Evolution*, **4**(7), pp. 675-683.

- ELDRIDGE, A., GUYOT, P., MOSCOSO, P., JOHNSTON, A., EYRE-WALKER, Y. and PECK, M., 2018. Sounding out ecoacoustic metrics: Avian species richness is predicted by acoustic indices in temperate but not tropical habitats. *Ecological Indicators*, **95**, pp. 939-952.
- FAGERLUND, S., 2007. Bird species recognition using support vector machines. *EURASIP Journal on Advances in Signal Processing*, **2007**, pp. 1-8.
- FARINA, A. and JAMES, P., 2016. The acoustic communities: Definition, description and ecological role. *Biosystems*, **147**, pp. 11-20.
- FOX, J. and WEISBERG, S., 2018. Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. *Journal of Statistical Software*, **87**, pp. 1-27.
- FROMMOLT, K. and TAUCHERT, K., 2014. Applying bioacoustic methods for long-term monitoring of a nocturnal wetland bird. *Ecological Informatics*, **21**, pp. 4-12.
- GARCIA, M. and FAVARO, L., 2017. Animal vocal communication: function, structures, and production mechanisms. *Current zoology*, **63**(4), pp. 417-419.
- GASC, A., FRANCOMANO, D., DUNNING, J.B. and PIJANOWSKI, B.C., 2017. Future directions for soundscape ecology: The importance of ornithological contributions. *The Auk: Ornithological Advances*, **134**(1), pp. 215-228.
- GEBERZAHN, N., GOYMANN, W., MUCK, C. and TEN CATE, C., 2009. Females alter their song when challenged in a sex-role reversed bird species. *Behavioral Ecology and Sociobiology*, **64**(2), pp. 193-204.
- HARDOUIN, L.A., ROBERT, D. and BRETAGNOLLE, V., 2008. A dusk chorus effect in a nocturnal bird: support for mate and rival assessment functions. *Behavioral Ecology and Sociobiology*, **62**(12), pp. 1909-1918.
- HARTING, F., 2019. DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models.[online] URL: <https://CRAN.R-project.org/package=DHARMA>, .

HAU, M., WIKELSKI, M., SOMA, K.K. and WINGFIELD, J.C., 2000. Testosterone and year-round territorial aggression in a tropical bird. *General and comparative endocrinology*, **117**(1), pp. 20-33.

HOPKINS, C.D., 1977. Electric communication. *How animals communicate*, **263**.

HOWELL, S.N., 1994. The specific status of Black-faced Antthrushes in Middle America. *Cotinga*, **1**, pp. 21-25.

ISLER, M.L., ISLER, P.R. and WHITNEY, B.M., 1998. Use of vocalizations to establish species limits in antbirds (Passeriformes: Thamnophilidae). *The Auk*, **115**(3), pp. 577-590.

JANČOVIČ, P. and KÖKÜER, M., 2011. Automatic detection and recognition of tonal bird sounds in noisy environments. *EURASIP Journal on Advances in Signal Processing*, **2011**, pp. 1-10.

KATZ, J., HAFNER, S.D. and DONOVAN, T., 2016. Tools for automated acoustic monitoring within the R package *monitoR*. *Bioacoustics*, **25**(2), pp. 197-210.

KEEN, S., ROSS, J.C., GRIFFITHS, E.T., LANZONE, M. and FARNSWORTH, A., 2014. A comparison of similarity-based approaches in the classification of flight calls of four species of North American wood-warblers (Parulidae). *Ecological Informatics*, **21**, pp. 25-33.

KIRSCHER, A.N., BLUMSTEIN, D.T., COHEN, R.E., BUERMANN, W., SMITH, T.B. and SLABBEKOORN, H., 2009. Birdsong tuned to the environment: green hylia song varies with elevation, tree cover, and noise. *Behavioral Ecology*, **20**(5), pp. 1089-1095.

KIRSCHER, A.N., CODY, M.L., HARLOW, Z.T., PROMPONAS, V.J., VALLEJO, E.E. and TAYLOR, C.E., 2011. Territorial dynamics of Mexican Ant-thrushes *Formicarius moniliger* revealed by individual recognition of their songs. *Ibis*, **153**(2), pp. 255-268.

KIRSCHER, A.N., EARL, D.A., YAO, Y., ESCOBAR, I.A., VILCHES, E., VALLEJO, E.E. and TAYLOR, C.E., 2009. Using songs to identify individual Mexican antthrush *Formicarius moniliger*: Comparison of four classification methods. *Bioacoustics*, **19**(1-2), pp. 1-20.

KIRSCHER, A.N., ZANTI, Z., HARLOW, Z.T., VALLEJO, E.E., CODY, M.L. and TAYLOR, C.E., 2020. Females don't always sing in response to male song, but when they do, they sing to males with higher-pitched songs. *Animal Behaviour*, **166**, pp. 129-138.

KOGAN, J.A. and MARGOLIASH, D., 1998. Automated recognition of bird song elements from continuous recordings using dynamic time warping and hidden Markov models: A comparative study. *The Journal of the Acoustical Society of America*, **103**(4), pp. 2185-2196.

KUMAR, A., 2003. Acoustic communication in birds. *Resonance*, **8**(6), pp. 44-55.

LOVELL, S.F. and LEIN, M.R., 2004. Song variation in a population of Alder Flycatchers. *Journal of Field Ornithology*, **75**(2), pp. 146-151.

MACE, R., 1989. The relationship between daily routines of singing and foraging: an experiment on captive great tits *Parus major*. *Ibis*, **131**(3), pp. 415-420.

MAZEROLLE, M.J., 2019. *AICcmodavg: model selection and multimodel inference based on (Q) AIC (c)*. R package version 2, .

MCLOUGHLIN, M.P., STEWART, R. and MCELLIGOTT, A.G., 2019. Automated bioacoustics: methods in ecology and conservation and their potential for animal welfare monitoring. *Journal of the Royal Society Interface*, **16**(155), pp. 20190225.

MENNILL, D.J. and VEHRENCAMP, S.L., 2005. Sex differences in singing and duetting behavior of neotropical rufous-and-white wrens (*Thryothorus rufalbus*). *The Auk*, **122**(1), pp. 175-186.

MORTON, E.S., 1975. Ecological sources of selection on avian sounds. *The American Naturalist*, **109**(965), pp. 17-34.

NAGUIB, M., 2006. Animal communication: overview.

NEAL, L., BRIGGS, F., RAICH, R. and FERN, X.Z., 2011. Time-frequency segmentation of bird song in noisy acoustic environments, *2011 IEEE International Conference on Acoustics, Speech and Signal Processing (ICASSP) 2011*, IEEE, pp. 2012-2015.

O'LEARY, M. and STUART, A., 2021. Singing behaviour of male Rufous Scrub-birds in the New South Wales Gloucester Tops.

ODOM, K.J. and BENEDICT, L., 2018. A call to document female bird songs: Applications for diverse fields. *The Auk: Ornithological Advances*, **135**(2), pp. 314-325.

ODOM, K.J., HALL, M.L., RIEBEL, K., OMLAND, K.E. and LANGMORE, N.E., 2014. Female song is widespread and ancestral in songbirds. *Nature Communications*, **5**(1), pp. 1-6.

ODOM, K.J., OMLAND, K.E., MCCAFFREY, D.R., MONROE, M.K., CHRISTHILF, J.L., ROBERTS, N.S. and LOGUE, D.M., 2016. Typical males and unconventional females: songs and singing behaviors of a tropical, duetting oriole in the breeding and non-breeding season. *Frontiers in Ecology and Evolution*, **4**, pp. 14.

PATCHETT, R., KIRSCHER, A.N., ROBINS KING, J., STYLES, P. and CRESSWELL, W., 2021. Female song in the Cyprus Wheatear *Oenanthe cypriaca*. *Journal of Ornithology*, **162**(4), pp. 1199-1204.

PENAR, W., MAGIERA, A. and KLOCEK, C., 2020. Applications of bioacoustics in animal ecology. *Ecological Complexity*, **43**, pp. 100847.

PENTERIANI, V., DELGADO, M.D.M., STIGLIANO, R., CAMPIONI, L. and SÁNCHEZ, M., 2014. Owl dusk chorus is related to the quality of individuals and nest-sites. *Ibis*, **156**(4), pp. 892-895.

POTAMITIS, I., NTALAMPIRAS, S., JAHN, O. and RIEDE, K., 2014. Automatic bird sound detection in long real-field recordings: Applications and tools. *Applied Acoustics*, **80**, pp. 1-9.

PRICE, J.J., 2009. Evolution and life-history correlates of female song in the New World blackbirds. *Behavioral Ecology*, **20**(5), pp. 967-977.

- QUISPE, R., SÈBE, F., DA SILVA, M.L. and GAHR, M., 2016. Dawn-song onset coincides with increased HVC androgen receptor expression but is decoupled from high circulating testosterone in an equatorial songbird. *Physiology & Behavior*, **156**, pp. 1-7.
- RITSCHARD, M., LAUCHT, S., DALE, J. and BRUMM, H., 2011. Enhanced testosterone levels affect singing motivation but not song structure and amplitude in Bengalese finches. *Physiology & Behavior*, **102**(1), pp. 30-35.
- SEDDON, N., 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution*, **59**(1), pp. 200-215.
- SEDDON, N., MERRILL, R.M. and TOBIAS, J.A., 2008. Sexually selected traits predict patterns of species richness in a diverse clade of suboscine birds. *The American Naturalist*, **171**(5), pp. 620-631.
- SEDDON, N. and TOBIAS, J.A., 2010. Character displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. *Proceedings of the Royal Society B: Biological Sciences*, **277**(1693), pp. 2475-2483.
- SEDDON, N. and TOBIAS, J.A., 2006. Duets defend mates in a suboscine passerine, the warbling antbird (*Hypocnemis cantator*). *Behavioral Ecology*, **17**(1), pp. 73-83.
- SHONFIELD, J. and BAYNE, E., 2017. Autonomous recording units in avian ecological research: current use and future applications. *Avian Conservation and Ecology*, **12**(1),.
- SLABBEKOORN, H., ELLERS, J. and SMITH, T.B., 2002. Birdsong and sound transmission: the benefits of reverberations. *The Condor*, **104**(3), pp. 564-573.
- SLATER, P.J. and MANN, N.I., 2004. Why do the females of many bird species sing in the tropics? *Journal of Avian Biology*, **35**(4), pp. 289-294.
- STANLEY, C.Q., WALTER, M.H., VENKATRAMAN, M.X. and WILKINSON, G.S., 2016. Insect noise avoidance in the dawn chorus of Neotropical birds. *Animal Behaviour*, **112**, pp. 255-265.

- STOWELL, D., WOOD, M., STYLIANOU, Y. and GLOTIN, H., 2016. Bird detection in audio: a survey and a challenge, *2016 IEEE 26th International Workshop on Machine Learning for Signal Processing (MLSP) 2016*, IEEE, pp. 1-6.
- SUEUR, J., AUBIN, T. and SIMONIS, C., 2008. Seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics*, **18**(2), pp. 213-226.
- SWISTON, K.A. and MENNILL, D.J., 2009. Comparison of manual and automated methods for identifying target sounds in audio recordings of Pileated, Pale-billed, and putative Ivory-billed woodpeckers. *Journal of Field Ornithology*, **80**(1), pp. 42-50.
- TAVES, M.D., SCHMIDT, K.L., RUHR, I.M., KAPUSTA, K., PRIOR, N.H. and SOMA, K.K., 2010. Steroid concentrations in plasma, whole blood and brain: effects of saline perfusion to remove blood contamination from brain. *PLoS One*, **5**(12), pp. e15727.
- TOBIAS, J., BRAUN, J., BRUMFIELD, R., DERRYBERRY, E., KIRSCHER, A. and SEDDON, N., 2012. The importance of Neotropical suboscine birds as study systems in ecology and evolution. *Ornitologia Neotropical*, **23**, pp. 259-272.
- TOBIAS, J., GAMARRA-TOLEDO, V., GARCÍA-OLAECHEA, D., PULGARIN, P. and SEDDON, N., 2011. Year-round resource defence and the evolution of male and female song in suboscine birds: Social armaments are mutual ornaments. *Journal of Evolutionary Biology*, **24**(10), pp. 2118-2138.
- TOBIAS, J.A. and SEDDON, N., 2009. Signal jamming mediates sexual conflict in a duetting bird. *Current Biology*, **19**(7), pp. 577-582.
- TOUCHTON, J.M., SEDDON, N. and TOBIAS, J.A., 2014. Captive rearing experiments confirm song development without learning in a tracheophone suboscine bird. *PLoS one*, **9**(4), pp. e95746.
- TRIFA, V.M., KIRSCHER, A.N., TAYLOR, C.E. and VALLEJO, E.E., 2008. Automated species recognition of antbirds in a Mexican rainforest using hidden Markov models. *The Journal of the Acoustical Society of America*, **123**(4), pp. 2424-2431.

- ULLOA, J.S., GASC, A., GAUCHER, P., AUBIN, T., RÉJOU-MÉCHAIN, M. and SUEUR, J., 2016. Screening large audio datasets to determine the time and space distribution of Screaming Piha birds in a tropical forest. *Ecological Informatics*, **31**, pp. 91-99.
- URIBARRI, G., RODRÍGUEZ-CAJARVILLE, M.J., TUBARO, P.L., GOLLER, F. and MINDLIN, G.B., 2020. Unusual avian vocal mechanism facilitates encoding of body size. *Physical Review Letters*, **124**(9), pp. 098101.
- WARNER, R.W., 1972. The anatomy of the syrinx in passerine birds. *Journal of zoology*, **168**(3), pp. 381-393.
- WICKHAM, H., 2016. *ggplot2: elegant graphics for data analysis*. springer.
- WILD, J.M., 1997. Functional anatomy of neural pathways contributing to the control of song production in birds. *European journal of morphology*, **35**(4), pp. 303-325.
- WILEY, R.H., 1991. Associations of song properties with habitats for territorial oscine birds of eastern North America. *The American Naturalist*, **138**(4), pp. 973-993.
- WOOD, C.M., POPESCU, V.D., KLINCK, H., KEANE, J.J., GUTIÉRREZ, R., SAWYER, S.C. and PEERY, M.Z., 2019. Detecting small changes in populations at landscape scales: A bioacoustic site-occupancy framework. *Ecological Indicators*, **98**, pp. 492-507.
- WREGE, P.H., ROWLAND, E.D., KEEN, S. and SHIU, Y., 2017. Acoustic monitoring for conservation in tropical forests: examples from forest elephants. *Methods in Ecology and Evolution*, **8**(10), pp. 1292-1301.
- YORK, J.E., RADFORD, A.N., DE VRIES, B., GROOTHUIS, T.G. and YOUNG, A.J., 2016. Dominance-related seasonal song production is unrelated to circulating testosterone in a subtropical songbird. *General and comparative endocrinology*, **233**, pp. 43-52.
- ZWART, M.C., BAKER, A., MCGOWAN, P.J. and WHITTINGHAM, M.J., 2014. The use of automated bioacoustic recorders to replace human wildlife surveys: an example using nightjars. *PloS one*, **9**(7), pp. e102770.