Influence of distance in the courtship song produced by the male zebra finch

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by

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Certificate

This is to certify that this dissertation entitled **Influence of distance in the courtship song produced by the male zebra finch** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Mr Ninad Thulkar** at Indian Institute of Science Education and Research under the supervision of **Dr Raghav Rajan**, Assistant Professor, Department of Biology, during the academic year 2017-2018.

Raghav Rajan

Dr Raghav Rajan

Assistant Professor

Department of Biology

Committee:

Dr Raghav Rajan

Prof Aurnab Ghose

This thesis is dedicated to my lab people

Declaration

I hereby declare that the matter embodied in the report entitled **Influence of distance in the courtship song produced by the male zebra finch** are the results of the work carried out by me at the Department of biology, Indian Institute of Science Education and Research, Pune, under the supervision of **Dr Raghav Rajan** and the same has not been submitted elsewhere for any other degree

Ninad Thulkar

10th April 2023

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Abstract

Many animals (including humans) change the loudness of their vocal signals according to distance from a receiver and background noise. The mechanisms underlying this change remain poorly understood. The song of the male zebra finch has many parallels to human speech and provides a good model to understand mechanisms involved in changing vocal signal loudness. Previous studies have shown that zebra finches increase song amplitude when distance from a female increases. However, these were recorded with a microphone fixed above the cage without controlling for changes in amplitude with changes in position of the bird relative to the microphone. More recent studies from our lab measured song amplitude with a microphone implanted on the head of the male zebra finch. The head implanted microphone showed no consistent trend in song amplitude with increasing distance from the female, but signals also showed considerable day-to-day variation suggesting a malfunction of the microphone. To eliminate the possibility of microphone malfunction and to record amplitude more accurately, I implanted birds with 2 microphones on their heads. Using this setup, I first showed that signals were correlated across the two microphones for the most part. However, there was some amount of uncorrelated noise in both microphones and I characterized some sources of this noise. Second, I did not find any significant change in song amplitude with changes in distance and/or background noise. Overall, these results demonstrate the potential of using a two-microphone system to further characterize vocal amplitude.

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Acknowledgments

I wish to express my deep gratitude to my supervisor, Dr Raghav Rajan, for his invaluable guidance and support. He has helped me through every stage of my project, and has always been very encouraging and approachable. Discussions with him were very insightful and helped keep me on track. I am indebted to him for creating a stimulating environment that facilitated my professional development.

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Contributions

Contributor name	Contributor role
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Raghav Rajan, Harini Suri, Ninad Thulkar, Anand Krishnan, Vidushi Sharma, Mahima Gautam	Methodology
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Raghav Rajan	Supervision
Raghav Rajan, Ninad Thulkar	Project administration
Raghav Rajan	Funding acquisition

This contributor syntax is based on the Journal of Cell Science CRediT Taxonomy[1].

[1] https://journals.biologists.com/jcs/pages/author-contributions

Chapter 1 Introduction

The fundamental idea of communication is for one animal to receive information via a signal produced by another animal, for mutual benefit. Animals use four major types of signals, which are: visual, auditory, tactile, and chemical signals, to communicate among themselves(Marler, 1967). Before reaching a recipient, the physical environment will significantly impact the signals a sender generates. The distance between the sender and receiver will also influence signal reception. For communication to remain effective, senders need to adapt their signals with increasing distance from a receiver.

Every type of signal has its own advantage to it, such as tactile and olfactory signals will be more advantageous for close proximity interactions. For example, ants use pheromone trails to follow each other(Fletcher & Brand, 1968), and baboons use touch to show affection and groom each other(Anthoney, 1968). For long-range communication, tactile communication will not be much more efficient than other signals. The chemical signal can be helpful along with visual and auditory signals for long-range animal communication. In the case of visual signals, animals try to maximise the contrast along with vigorous movements to grasp the attention of another animal. Peacocks use their elaborate tail during their courtship rituals(Davison, 1983), and fireflies glow in the dark to attract their mates(Lloyd, 1973). But the effectiveness of this signal can easily be obliterated by a non-homogeneous background. Both the selected examples will be well suited if they are performed under certain environmental conditions. Chemical and auditory cues bypass those obstacles more easily than visual signals. Thus, visual cues are also not suitable for long-range communication. In higher-order animals, communication with chemical signals is less common compared to auditory signals. Hence, primates prefer vocal signals to interact with one another for close and long-range communication(Marler, 1967). It can be produced in a variety of ways to signal any kind of distress, danger, affection, or any other kind of message. Therefore, the auditory signal is more advantageous than other types of signals for a in depicting these diverse ranges of conditions.

A human language is a more advanced way of communication in which various words and phrases are put together in certain ways to express complicated signals in an easy manner. Although primates don't have complex vocalisations like human language, but many primates chose vocal communication over other signals to interact among themselves. Primates along with the other higher-order animals incorporate their other sense organs to properly interact with one another. When animals try to interact, they unconsciously change their vocal amplitude according to their immediate environmental conditions. In other words, many animals adjust their vocal signal if the receiver is further awa Coen et al., 2016; Cynx & Gell, 2004; Dabelsteen et al., 1998) y or if there is a lot of background noise in their immediate environment (Lombard effect)(Brenowitz, 1986; Coen et al., 2016; Cynx et al., 1998; Dabelsteen et al., 1998).

In both situations, every other animal including primates and humans, their first instinct was to increase the amplitude of their vocal signal. Even small kids (age three years) know that they need to increase their vocal intensity if the listener is farther away(Johnson et al., 1981). The Lombard effect can also be seen in many other animals, where the animals try to increase the amplitude of their vocal signal on a voluntary basis. Male green frog Rana clamitans(Owen & Gordon, 2005) alter the amplitude of their aggressive calls(Wells & Schwartz, 1984) based on the stimulus intensity. Amphibians are also capable of changing the intensity of courtship signals, which was later on observed in the rest of the vertebrates(Roy, 1996). Similar to humans, the Japanese quail Coturnix coturnix japonica also increases the amplitude of its vocal signal, if background noise exists around them(Potash, 2013), for selective hearing. The Lombard effect is not just restricted to the animals which are interacting vocally. Other animals who use different signals to communicate also happens to follow it. Such as the fiddler crab Uca perplexa changes the intensity of the claw waving display if the receiver is further away(How et al., 2008)

To understand the working of complex human language, we need a simple model organism to test those hypotheses along with some modifications. Therefore, songbirds are considered a good model organism for understanding the learning of complex vocal signals(How et al., 2008). Songbirds majorly interact among themselves during their courtship rituals(Soha & Peters, 2015). As a part of their courtship rituals, almost all the songbirds will sing a song, which is easier for us to study vocal communication in animals. Among all the songbirds, zebra finches are the most commonly used songbird(Nottebohm & Arnold, 1976) because of three major factors:

The first and most important one for choosing the zebra finch over other songbirds is that they have a stereotyped crystallised song throughout their entire lifetime(Lachlan et al., 2016; Soha & Peters, 2015). That the male zebra finch will sing the same song every other time whenever he wants to mate with the other female. The second reason is that they can be bred in captivity and thus are the perfect model organism to perform experiments in laboratory conditions. The third one is that learning courtship songs in juvenile zebra finches is very much similar to human speech learning(*BIRDSONG AND HUMAN SPEECH: Common Themes and Mechanisms*, 1999; Soha & Peters, 2015).

Only male birds produce a courtship song in the zebra finches, and female birds only produces call(Riebel et al., 2002). Songs produced by the male zebra finch are classified as directed and undirected(Price, 1979; Zann et al., 1996). A directed song is further distinguished from an undirected song by observing some of the song features and the posture of the finch. The directed song contains a higher number of Introductory notes (INs) and motifs, with a more rigid element sequence with a faster tempo of the song(Sossinka & Böhner, 1980).

Therefore, a zebra finch song starts with INs, followed by a few motifs, to form a bout. Between the two bouts, there is a long pause of more than two seconds or less and a short pause of half a second that can be seen in between the two motifs within a single bout. A motif consists of some syllables produced in the same order whenever the bird intends to sing a song(Price, 1979). Syllables are the smallest continuous component of the song, and INs are the series of identical repeats produced prior to the start of the song.

Vocal signals are visually analysed by representing them as a spectrogram, which is a visual way to represent the signal strength over time at various frequencies present in the particular waveform. The intensity of the sound can be represented by the darker shade of the colour code, which is used to represent the spectrogram. So, representing the song of a zebra finch on the spectrogram to label and analyse is a widely used method(Price, 1979; Sossinka & Böhner, 1980).

Since zebra finches are considered a good model organism for Human learning it is necessary to see if the Lombard effect and selective hearing can be seen in zebra finches or not. And according to previous studies(Brumm & Slater, 2006; Suri & Rajan, 2018), zebra finches follow the principles of the Lombard effect, i.e., if the female bird is farther away from the male bird(Cynx & Gell, 2004) and when there is a cocktail party like situations(Cynx et al., 1998) to convey their message more conveniently. But all the studies failed to quantify accurately the change in amplitude made by a male zebra finch in this situation.

In earlier studies, researchers tried to record the zebra finch song in captivity, where the male and female birds are placed in front of each other. A mic is placed a few centimetres above the cage. They used an omnidirectional mic, which is placed in such a way that it is equidistant from both birds(Brumm & Slater, 2006; Cynx et al., 1998; Cynx & Gell, 2004). But placing the mic a few centimetres above the cage will not help us to quantify the change made by the bird, as the vocal intensity is getting compensated because of the distance in between mic and beak of the bird and we will not be able to track the exact changes made by the bird(Michael et al., 1995). So, another study from our lab(Suri & Rajan, 2018) tried to record the amplitude of the bird's song by implanting a mic on the bird's head. The results of that study show that there is a change in the amplitude of the bird's song. While quantifying the change, one can observe a drastic change in the amplitude of the bird's song from one day to another. It is not clear if this change was due to malfunction of the mic or due to the bird changing its song amplitude from day-to-day.

To tackle this problem, I implanted two mics on the bird's head. The reason for implanting two mics on the bird's head is that the two mics will record the same courtship song with an almost similar amplitude level. There can be a minute difference in the amplitude level of the song captured by the two mics. If one of the mics is not working properly, then there will be a huge difference between the amplitude level of the song captured by the two mics. If the two mics show a correlated increase in amplitude, then we can certainly say that zebra finches can also drastically change the amplitude level of their courtship song.

Therefore, the aim of this project is to implant two mics on a bird's head (one beside the other) and record the amplitude level of the courtship song under two different conditions

1. When the female bird is at different distance (00 and 20cm) away from the male.
2. When there is different levels of background noise.

Chapter 2 Methods

All procedures at IISER Pune were conducted after approval from the Institute Animal Ethics Committee (IAEC), IISER Pune and were in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi.

This study used ten adult male zebra finches and ten female zebra finches (>120 days post-hatch). These birds were either purchased from an outside source or bred in the bird colony at IISER Pune. All birds were housed in group cages in a colony with a 14-hour (light): 10-hour (dark) cycle. Before the start of the experiment, all birds were first separated into individual cages and were isolated in a sound-attenuated chamber for 2-7 days prior to the start of the experiment.

Head-fixed Microphone

To accurately record the bird's song, we surgically implanted two microphones (CUI, CME-1538-100LB purchased from Mouser Electronics, TX) on the bird's head by removing the upper layer of the skull. Because of the bird's vigorous movements, these mics were soldered onto the connector and then glued properly so the soldered wire should not come out. Two mics are soldered onto the connector, and two mics will capture the same song.

The second mic is soldered just behind the first mic onto the connector in the same orientation as of the first one. In other words, the positive terminal of both mics will be right just behind the other, and the same is true for the ground terminal.

Dental cement (DPI Cold Cure) was used to implant the mic on the bird's head, adding some weight to the bird's head. The weight of all these things were less than 1 gram, measured after the mics fell and were recovered at the end of the experiment.

The connector attached at the other end of the copper wire perfectly fits into the connector soldered to the mic implanted on the bird's head. While inserting one connector (copper wire) into the connector of the mic, we ensured that it is not loose and will not come off while recording the bird's song.

Surgery:

All surgery related equipment like a scalpel blade, forceps, scissors, etc were sterilised by placing them in a hot bead steriliser (Fine Science Tools) for a few minutes and then cleaned thoroughly with 99.9% ethanol.

An oral analgesic (meloxicam, 12.5mg/kg) was administered at least 1 hour before the surgery. Within that 1 hour of duration, the bird was not allowed to eat anything other than water. This was done by removing the seed cup. Birds were anesthetised with 4% isoflurane and an absence of response to toe pinch was used as an indicator of surgical level of anaesthesia. The bird was put in a 'bird jacket' and placed in the stereotaxic apparatus. Anaesthesia was maintained with 1-4% isoflurane, while performing the surgery on the bird, depending on the response of the bird. For that, respiration was constantly monitored and isoflurane levels were adjusted to ensure surgical plane of anaesthesia and regular breathing pattern. In addition, a heat lamp was placed nearby to keep the bird warm (as thermoregulation does not work well during anaesthesia).

To perform the surgery on the bird, we needed to fix his head onto the stereotaxic apparatus, so that it will remain intact onto the stereotaxic apparatus. To fix the head properly, the feathers over the ear pinnae are trimmed with the help of surgical scissors for ease to fix the bird's head using the ear bars onto the stereotaxic apparatus. The bird's head was held in a fixed position with the help of beak and ear bars. Further, the bird's body was held in place by taping the jacket onto the stereotaxic apparatus. Feathers on the scalp of the bird were plucked out for accessing the skull. The layer of skin on the skull was anesthetized by injecting a local anaesthetic, lignocaine (25-50 microliter).

The skull is exposed by removing the layer of skin over it. To make the dental cement stick to the skull, the skull is roughened before fixing the mic. The combined weight of a custom-made mic and the dental cement was <1 gram, which is tolerated by a 13-17 gram of zebra finch.

After the completion of the surgery, the bird was removed from the stereotaxic apparatus and placed back into its cage. Once the bird was completely awake it was given, 100 microliters of dextrose to help in recovery process. The bird was kept under an observation in a sound-attenuated chamber for two days. If the bird is fit and fine after the observatory period, only then, the bird will be used for the experimental recording.

Figure 1: Picture of the bird after successful mic implantation of the two mics and the connector attached. The pink layer is the dental cement holding and covering the two mics on the head of the bird.

Experimental setup:

To connect the head-implanted mic to the bird's head, a thin copper wire with the connectors was made. Another end of that thin copper wire was connected to a frictionless commutator, which will rotate along with the torsional strain of the wire to avoid restricting the moment of the bird within the cage. Another end of the commutator is connected to the audio jack of the PC, which will convert the audio signals into an electrical signal while recording the bird's song. A custom python based program was used to record audio signals.

To avoid any disturbance from the electromagnetic signals from the power line, we tried to shield and ground the wire which is connecting the commutator to the PC. Similarly, the experimental cage will have the Faraday cage effect, which is also grounded along with the shielded wire. While recording, another cage was kept beside the male experimental cage to keep a female bird. Both the cages were kept on the table beside the PC, to record the directed song of the male zebra finch.

A Bluetooth speaker was kept at the back of the cage in between the two experimental cages to play white noise and colony noise during the experiment. The loudness of the speaker was kept at 10% of its loudness which was around 55-60dB, with the help of decibel meter. The rest of the sides were covered by a white sheet of paper, so that two birds, won't be scared of wires.

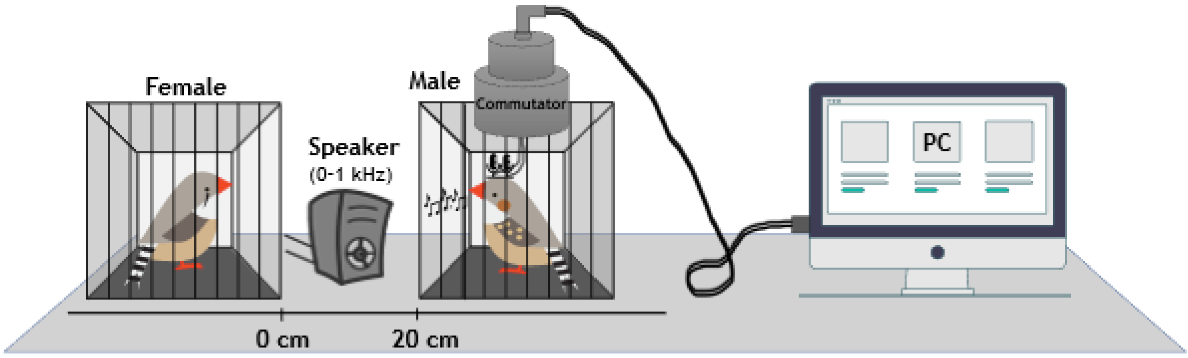

Figure 2: Representation of the experimental setup kept on the top of the table, along with the PC kept beside the cage to record the song of the bird. The speaker was kept behind the cages and within the wall an electrical cable was fixed within the wall the for the power supply to the PC. Credits: Anand Krishnan, Pradeep Trimbake (for animated finch).

Experimental procedure:

Habituation

When the bird was kept in a sound-attenuated chamber for observation, the bird eventually learns to bear the mic's weight while recovering from the surgery. The observation period for the bird was for two days, during which the singing of song and diet of the bird gets back to normal. If due to some reason, it is not back to normal, then the observation period is extended until the bird returns to his fully fit and fine state.

The next step is to learn to bear the mic's weight when the connector is attached to it. For the initial days of the training phase, a bird was kept in the experimental cage with the connector attached to the mic for the rest of the day so that the bird gets acclimatised to the experimental setup.

During this time, the recording was kept on, to see at what time that male bird is most active. That time slot will be chosen for that bird to record, which is also known as active hours of the bird. At most, six training sessions will occur during the active hours, during which the bird must learn to respond for at least four sessions to proceed to the next stage of the training phase.

Training

The male bird must learn to respond to the female bird in all four conditions within the time span of 5 mins, during the active hours. No food or water cups were kept in either of the experimental cages, and singing a song is an energy-consuming process for the male birds. So, if the bird sings a song for the female bird, then the male bird was moved back into his original cage, where he can access food and water. As part of the reward, his cage was moved back into the colony, where other birds were kept in separate cages.

If the male bird don't respond or sing to the female bird, he was left in an experimental cage, where he would not have access to food and water as a part of the punishment. The connector was left connected to the mic, and the lights of an experimental room are turned off for half an hour.

During the training phase, the male bird had to sing a song for the female bird, in the most basic condition, i.e., for 00cm with no noise. Gradually, the bird learns to sing a song for the female in all other experimental conditions. The final stage of the training phase for the bird was to learn to sing a song for female bird when the cages were 20cm apart in the presence of white noise. After that, the bird was ready for the experiment. The number of days required for the bird to train completely for the experiment depends totally on the behavioural responses of the male bird, which may take a month or two to make one bird fully ready for the experiment.

Recording of bird:

Before the start of the recoding, the male bird was shifted to the male experimental cage (during the active hours), and the connector was plugged on to the mic. The bird would be held in front of the Bluetooth speaker, producing twelve beep sounds from 500Hz to 6000Hz (audible range of zebra finch), with an increment of 500Hz after each beep. This was our control experiment which would let us know if the two mics on the bird's head were working properly or not. A female bird should be placed in the female experimental cage. While keeping the female bird into the female cage, the lights of the experimental room had to be switched OFF.

After the start of the recording, lights were switched ON, and he starts to sing, immediately after sighting the female bird next to his cage. Based on the response of the male bird during the 5 minutes while recording, he would get either get reward or punishment for the next half an hour. Rest of the sessions were also recorded in the similar manner.

For one day of recording, there should not be more than six sessions, or else it may exceed the time window of active hours, for the rest of the sessions. For a good sample size, ten days of data will be enough for each session, where the bird must respond to every other session of that particular day.

As two connectors are connected, the circuit is completed, in which the two mics are connected to the USB sound card, which is attached to the PC for recording purposes. We capture the male bird's song using the PyCBS (custom-made python code) recorded at a 44100Hz sampling rate.

Song Analysis:

The recorded sound is then labelled using the AutoSongSegmentLabel (ASSL; custom-made MATLAB code). Using this code, we annotated the syllables produced by the bird in his song. Distinct syllables were annotated differently based on the spectrogram and voltage fluctuation captured while recording. To annotate the syllables, a distinct amplitude threshold is selected for every bird based on the visual inspection of a few song files. The selected threshold will then be kept constant for all the recorded sessions for a given bird.

Individual syllables were identified visually to make a template file for a given bird to make the labelling more prominent and accurate. The code labels the rest of the recorded files using the template file and then this was checked manually for consistency.

Before labelling the recording of any one mic, the recording of both mics was synchronised using SynchroniseWaveFiles_ForTwoMics (custom-made MATLAB code). This code synchronised the recorded data of the two files by aligning the first few syllables from the two files. Since the two mics will capture the same song, the syllables captured by the other mic will also be the same. So, the song captured by the other mic is labelled using MakeNoteFiles_ForOtherMic (another custom-made MATLAB code) using the onset-offset data from the first mic.

When the labelling is finished, ASSL will give a text file mentioning every feature of the labelled syllables. From this data, the Log and RMS Amplitude of the labelled syllables is used for further analysis.

Simulating a mathematical model to get the correlation between the two mics

Based on the preliminary data, I tried writing a MATLAB code to simulate some desired plots for examining the results obtained from the first bird. This mathematical model would help me get the plots for an ideal situation if the two mics were correlated to each other. In addition, this model was also capable of generating the plot of the two mics if one of the two mics was capturing the random noise when both were perfectly correlated to each other.

Troubleshooting experiment

We tried performing some playback experiments to find the cause of the capture of random noise detected by either of the two mics. For this, we kept the spare mics in a hanging position just above a speaker placed inside the middle of the male bird's experimental cage.

For the playback experiment-1, I tried keeping the two mics in all possible orientations (six different orientations) while recording from it. While performing this experiment, we used a Bluetooth speaker to play an audio file recorded from some other bird.

In the playback experiment-2, I used a simple speaker and a new pair of two mics which would later be used to implant the bird's head. An audio file was chosen from some random bird, which had a single bout in it. That bout was processed in MATLAB to increase the amplitude level by 2, 5 and 10 times its earlier level. During the playback experiment, the modified audio file was played from the simple speaker for two sessions. The difference between the two sessions was the presence and absence of shielding of the wire that was connecting the commutator to the PC.

There was one more troubleshooting experiment where I suspected that the connector might be loose and because that, it may have been capturing the random noise while performing the experiment. That time, I avoided to unplug the connector from the mic, which completes the circuit with the PC for recording purpose. When the bird was kept in reward, instead of placing him back into his original cage, I placed the seed and water cup within the experimental cage only, in the presence of light. Since, singing a courtship song was an energy consuming process for the bird, he would feed himself while enjoying the half an hour of reward time.

Chapter 3 Results

The log amplitude captured by both mics were positively correlated

It had been observed that there was a drastic decrease in the amplitude of the syllable on a certain day for the same condition in previous studies (Suri & Rajan, 2018). Before the start of the experiment, I decided to check if the two mics were correlated or not. For this, I plotted the Log amplitude of a single syllable across the mic1 vs mic2 plot, where different days and conditions were represented by different colours and shapes, respectively. From the graph, it was observed that the two mics were correlated for that particular day for all the four conditions, but unfortunately, they w

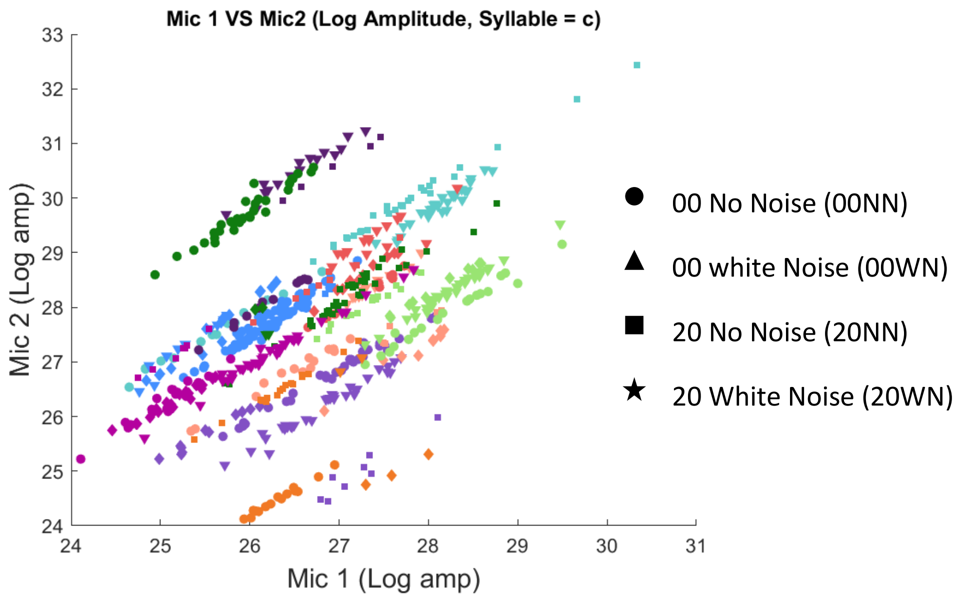

Figure 3: The Log amplitude plot for c-syllable.
The Log amplitude values of the mentioned syllable for the two mics were represented. Different colours were representing the days and four different conditions were represented by four different shapes. Each point depicts the occurrence of the syllable with the average value of that syllable.

Simulation of log amplitude values for the two mics

As the two mics were not correlated across different days, I hypothesised that some random noise could have been captured along with the song of the bird. To verify that, I simulated some plots on MATLAB where the two graphs were depicting how it will look if the two mics correlated and uncorrelated. From the previous experiment, it was observed that the two mics were correlated, so I added some random number in equation of the graph which was depicting the prefect correlation between the two mics. From the simulated graphs, it was confirmed that some amount of random noise was being capture along with the song of the bird.

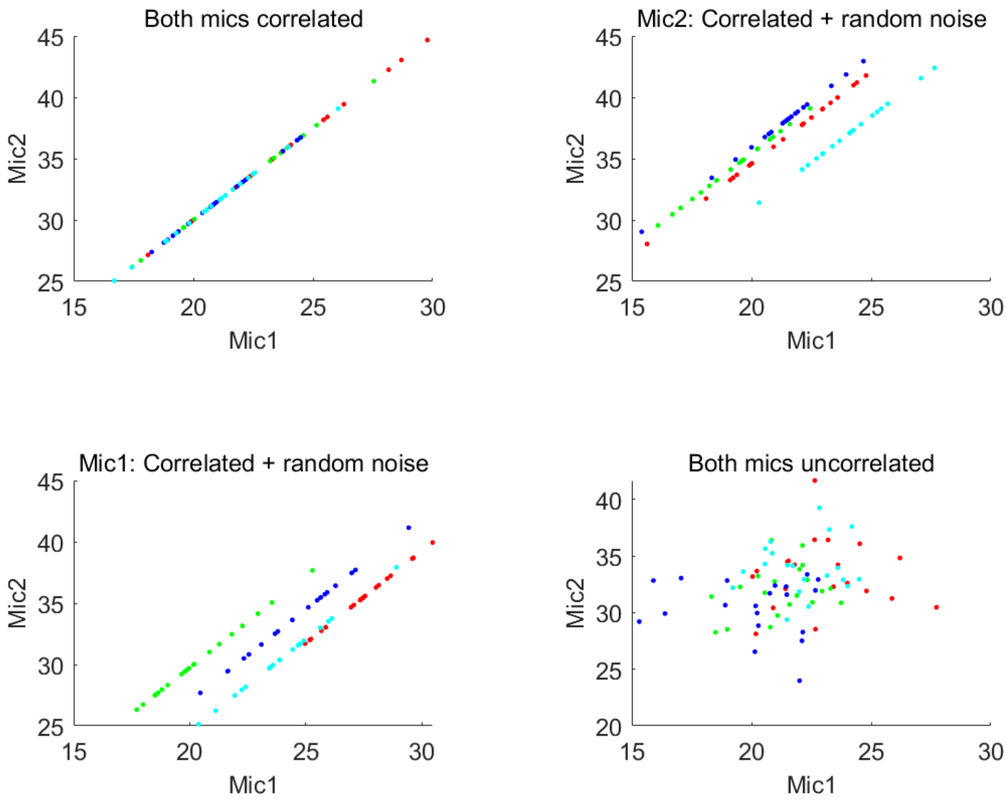

Figure 4: Mathematical model for the Log Amplitude values of mic1 vs mic2, where the mic2 values were 1.5 times of mic1 Log amplitude values. Different colour represents each session, and an individual dot represented a single syllable.

From the simulated graph, if the two mics were perfectly correlated, then all the points must lie within the diagonal axis. If one of the two mics must be capturing some random noise along with the bird song, then there would be shift along the diagonal axis for the particular session. And if the two mics were not at all correlated, then the dots would be spread across the XY-plane for the two mics. From the simulated mathematical model, I concluded that random noise was captured by either of the two mics, which must be responsible for the shift of syllable points throughout the particular session.

Playback experiments to examine random noise captured by the two mics

I performed some audio playback experiments using an extra pair of mics to detect and reduce the noise captured by the two mics along with a small speaker. While performing those playback experiments, it was kept inside the cage exactly at the centre of the cage, and the two mics were hanging a few centimetres above the speaker.

Shielding of wires could reduce the spread of points from the diagonal axis for the two mics within a session

Since, it was confirmed from the simulation that some random noise was being captured along with the song of the bird. I speculated that the electromagnetic radiation from the electric power line to the PC might hinder with the recorded data. The wire which was connecting the commutator to the PC was carrying the electrical data (non-digitalised electrical data) was more prone to disturbance. To avoid that electromagnetic radiation, I covered the above mentioned wire with the aluminium foil, so that whatever charges that are hindering the recorded data, will reside on the surface of the wire. Therefore, I performed two playback experiment in with shielded and unshielded wire. From the graph, it was seen that my speculation about the electromagnetic effect was correct. From the graph, it was observed that shielding of wire, reduces the capture of noise up to a large extent. But even after that even after that the was some level of random noise being captured.

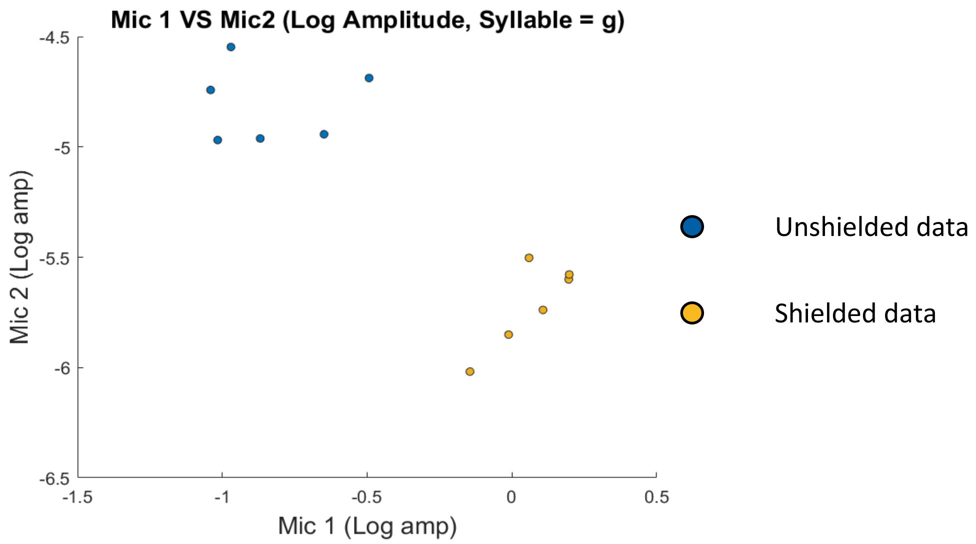

Figure 5: Log amplitude plots for playback experiment-1, where the two sessions were recorded using the shielded and unshielded wires (connecting to the commutator to the PC). For both the sessions, the distance between the two mics and speaker was kept constant.

Bending the wires of the two mics was responsible for the shift of amplitude values across the two mics

As the male bird tends to hop to-and-fro while singing a courtship song for a female. At the time. I observed that the amplitude is getting fluctuated similar to the hopping behaviour. Therefore, I hypothesised that the deviation of points from the diagonal axis was because of the bend in the wires of the two mics. For that, I decided to perform another playback experiment, where the dummy mics were kept in a different orientation just above the speaker.

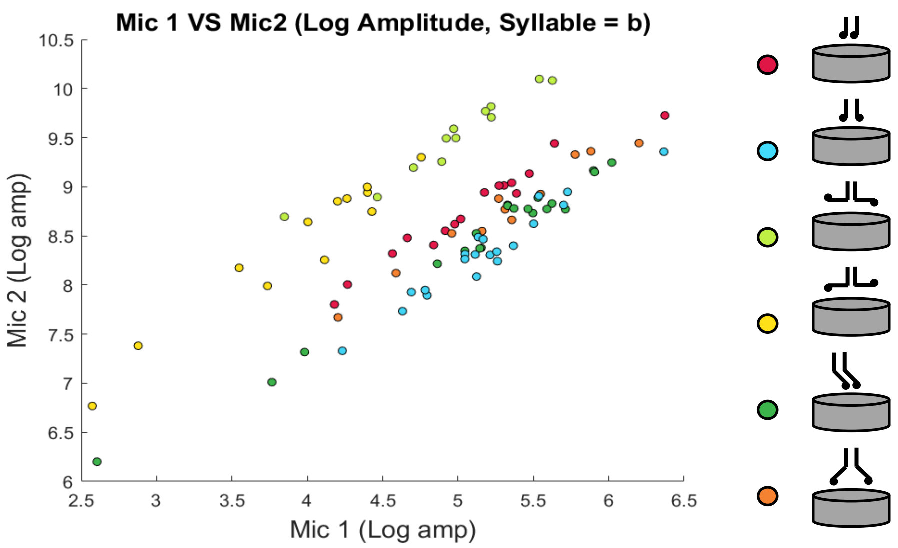

Figure 6: Log amplitude plot for the playback experiment-2, where the *bending the wires of the two mics was responsible for the shift in points across diagonal axis. Amplitude values of a single syllable for the two mics was shifted from the diagonal axis if they were bend up to certain extent. Here the lime and yellow-coloured sessions had bent in their wires around 90 degrees.*

During the session-3 (lime) and 4 (yellow), the wires were bent around 90°, were correlated, but at the same time it was shifter from the diagonal axis. For the rest of the sessions, almost all the points were lying on the diagonal axis. From this playback experiment, I confirmed that the shaking or bending of wires must be avoid to reduce the capture of random noise along with the song of the bird. To avoid the bending of wire, I tried to cover wires coming out from the speaker with the dental cement which would restrict bending of it.

Reconnecting mics multiple times did not affect amplitude measurement

I wanted to test if connecting and disconnecting the two mics causes a systematic shift in the amplitude recorded by the mic. Therefore, I recorded four sessions of one day for a bird without unplugging the connector from the two mics. In between the two sessions, I haven't unplugged the two mics. During that time, I was keeping the seed and water cups inside experimental cage as part of his reward. From this, I get to know that there still there was the shit was still there in the amplitude recorded by the two mics. Which concludes that the systematic shift which I was observing was not because of the plugging and unplugging of the connector to the two mics implanted on the bird's head.

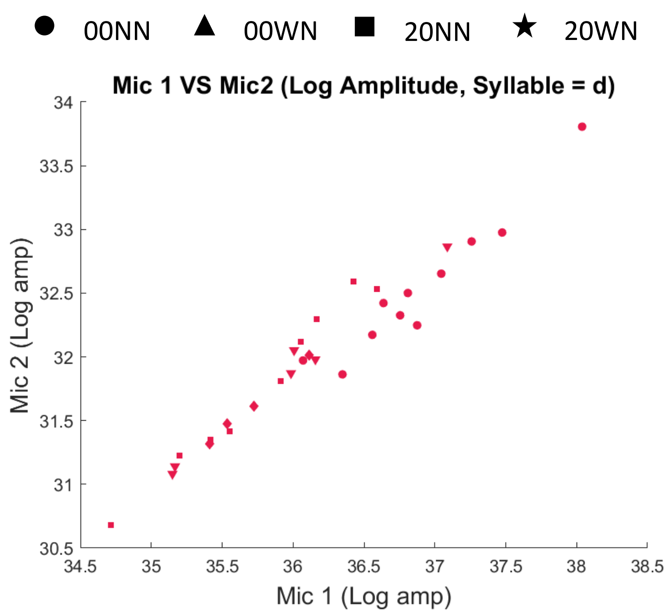

Figure 7: Reconnecting mics multiple times did not affect amplitude measurement.
Each dot represented the average value of a log amplitude of a single syllable. Shapes were representing the conditions.

A significant reduction of spread of the Log amplitude values across the diagonal axis

I modified the experimental setup with the help of those playback experiments, and recorded the second bird. To observe a distinct change within for the syllable values, I recorded the same bird for the next five days with the shielded setup. By implementing all the modification to the experimental setup, I was able to reduce the spread across the diagonal axis up to much greater extent. From the plot I concluded that shielding helped me to reduce the spread across the diagonal line for that particular day. I assumed that the little spread across the diagonal line will always be there in the real world. I had to quantify the error for a single day along with the shift observed across the rest of the days.

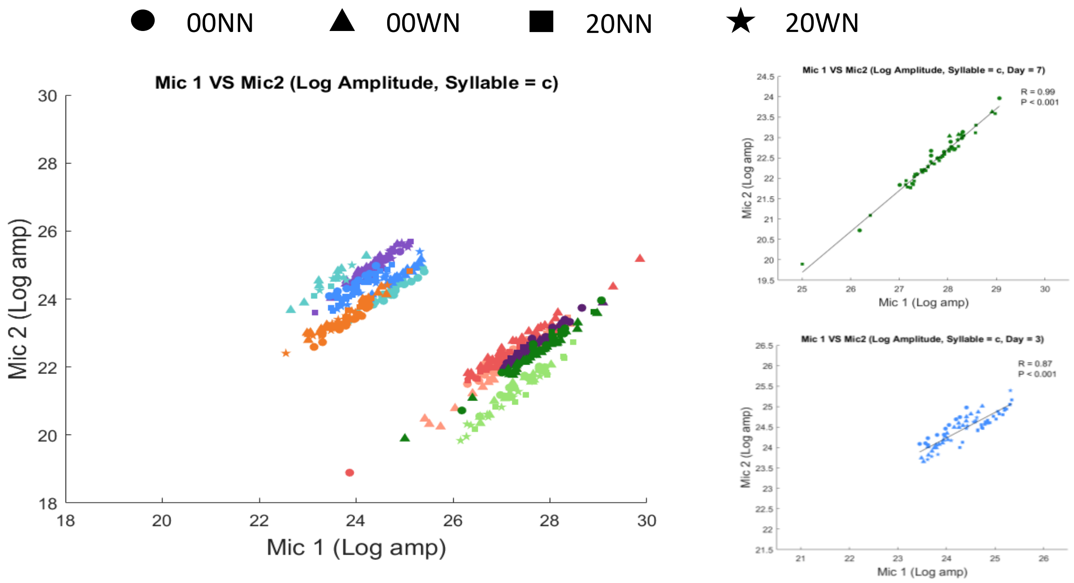

Figure 8: A significant reduction of the spread of the Log amplitude values across the diagonal axis. Log Amplitude plot for the second bird, where different days and colours were represented by different colours and shapes, respectively. The upper cluster in the bigger plot represents the five-day data of the unshielded setup, and the lower cluster represents the five days of data obtained from the shielded setup.

The Log amplitude values of both mics were correlated for a given day

Before quantifying the error, I observed decided to observe the shift of a single syllable across the four conditions within a day. Therefore, I plotted the average amplitude of each syllable for mic1 and 2, including the four conditions in it. From the plots, there was a distinct amount of shift across the four conditions within a day. By then, I could not figure out any other way to reduce the shift across the four conditions. Therefore, I decided to quantify that as well along with, the spread of the Log amplitude values for a given day.

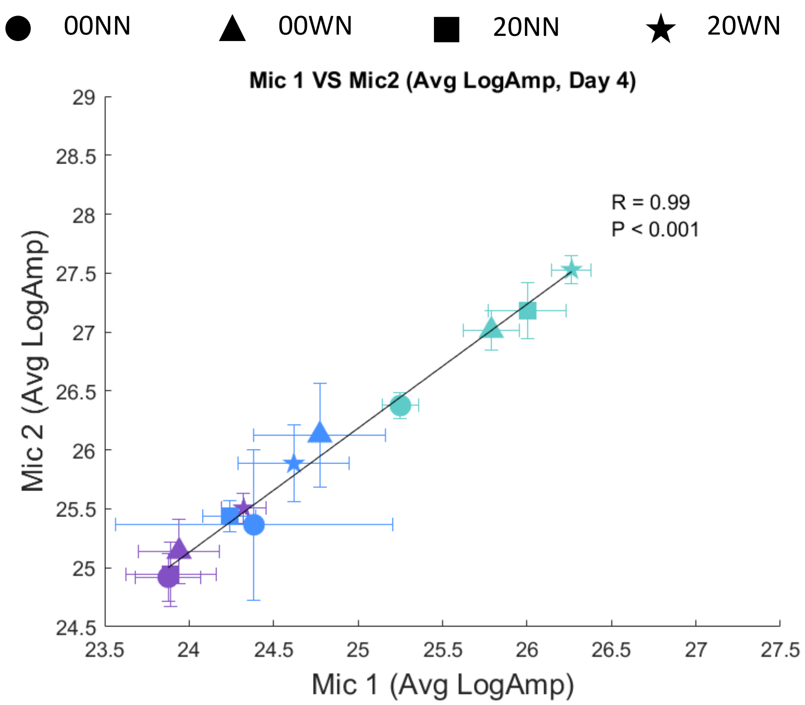

Figure 9: The Log amplitude values of both mics were correlated for a given day
Average plots across mic1 and mic2, where all the Log amplitude values for a unique set of syllables were merged into a single value for 3rd bird. Each colour represents an independent syllable used by the bird in his song during the four different conditions. The error bars were depicting the standard error for that particular point.

The Log amplitude values varies across days for both mics, irrespective of condition

To quantify the change that occurred across the days for both mics, I plot the range of average value of syllables along with the range of it for the different sessions of that day. Since, I hypothesised that the 00NN condition would be the most basic condition among the rest of the conditions. So, I normalised the rest of the sessions with 00NN (most basic condition) from the same graph. I observe some trend followed in the graph plotted and concluded that there was no correlation for a unique syllable across the four conditions for all days.

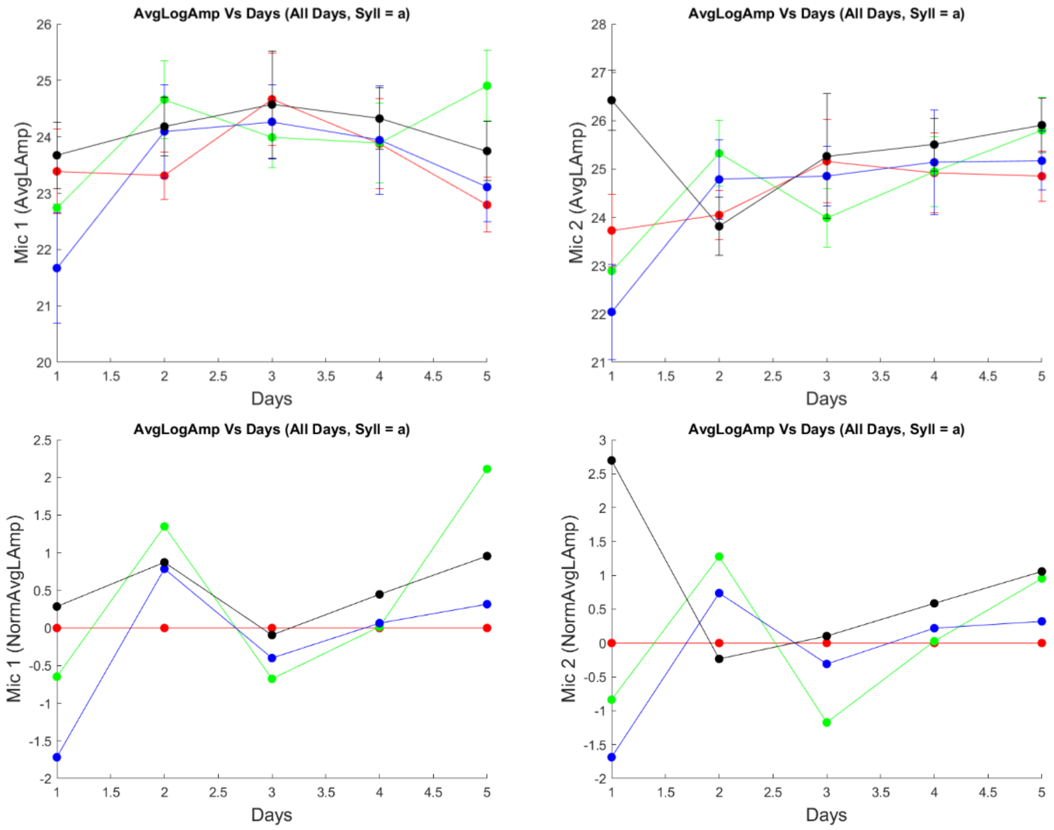

Figure 10: The Log amplitude values vary across days for both mics, irrespective of the condition [Upper set of graphs] Representation of the average Log amplitude values of a unique set of syllables for each day plotted across days across the two mics, differently for mic1 and mic2. [Lower set of graphs] Representation of the same data in a normalised format according to the 00NN condition. Each colour represented the following conditions: red-00NN, blue-00WN, green-20NN, and black-20WN.

Similar trend for variance between the two mics for all the syllables

As there was no trend seen across the four conditions for all day for the two mics, I decided to focus on the variance among the two mics for the same type of graphs which was having a similar kind of variance between the two mics. Therefore, I concluded that the two mics were working fine, and the difference in the amplitude level for the two mics was because of some other factor.

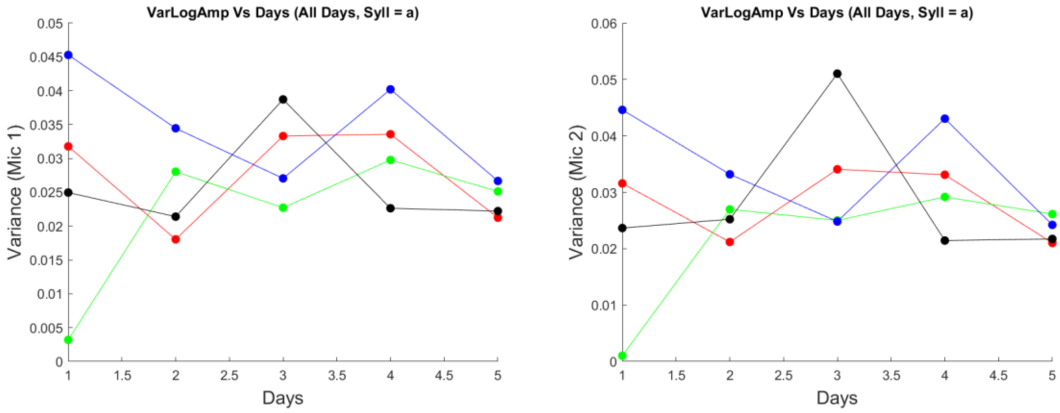

Figure 11: Similar trend for variance between the two mics for all the syllables
The variance of average Log amplitude values for each day was plotted for a single syllable differently for mic1 and mic2. Each point represents the average value of the syllable (mentioned in the title) for the mentioned day on the X-axis. Each colour represented the following conditions: red-00NN, blue-00WN, green-20NN, and black-20WN.

Similar trend of variance level for the Log amplitude of the two mics across different condition

From the beginning, I was looking for some trend across the four different conditions to a unique syllable for different days. In the search of some specific change across the four conditions, the scatter plot was not enough to observe whether there was an increase or decrease among the four conditions. Hence, I plotted the same data in a box plot format where the average value of a particular syllable was clubbed together for one day, and was joined by a line across all four different conditions. Because of that I got a similar trend for the variance level of the Log amplitude data of each syllable across the two mics. So, I concluded from the variance level recorded from both the mics that the two mics were correlated and working fine. Different syllables exhibit different trends in variance across days and conditions. That was observed by the difference in the mean and standard deviation of variance across conditions for different syllables.

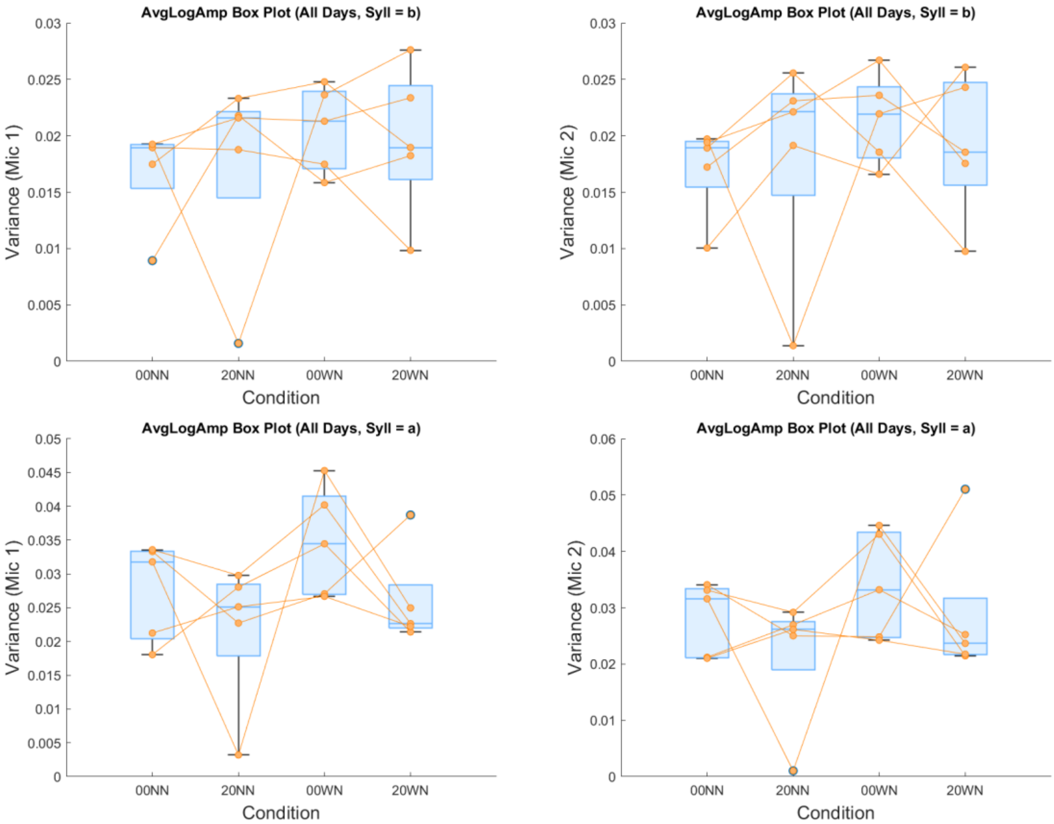

Figure 12: Similar trend of variance level of the Log amplitude of the two mics across different condition Representation of the variance of the average log amplitude values across the four different conditions for a single syllable. Each syllable inferred a different kind of trend across the four conditions, and the trend for mic1 and mic2 was the same for each syllable. Each point represents the average variance value of log amplitude for a single day.

Similar trend across conditions was observed for both mics

At that time, I focused on finding some similar trend for the Log amplitude values of the two mics across all conditions. To get any trend, I observed the change in the average Log amplitude values of all the syllables across different conditions for a single day. To view the results more accurately, I normalised the data of all the syllables according to the basic condition of my hypothesis. The graph points that, the trend for each syllable across all the conditions for one day was preserved in both mics, which meant that the two mics implanted on the bird's head were correlated and were working properly.

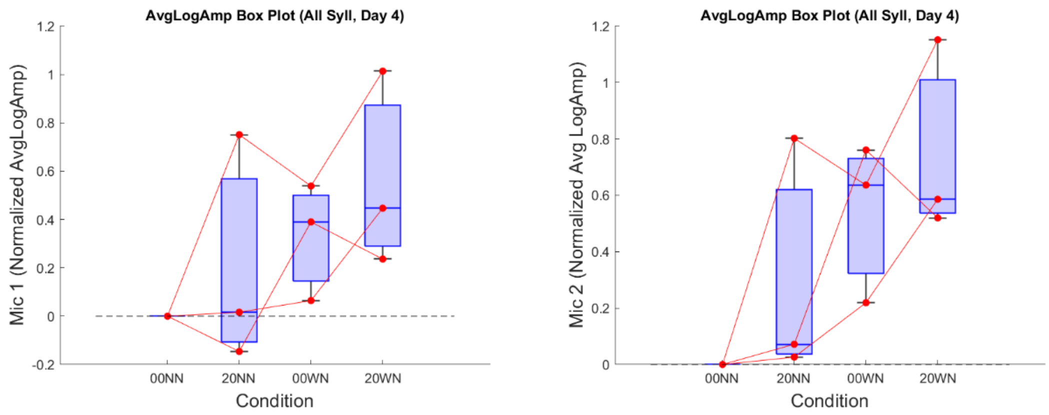

Figure 13: Similar trend across conditions was observed for both mics
The variance of the average Log amplitude was normalised across the four different conditions for a single day. Each dot represents the variance of average Log amplitude values of that particular condition of that day.

Minimal increase was seen across the conditions for each syllable, exhibiting different kind of for each syllable

It was observed that there had been a similar kind of trend across different days and different conditions for a single syllable. To observe whether different syllables used in the song were exhibiting different levels of amplitude, I plot all the syllable data in one plot. Therefore, I combined the Log amplitude data of all the days for one syllable of that particular condition. From the graph it was observed that different syllables were exhibiting different levels of amplitudes and the amplitude fluctuation because of the four different conditions was minimal. Each syllable was exhibiting a different kind of increase for the four conditions. Not a single syllable was exhibiting a similar kind of increase in the Log amplitude. Even some syllables were showing a similar type of increase in the Log amplitude, the relative increase within the four conditions might differ.

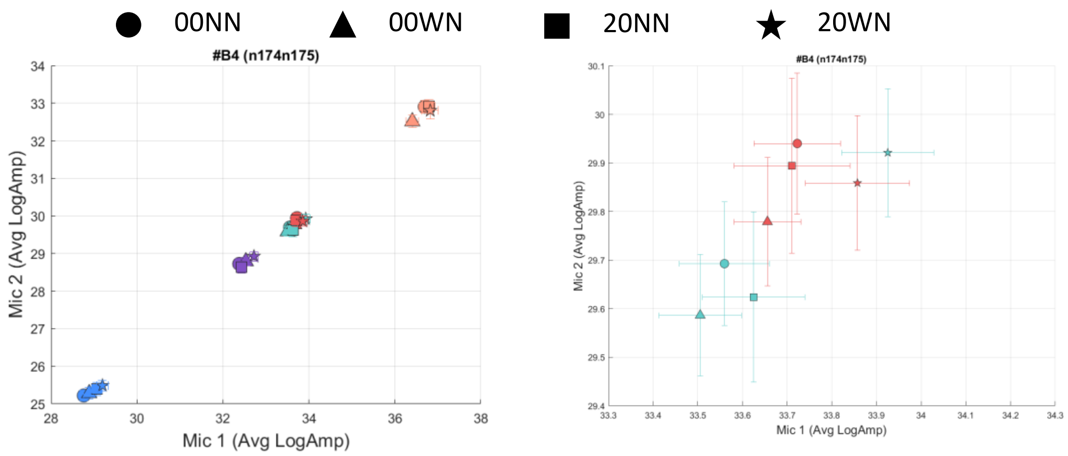

Figure 14: Minimal amount of increase in the Log amplitude for each syllable across different conditions
Each colour is representing a unique syllable which were repeatedly used in the song sung by the bird and different shapes are representing the four different conditions, i.e., circle: 00NN, triangle: 00WN, square: 20NN, star: 20WN.

Similarly, a minimalistic change in amplitude was observed across different conditions

Different syllables from the same bird had different trend, but there might be similar trend for the song of each bird across four different conditions. Therefore, I combined that syllable data to a song data by averaging the Log amplitude of all the syllables from the song of each bird across four different conditions. Each bird was making a very less change in the amplitude across different conditions, and the change was around 0.5-1dB. The change observed across condition for each bird was hard to observe and quantify the change made by the bird.

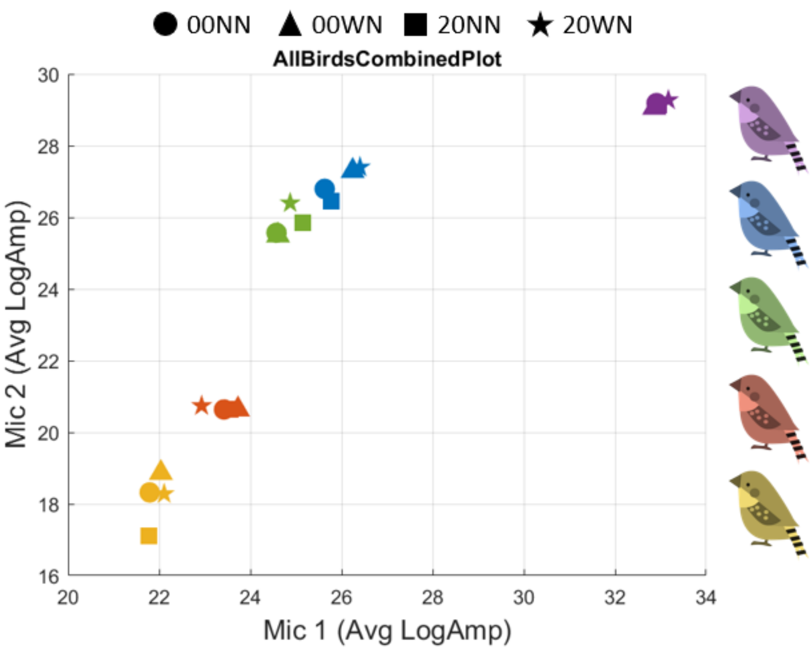

Figure 15: Across birds, the amplitude value for both mics were correlated, irrespective of the four conditions.
Representation of the change in the average Log amplitude values of the song for each bird across the two mics. Each point contains the average value of all the syllables and days of one particular condition of that bird

Amplitude value differ less across conditions

Since was hard to observe and quantify the change made by each bird in their song across different conditions, hence I plot the same thing in box plot. In the box plot, the average Log amplitude of the song of each bird across different condition, separately for the two mics. To quantify the amount of change made by the bird in across each condition, I did the normalisation across the basic condition. It had been observed that the change made by each bird was not very significant across different conditions as the change observed by the two mics were around 0.2-1dB.

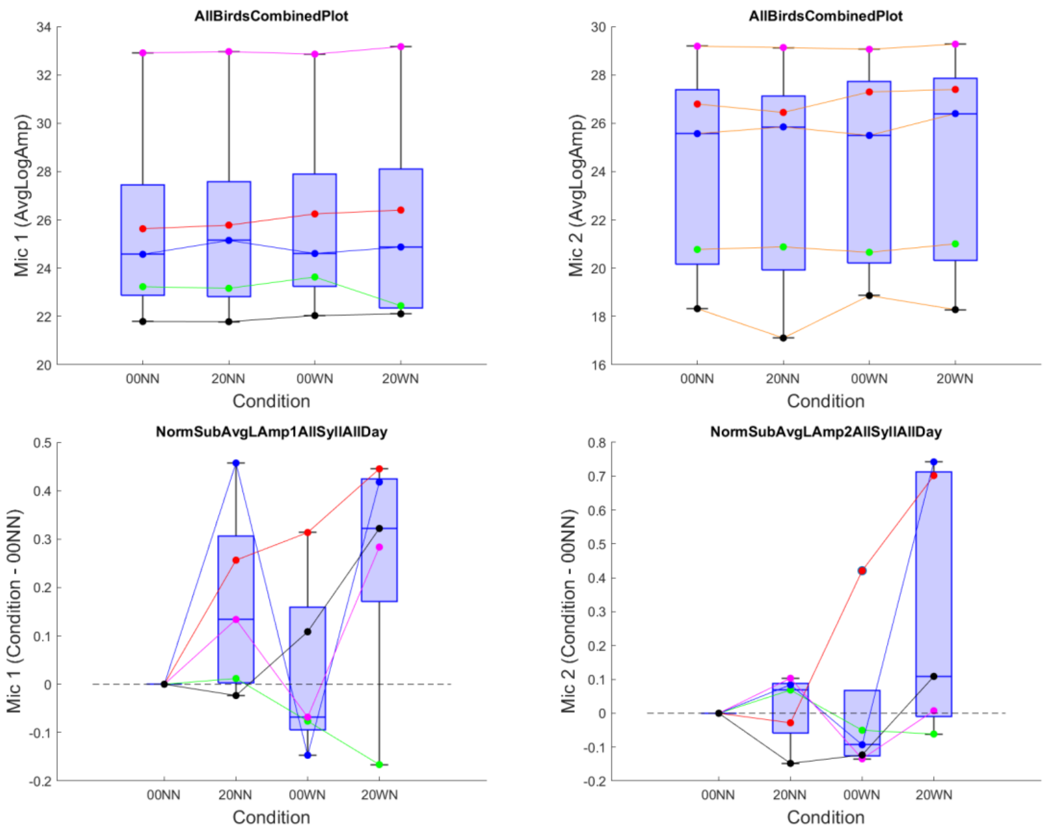

Figure 16: The Log amplitude value difference across conditions was less
Comparison of the change in the average Log amplitude values of the song for each bird across all four conditions. Each bird was represented by a unique colour, which was joined by the lines across the conditions. The upper set of graphs denotes the actual average Log amplitude values for each condition. The lower set of graphs represents the normalisation of the graphs represented above graphs for the two mics.

Minimal level of increase in amplitude of song across the extreme conditions

Till now it was clear that there is a minor increase the amplitude of bird's song in absolute terms. In order to quantify the amount of change at which the bird intends to increase the amplitude of the song specifically for the four different conditions, I compared the amount change made in the song for every bird. To compare the change made by each bird, I normalised the amplitude values of the song for each bird produced in four different, by dividing it with the basic condition (00NN) according to my hypothesis. Each bird was normalised separately, which was reduced to one, and thus was easier for me to compared the data obtained from all the birds. For quantification purpose, I averaged out all the data of different birds for the same conditions to check the tendency of the zebra finch for those particular conditions. From the average data I found out that there was an increase in the amplitude of the song as the complexity of the condition increases. To conclude from this project, male zebra finch would increase around 1.2% of their amplitude if the female bird was moved to 20cm away from the bird, in the presence of background noise which was 60-70dB loud.

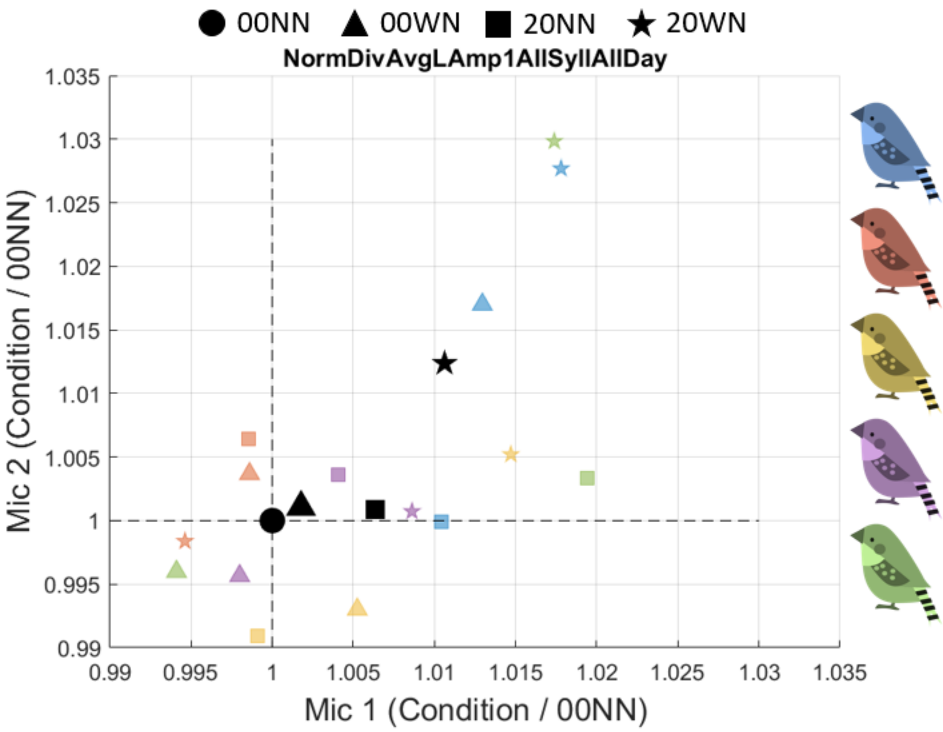

Figure 17: Relative increase in the amplitude level of the song sung by each bird in different conditions, which was normalised with the basic 00NN condition.
Each bird was is represented by different colour and the log amplitude level at which the bird was singing in different conditions were represented by the same colour of points. Different shapes were representing different conditions at which the birds were kept while recording the song. The dark black coloured shapes were representing the average of value of that particular condition.

Chapter 4 Discussion

I hypothesised that the bird would increase the amplitude of the song as the condition becomes more and more difficult to communicate with the other bird. To test this hypothesis, I placed a male and female birds separately in a small cage placed far and near the male bird's cage in the presence and absence of white noise. Here, different conditions represent different kinds of situations where the male bird might need to adjust the loudness of the song in order to communicate with the female bird, successfully. The outcome of this project infers that, each syllable is exhibiting a different level of amplitude, and each syllable behaves independently to the condition, in order to increase amplitude of the song with respect to different conditions. The change observed for each syllable across the four different conditions can either be an increasing or decreasing trend across the four conditions at which the bird was placed. This increasing and decreasing trend is independent of the four situations in which I hypothesised that the bird would increase the amplitude level for each syllable as the condition becomes harsher. The change made by the bird in the Log amplitude values for each syllable lies within the range of 0.2-1.8 dB.

Since the two mics implanted on the bird's head were not perfectly correlated, there was always a fluctuation between the Log amplitude values of both the mics, which was also around 0.2-1.8 dB values. But the change in variance and average values of the Log amplitude is similar across the two mics for the four conditions and different across days. As the fluctuation made by the mic is more or less similar to the changes made by the bird, the quantified values obtained from the results cannot be trusted fully. In some of the plots, a trend for the two mics was reversed, which can be because of the bending of wires of the two mics. There were two points at which the mic connection could have been disrupted. One was that the connector attached to the mic might get loose because of the bird's dancing movement while singing a song. Or else, a loose connection between the sound card and the PC could be the factor for this change, which can easily get disconnected even with a slight jerk to the table, where the PC was kept.

In the earlier study, similar results were obtained, where the male bird was changing the amplitude within the same range when only one mic was implanted on the head of the bird(Suri & Rajan, 2018). Even in other studies, it was observed and proved that zebra finches follow the concept of the Lombard effect(Brumm & Slater, 2006; Cynx & Gell, 2004; Loning et al., 2022). In another study(Cynx et al., 1998), it was shown that they change the amplitude of the song according to the noisy environmental conditions. My results coincide with the results obtained in the previous studies where the bird seems to change the amplitude of the song according to environmental changes. Since the bird was not responding to the larger distance, this study was restricted to at most 20cm with noise condition, which is very less compared to other studies(Loning et al., 2022), where the change in amplitude was measured for even more distances.

From these observations, one can shield both the wires separately, which connect the commutator to the PC, with good quality sound cards for recording the song of the bird. This will help reduce the noise captured by the two mics while recording. While performing this experiment, one can also measure the air pressure within the lungs and the syringes of the bird to get the correlation between the song's amplitude and the bird's capacity to increase the song's amplitude. Prior to the implantation of the mic, one can perform different types of playback experiments for checking the proper working of the experimental setup and the two mics. The results of this study depicts that the bird might be changing the level of amplitude but the same amount of change was also observed across the two mics, which was around 0.2-1.8 dB for the four conditions. Since the two bird were not separated by a small distance, and the loudness of the background noise was also very less, the male bird might have felt to increase the amplitude around 1.2% than their usual level of loudness.

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