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QUANTIFYING SPECTRAL AND TEMPORAL FEATURES OF BIRDSONGS USING A LIGHT-WEIGHT IMPLANTABLE DEVICE

by
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ABSTRACT
OF THE THESIS OF

Paul Joseph Chaptini for Master of Science
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Title**:** Quantifying Spectral And Temporal Features Of Birdsongs Using A Light-weight Implantable Device.

Social cues modulate all sorts of communication in a wide range of species, and these modulatory changes make communication signals more salient. The songbird vocal system has emerged as the best-developed model for the neural basis of vocal communication, speech acquisition and production since songbirds are among the very few animals that learn their vocalizations through vocal imitation, like humans. In order to better understand the basis of vocal communication in a social context, it is important to observe and collect data from specimen in natural, stress-free environment, but most importantly at individual-level. While microphones are a good tool to record the vocalizations of songbirds in isolation, they are not able to record the vocalizations of multiple birds within the same environment due to sound cross-contamination. In this work, we aim to implant piezoelectric accelerometers on the skulls of songbirds which record bone-conducted recordings that are uncontaminated by airborne sounds, thereby rendering the study of vocal communication of multiple songbirds in the same environment possible. The implantable accelerometer along with its required circuitry for signal processing and filtering would allow for recordings of free-moving, single bird vocalizations in group settings and under different study conditions. This approach makes it possible to obtain vocalizations irrespectively of conspecific and background noises. The aim is to study the changes in the temporal and spectral features of song (syllables' duration, pitch, frequency modulation, entropy, etc...) using accelerometers under various conditions, and compare these bone-conduction recordings to the standard microphone recordings in an attempt to assess the integrity and efficiency of the implantable device.

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ABBREVIATIONS

- HVC$_{RA}$: RA area-projecting neurons of the HVC nucleus.
- HVC$_{X}$: X-projecting neurons of the HVC nucleus.
- HVC$_{int}$: HVC interneurons.
- nXIIts: Tracheosynrigeal portion of the hypoglossal nucleus
- DLM: Medial portion of the dorsolateral nucleus of the thalamus
- LMAN: Anterior neostriatum
- NMDA: N-Methyl-D-aspartic acid
- AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
- EEG: Electroencephalogram
- FF: Fundamental Frequency
- ADC: Analog-Digital convertor
- PCB: Printed Circuit Board

CHAPTER I

INTRODUCTION

A. Introduction

The capture and assessment of vocalizations from free-moving, undisturbed songbirds are key factors to the correct understanding and analysis of the species' vocal behaviors. Zebra finches are small dimorphic birds commonly studied for their audition-dependent vocal plasticity (Konishi, 1965) , they exhibit elaborate patterned vocal sequences denoted by rhythms and pitches that arise from a set of forebrain nuclei commonly known as the "song system" (Margoliash and Schmidt, 2010). In order to comprehend the different factors at play when it comes to vocal shaping and behavior, it is preferable to obtain recordings from undisturbed specimen in a natural setting (Brown et al., 2013). We aim to implant hermetically sealed piezoelectric BU accelerometers (Knowles Acoustics) on the skulls of songbirds that are light weight (0.28 grams) and record bone-conducted vocalizations that are uncontaminated by airborne sounds. The usage of the minimally-invasive accelerometers in the field has made it possible bypass the behavioral and locomotor side-effects brought by larger, more constraining recording devices (Gill et al., 2016). Birds are then tethered using a cable to an amplifier via a slip ring commutator and the recordings are then digitally saved to a computer disk.

In this proposal, we will be using the BU accelerometers in conjunction with a custom-made PCB board that will be connected to the implanted accelerometer. Vocalizations from accelerometer-implanted male zebra finches are collected and the spectral and temporal features (such as pitch, frequency modulation and syllable duration) of the bone-conducted vocalizations are analyzed.

Such devices might have various implementations and executions based on the studies at hand; those might range from behavioral studies to brain wave recordings, all of which having the potential to create meaningful conclusions that might be extended to human vocal, or auditory learning sets of behavior.

B. Study Aims

A multitude of tools have been engineered throughout the years in order to better grasp the fundamentals of animal speech and behavior. Notably, optogenetics and similar neuromodulation tools, along with microphone fitted backpacks and other wireless, battery free recording devices have been put to use in an effort to better understand the underlying mechanisms behind vocal communications in zebra finches. Most efforts, however, seem to be pointed towards studying the behavioral changes associated with the implantation of such devices, while the underlying vocal behavior of the specimen in groups and in natural settings tend to remain as a secondary, background aim to the study (Ausra et al., 2021; Gill et al., 2016; Tremere et al., 2010). Here, the main goal is to be able to collect high-quality bone-conducted sounds from male zebra finches, hopefully without the need to tether the birds to commutators although initially we will be doing so to test our setup. The purpose is to establish a clear distinction between the changes in the temporal and spectral features of song (syllables' duration, pitch, frequency modulation, entropy, etc...) using ACC recordings in comparison to microphone recordings. Those experiments will be based on biosignal processing and analysis of the recorded vocalizations obtained by the implantation of the accelerometer and related devices.

In order to achieve this, we decided to use a piezoelectric accelerometer encased in a Bluetooth-enabled wearable device that can record, process, and transfer vocalization samples. The implantable accelerometer along with the required circuitry for signal processing and filtering would allow for recordings of free-moving, single bird vocalizations in group settings and under different study conditions.

CHAPTER II

BACKGROUND

A. Song learning and production in songbirds

Vocal communication is a critical aspect of behavior in most species' social environment. Through auditory feedback, songbirds develop their songs and maintain them throughout adult life. This act of assimilation via auditory feedback is the principal factor affecting the correct development and sustain of learned song as proven by experiments carried on deaf birds, which further highlight the necessity of audition-dependent learning (Nordeen and Nordeen, 1992; Nottebohm et al., 1986). Humans and songbirds share some similarities when it comes to vocal learning and communication, they are both prone to the influence of their peers and parents starting from a young age, constituting a critical learning period for both species (Marler, 1970). The zebra finch, with its ability to produce distinct syllables that help shape the repeating sound sequences known as motifs, has proved to be a notable study specimen when it comes to speech learning and vocalization experiments. That singing behavior not only has the mating purpose of attracting females, but is also sometimes associated with territorial calls and signals (Wiley, 1996). Moreover, song assimilation in the zebra finch happens in multiple steps throughout its first few months post-hatching, akin to speech development starting well into infancy, and extending further into the first few developmental stages of children, with the sub-song stage in songbirds paralleling the babbling stage in humans. However, compared to other species of songbirds like the canary, zebra finches are capable of song learning and assimilation only during their first year of life, and are thus classified as age-limited learners. Zebra finches undergo two known stages of song acquisition, the first one occurring during juvenile development involving song assimilation and creation of a specific template in the brain, this is known as the sensory learning phase, and is followed by the sensory-motor learning phase where song trials occur and are continuously compared to the previously established template with song adjustments done in parallel, culminating in song crystallization (Konishi, 1965).

As such, scientists have tried to elucidate both vocal and motor speech formation and learning in Humans through studies of the underlying neural and behavioral vocal pathways in songbirds. It is possible to study and learn from this model thanks to the songbirds' innate abilities to not only copy, but recreate conspecific behaviors and song patterns acquired from social and behavioral cues provided by their environment. The similarities between speech acquisition in both songbirds and humans also extend to their need for practice and to the provision of correct auditory input, while the simplicity of this physiological pathway in the zebra finch relative to the one in humans further lends itself to such studies (Solis and Perkel, 2005). Vocal learning also possesses underlying temporal and spectral structures that are prone to error as shown by continuous delayed auditory feedback experiments in songbirds leading to speech dysfluency (Yates, 1963). In songbirds, those vocal gestures arise from a set of forebrain nuclei better known as the "song system". Two main interconnected pathways are present in this system, notably the descending motor pathway responsible for the motor behavior underlying song production, and the anterior forebrain pathway responsible for early song assimilation and development (Nottebohm et al., 1986). Nucleus HVC in the descending motor pathway possesses three distinct types of communicating neurons, those projecting to the robust nucleus of arcopallium (HVC_{RA}), those projecting to Area X (HVC_X) , and interneurons (HVC_{INT}), with each of those HVC neurons having different functions and cellular properties (Mooney and Prather, 2005). The HVC is a telencephalic nucleus that plays a critical role in song associative assimilation and production, as shown by adult birds' inability to sing once their HVC was bilaterally lesioned at the onset of song-learning (Gentner et al., 2000).

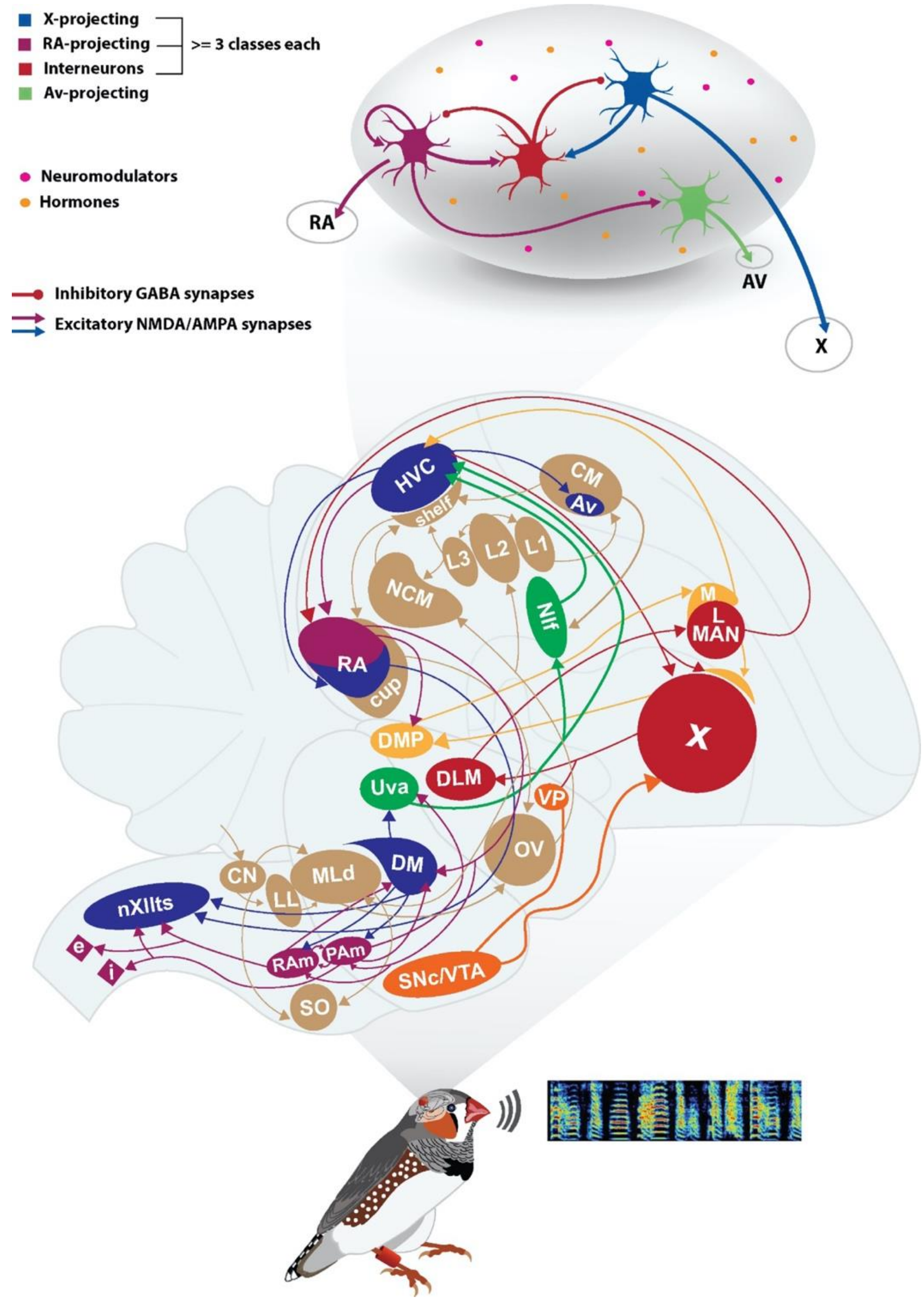


Figure 1 Schematic of a male zebra finch along with a sample spectrogram of a song showing frequency of sound versus time are shown in the lower panel. Reasonably complete song system and auditory system pathways are shown in the middle panel. The vocal motor pathway (VMP, blue color) contains circuits that directly pattern song output. Incoming sensory information is processed by HVC and NIf (green color), and HVC and RA shape motor sequences that project out to the peripheral vocal organs, the syrinx and respiratory muscles, via the hindbrain nucleus nXIIts and brainstem respiratory nuclei RAm and PAm (purple color). The anterior forebrain loop (AFP, red color) pathway contains circuits that are important for song learning and song variability. VTA sends dopaminergic inputs to area X. The upper panel shows a schematic of HVC's internal circuitry. HVC includes multiple

types of HVC$_{X}$ neurons that project to area X, HVC$_{RA}$ neurons that projects to nucleus RA, HVC interneurons, and a very sparse population of HVC$_{Av}$ that projects to nucleus Avalanche. The nucleus is bathed with a wide set of hormones and neuromodulators (colored circles). X-projecting and RA-projecting neurons are known to excite interneurons via NMDA and AMPA synapses, while interneurons in their turn inhibit both classes of projecting neurons via GABA synapses. Moreover, HVC$_{RA}$ neurons send excitatory projections onto HVC$_{AV}$ neurons. (Adopted from Daou et al. 2021)

HVC$_{RA}$ and HVC$_{X}$ neurons are associated with excitatory inputs to HVC$_{INT}$ neurons that, by mediation from NMDA and AMPA currents, are in turn responsible for inhibiting both HVC$_{RA}$ and HVC$_{X}$ neurons (Mooney and Prather, 2005). During song production, HVC$_{RA}$ neurons have been shown to fire once, in a time-dependent manner, while HVC$_{INT}$ neurons fired densely and at higher frequencies compared to both HVC$_{RA}$ and HVC$_{X}$ neurons (Hahnloser et al., 2002). All those types of neurons function in tandem in order to give rise to the corresponding timely syllables and notes, characteristic of male zebra finches' songs.

B. Recording devices

Studying the different factors that help shape vocal communication is a necessary step to undertake in order to better understand the modalities behind behavior-mediated vocalizations. In that aspect, the key is to obtain reliable information from each individual specimen under natural conditions, without having to rely on constraining devices or settings that could affect the recordings, and by extension the resulting data. Observing animals for more than a few moments in their natural habitats and under free-roaming conditions is a challenge posed not only by the inherent presence of the observer, but also by technological limitations and observational bias. Animals often perceive humans as predators, as such, it is easier to trigger flight mechanisms and elusive behaviors that are not suitable for observational experiments and data collection (Caro, 1999; Clarke, 1990; Schneirla, 1950). However, in some cases, habituating an animal to human presence has been possible, but it requires further deterministic studies and continued strenuous labor (Jack et al., 2008). Habituation also does not remove the consequential possibility of a change in behavioral interactions when studying species in groups or in the presence of a mating partner in a cage. In fact, Elie et al. observed that, compared to birds in social settings, isolated male finches sang more and had more vocalization changes (Elie et al., 2015). On the other hand, human bias manifests through the tendency of directly observing certain specific behaviors and events while inadvertently, or willingly missing others (Altmann, 1974). Furthermore, there exist a multitude of other limitations that prevent accurate recording and observational analysis of some specimen. Considering the need to observe animals in stress-free and natural environments, keeping track of their location represents another hurdle to be overcome. This was achieved through the use of geomagnetic loggers, thermal sensors and passive or active transponder tags that helped solve spatiotemporal constraints, and permitted an easier, more accurate way of locating and following a specimen over time (Cagnacci et al., 2010; Tomkiewicz et al., 2010). With this comes the issue of data collection and recording, and more importantly, of maintaining a certain level of fidelity when recordings are being made over a distance compared to directly in an enclosure. Using the standard stationary recording apparatus does not allow for the collection of individual-level recordings, but rather for the integration of multiple vocalizations from different specimen in captivity. Cables and tethering required for recording in enclosure settings also tend to affect the behavior of the specimen and do not always allow for free-movement because of the constraints brought by the technological limitations of having multiple recording components linked together, thus tempering with the birds' ability to move.

Accelerometers and their associated tools allowed us to circumvent this issue, by providing clearer recordings all while eliminating the need for cumbersome and protruding cables and associated devices (Hiryu et al., 2007). Zebra finches only having about 0.825 cm^2 of available head space, using small implantable devices might help remedy some of the problems initially observed when trying to obtain clear recordings from individual freely-moving songbirds. Tools such as accelerometers and optogenetic platforms are being engineered with the idea of being weightless and flexible means to better study vocal communication and its underlying mechanisms in small birds and other animals (Ausra et al., 2021; Gill et al., 2016).

Accelerometers are known as spring-like, piezoelectric devices, are fitted by sensors and capable of deformation, which when put under certain conditions, can generate representative wave-like voltage signals. The sensors can potentially capture and sample widely ranged frequencies, with the obtained signal capable of being transferred and converted into the appropriate output (Eisenring et al., 2022). Implanted accelerometers register the vibrations (acceleration) representing vocal outputs in the bird, allowing us to convert the recorded physical frequencies into the desired vocalizations (sound) on an individual-based level (Fig. 2). However, the implantation of accelerometers requires surgical procedure which might not always be safe for the specimen, especially following anesthesia (Schregardus et al., 2006).

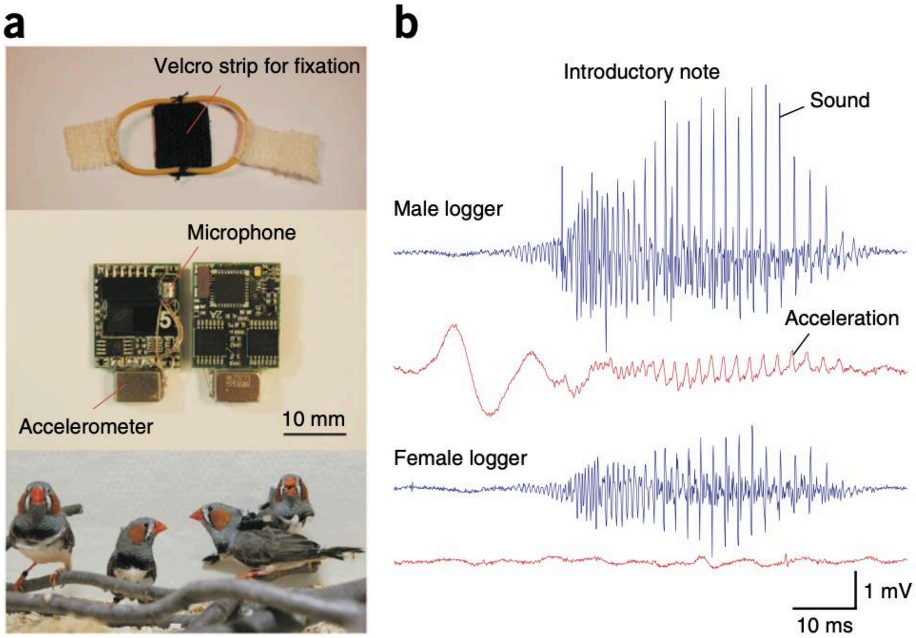


Figure 2 A wearable sound and acceleration logger for recording individual vocalizations in a group of songbirds. (a) Harness with Velcro strip for rapid fixation of the logger to the back of the bird (top), top and bottom views of Neurologger 2A used for sound and acceleration recording (center), and four zebra finches with the backpacks during the experiment (bottom). (b) Raw records of an introductory note recorded with backpacks placed on the singing male (top) and a listening female (bottom) (Adopted from Anisimov, 2014).

Miniature tools akin to the accelerometer are being used in order to record EEG signals, leading the way to a more accurate unraveling of the neural mechanisms underlying some aspects of behavior. Some other similar devices were deployed to make more sensitive measurements of the specimens' internal state mainly by recording body temperature and heart rate, while other tools like pressure and light sensors were used to better understand the physical environment and strains the specimen would experience in the wild (Greif and Yovel, 2019; Hughey et al., 2018).

Power management along with data communication are both important aspects of wearable devices that should be taken into consideration during the engineering development of those instruments. Telemetric challenges involving the transfer of the recorded data via Bluetooth or other low-power consumption methods have been studied in order to create lighter, smaller and more efficient microphones and transmitters that would allow for implantation on smaller species like the zebra finch, all while providing adequate recording capacities at longer distances (Magno et al., 2020). Ausra et. al. 2021 showcased the use of data uplink from infrared communication with its miniature footprint and its minimal number of required components and valued it as an appropriate data exchange method for use in smaller animal species. Additionally, they have looked at ways to overcome the heavy power-consumption needed by the implanted devices as well as their limited energy-harvesting potential, and found that by continuously harvesting power wirelessly through resonant magnetic coupling, they could bypass the need for batteries and provide the device with the appropriate current. Other power-hungry processes, such as data uplink, were dealt with by introducing a capacitor bank having a capacitive storage of 5.6V, which was able to provide the necessary energy for use during those high-powered events. Considering the limited head-space available in the songbird specimen, it was also necessary for them to limit the size of that capacitor bank, hence also limiting the amount of energy stored. As such, they devised a way to manage data uplink by splitting the obtained data into two 8-bits components, thus shortening the sending event length and decreasing the amount of energy needed, the 8-bit components were then recombined at the level of the receiver, where energy consumption and device size did not matter. It is important to keep in mind that similar power delivery methods experience some drop-offs with increased distance from their power-provider, thus limiting their use to smaller enclosures or arenas, with a big dependency on antenna design and location of the receiver.

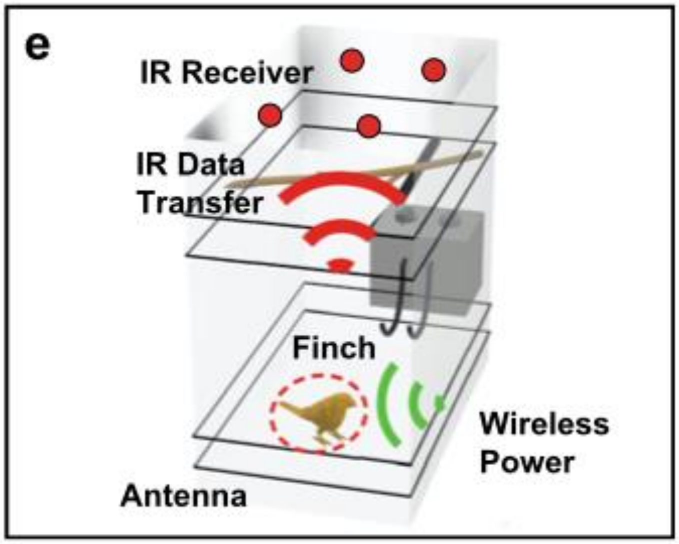


*Figure 3 Multimodal optogenetic stimulator and thermography device characterization.**e.** Rendering of experimental arena showing data uplink and power delivery to animal with implanted device. (Adopted from Ausra, 2021)*

When studying the singing behavior of songbirds in groups, it is fundamental to capture the vocalizations of every bird independently, thus disregarding conspecific songs and sets of vocal behaviors when needed. Accelerometers allow this by capturing individual-specific frequencies and transmitting them to a receiver where they can be converted to acoustic signals. This transmission needs to be done specifically, and in a non-overlapping manner as to allow for parallel single channel recordings and characterization of the individual vocalization in a group environment (Gill et al., 2016). Accelerometers are also used to determine and quantify flight behaviors along with their energetic expenditure, with even flock dynamics capable of affecting energy cost during flight being scrutinized via this recording method (Sur et al., 2017; Taylor et al., 2019).

C. Behavioral Studies

1. Device related behavioral changes

The goal behind the creation of miniature devices capable of measuring physical and behavioral changes in animals is to achieve such observations without having to subject the specimen to external stresses that may lead to biased results. Accelerometers, optogenetic tools, microphones, and circuit-fitted backpacks have all been used in order to better understand the different modalities underlying behaviors and processes exhibited during an animal's daily activities. However, the implantation of such tools, although miniature in size and not physically limiting, lead to some clear behavioral changes that have been studied and quantified in recent literature. Gill et al. evaluated the impact that battery exchange and backpack attachment had on zebra finches by measuring the birds' individual calling along with their locomotor activity in a standardized environment. The baseline of calling behaviors and locomotor activities they considered was obtained through observations of the birds prior to backpack attachment. A stable plateau of calling activities in all birds was reached on day 7, and it was decided to use this value as an indicator for habituation. This baseline was used in order to determine the effect of physical handling in a study that lasted twenty days and involved recording and tracking of both vocal and locomotor behaviors 4 hours after 'lights on' in sound-proof chambers housing seven male birds. Locomotor activity was evaluated based on quantification of automated motion-detected multi-channel videos, whereas continuous video recordings were initiated once the bird moved, stopping only when no movements were detected for a single duration of 5 seconds.

Statistically relevant differences between the baseline and the observed behaviors post-handling have shown that both backpack attachment and battery exchange truly affected the number of calls they made as well as their physical activity in the cage. As seen in Figure 4, the constraints of physical handling only showed during the first day post-attachment, with the birds recovering shortly after.

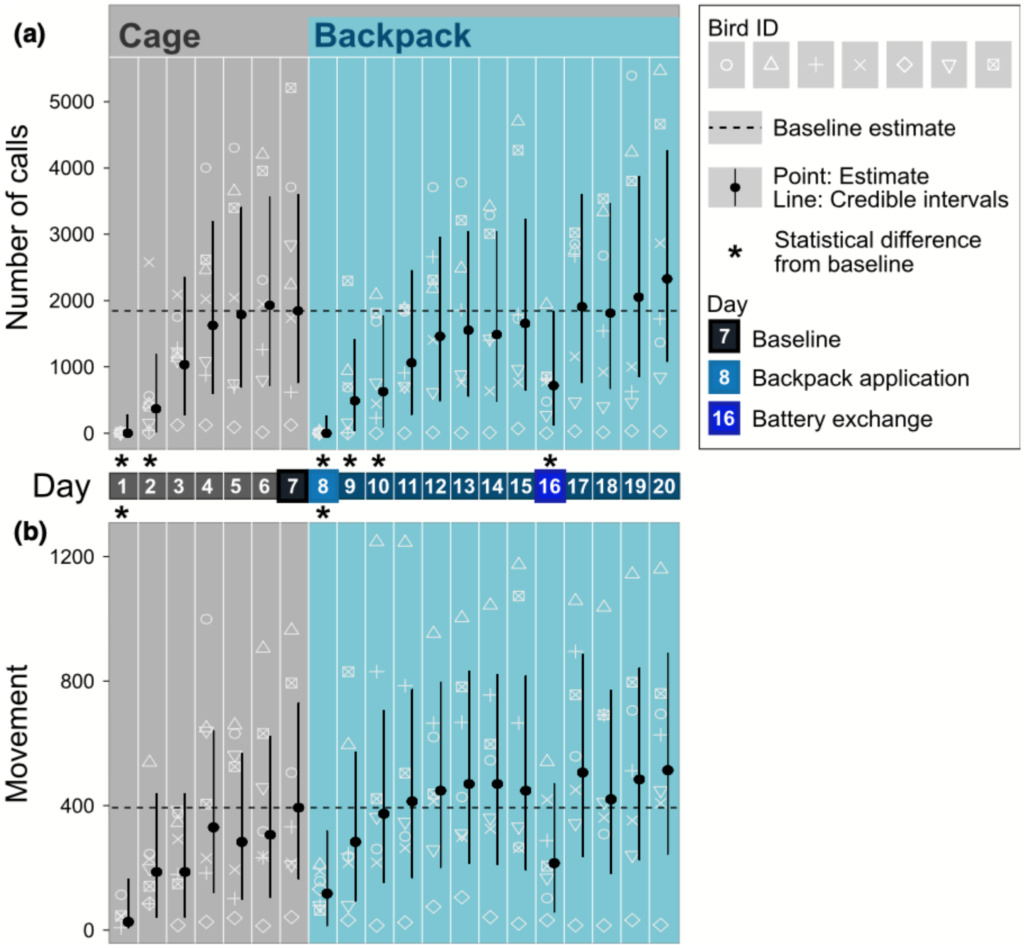


Figure 4 Calling behaviour (a) and movement (b) before and after backpack attachment. (a) Number of calls and (b) amount of movement (= duration of motion-detected video recordings) for each day before (grey background) and after backpack attachment (turquoise background). Black points and vertical lines indicate Bayesian estimates and credible inter- vals (CrI). Dashed horizontal black lines show model estimate of calling activity (a) or of movement (b) on day 7 (= baseline). If credible intervals of 1 day do not overlap with this line in the same graph, there is a difference between the amount of calling (a) or of movement (b) compared to the baseline. Data points from different individuals are represented by different white empty shapes (Bird ID). (Adopted from Gill et al. 2016)

Bird activity and singing behaviors seemed to return to baseline levels approximately two days after handling, with no statistically significant changes happening during the next few days.

Behavioral changes related to the implantation of accelerometers might also limit recording and power transfer efficiency, as seen in Ausra et al. where transfer of power to the implanted wireless device via magnetic resonance needed to be achieved locally and in a predetermined environment. This was achieved by using spatial position analysis capable of tracking the animal's movement, leading to the creation of a heat map in the boundaries of the enclosure (Fig. 5), characterizing the behavioral patterns of the specimen and thus facilitating antenna positioning and maximizing power transfer efficiency (Ausra et al., 2021).

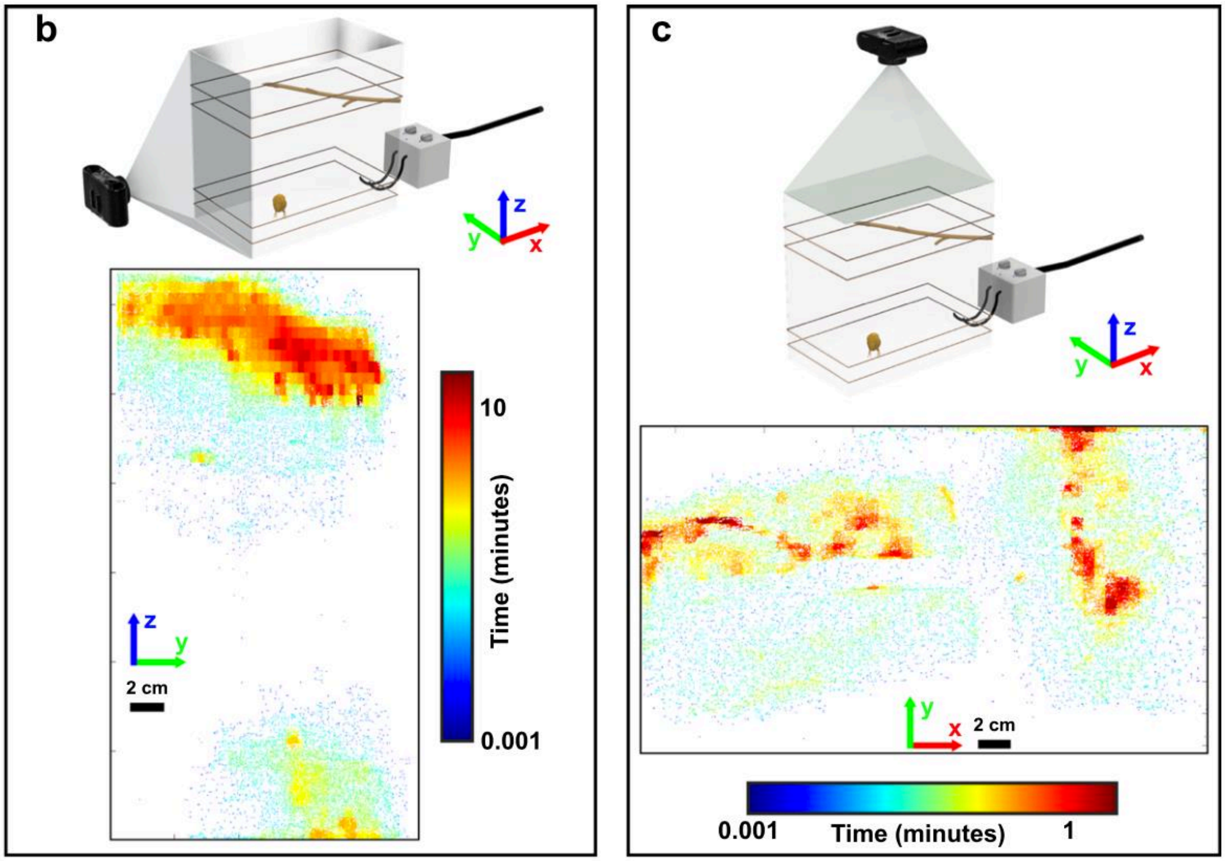


Figure 5 Behavior guided primary antenna design and secondary antenna optimization. A Workflow diagram showing behavior guided antenna design for resonant magnetic coupling-based devices. b Rendered schematic of side camera recording setup (top) and heat map indicating spatial location of the finch. c Rendering of cage with top camera (top) and heat map indicating spatial location.

(Adopted from Ausra et al. 2021)

Since the goal of using novel, and miniature implantable technologies is to permit observations and recordings to be done in the most natural, stress free environment possible, it is also important to determine the effect that such devices have on the reproductive and mating behaviors of songbirds in community. Ausra et al. used birds that had already reached sexual maturity, and fitted them with a backpack similar to the one they used in their previous experiment. By measuring the mean clutch size of the eggs, and comparing that number to the mean clutch size of eggs from breeding colonies without an attached backpack, they determined that handling and implantation of miniature devices did not impede on successful reproduction in the long run.

Moreover, it was determined by Soul et al. that there was a distinguishable difference between zebra finch calls in non-stressful conditions and calls emitted under stressful conditions. Calls from male and female birds were also prone to distinction, but not to discrimination as they found that each call was highly individualized and that emotional status did not affect song perception from other songbirds (Soula et al., 2019). Potential stressors associated with the attachment of an accelerometer or similar devices would therefore not be expected to impact mating calls and female-male behaviors.

All in all, while it was determined that implantation of devices and physical handling of the bird specimen lead to a decrease in their normal vocal and locomotor behavior, experimental observations proved that this change did not last for more than a few days, after which the songbirds would have had time to get habituated to their new circumstances, and would thus start exhibiting normal behaviors again. Accelerometers and their associated miniature devices would therefore not be considered a burden on the bird, and would permit for more accurate and representative recordings of the Zebra Finchs' behavior in a natural environment.

2. *Changes in spectral and temporal features of song*

Song learning, in parallel to language acquisition, is rooted in social processes that greatly influence the sequencing, acoustic structure, and trajectory of an adult birds' song by modulating various sensorimotor mechanisms throughout development (Chen et al., 2016; Reader, 2016; Riebel, 2009).

Taeniopygia guttata, also known as the zebra finch, is capable of modulating the different spectral and temporal features of their song in order to better tailor them to specific social environments and events, for example, such optimizations in song structure help exacerbate a male finch's attractiveness during mating (Woolley and Doupe, 2008). The zebra finch song comprises many different structures and vocal elements that help shape and distinguish song production depending on social contexts and environmental cues (James et al., 2018; James and Sakata, 2015). Those vocal features include syllables, gaps, bouts, and motifs, which are the fundamental units of songs. Syllables represent the structural elements of the song and are separated by the gaps, being the small silent intervals between different sung syllables, and the song is produced in bouts, or in adjoining instances of vocalizations with separated syllables. Motifs contain stereotyped sequences of syllables that can help us distinguish and identify different motifs, those syllables in the motif differ from standalone syllables in that they are comprised of shorter gap intervals (James and Sakata, 2019).

Following song crystallization, zebra finches were shown to retain the same motifs throughout their life (Fig. 6). James et al. demonstrated that socially tutored birds, and some non-socially tutored birds presented identical motifs, in this case "abcde", both when they were young and again when studied after a certain interval of time as shown in the spectrogram.

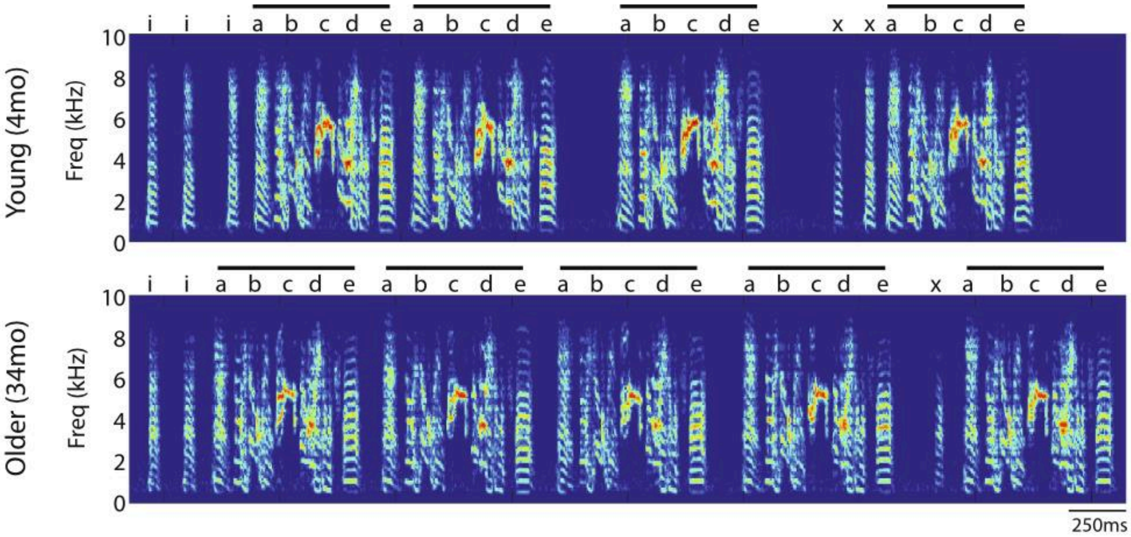


Figure 6 . Examples of young (top) and older (bottom) adult recordings of a socially tutored zebra finch. Above each spectrogram (time on the x-axis, frequency on the y-axis, color indicates intensity) is the arbitrary labelling scheme for this bird. (James and Sakata, 2019)

Alternatively, other non-socially tutored birds (tutored via song-playback) were observed to have had substantial modifications to the general structural integrity of syllables as well as some additions or deletions from the motif studied. When comparing birds that were socially tutored to birds that were not, they noticed that some experienced a change in the spectral structure of their song after a certain interval of time, thus proving that social factors affect the retention and crystallization of a song's modalities over time. Those acoustic changes are apparent in the figure below, where a syllable was dropped (here syllable "g") and replaced at the end of the motif by another novel syllable denoted as syllable "h", while the acoustic structure of the socially tutored bird's song remained stable across context and with age. Moreover, it was observed that the fundamental frequency (FF) of syllables, along with the bout duration and motif repetition varied significantly in birds whose songs were female-directed when compared to undirected bird songs. Other studies also noted that male Zebra finch songs appeared to be faster, with more stereotypical structures when they were directed towards female finches (Sakata et al., 2008; Woolley and Doupe, 2008).

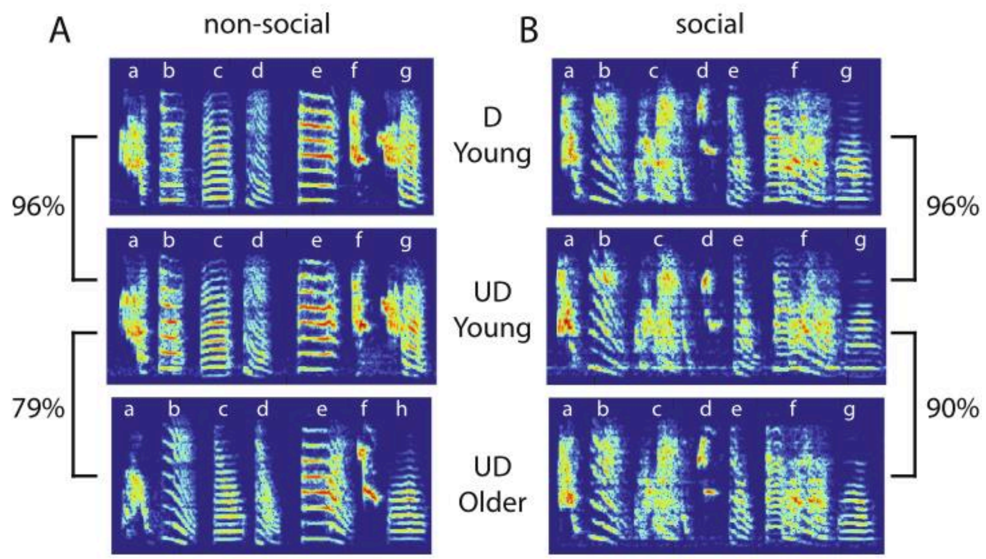


Figure 7 Examples of age and context-dependent changes to motifs.Examples of an in- dividual bird's motifs in each condition: Undirected (UD) song as a young adult (middle), female-directed (FD) song as a young adult (top) and UD song as an older adult (bottom). On the top portion of each spectrogram are the labels assigned to each syllable (the labels themselves are arbitrary). Percentages indicate the quantitative acoustic similarity across context (UD young to D young) and age (UD young to UD older). (James and Sakata, 2019)

So while the spectral and temporal features of songs do not necessarily change under normal conditions, they have been prone to variations when subject to the presence of female specimen. The coordination between partners throughout incubation periods has been shown to possess underlying calling behaviors that are affected by the way parental care is "negotiated" between the male and female finch (Boucaud et al., 2016). Late return of the male finch to the nest was correlated with higher call rate and shorter duets with the female, while higher call rates from the male lead to shorter incubation bouts. Moreover, female incubation decreased with fewer male calls during the duetting phase. This demonstrates the importance of vocal exchange between Zebra finch partners and highlights some of the vital aspects of song features and modulation in communication, social, and mating behaviors in natural settings.

CHAPTER III

METHODOLOGY

A. Accelerometer, PCB & Backpack

Using indirect recording tools like microphones would tamper with our ability to obtain individual-specific vocalizations, and would include the totality of background noises and conspecific vocalizations from the target birds' environment. Alternatively, direct measurements using implantable accelerometers capable of recording vocalizations through bone conduction would be the most fitting tools for recordings that need to be obtained individually from free-living animals in natural settings. To this end, we used an accelerometer and coupled it to a backpack containing a miniature board capable of receiving, analyzing, and then saving the vocalization recorded. Attachment of those components to the birds are necessary for the provision of accurate, individual-specific signals.

In this study, we used a hermetically sealed BU series accelerometer (Knowles Acoustics) weighing 0.28 grams and featuring broadband output along with a wide frequency range (Fig. 8).

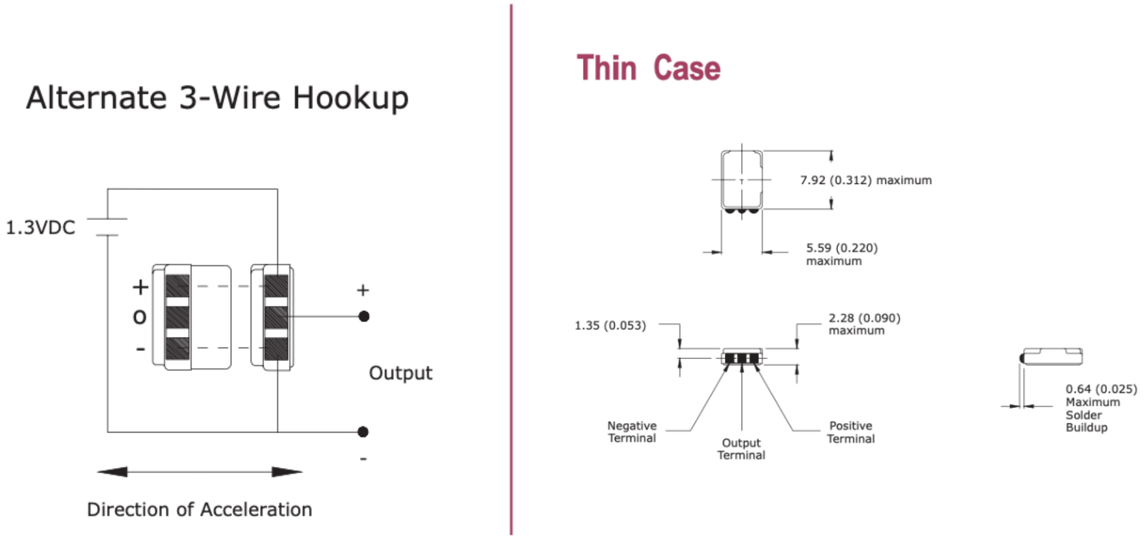


Figure 8 Wire Hookup and Outline drawing of the BU series accelerometer used.

Performance wise, this accelerometer possesses a sensitivity tolerance of around 4.5 dB at 1000 Hz and can be powered by direct current between 1.5 and 10.0 Volts. Moreover, it has a current conduction capability of a maximum of 50 uA, as well as an output impedance ranging from 4900 to 5500 ohms. This 3-wire hookup accelerometer allows us to obtain recordings that are not subject to noise other than low frequency signals caused by the birds' own movements, thus acting as a first "filter" towards the obtention of a clear signal (Fig. 9).

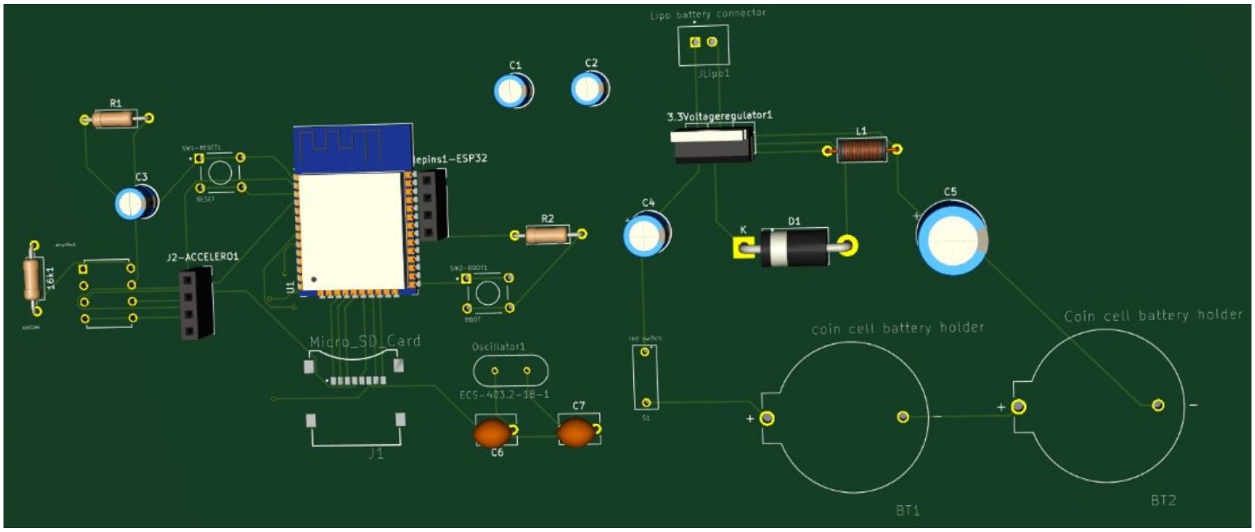


Figure 9 KiCad generated 3D footprint of the circuit board to be tested and used in the backpack.

When it comes to the circuit board, it was first decided to run Arduino software on an ESP32 Wroom 32 microcontroller that was programmed to run using a BOOT and RESET buttons, this ESP32 works in conjunction with a Crystal oscillator (Quartz HC49U) 40MHZ. The Arduino-SDK was chosen specifically thanks to the versatility it offers and its practicality in case of a change in microcontroller design.

The accelerometer takes in the vibration and translates them into a voltage signal that can be measured and detected. But that voltage provided is in the range of 0.2-0.4 V with the output to ESP32 needing to be about 3V max, so we had to amplify this signal. To achieve that, we used an amplifier (IC AD623AN) that supports a single supply of 3.0 V and that leaves all the variations, forms and integrity of the signal intact, but amplifies it by the programmed gain before feeding it back to the ESP. This amplifier is designed to provide accurate gains using 0.1%-1% tolerance resistors. Our accelerometer is connected to the microcontroller along with other components, and feeds the measured signal into the ESP32 where it is stored digitally after passing by the ADC thus undergoing analog to digital conversion. The microcontroller does not save all the signals sent by the accelerometer, instead, we get the power of the received signal at a predetermined frequency and compare it to the total power, if this component in frequency contributes the most to the signal (or if the ratio of that power) is above a certain value (about 0.2) then it means that there is significant power which is representative of bird vocalization, and it is only then that the signal gets stored in the micro SD card.

Furthermore, the PCB contains a microSD card socket that interfaces directly with the ESP32 microcontroller, and is used to store the recorded vocalization for future analysis (Fig. 10). Prior to safeguarding those signals however, another filter responsible for discriminating bird vocalizations was designed and implemented into the microcontroller used in conjunction with the accelerometer. That bandpass filter with cutoff frequency 400 Hz was created using an online tool and tested prior to implementation in the microcontroller.

ESP32 operates at 3.3 Volts and is connected to a dropdown regulator (LM2576) that acts on regulating the voltage supplied to the microcontroller from the power source. This regulator can output 3.3V and 0.5A which is appropriate for our ESP32 microcontroller. Decoupling capacitors were also added to stabilize the voltage supply and avoid fluctuations. The final power source in the backpack has not been determined yet but it is currently possible to use either LifePO4 batteries with a working voltage of 3.0V-3.2V or Lipo battery/Li-ion with a working voltage of 3.7V. In case the final design uses LifePO4 batteries, there will be no need for the voltage regulator interface.

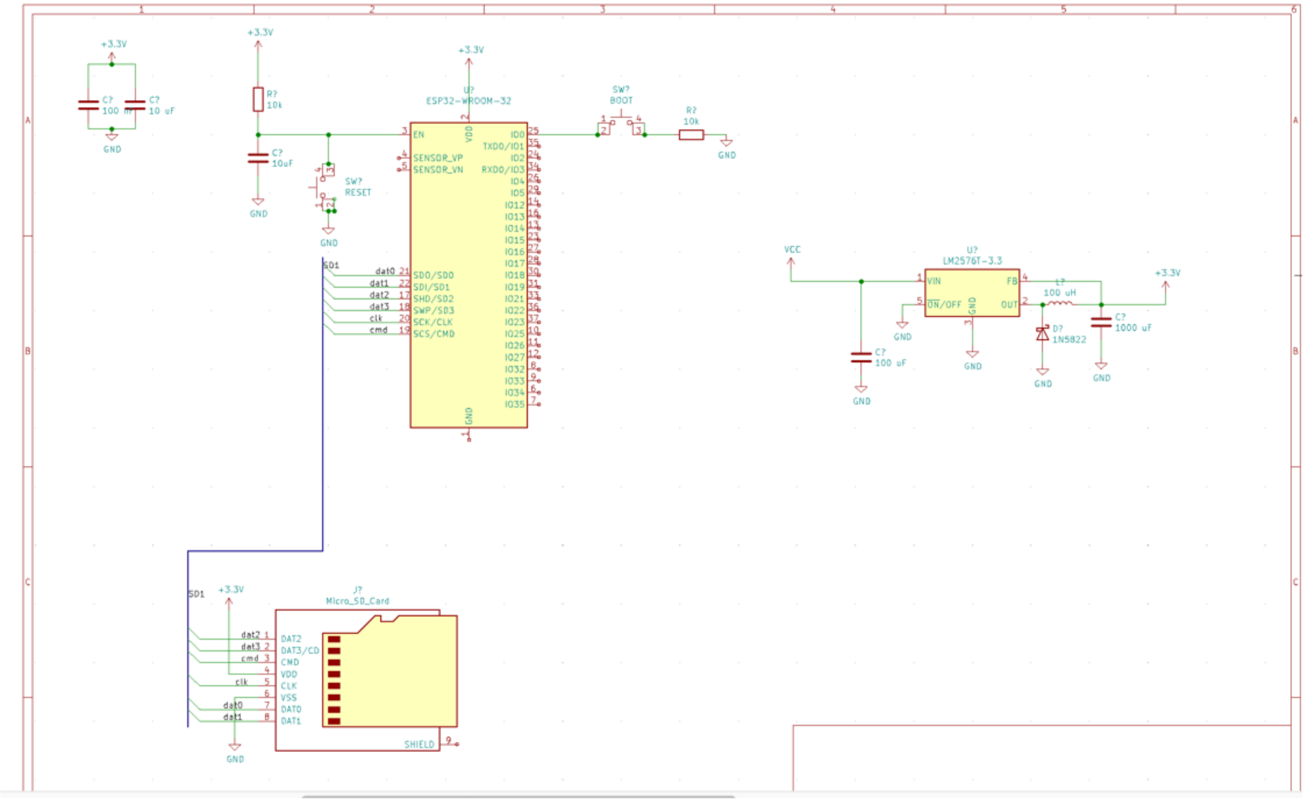


Figure 10 Different components included in the PCB along with their footprints

B. Surgeries

The use of an accelerometer to obtain bird-specific recordings is a common technique in the field of neuroscience. The accelerometer measures the movements of the skull and provides valuable information about the bird's behavior, including vocalizations and head movements. In order to affix the accelerometer onto the skull of the zebra finch, we conducted the following surgical procedure. The following surgery was performed on an overdosed dead bird for demonstration purposes.

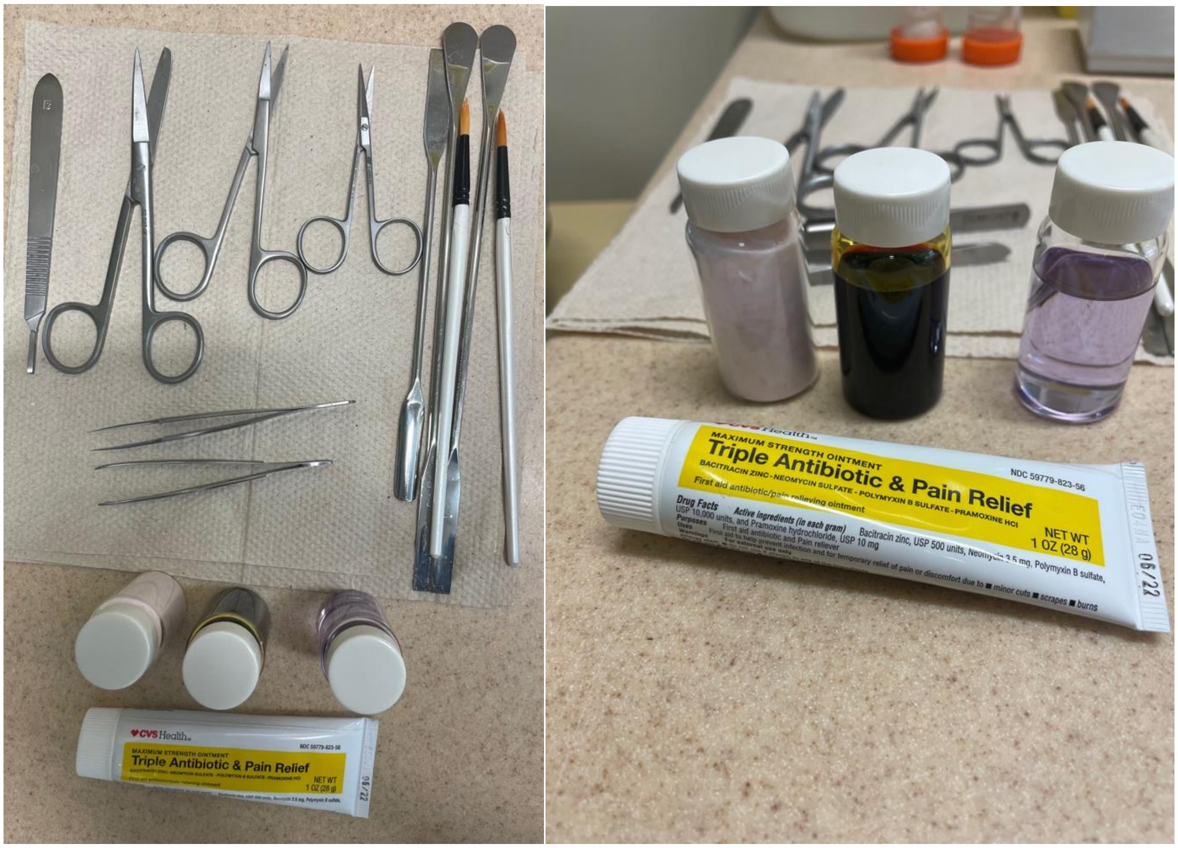


Figure 11 Surgical material, dental cement mixture, and anesthetic used during the surgery.

Before the surgery, the bird was placed under general Isoflurane anesthesia. Isoflurane is a commonly used inhalational anesthetic that causes reversible loss of consciousness and reflexes and is often used in animals because its rapid onset of action and short recovery time. The feathers on and around a specific section of the bird's skull were plucked to provide better access to the skull, to help with the application of the anesthetic and later on, the accelerometer (Fig. 12).

Figure 12 Plucked area of the bird skull.

Next, in an effort to reduce the risk of complications associated with prolonged general anesthesia, we used a local anesthetic gel called Lidocaine, since placing the bird under general anesthesia for too long has shown to be correlated with lower chances of survival post-surgery. Lidocaine is a fast-acting local anesthetic that blocks nerve impulses and provides pain relief. It was applied to the exposed skin of the head using a syringe to numb the area and minimize the bird's discomfort. Equithesin being a combination of three anesthetic agents, including chloral hydrate, magnesium sulfate, and pentobarbital, that act together to provide anesthesia, was also used in droplets as an additional protective measure (Fig. 13).

Figure 13 Application of Lidocaine using a syringe to the exposed skin of the bird.

The surgical procedure involved sectioning the skin using surgical scissors to expose the skull, thus, the first layer of the skull was then targeted for removal using a surgical blade (Fig. 14). Shaving the first layer of the skull is a critical step in the procedure, as it allowed access to the second spongy layer of the cranium where the accelerometer was going to be attached, this second spongy layer of the cranium provides a secure attachment point for the accelerometer.

Figure 14 First layer of the skull successfully removed, spongy layer exposed.

To attach the accelerometer, a dental cement mixture prepared in the lab was used, this dental cement is commonly used in surgical procedures as it provides a strong and secure attachment (Fig. 15). The accelerometer was then affixed to the second spongy layer of the cranium, and the dental cement was allowed to set (Fig. 16) .

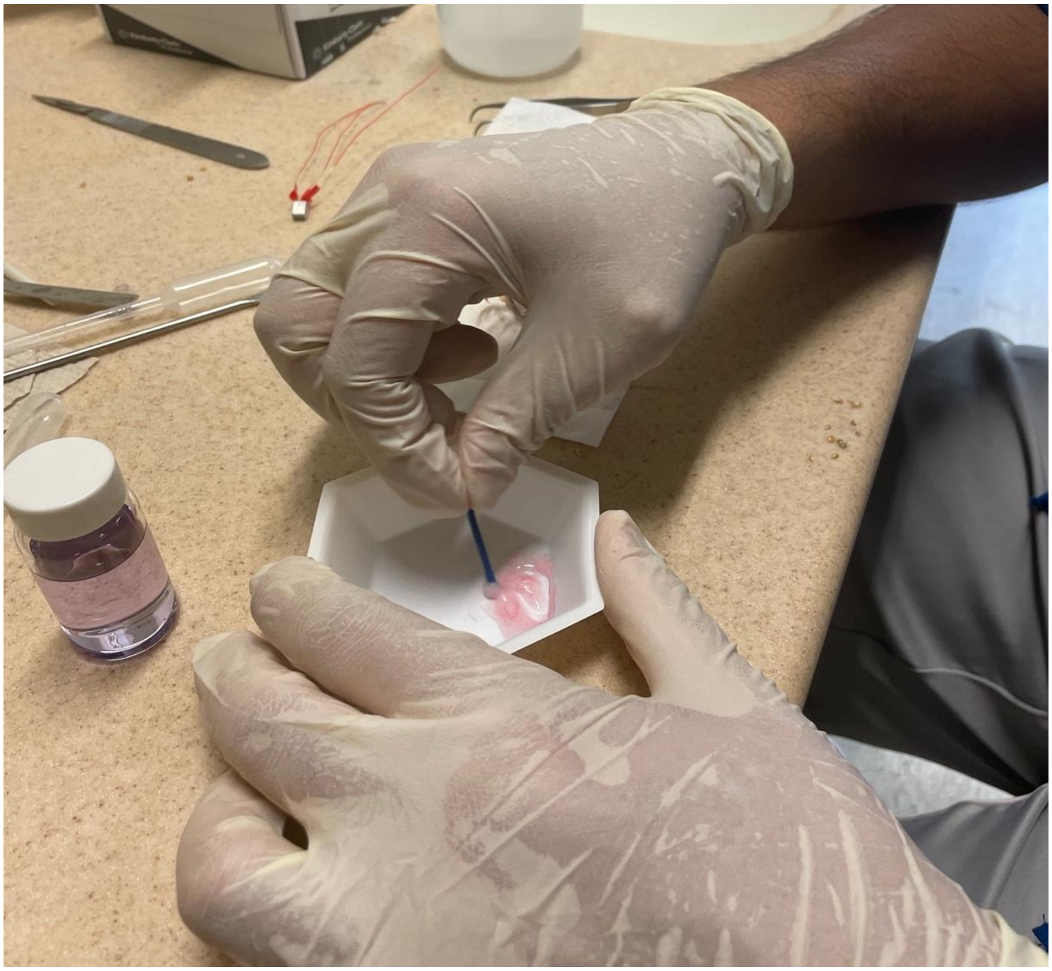


Figure 15 Preparation of the dental cement mixture.

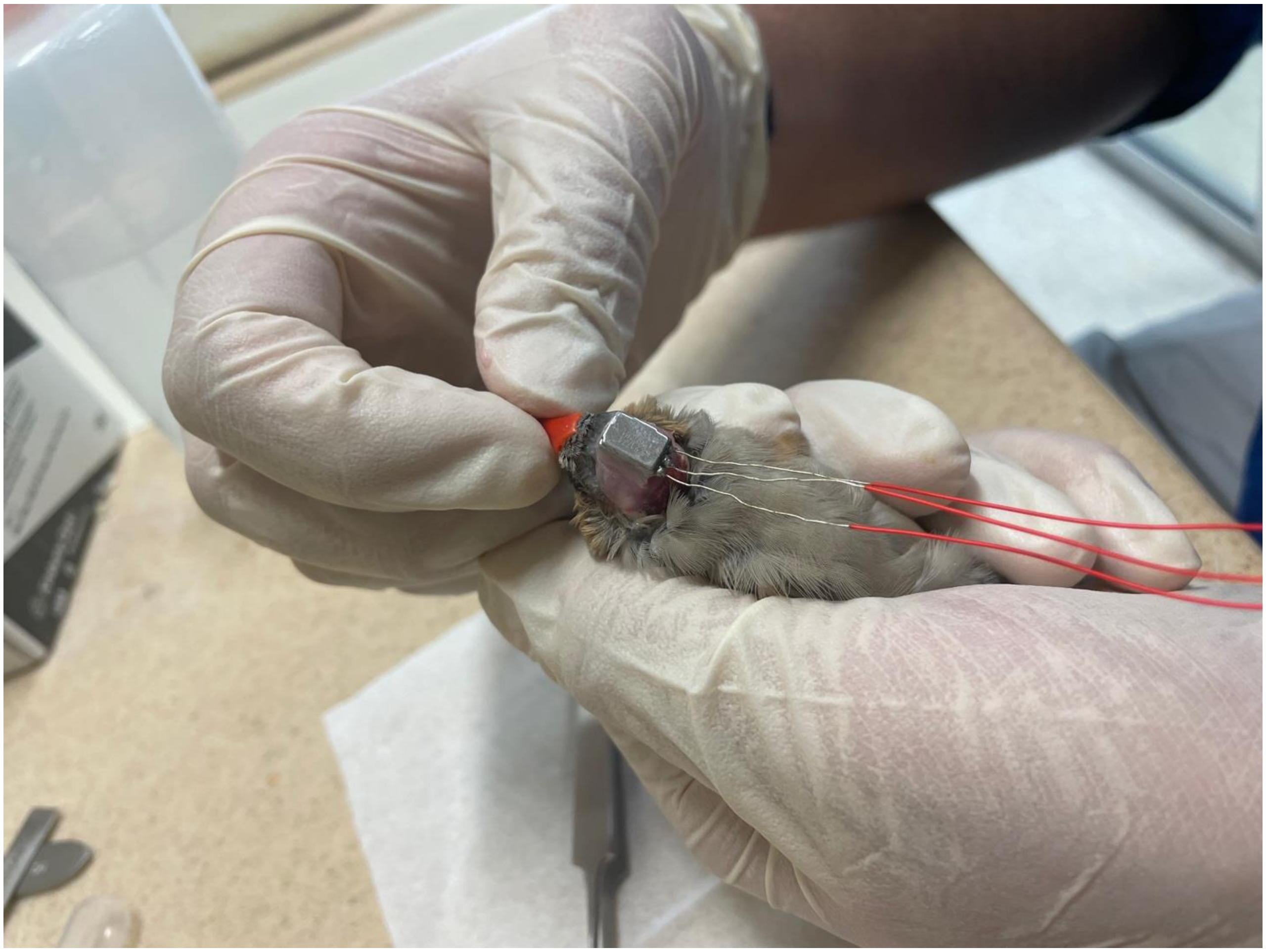


Figure 16 Accelerometer successfully affixed to the second layer of the skull.

Following the surgery, the bird was placed back into its cage to recover and was provided with food and water during the recovery period to ensure that it regained its strength and recovered from the surgery without complications. This procedure allowed us to collect vocalization data from the accelerometer, which we then compared to data obtained from more traditional methods, notably through microphone recordings, and this for two distinct birds which were labeled as Orange bird and White bird.

C. Software and Analysis

In order to analyze the data collected by both the accelerometer and the microphone, we used a software called SAP, or Sound Analysis Pro (Fig. 17), which provides a user-friendly interface allowing users to import and visualize sound recordings, apply filters, segment and annotate sounds, and generate various analyses, such as spectrograms, power spectra, and pitch tracks.

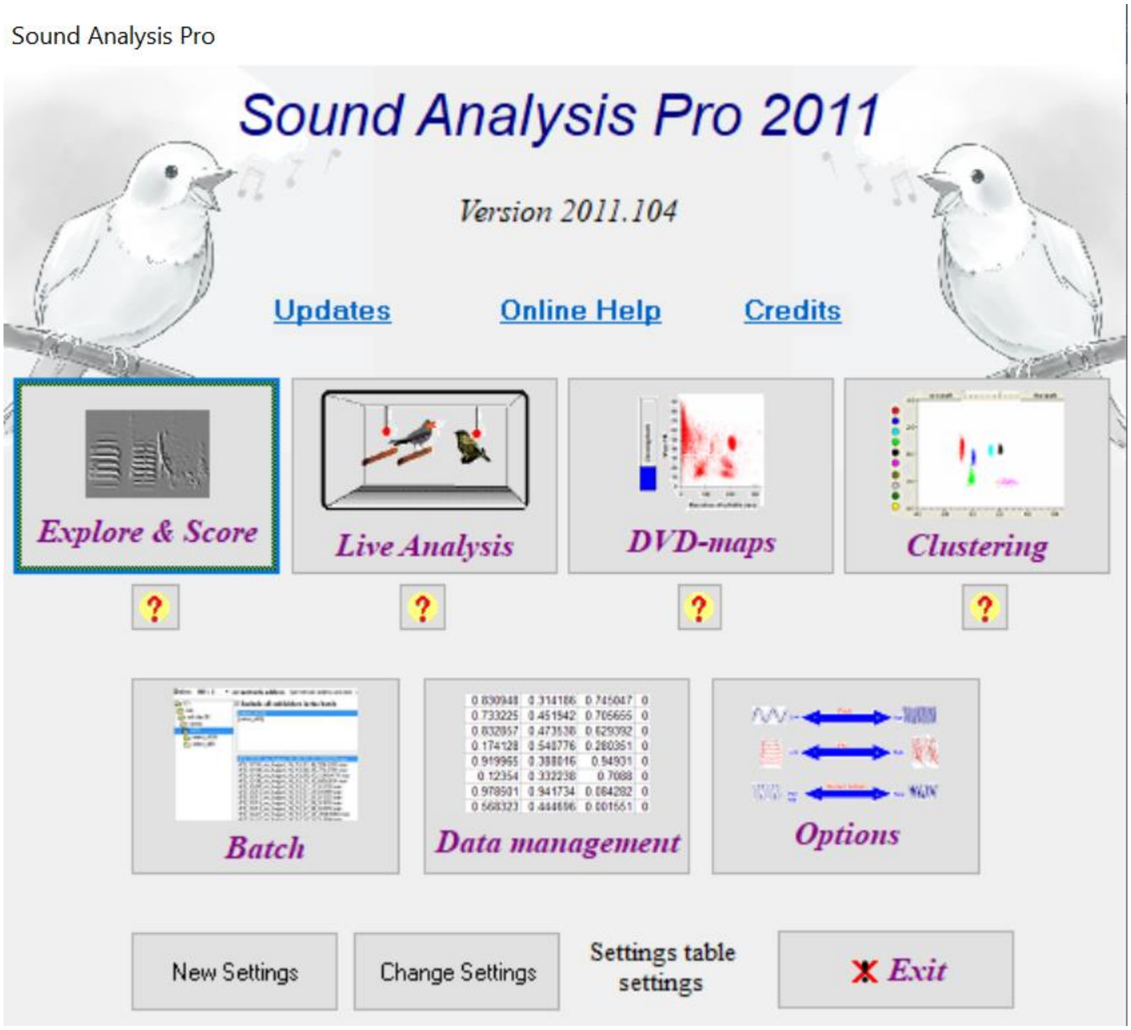
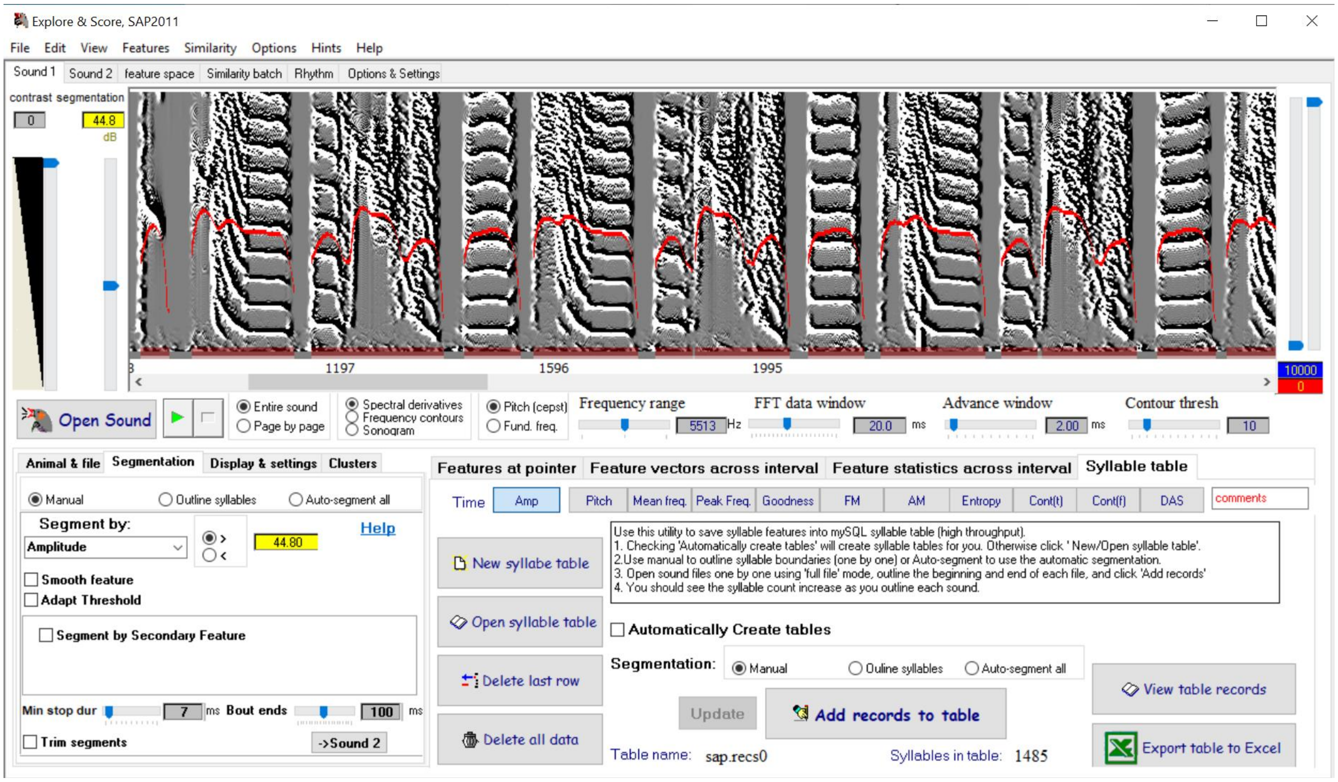


Figure 17 . Sound Analysis Pro software.

SAP (Sound Analysis Pro) was developed by the Bioacoustics Research Program at the Cornell Lab of Ornithology for analyzing and visualizing sound recordings, and is built on a digital signal processing (DSP) engine named ztBirdEngine, capable of handling sound input from up to 10 channels and performing real-time signal processing, recording, and playback control. SAP is designed for researchers and conservation biologists who want to analyze and understand the sounds made by animals, particularly birds, but can also be used for any kind of acoustic analysis. Some of the key features of SAP include a user-friendly interface that allows users to import and visualize sound recordings, apply filters, segment and annotate sounds, and generate various analyses, such as spectrograms, power spectra, and pitch tracks as well as a range of tools and algorithms for automating sound analysis, such as automated sound classification, clustering, and pattern recognition.



contrast segmentation: 0 / 44.8 dB

● Entire sound ○ Page by page

● Spectral derivatives ○ Frequency contours ○ Sonogram

● Pitch (cepst) ○ Fund. freq.

Frequency range: 5513 Hz

FFT data window: 20.0 ms

Advance window: 2.00 ms

Contour thresh: 10

● Manual ○ Outline syllables ○ Auto-segment all

Segment by: Amplitude ● > ○ < 44.80

☐ Smooth feature

☐ Adapt Threshold

☐ Segment by Secondary Feature

Min stop dur 7 ms Bout ends 100 ms

☐ Trim segments

Use this utility to save syllable features into mySQL syllable table (high throughput).
1. Checking 'Automatically create tables' will create syllable tables for you. Otherwise click ' New/Open syllable table'.
2.Use manual to outline syllable boundaries (one by one) or Auto-segment to use the automatic segmentation.
3. Open sound files one by one using 'full file' mode, outline the beginning and end of each file, and click 'Add records'
4. You should see the syllable count increase as you outline each sound.

☐ Automatically Create tables

Segmentation: ● Manual ○ Ouline syllables ○ Auto-segment all

Table name: sap.recs0

Syllables in table: 1485

Figure 18 Explore & Score Feature of SAP.

For this study, SAP, and more precisely its Explore & Score feature (Fig. 18) was used in order to better understand the changes in the temporal and spectral features of song such as syllables' duration, pitch, frequency modulation, entropy, etc…

The SAP (Sound Analysis Pro) software offers an Explore and Score feature that is specifically designed to assist users in identifying and analyzing specific sound events within a vast dataset, which proves to be the most appropriate for the goal of this study. This feature is particularly helpful when analyzing animal vocalizations, such as bird songs or bat calls, where multiple vocalizations of interest may be present in the sound recordings.

This feature comprises two primary steps that are intended to facilitate the identification and labeling of vocalizations of interest, followed by their automatic scoring. During the exploration phase, we visualized the waveform and spectrogram of sound recordings, and marked the start and endpoints of the vocalizations we wished to examine. These segments can then be assigned a label, such as "chirp" or "trill," to make them easily recognizable.

Once all the vocalizations of interest have been labeled, we then moved on to the scoring phase, where SAP automatically analyzes the sound recordings and scores the occurrence of each labeled vocalization. This algorithmic scoring process bases itself on spectral and temporal features to match the labeled vocalizations with the sound events detected in the recordings.

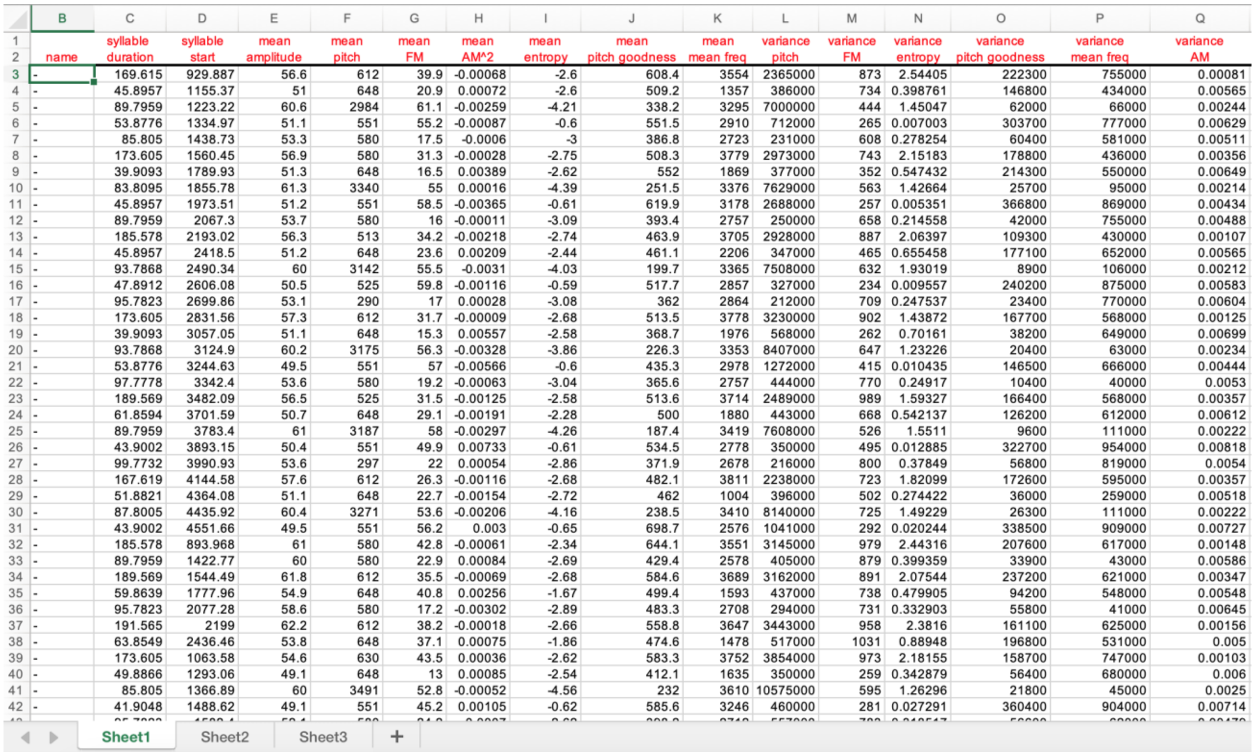
By using the Explore and Score feature, we are able to efficiently and accurately analyze vast datasets containing numerous sound events of interest., thus significantly reducing the time and effort required for manual analysis, providing valuable insights into the behavior and communication of the zebra finch.

Following this first analysis, the different data sets obtained from 2 distinct bird recordings were aggregated into Excel files, which were subsequently run through the following MATLAB code allowing us to visualize the data via graphs.

```matlab
% Setup the Import Options and import the data
opts = spreadsheetImportOptions("NumVariables", 24);%24 is the number of
columns inside the Excel file that can vary

% Specify sheet and range
% choose the biggest data range depending on the Excel files, change B and
% Y accordingly
opts.Sheet = "Sheet1";
opts.DataRange = "B2495:Y15";% the letter is the column and the number is the
row

% Specify column names and types
% make sure that the number of things in variable names equal those in
% variable types
% check for correct order, number of variables
opts.VariableNames = ["name", "duration", "start", "amplitude", "pitch",
"FM", "AM2", "entropy", "pitchgoodness", "meanfreq", "pitch_1", "FM_1",
"entropy_1", "pitchgoodness_1", "meanfreq_1", "AM", "month", "day", "hour",
"minute", "second", "cluster", "filename", "comments"];
opts.VariableTypes = ["double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double"];
```

```
% Import the data, by default as a table but we need it as matrix
% Change the name of the Excel file,the folder
% Make sure the Excel and Matlab files are in the same folder
OrangetableA1 = readtable("/Users/apple/Desktop/Thesis research/New
Tables/orangeA_table.xls", opts, "UseExcel", false);
OrangetableM1 = readtable("/Users/apple/Desktop/Thesis research/New
Tables/orangeB_table.xls", opts, "UseExcel", false);

% Convert to output type
% We need to convert from a table to a matrix because scatter reads only
% matrices
OrangetableA1 = table2array(OrangetableA1);
OrangetableM1 = table2array(OrangetableM1);
 % Clear temporary variables
clear opts

figure(1);
scatter(OrangetableA1(:,2),OrangetableA1(:,8),'blue');%first one is X second
one is Y
title('Accelerometer Data Orange');
xlabel('Syllable Duration');%make sure the units are the same in the
different plots
ylabel('Mean Entropy');

figure(2);
scatter(OrangetableM1(:,2),OrangetableM1(:,8),'green');%:,2 means the whole
of column 2
title('Microphone Data Orange');
xlabel('Syllable Duration');
ylabel('Mean Entropy');

figure(3);
scatter(OrangetableA1(:,2),OrangetableA1(:,8),'blue');
hold on;
scatter(OrangetableM1(:,2),OrangetableM1(:,8),'green');
title('Accelerometer+Microphone Data Orange');
xlabel('Syllable Entropy');
ylabel('Mean FM');
hold off;
```

The obtained Excel file (Fig. 19) contains information about the syllable duration, syllable start, mean amplitude, mean pitch, mean FM, mean entropy, mean pitch goodness, mean frequency, variance pitch, variance FM, variance entropy, variance pitch goodness, variance mean frequency, variance AM. For the purpose of this study, we focused on Syllable Duration, Mean FM, Mean Pitch, and Mean Entropy.



	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		syllable	syllable	mean	mean	mean	mean	mean	mean	mean	variance	variance	variance	variance	variance	variance
2	name	duration	start	amplitude	pitch	FM	AM^2	entropy	pitch goodness	mean freq	pitch	FM	entropy	pitch goodness	mean freq	AM
3	-	169.615	929.887	56.6	612	39.9	-0.00068	-2.6	608.4	3554	2365000	873	2.54405	222300	755000	0.00081
4	-	45.8957	1155.37	51	648	20.9	0.00072	-2.6	509.2	1357	386000	734	0.398761	146800	434000	0.00565
5	-	89.7959	1223.22	60.6	2984	61.1	-0.00259	-4.21	338.2	3295	7000000	444	1.45047	62000	66000	0.00244
6	-	53.8776	1334.97	51.1	551	55.2	-0.00087	-0.6	551.5	2910	712000	265	0.007003	303700	777000	0.00629
7	-	85.805	1438.73	53.3	580	17.5	-0.0006	-3	386.8	2723	231000	608	0.278254	60400	581000	0.00511
8	-	173.605	1560.45	56.9	580	31.3	-0.00028	-2.75	508.3	3779	2973000	743	2.15183	178800	436000	0.00356
9	-	39.9093	1789.93	51.3	648	16.5	0.00389	-2.62	552	1869	377000	352	0.547432	214300	550000	0.00649
10	-	83.8095	1855.78	61.3	3340	55	0.00016	-4.39	251.5	3376	7629000	563	1.42664	25700	95000	0.00214
11	-	45.8957	1973.51	51.2	551	58.5	-0.00365	-0.61	619.9	3178	2688000	257	0.005351	366800	869000	0.00434
12	-	89.7959	2067.3	53.7	580	16	-0.00011	-3.09	393.4	2757	250000	658	0.214558	42000	755000	0.00488
13	-	185.578	2193.02	56.3	513	34.2	-0.00218	-2.74	463.9	3705	2928000	887	2.06397	109300	430000	0.00107
14	-	45.8957	2418.5	51.2	648	23.6	0.00209	-2.44	461.1	2206	347000	465	0.655458	177100	652000	0.00565
15	-	93.7868	2490.34	60	3142	55.5	-0.0031	-4.03	199.7	3365	7508000	632	1.93019	8900	106000	0.00212
16	-	47.8912	2606.08	50.5	525	59.8	-0.00116	-0.59	517.7	2857	327000	234	0.009557	240200	875000	0.00583
17	-	95.7823	2699.86	53.1	290	17	0.00028	-3.08	362	2864	212000	709	0.247537	23400	770000	0.00604
18	-	173.605	2831.56	57.3	612	31.7	-0.00009	-2.68	513.5	3778	3230000	902	1.43872	167700	568000	0.00125
19	-	39.9093	3057.05	51.1	648	15.3	0.00557	-2.58	368.7	1976	568000	262	0.70161	38200	649000	0.00699
20	-	93.7868	3124.9	60.2	3175	56.3	-0.00328	-3.86	226.3	3353	8407000	647	1.23226	20400	63000	0.00234
21	-	53.8776	3244.63	49.5	551	57	-0.00566	-0.6	435.3	2978	1272000	415	0.010435	146500	666000	0.00444
22	-	97.7778	3342.4	53.6	580	19.2	-0.00063	-3.04	365.6	2757	444000	770	0.24917	10400	40000	0.0053
23	-	189.569	3482.09	56.5	525	31.5	-0.00125	-2.58	513.6	3714	2489000	989	1.59327	166400	568000	0.00357
24	-	61.8594	3701.59	50.7	648	29.1	-0.00191	-2.28	500	1880	443000	668	0.542137	126200	612000	0.00612
25	-	89.7959	3783.4	61	3187	58	-0.00297	-4.26	187.4	3419	7608000	526	1.5511	9600	111000	0.00222
26	-	43.9002	3893.15	50.4	551	49.9	0.00733	-0.61	534.5	2778	350000	495	0.012885	322700	954000	0.00818
27	-	99.7732	3990.93	53.6	297	22	0.00054	-2.86	371.9	2678	216000	800	0.37849	56800	819000	0.0054
28	-	167.619	4144.58	57.6	612	26.3	-0.00116	-2.68	482.1	3811	2238000	723	1.82099	172600	595000	0.00357
29	-	51.8821	4364.08	51.1	648	22.7	-0.00154	-2.72	462	1004	396000	502	0.274422	36000	259000	0.00518
30	-	87.8005	4435.92	60.4	3271	53.6	-0.00206	-4.16	238.5	3410	8140000	725	1.49229	26300	111000	0.00222
31	-	43.9002	4551.66	49.5	551	56.2	0.003	-0.65	698.7	2576	1041000	292	0.020244	338500	909000	0.00727
32	-	185.578	893.968	61	580	42.8	-0.00061	-2.34	644.1	3551	3145000	979	2.44316	207600	617000	0.00148
33	-	89.7959	1422.77	60	580	22.9	0.00084	-2.69	429.4	2578	405000	879	0.399359	33900	43000	0.00586
34	-	189.569	1544.49	61.8	612	35.5	-0.00069	-2.68	584.6	3689	3162000	891	2.07544	237200	621000	0.00347
35	-	59.8639	1777.96	54.9	648	40.8	0.00256	-1.67	499.4	1593	437000	738	0.479905	94200	548000	0.00548
36	-	95.7823	2077.28	58.6	580	17.2	-0.00302	-2.89	483.3	2708	294000	731	0.332903	55800	41000	0.00645
37	-	191.565	2199	62.2	612	38.2	-0.00018	-2.66	558.8	3647	3443000	958	2.3816	161100	625000	0.00156
38	-	63.8549	2436.46	53.8	648	37.1	0.00075	-1.86	474.6	1478	517000	1031	0.88948	196800	531000	0.005
39	-	173.605	1063.58	54.6	630	43.5	0.00036	-2.62	583.3	3752	3854000	973	2.18155	158700	747000	0.00103
40	-	49.8866	1293.06	49.1	648	13	0.00085	-2.54	412.1	1635	350000	259	0.342879	56400	680000	0.006
41	-	85.805	1366.89	60	3491	52.8	-0.00052	-4.56	232	3610	10575000	595	1.26296	21800	45000	0.0025
42	-	41.9048	1488.62	49.1	551	45.2	0.00105	-0.62	585.6	3246	460000	281	0.027291	360400	904000	0.00714

Figure 19 Excel File obtained following the analysis on SAP software.

CHAPTER IV

ANALYSIS & RESULTS

The scatter plots below (Fig. 20, Fig. 21) display the superimposition of two data sets, one obtained from the accelerometer and the other from the microphone of the "Orange" bird surgeries surgeries and analyses. The green dots represent the vocalization data obtained from the microphone while the blue dots represent those obtained from the accelerometer. The resulting plots suggest that there is a strong positive correlation between the two sets of data, as they tend to cluster together throughout the plot, indicating that there is a correlation between the bird's vocalizations as recorded with the microphone and accelerometer. Furthermore, this correlation between the data sets is maintained when the syllable duration is plotted against the Mean FM and Mean Entropy, maintaining similarities in both shape, and positioning, indicating that the accelerometer data is reliable and consistent.

Thus, the accelerometer proves to be an efficient method of recording, displaying, and representing bird vocalizations. The consistent positioning and lack of shape variability in the scatter plots demonstrate that the data obtained from the accelerometer is representative of the correct vocalizations of the bird, as they are. This consistency in shape and positioning suggests that the accelerometer is an efficient and accurate method of recording bird vocalizations and can be used to provide valuable insights into the relationship between a bird's physical movements and its vocalizations.

Overall, this analysis of the scatter plot suggests that using the accelerometer as a method for recording bird vocalizations is an effective and reliable technique, when compared to the alternative method of using a microphone as a recording tool. The uniformity of shape in the data between the scatter plots indicates that the data points are evenly distributed and that there are no significant outliers that may affect the correlation between the two data sets, cementing the accelerometer as an efficient, sure, and accurate way to record bird vocalizations.

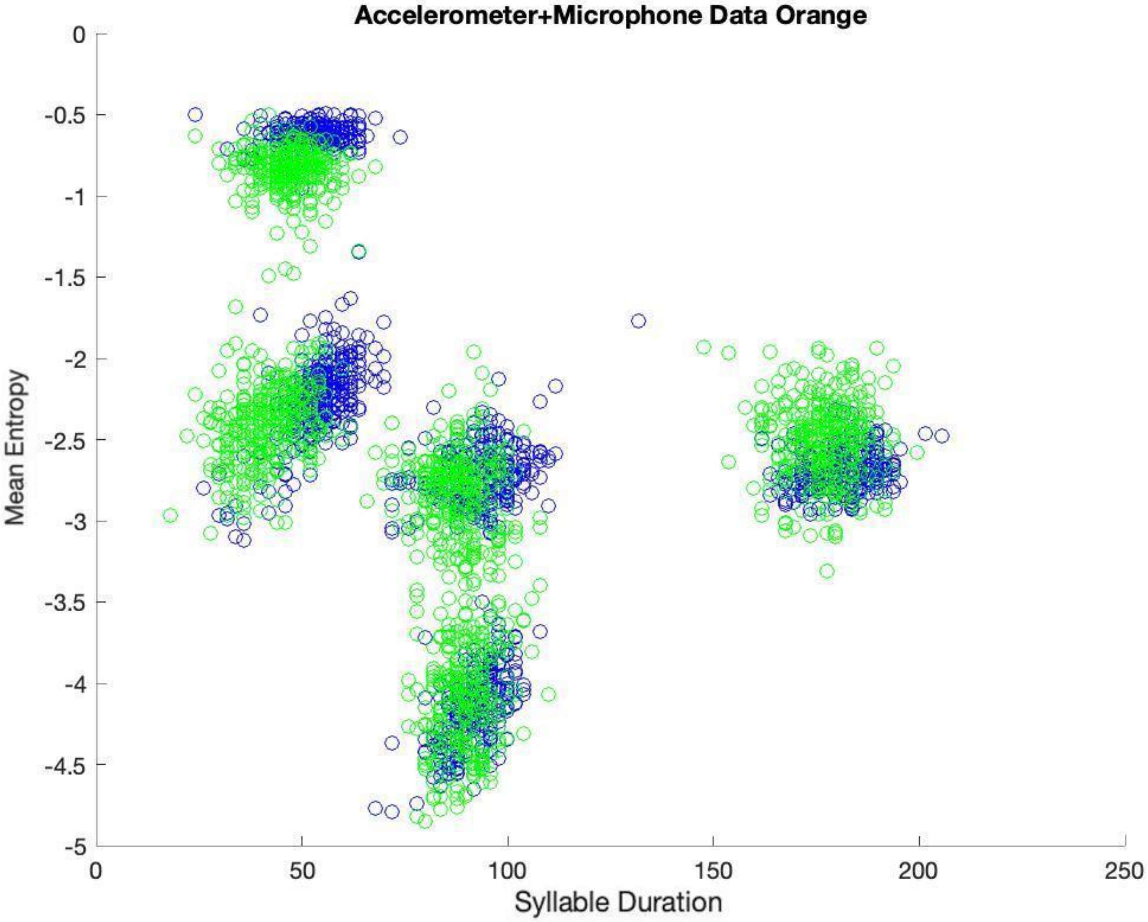


Figure 20 . Scatter plot of mean entropy versus syllable duration representing the superimposed data of the Orange bird for accelerometer recordings (blue) and the microphone recordings (green). Notice the accurate spectral (mean entropy) and temporal (syllable duration) features when comparing the two methods of recording sound.

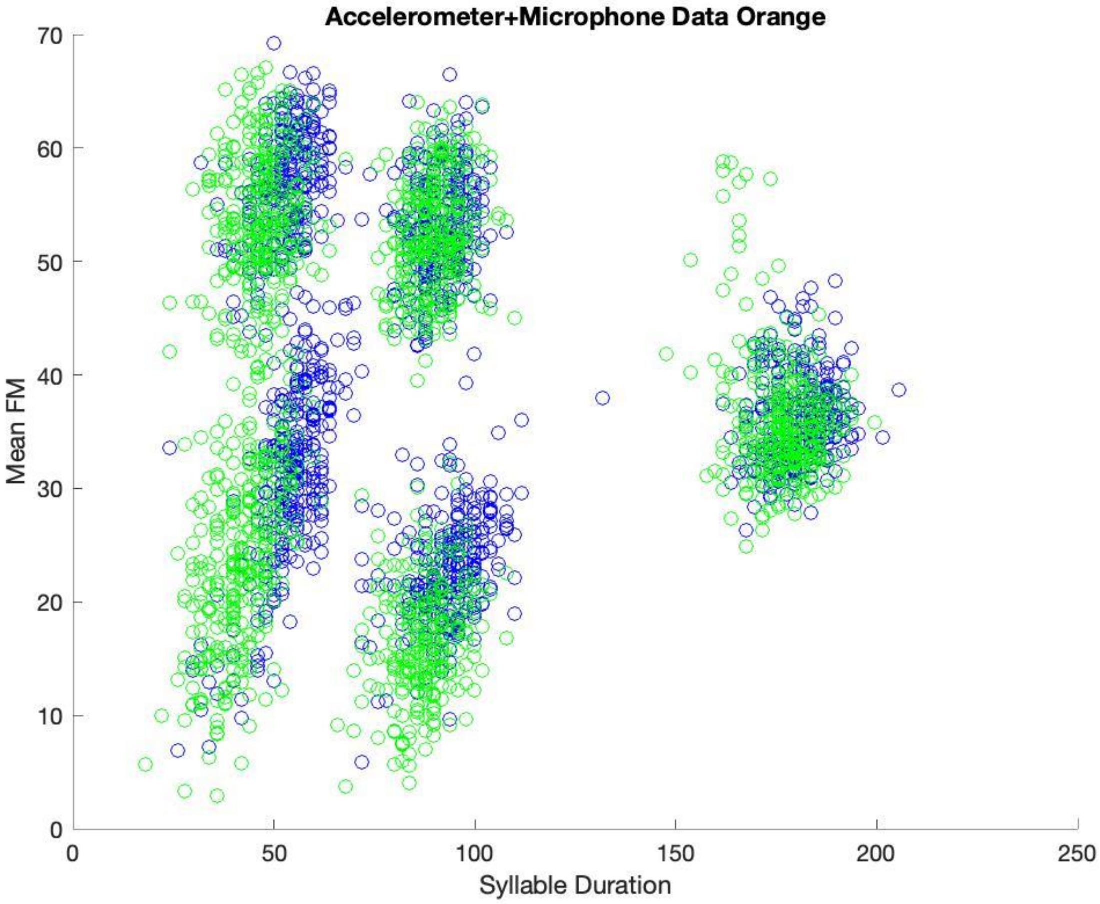


Figure 21 Scatter plot of mean frequency modulation versus syllable duration representing the superimposed data of the Orange bird for accelerometer recordings (blue) and the microphone recordings (green). Notice the accurate spectral (mean FM) and temporal (syllable duration) features when comparing the two methods of recording sound.

The first graphs obtained for the White bird are shown below, representing the superimposition of both the accelerometer and microphone data that were obtained following the same surgeries and analyses as those for the "Orange" bird (Fig. 22, Fig. 23). The superimposed scatter plot displays both distinct data sets, with the green representing the vocalization obtained from the microphone and the blue representing those obtained from the accelerometer. Upon first glance, we can see that both data sets tend to cluster together throughout the plot, suggesting a strong positive correlation between the data points of the two sets. This correlation is further maintained when the Mean FM, and Mean Entropy are plotted against the syllable duration. The similar positioning and lack of shape variability in the scatter plots demonstrate the reliability and consistency of the data obtained from the accelerometer, proving that this method of recording would be both efficient and representative of the correct vocalizations of the bird.

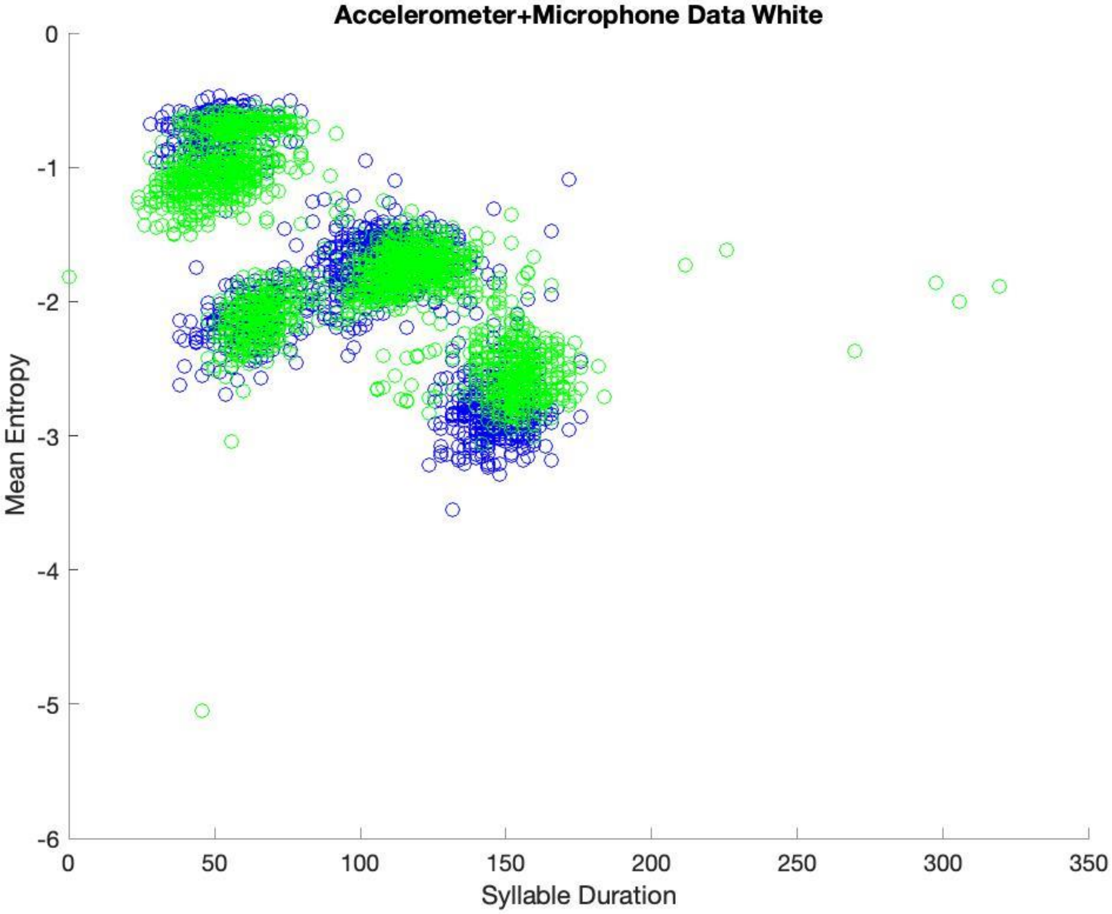


Figure 22 Scatter plot of mean entropy versus syllable duration representing the superimposed data of the White bird for accelerometer recordings (blue) and the microphone recordings (green). Notice the accurate spectral (mean entropy) and temporal (syllable duration) features when comparing the two methods of recording sound.

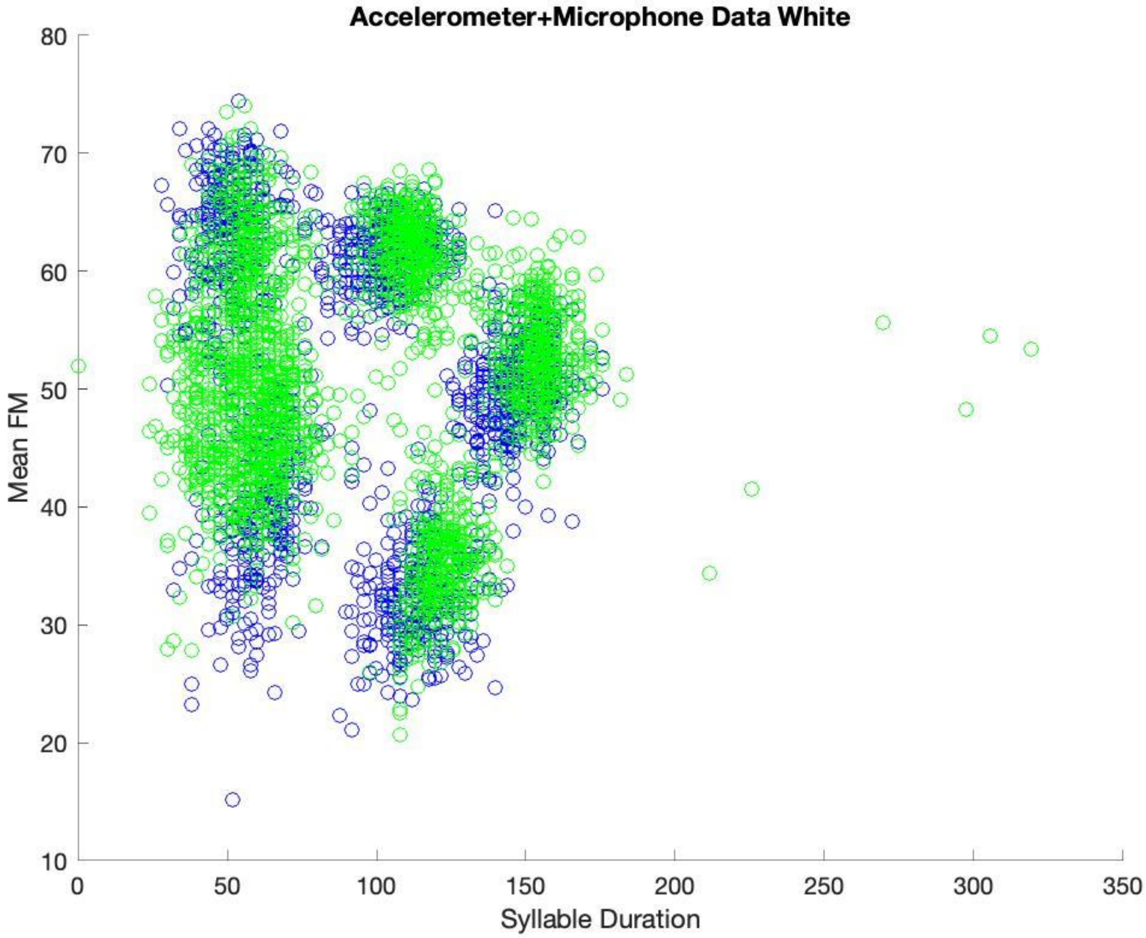


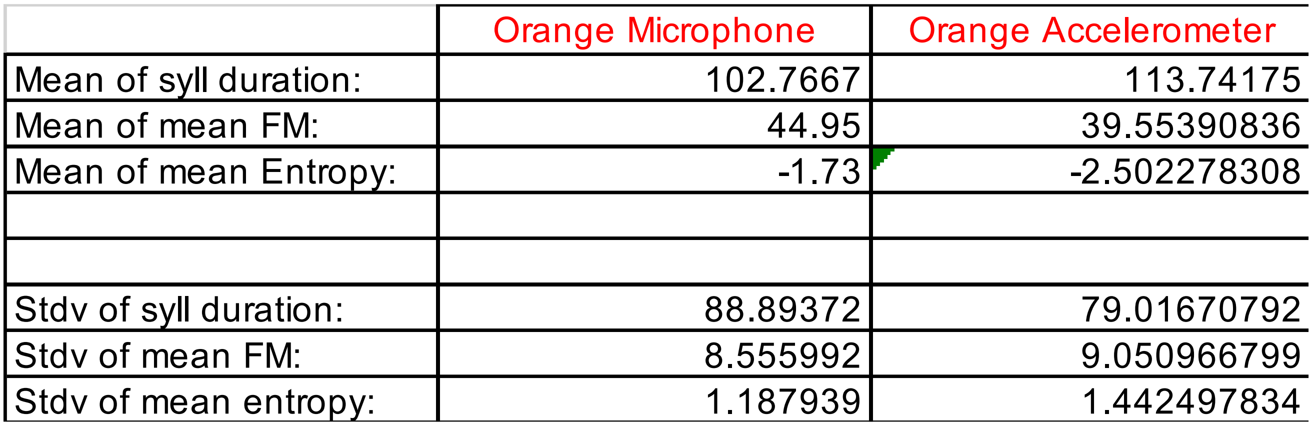
Figure 23 Scatter plot of mean frequency modulation versus syllable duration representing the superimposed data of the White bird for accelerometer recordings (blue) and the microphone recordings (green). Notice the accurate spectral (mean FM) and temporal (syllable duration) features when comparing the two methods of recording sound.

A. Statistical Analysis for the Orange data set

In order to better study the impact of the different recording methods on the vocalizations elicited, we measured the means and standard deviations of each of syllables duration, mean frequency modulation and mean entropy for the entirety of the data set in a specific bird (Fig. 24) and then for each syllable alone in a single bird (Fig. 25), both for accelerotmeter and for microphone recordings. These spectral and temporal features uniquely identify syllables (Figs. 20-23) in both birds as obtained by SAP (see Methods). Furthermore, the statistical analyses were conducted separately on the segmented data to more accurately portray the impact the accelerometer recordings might have had on vocalizations.

Fig. 24 shows the quantification for the entirety of the data set for the Orange bird, for both microphone and accelerometer recordings. We used the two-sample Kolmogorov-Smirnov test as a nonparametric hypothesis test that evaluates the difference between the cdfs of the distributions of the two sample data vectors for each of the microphone and accelerometer data. Notice the tight correspondence and similarity in the measured responses despite the fact that we are averaging across the totality of the data set (p = 0.98, showing that the two sets of data are identical and supporting the null hypothesis that there is no difference). The differences in some the measures are due to the fact that the recordings exhibit intrinsic noise that the microphone detects when the birds is pecking or perching or flicking his wings, etc.. These sounds can not be filtered out without the need for a intensive manual labor work that goes through every song.

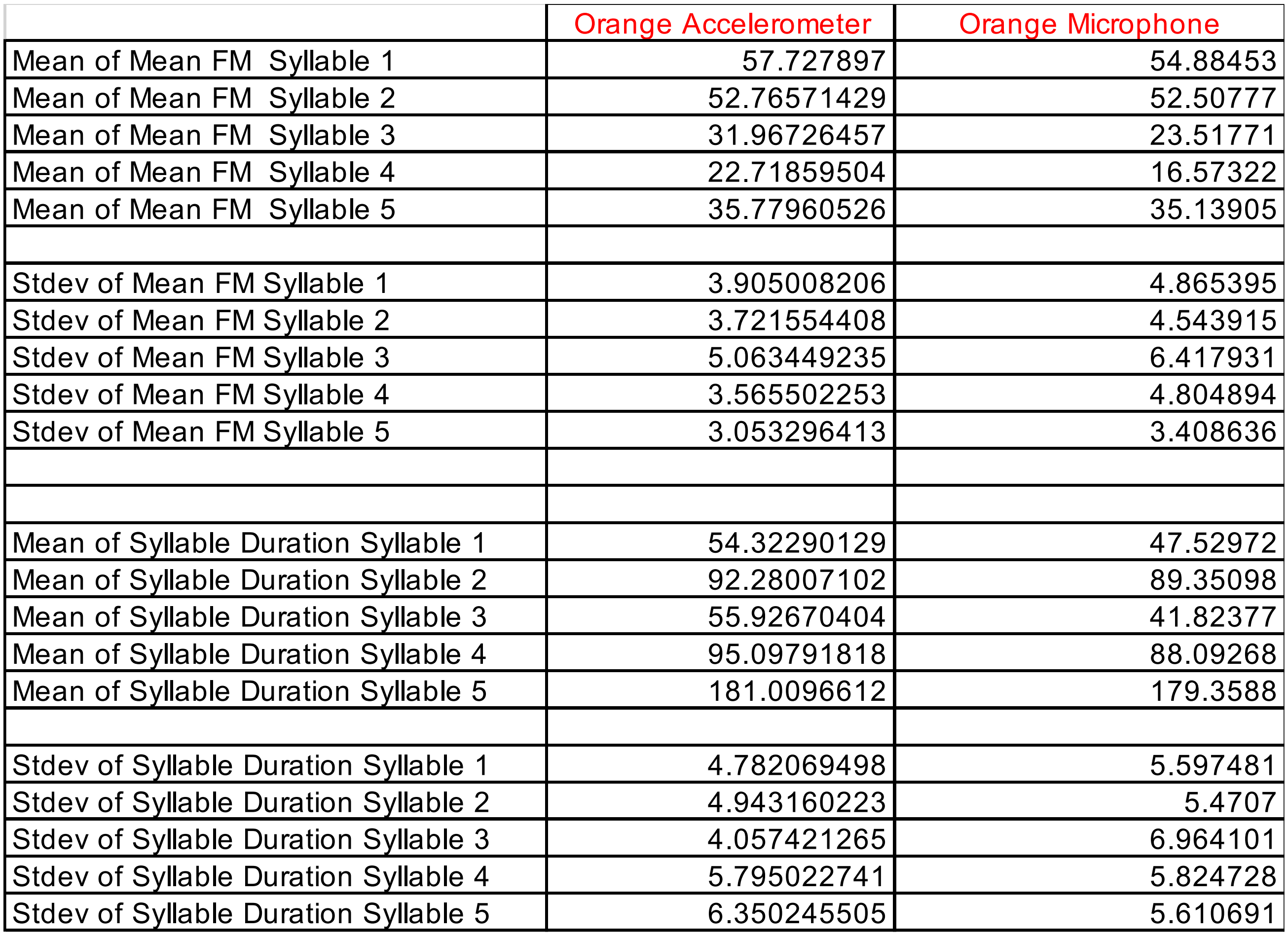
Table 1 Means and standard deviations for syllable durations, mean frequency modulation, and mean entropy of the entirety of the dataset for the Orange bird collected simultaneously using both accelerometer and microphone recordings. Two-sample Kolmogorov-Smirnov test (p-value: 0.98, h:0, ks2stat: 0.1667) shows that the two sets of data are identical, supporting the null hypothesis that there is no difference.



	Orange Microphone	Orange Accelerometer
Mean of syll duration:	102.7667	113.74175
Mean of mean FM:	44.95	39.55390836
Mean of mean Entropy:	-1.73	-2.502278308
Stdv of syll duration:	88.89372	79.01670792
Stdv of mean FM:	8.555992	9.050966799
Stdv of mean entropy:	1.187939	1.442497834

This statistical analysis further ascertains that the accelerometer is an efficient, and accurate recording method, as seen through the statistical results, showing that there is a strong positive correlation between the data sets representing the accelerometer and microphone. Those same overall results are also maintained when considering every syllable independently, with not enough evidence to suggest that the samples come from different distributions.

Table 2 Excel Table containing Mean of Mean Frequency Modulation, Standard Deviation of Mean Frequency Modulation, Mean of Syllable Duration, Standard Deviation of Syllable Duration of the five distinct Syllables recorded using both Accelerometer and Microphone for the Orange Bird. Two-sample Kolmogorov-smirnov test (p-value: 0.999 , h:0, k2stat :0.1) shows that the two sets of data are identical , supporting the null hypothesis that there is no difference.



	Orange Accelerometer	Orange Microphone
Mean of Mean FM Syllable 1	57.727897	54.88453
Mean of Mean FM Syllable 2	52.76571429	52.50777
Mean of Mean FM Syllable 3	31.96726457	23.51771
Mean of Mean FM Syllable 4	22.71859504	16.57322
Mean of Mean FM Syllable 5	35.77960526	35.13905
Stdev of Mean FM Syllable 1	3.905008206	4.865395
Stdev of Mean FM Syllable 2	3.721554408	4.543915
Stdev of Mean FM Syllable 3	5.063449235	6.417931
Stdev of Mean FM Syllable 4	3.565502253	4.804894
Stdev of Mean FM Syllable 5	3.053296413	3.408636
Mean of Syllable Duration Syllable 1	54.32290129	47.52972
Mean of Syllable Duration Syllable 2	92.28007102	89.35098
Mean of Syllable Duration Syllable 3	55.92670404	41.82377
Mean of Syllable Duration Syllable 4	95.09791818	88.09268
Mean of Syllable Duration Syllable 5	181.0096612	179.3588
Stdev of Syllable Duration Syllable 1	4.782069498	5.597481
Stdev of Syllable Duration Syllable 2	4.943160223	5.4707
Stdev of Syllable Duration Syllable 3	4.057421265	6.964101
Stdev of Syllable Duration Syllable 4	5.795022741	5.824728
Stdev of Syllable Duration Syllable 5	6.350245505	5.610691

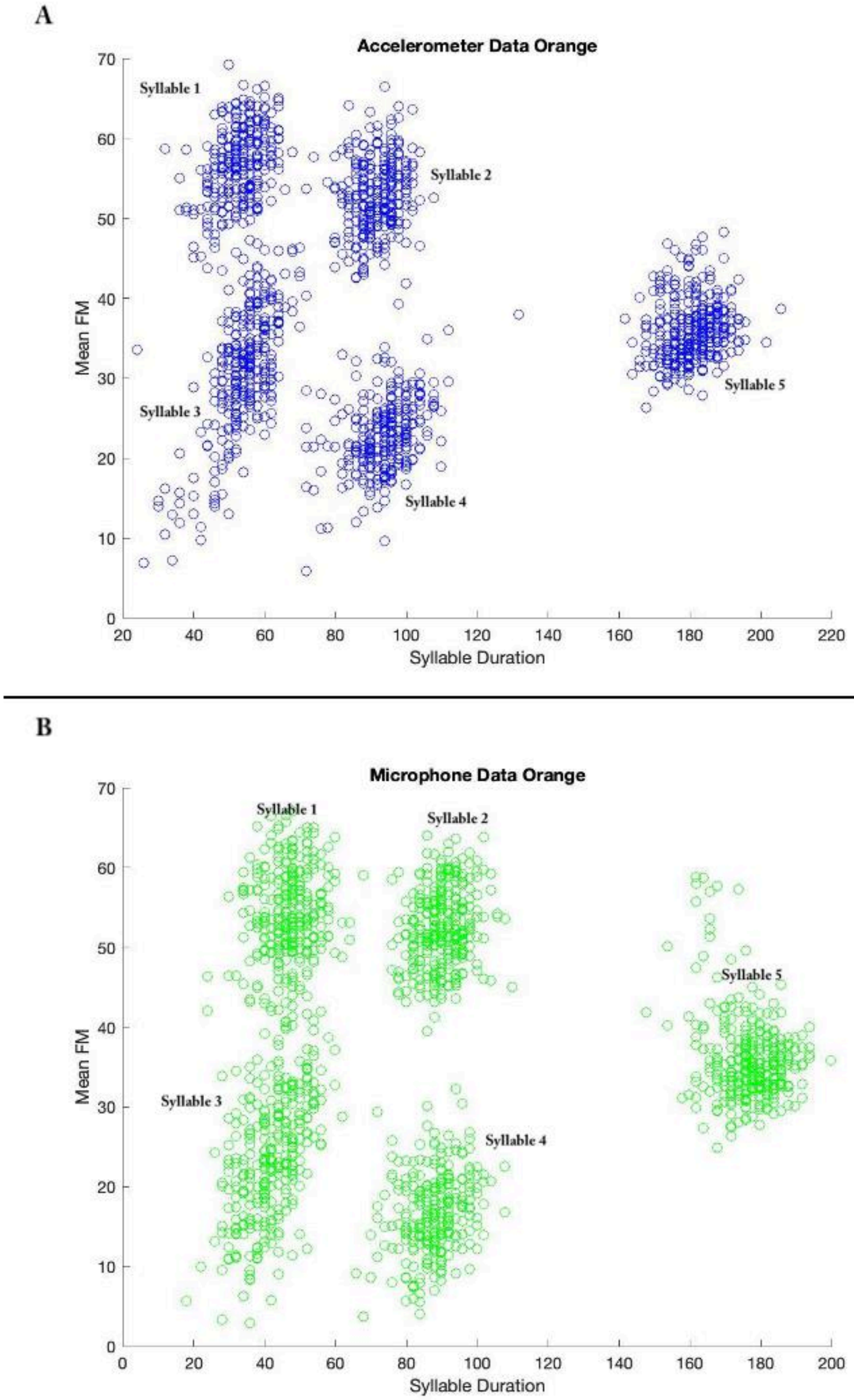
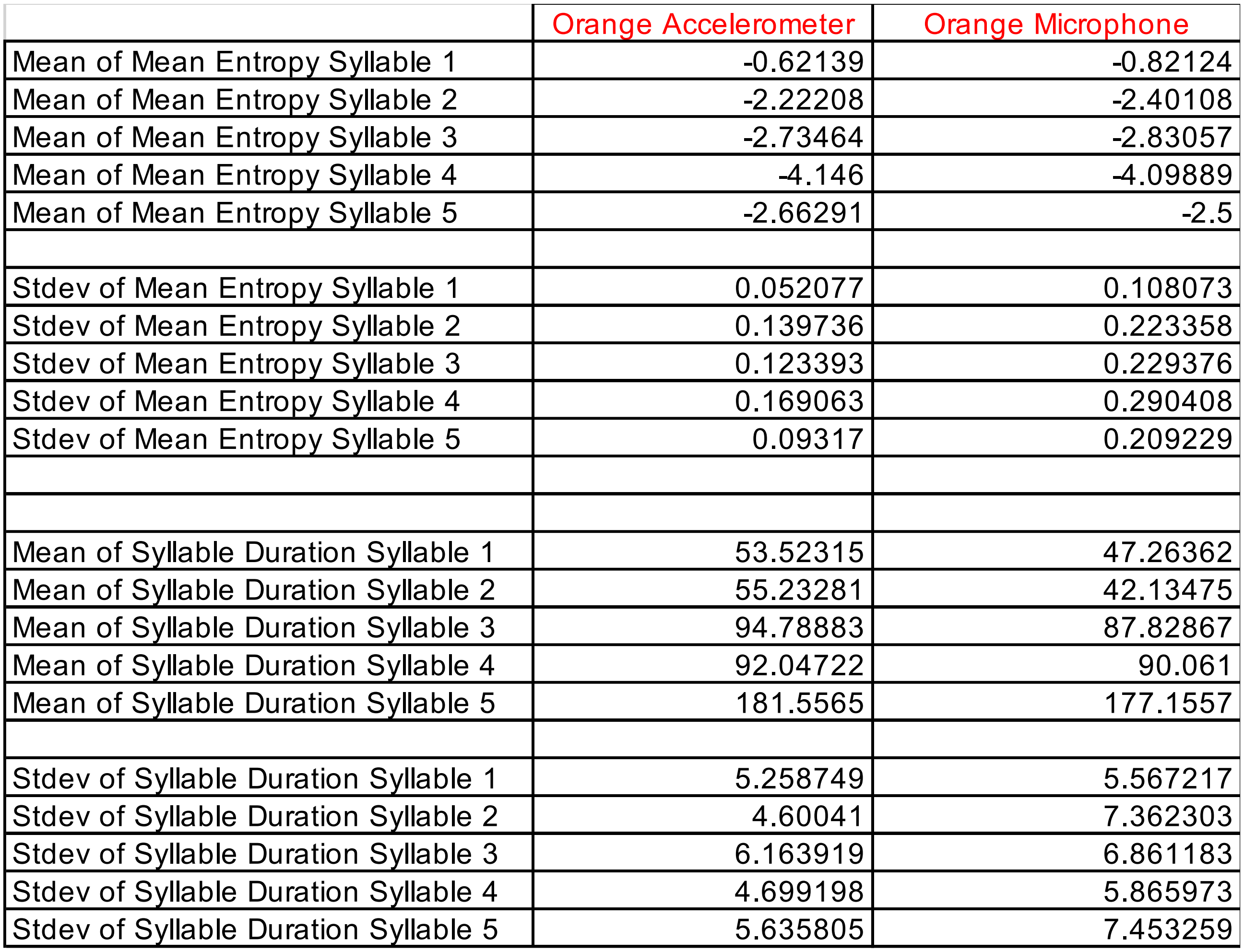


Figure 24 Fig. 25. A, Scatter plotof Mean Frequency Modulation vs Syllable Duration of the Orange bird for the Accelerometer recordings (blue) with distinguishable and annotated Syllables. ***B****,Scatter plot of Mean Frequency Modulation vs Syllable Duration of the Orange bird for the Microphone recordings (green) with distinguishable and annotated Syllables.*

Table 3 Excel Table containing Mean of Mean Entropy, Standard Deviation of Mean Entropy, Mean of Syllable Duration, Standard Deviation of Syllable Duration of the five distinct Syllables recorded using both Accelerometer and Microphone for the Orange bird. Two-Sample Kolmogorov-Smirnov test (p-value :0.771, h:0, kstat: 0.200) shows that the two sets of data are identical, supporting the null hypothesis that there is no difference.



	Orange Accelerometer	Orange Microphone
Mean of Mean Entropy Syllable 1	-0.62139	-0.82124
Mean of Mean Entropy Syllable 2	-2.22208	-2.40108
Mean of Mean Entropy Syllable 3	-2.73464	-2.83057
Mean of Mean Entropy Syllable 4	-4.146	-4.09889
Mean of Mean Entropy Syllable 5	-2.66291	-2.5
Stdev of Mean Entropy Syllable 1	0.052077	0.108073
Stdev of Mean Entropy Syllable 2	0.139736	0.223358
Stdev of Mean Entropy Syllable 3	0.123393	0.229376
Stdev of Mean Entropy Syllable 4	0.169063	0.290408
Stdev of Mean Entropy Syllable 5	0.09317	0.209229
Mean of Syllable Duration Syllable 1	53.52315	47.26362
Mean of Syllable Duration Syllable 2	55.23281	42.13475
Mean of Syllable Duration Syllable 3	94.78883	87.82867
Mean of Syllable Duration Syllable 4	92.04722	90.061
Mean of Syllable Duration Syllable 5	181.5565	177.1557
Stdev of Syllable Duration Syllable 1	5.258749	5.567217
Stdev of Syllable Duration Syllable 2	4.60041	7.362303
Stdev of Syllable Duration Syllable 3	6.163919	6.861183
Stdev of Syllable Duration Syllable 4	4.699198	5.865973
Stdev of Syllable Duration Syllable 5	5.635805	7.453259

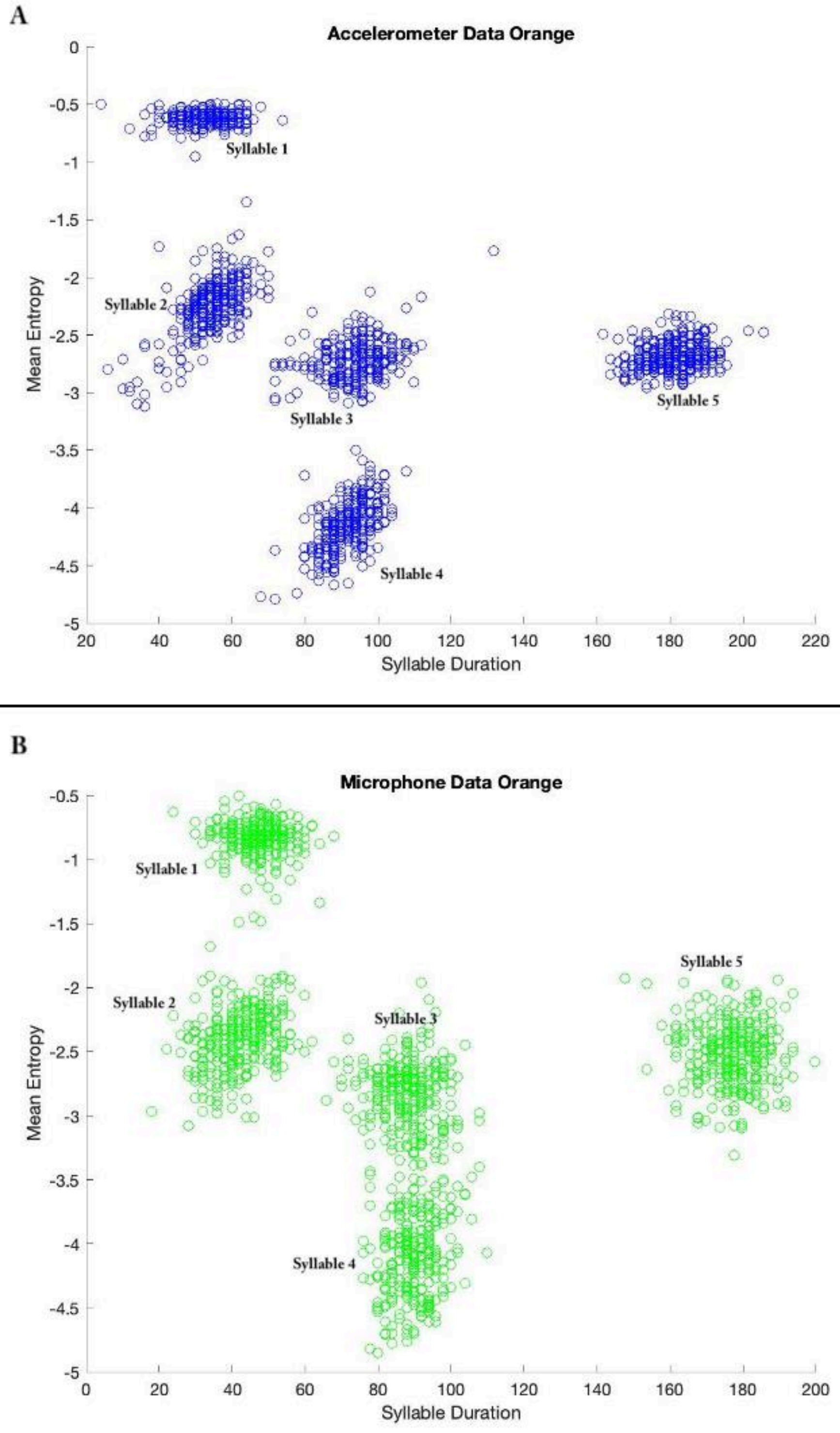


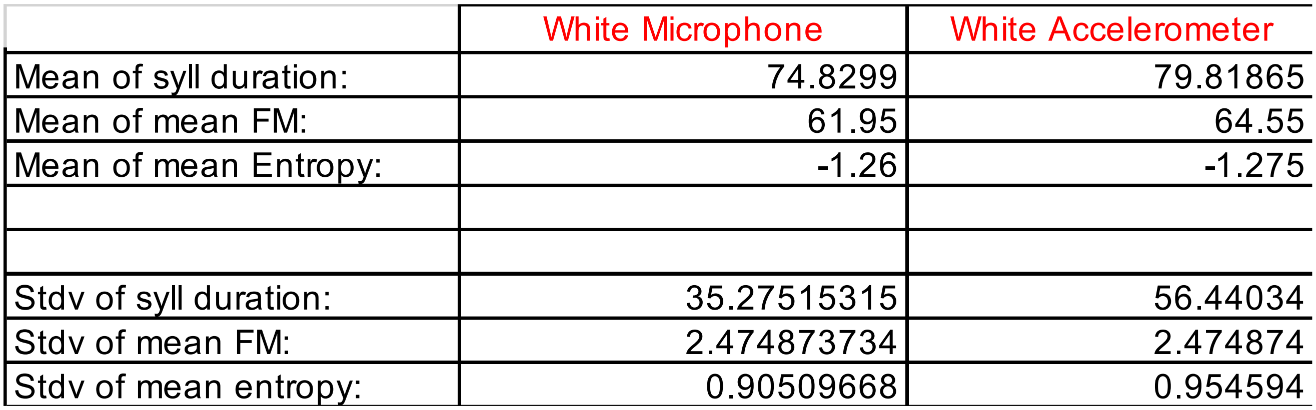
*Figure 25 A, Scatter plot of Mean Entropy vs Syllable Duration of the Orange bird for the Accelerometer recordings (blue) with distinguishable and annotated Syllables.**B**, Scatter plot of Mean Entropy vs Syllable Duration of the Orange bird for the Microphone recordings (green) with distinguishable and annotated Syllables.*

B. Statistical Analysis for the White data set

Moreover, to better study the impact of the different recording methods on the vocalizations elicited for the white bird, we followed the same procedure as previously stated and measured the means and standard deviations of each of syllables duration, mean frequency modulation and mean entropy for the entirety of the data set in a specific bird (Fig. 29) and then for each syllable alone in a single bird (Fig. 30), both for accelerotmeter and for microphone recordings. The syllables annotated are uniquely identified by their spectral and temporal in both birds as obtained by SAP (see Methods). As a way to better and more accurately portray the impact the accelerometer recordings might have had on vocalizations, the statistical analyses were conducted separately on the different segemented data.

Fig. 29 represents the quantification for the entirety of the data set for the White bird, for both microphone and accelerometer recordings. We employed the same two-sample Kolmogorov-Smirnov test as the one for the Orange data set to evaluate the difference between the cdfs of the distributions of the two sample data vectors for each of the microphone and accelerometer data. Interestingly, there is a tight correspondence and similarity in the measured responses despite the fact that we are averaging across the totality of the data set ($p = 1.0$, further proving that the two sets of data are identical and supporting the null hypothesis that there is no significant differences between them). This result implies that the differences observed between the two datasets are likely due to random sampling variability, here due to the physical movements and actions of the bird, rather than a systematic difference between the underlying distributions.

Table 4 Excel Table containing Mean of Syllable Duration, Mean of Mean Frequency Modulation, Mean of Mean Entropy, Standard Deviation of Syllable Duration, Standard Deviation of Mean Frequency Modulation, and Standard Deviation of Mean Entropy of the data collected using both Accelerometer and Microphone for the White bird. Two-sample Kolmogorov-Smirnov test (p-value : 1.0, h:0, kstat : 0.125) shows that the two sets of data are identical, supporting the null hypothesis that there is no difference.



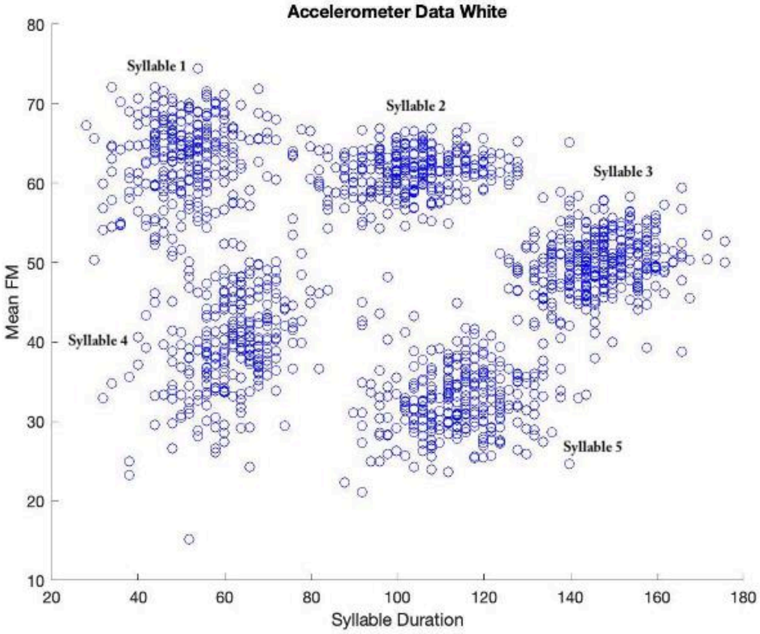
	White Microphone	White Accelerometer
Mean of syll duration:	74.8299	79.81865
Mean of mean FM:	61.95	64.55
Mean of mean Entropy:	-1.26	-1.275
Stdv of syll duration:	35.27515315	56.44034
Stdv of mean FM:	2.474873734	2.474874
Stdv of mean entropy:	0.90509668	0.954594

The statistical analysis conducted provides additional evidence to support the idea that the accelerometer is a highly effective and precise recording method. The results indicate that the data sets collected from the accelerometer and the microphone are drawn from the same distribution, which further supports the reliability and consistency of the accelerometer data. This same conclusion is maintained when examining each individual syllable independently, as the two sets of data are shown to be identical, supporting the null hypothesis that there is no difference observed between Syllables 1, 2, 3, 4, and 5 of the White bird when comparing the different recording techniques. These findings provide strong support for the use of accelerometers as a reliable method for recording bird vocalizations, as they provide highly accurate and consistent data that can be analyzed and interpreted with confidence.

Table 5 Excel Table containing Mean of Mean Frequency Modulation, Standard Deviation of Mean Frequency Modulation, Mean of Syllable Duration, Standard Deviation of Syllable Duration of the five distinct Syllables recorded using both Accelerometer and Microphone for the White bird. Two-sample Kolmogorov-Smirnov test (p-value:0.999, h:0, ks2stat: 0.100) shows that the two sets of data are identical , supporting the null hypothesis that there is no difference.

	White Accelerometer	White Microphone
Mean of Mean FM Syllable 1	63.80047	58.25136
Mean of Mean FM Syllable 2	61.49097	62.15829
Mean of Mean FM Syllable 3	50.24611	52.92968
Mean of Mean FM Syllable 4	40.37039	44.54352
Mean of Mean FM Syllable 5	32.48678	33.99964
Stdev of Mean FM Syllable 1	4.172378	5.421181
Stdev of Mean FM Syllable 2	2.49016	2.634525
Stdev of Mean FM Syllable 3	3.176672	3.638788
Stdev of Mean FM Syllable 4	4.813418	3.476459
Stdev of Mean FM Syllable 5	3.516082	2.522627
Mean of Syllable Duration Syllable 1	51.37162	55.9196
Mean of Syllable Duration Syllable 2	102.4315	109.2063
Mean of Syllable Duration Syllable 3	146.8251	151.898
Mean of Syllable Duration Syllable 4	61.77377	56.72383
Mean of Syllable Duration Syllable 5	114.726	122.2761
Stdev of Syllable Duration Syllable 1	5.569234	8.772098
Stdev of Syllable Duration Syllable 2	9.197407	6.627161
Stdev of Syllable Duration Syllable 3	7.725294	9.654779
Stdev of Syllable Duration Syllable 4	7.81845	10.23305
Stdev of Syllable Duration Syllable 5	7.355452	7.326173

A



B

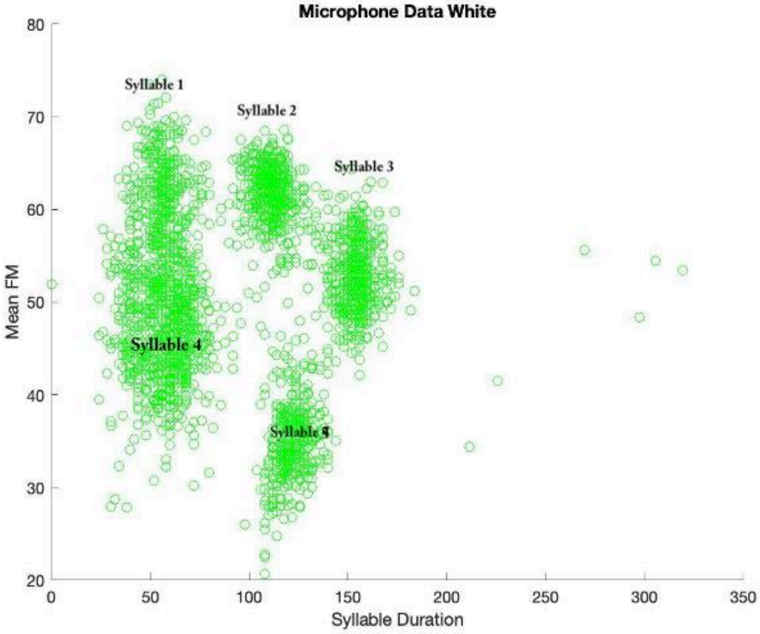
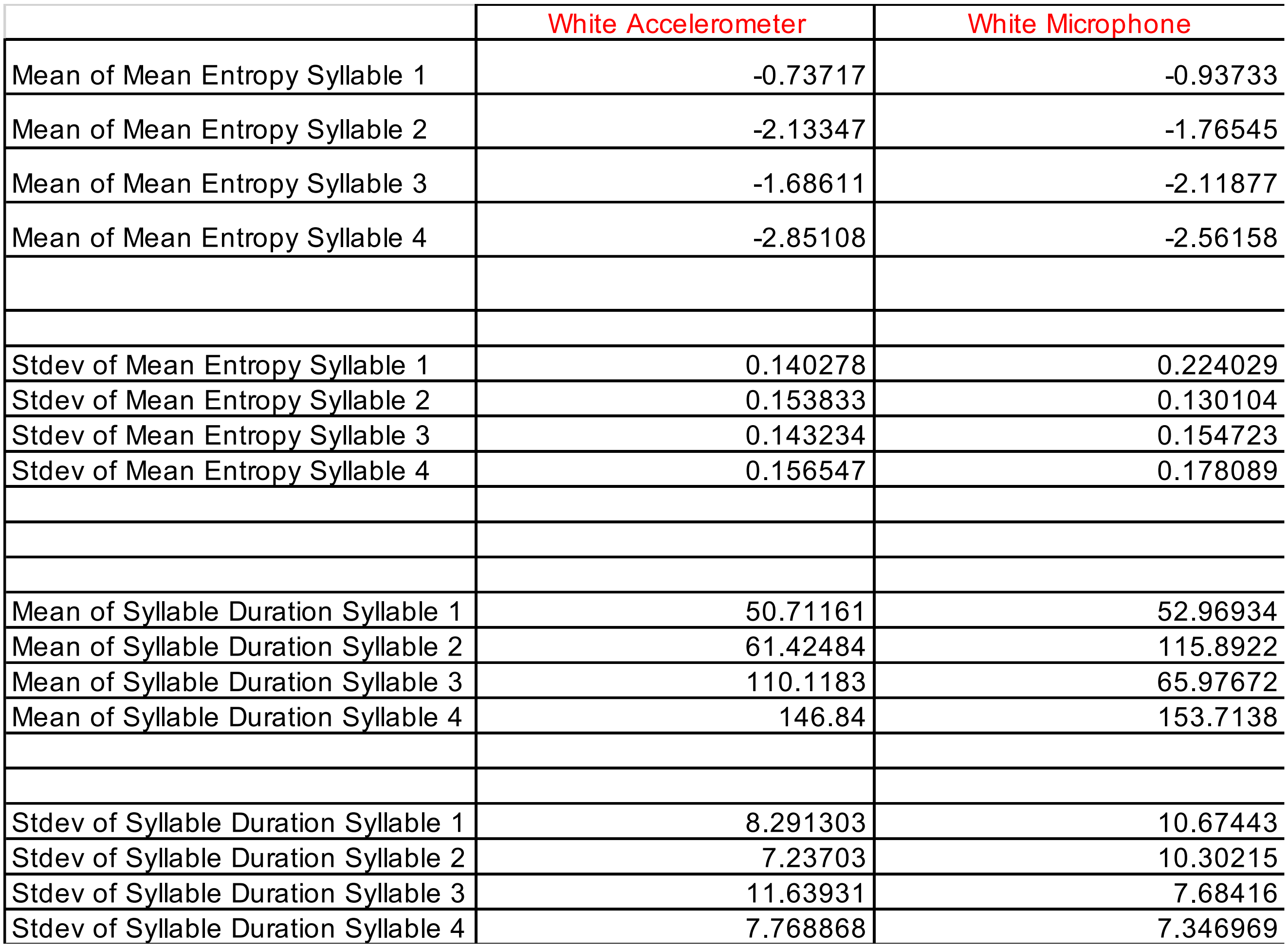


*Figure 26 A, Scatter plot of Mean Frequency Modulation vs Syllable Duration of the White bird for the Accelerometer recordings (blue) with distinguishable and annotated Syllables.**B**, Scatter plot of Mean Frequency Modulation vs Syllable Duration of the White bird for the Microphone recordings (green) with distinguishable and annotated Syllables.*

Table 6 Excel Table containing Mean of Mean Entropy, Standard Deviation of Mean Entropy, Mean of Syllable Duration, Standard Deviation of Syllable Duration of the five distinct Syllables recorded using both Accelerometer and Microphone for the White bird. Two-sample Kolmogorov-Smirnov test (p-value:0.999, h:0, ks2stat:0.125) shows that the two sets of data are identical, supporting the null hypothesis that there is no difference.



	White Accelerometer	White Microphone
Mean of Mean Entropy Syllable 1	-0.73717	-0.93733
Mean of Mean Entropy Syllable 2	-2.13347	-1.76545
Mean of Mean Entropy Syllable 3	-1.68611	-2.11877
Mean of Mean Entropy Syllable 4	-2.85108	-2.56158
Stdev of Mean Entropy Syllable 1	0.140278	0.224029
Stdev of Mean Entropy Syllable 2	0.153833	0.130104
Stdev of Mean Entropy Syllable 3	0.143234	0.154723
Stdev of Mean Entropy Syllable 4	0.156547	0.178089
Mean of Syllable Duration Syllable 1	50.71161	52.96934
Mean of Syllable Duration Syllable 2	61.42484	115.8922
Mean of Syllable Duration Syllable 3	110.1183	65.97672
Mean of Syllable Duration Syllable 4	146.84	153.7138
Stdev of Syllable Duration Syllable 1	8.291303	10.67443
Stdev of Syllable Duration Syllable 2	7.23703	10.30215
Stdev of Syllable Duration Syllable 3	11.63931	7.68416
Stdev of Syllable Duration Syllable 4	7.768868	7.346969

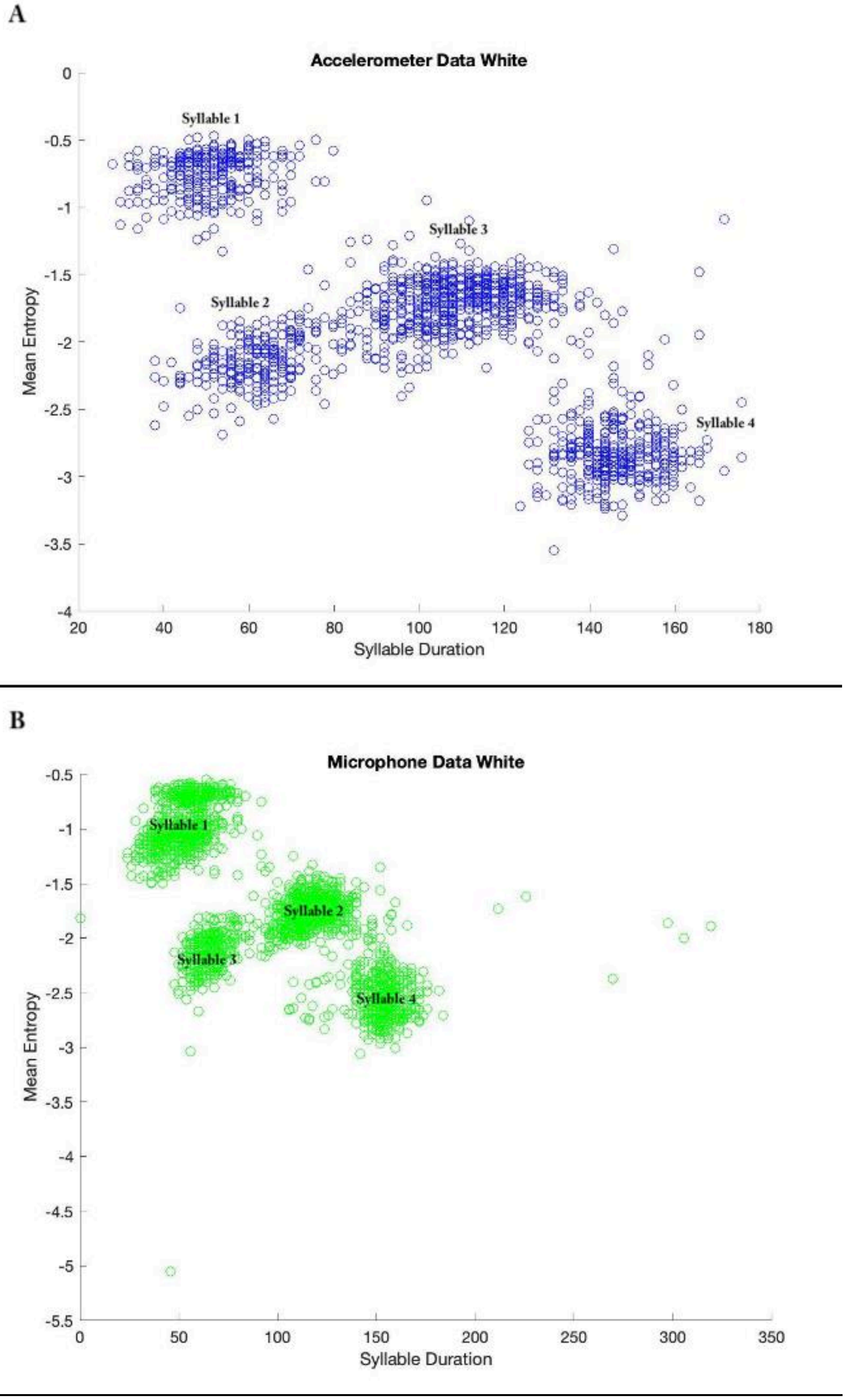


*Figure 27 A, Scatter plot of Mean Entropy vs Syllable Duration of the White bird for the Accelerometer recordings (blue) with distinguishable and annotated Syllables.**B**, Scatter plot of Mean Entropy vs Syllable Duration of the White bird for the Microphone recordings (green) with distinguishable and annotated Syllables.*

CHAPTER V

CONCLUSION

We have presented concrete proof that the use of an Accelerometer for recording of bird vocalizations is as reliable as the use of more traditional methods such as the Microphone.
First, by setting up successful surgeries, we were able to implant said accelerometers onto the bird's skull, allowing us to record the mechanical waves propagating as a result of vocalizations, we were then able to collect those signals from the accelerometer and convert them into sound waves. Those sound files were then analyzed using SAP Software, and converted into data points that were then stored in an Excel table for analysis using the written MATLAB code. Following the obtention of scatter plots, both statistical and visual analyses were undertaken in order to measure whether or not there would be any significant difference between our two recording methods. Through two-sample Kolmogorov-Smirnov tests, we found that our two sample data sets are drawn from the same distribution, thus supporting accelerometers as a reliable, efficient, and safe way of recording.

In closing, the work presented in this dissertation has the potential to impact both research and clinical applications, not only in the field of songbird research, but also in matters of troubled speech development, and impairments. This will hopefully pave the way for more integrative, fast, and efficient experiments, thus helping us provide a more profound understanding of the underlying neural and behavioral processes of song and speech formation, retention, and development.

By creating a small backpack and helmet with integrated accelerometers, and their associated wireless components, one can only hope to further delve into the elusive world of songbirds with the hopes of better understanding the neural intricacies behind their songs.

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