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INTERNSHIP REPORT

MORPHO-FUNCTIONAL STUDY OF BIRDS VOCAL SYSTEM:

INFLUENCE OF THE PHYLOGENY, SIZE AND NOCTURNALITY ON ACOUSTIC PARAMETERS

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From 01/09/2020 To 31/12/2020

Acknowledgments

Firstly, I would like to communicate my special thanks of gratitude to Dr. Pauline Provini, my internship supervisor, who provided me with the golden opportunity to do this internship to understand the mechanisms by which birds are producing vocalizations. She was an excellent mentor and passionate teacher. She put a lot of time and effort into planning my internship and I am so grateful that she did because she offered me this amazing experience. The time spent working with her helped me realize that my true calling is to work with birds. Whether it is in morphofunctional biology to understand their physiology, or systematics to acknowledge the ties of kinship and filiation, or evolutionary ecology to comprehend the way they interact with their environments, it does not really matter because what I want is to study birds in their globality.

She also helped me face and reconnect with my ethic. Before this internship, I thought that working on experiments that require the death of the animals does not bugger me. The truth is that it does! I do not want to be involved in any experiments involving the death of a living being. I would have never figured this out this early in my life without Pauline Provini. So I would like to thank her again for helping me find my way.

Secondly, I want to thank Dr. Rachel Olson, and every researcher and staff member at CRI, who were so nice to me and made me feel welcome in the team. Even though I was already a student at CRI, I discovered an entirely new universe that I had no idea existed. It was a true rediscovery to start spending time in new social circles like other interns or even researchers. When I was a student, I used to hang out with other students, sometimes teachers but that was it. Now I became familiar with a majority of the CRI community and I am truly grateful for it.

In spite of the COVID-19 crisis that made me telecommute for a fair amount of time during the internship, I still felt an important asset to the team and know I will be missed when my contract ends. It was a pleasure to work with you all.

MORPHO-FUNCTIONAL STUDY OF BIRDS VOCAL SYSTEM: INFLUENCE OF THE PHYLOGENY, SIZE AND NOCTURNALITY ON ACOUSTIC PARAMETERS

Abstract

Birds are able to produce a great diversity of sounds. They vocalize with a specific vocal organ, the syrinx, producing the primary vibration. However, it is not fully understood how the produced sound is filtered afterward by the vocal tract (trachea, upper part of the esophagus, oral cavity, and beak). The only birds capable of mimicking human speech belong to the order of Psittaciformes (parrots) and Passeriformes (songbirds). As they are sister-groups, we hypothesized that the phylogeny influences acoustic parameters. To test this, we selected sounds produced by at least one species per bird family, using bird recordings from open online libraries. We extracted relevant acoustic parameters (such as mean, maximal and minimal frequency, and amplitude) and mapped these parameters on the phylogenetic tree of the studied birds to assess the influence of the phylogeny, but also the size and nocturnality, on acoustic parameters. We observe that songbirds, hummingbirds and parrots can produce higher maximum pitch and louder sounds than the majority of birds. Because songbirds and parrots are sister-groups, and because the vocal organ of hummingbirds shows convergence with songbirds, there might be a phylogenetic relation between maximum pitch and vocal organ morphology. There does not appear, however, to be any correlation between loudness and phylogeny. Results rather suggest that body mass explains loudness repartitions in birds. There is also a negative correlation between nocturnal vision and both the maximum pitch and maximum loudness. The trends show that maximum loudness seems to be related to body mass and maximum pitch seems to depend on phylogenetic proximity and vocal organ morphology.

In parallel, I was involved in the development of an experimental protocol to record vocalizing birds to quantify the movements between the syrinx structures and the rest of the vocal tract in order to convey a biomechanical study on bird vocalisation

Keywords

Bird vocalization, Acoustic parameters, Birds phylogeny, Vocal tract, Syrinx

ÉTUDE MORPHO-FONCTIONNELLE DU SYSTÈME VOCAL DES OISEAUX: INFLUENCE DE LA PHYLOGÉNIE, DE LA TAILLE, ET DU MODE DE VIE NOCTURNE SUR LES PARAMÈTRES ACOUSTIQUES

Préambule

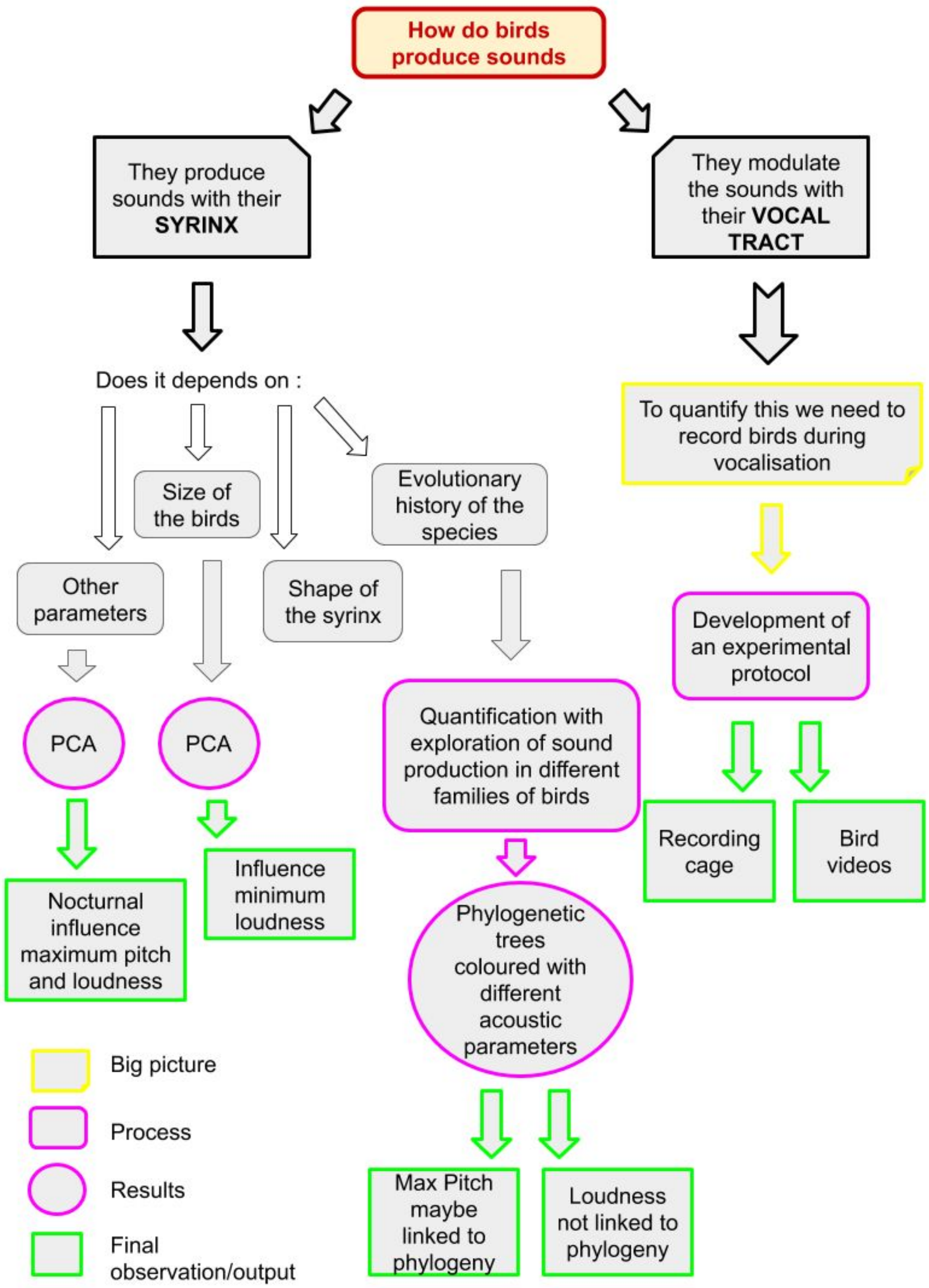
Les oiseaux sont capables de produire une grande diversité de sons. Ils vocalisent en utilisant un organe unique à cet effet, le syrinx, produisant la vibration primaire. Malheureusement, la manière dont ils filtrent le son après sa production par le syrinx reste un mystère. On sait néanmoins que la trachée, la partie supérieure de l'œsophage, la cavité orale et le bec sont impliqués. Les seuls oiseaux capables d'imiter la parole humaine appartiennent aux ordres des Psittaciformes (perroquets) et Passeriformes (passereaux). Comme ils sont des groupes-frères, on conjecture ici que la phylogénie influence les paramètres acoustiques. Pour tester cette hypothèse, nous avons sélectionné des sons produits par au moins une espèce d'oiseaux par famille, en utilisant des enregistrements provenant de librairies libres de droit en ligne. Nous avons extrait les paramètres acoustiques importants, tels que la fréquence et l'amplitude moyenne, maximum, et minimum, dans le but de superposer ces paramètres à un arbre phylogénétique des oiseaux étudiés ici pour comprendre l'influence de la phylogénie, mais aussi de la taille et du mode de vie nocturne, sur les paramètres acoustiques. Nous observons que les passereaux, les colibris et les perroquets ont la capacité de produire des sons de hauteur maximum et d'intensité supérieure à la majorité des oiseaux. Parce que les passereaux et les perroquets sont des groupes-frères, et parce que les organes vocaux des colibris ont convergé vers ceux des passereaux, il existe peut être une relation phylogénétique entre la hauteur maximum d'un son et la morphologie des organes vocaux de l'oiseau. Cependant, aucune corrélation n'apparaît entre la phylogénie et l'intensité du son. Les résultats suggèrent plutôt que la taille explique la répartition de l'intensité du son chez les oiseaux. Il y a également une corrélation négative entre la vision nocturne et la hauteur et l'intensité maximum du son. Les résultats laissent à penser que l'intensité maximum du son produit par un oiseau dépend de sa taille, et la hauteur maximum du son produit dépend de la proximité phylogénétique et de la morphologie de l'organe vocal des oiseaux.

En parallèle, j'ai été impliquée dans le développement d'un protocole expérimental pour enregistrer des oiseaux qui vocalisent dans le but de quantifier le mouvement entre le syrinx et les organes vocaux impliqués dans la modulation du son pour effectuer une étude biomécanique de la vocalisation chez les oiseaux.

Mots clefs

Vocalisation chez les oiseaux, Paramètre acoustique, Phylogénie des oiseaux, Organes vocaux, Syrinx

Graphical abstract



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Introduction

Birds and humans share a common characteristic, they are both able to produce a remarkable diversity of sounds. The comparison goes even further ; some birds belonging to the family of parrots and songbirds have the ability to mimic human voices (1). However, birds and mammals differ in the way they produce sounds (2). Birds vocalize using a special organ, the syrinx, producing the primary vibration. Like mammals, birds also possess a larynx (organ producing the primary vibration in mammals). However, it is not used in the process of sound production (3).

The field of birdsong study has been left unnoticed for a long time. Even though most research focuses on the production of sound by the syrinx, there is “few data on the relationship between amplitude and frequency control in the songbird syrinx” (4), Nemeth et al., 2013. After the primary sound was produced by the syrinx, it is modulated by the rest of the vocal tract. The vocal tract is composed of every morphological feature involved in filtering the primary vibration (5). We can denote the trachea, upper part of the esophagus, oral cavity, and beak. Different theories exist on complex sounds production. One school of thought defends that sound diversity comes from its production at the sound source (6). Others debate that the modulation of the sound after its creation has an important role to play (7–9). Because most research focuses on the production of sound by the syrinx, those modulations have been overlooked for a long time.

Pauline PROVINI’s project is to bring a better understanding of the way the vocal tract filters the sound after its production in order to produce bio-inspired vocal prostheses to improve the voice quality of people with laryngectomies. Initially, I was supposed to work on a prototype of the prostheses. However, because of the COVID-19 crisis, there was no preliminary data available to do so at the beginning of my internship. So, I started working on two alternative projects simultaneously. One of my goals was to analyze the data I gathered during the SCORE project. As a reminder, during the SCORE project, I collected bird recordings and extracted from each sound basic parameters like the frequency, the amplitude, and so on. In parallel, I was involved in the development of an experimental protocol to record vocalizing birds. Both of these projects are important to better understand the diversity of bird sound and try to understand the relationship between the bird vocal tract structure and the sound they produce.

To better understand both these projects, I inquired about sound characteristics and sound production in birds. On a physical approach, sound is defined as a vibration that propagates through media as a mechanical wave (10,11). The medium is defined as an elastic matter that can be gaseous, liquid, or solid, like air or water. To create sound, we need a source (here the bird’s syrinx). The sound created will initiate a disturbance, called vibration, in the surrounding media. While the source keeps emitting disturbance to the surrounding, the medium will continue to vibrate, thus creating a mechanical wave that propagates at sound speed. Sound speed varies in function of the medium crossed, the temperature, and the pressure.

To study bird sound, we need to identify a cyclic part in the song that is called a syllable. Once we isolate the syllable of interest, we can study its characteristics. Sound characteristics can be visualized using a spectrogram. By decomposing the original sound spectrogram into multiple harmonics we can find all the sinusoidal sound waves that form the original sound. Sinusoidal sound waves are easier to study than rough sound waves. Sound waves are defined by their frequency (inverse of the wavelength), amplitude (equivalent to sound pressure and intensity), speed (depends on the medium), and direction (linked to spatial location).

To study sounds, scientists use subjective notions that do not have a purely objective physical consensus like pitch, loudness, timbre, and duration. Those notions depend on objective physical parameters such as frequency, amplitude, higher harmonics, and speed, respectively. (see Figure 1) On the fundamental harmonic of the soundwave, which is assumed to be a simplified (because sinusoidal) version of the original sound, we can study the frequency, the amplitude, and the speed. However, the timbre can only be noticed by looking at the higher harmonics. (eg. see Annex 1)

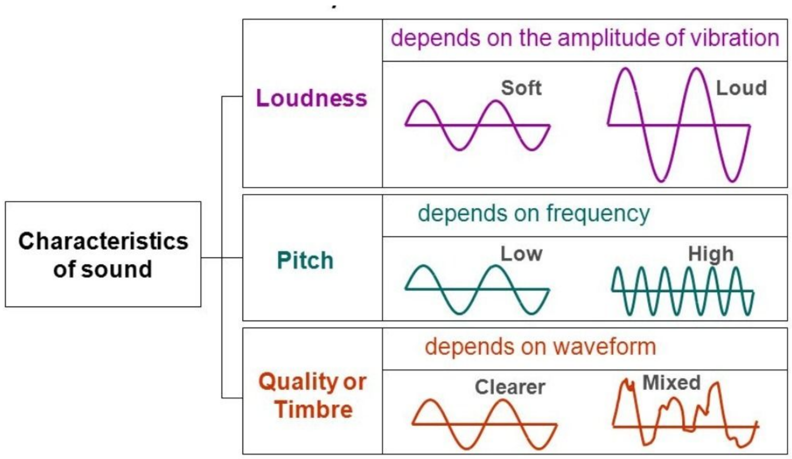

Figure 1 : Sum up scheme (the scheme misses a representation of duration)
https://brainly.in/question/2198002

In birds, sound waves are produced by the vibration of the membranes of the syrinx. Those membranes, the lateral tympaniforme membrane, the medial tympaniforme membrane, and the labia, are part of the syrinx soft tissues. The other soft tissues present in the sound source are the bronchi, the muscles, the connective tissues, the nerves, and the blood vessels. The syrinx is also composed of cartilaginous tissues like tracheal, syringeal, and bronchial rings, the tympanum, and the pessulus (12) (see Figure 2).

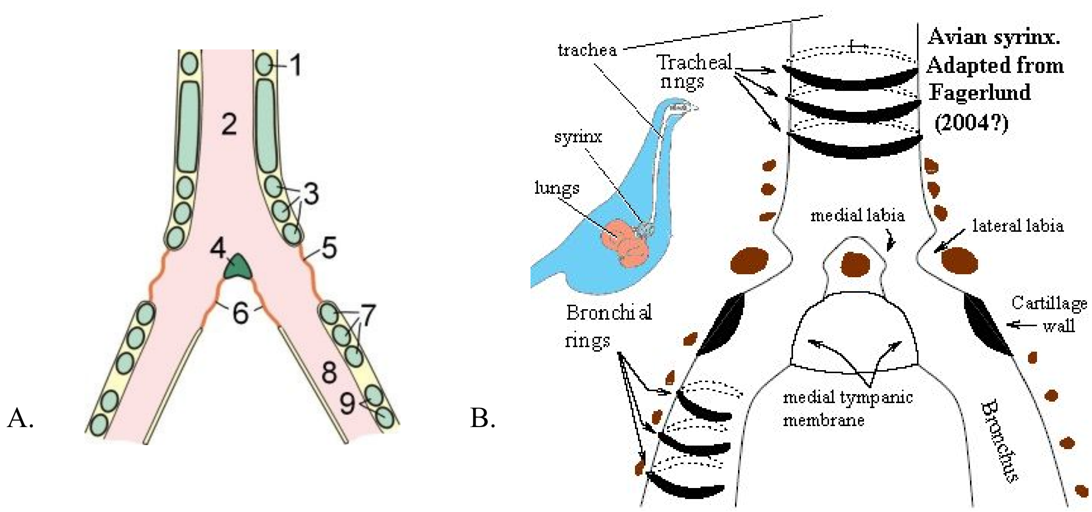

Figure 2 : Schematic drawing of an avian syrinx of tracheobronchial type

Figure A : 1: last free cartilaginous tracheal ring, 2: tympanum, 3: first group of syringeal rings, 4: pessulus, 5: membrana tympaniformis lateralis, 6: membrana tympaniformis medialis, 7: second group of syringeal rings, 8: main bronchus, 9: bronchial cartilage

https://en.wikipedia.org/wiki/Syrinx_(bird_anatomy)#/media/File:Syrinx.jpg

Figure B : Showcase the different type of membrane that vibrates in the syrinx

http://speakbeakblog.blogspot.com/2008/08/remarkable-sounds-of-birds.html

Lateral typaniforme membranes are controlled by syringeal muscles and appear to be the main sound generators (13). Oscillation of the typaniforme membranes and labia is induced by passing airflow and produces the sounds. Thus, the vibration is determined by the air pressure in the air sac (cavities with a constant presence of air that helps birds to fly and allow a constant airflow in the respiratory system) driving air through the syrinx (12). The membranes shape, tension, thickness, and the bronchial openings are controlled by muscles and can alter the sound (2).

There are three different types of syrinx morphology: the tracheobronchial syrinx, located at the lower end of the trachea and the upper parts of the bronchi, the bronchial syrinx, located in the bronchi, and the tracheal syrinx, located in the trachea (14). The tracheobronchial syrinx is the most common type in bird species (15). It is found in Passeriformes (songbirds) (16), in some Psittaciformes (parrots) (17), in Galliformes (landfowl) (16), and more. Because of its position, in the middle of the tracheal bifurcation, it allows for muscles on the left and right branches to modulate vibrations independently from one another, thus allowing some birds like songbirds to produce more than one sound at a time (18,19).

Humans can alter their pitch, loudness, duration, and timbre using both their larynx and vocal tract. The larynx is responsible for sound production, which is articulated afterward into words by the vocal tract (9). Birds have a higher frequency span than humans, and organs adapted to sound production across this frequency range. Birds' position of the syrinx seems to offer a biomechanical advantage for sound production over the larynx (3). The long vocal tract of birds positioned above the sound source facilitates the tuning between fundamental frequency and the vocal tract resonance (3). Songbirds also have an increased frequency range due to their bifurcated sound-source (20). The right branch produces sounds with fundamental frequencies higher than 4kHz, frequency below this is sung by the left branch. This allows passerine bird songs to contain upward and downward frequency sweeps that can cover up to 2 octaves (interval between one musical pitch and another with double its frequency) (20). Their bifurcated syrinx gives them the possibility to produce sound from two different sound sources, thus producing more than one sound at a time (18,19). Furthermore, birds' air sac allows for constant and unidirectional airflow through the syrinx that enables birdsong to be louder (7) and to last longer (21). All these adaptations permit the vocal register of birds, from what we know, to go from 100Hz to 12 000Hz (4,20,22–25). For comparison, the average vocal range of an adult human, on the other hand, spans from 85Hz to 255Hz (26,27).

Despite the knowledge of the birds' syrinx morphology and the mechanisms involved in sound production, it is not fully understood how the primary vibration is articulated into syllables afterward. The sound produced by the syrinx is filtered afterward by the vocal tract. We know that in songbirds, motions of the vocal tract help modulate the sound (9,28). When producing low pitch sounds, the throat and breast area expend visually, and the beak stays fairly closed. Conversely, while making high pitch sounds, the neck elongates and the beak opens widely. Parrots, in contrast, modulate the sound using their tongue and beak as a vocal articulator, in a similar way humans do (8). However, both parrots and songbirds seem to modulate the sound by changing the length of their vocal tract and the opening of their beak (8,9).

Little is known about bird sound and bird vocal organs evolutions (29). Different morphologies have been described in a relatively large number of living and extinct birds (29). However, no morpho-functional study has been performed for now. The questions of the relationship between the vocal organs shapes and acoustic characteristics stays open.

During my internship, I conducted a morphofunctional study to look for convergence in the produced sounds among 130 different bird species. To do so, I used the principal component analysis (PCA) on sound characteristics, such as the maximal frequency and amplitude, of multiple bird species per group using recordings from open online libraries. I will interpret the results using morphological parameters, such as the size of the bird, or its position in the phylogeny, to see if a given sound can be determined by the size of the bird, or depends on the bird family.

To determine how the different parts of the vocal tract modulate sound in songbird and non-songbird species, Pauline Provini's team want to film birds vocalizing with light cameras and synchronously record their sound with microphones to quantify the motions of the beak, neck, and pouch, while birds produced different sounds. I will assess here the development of the experimental protocol to record vocalizing birds and present preliminary data I gathered during my internship.

I also submitted an abstract as a first autor for a talk at the international 2021 SICB conference cycle (SOCIETY FOR INTEGRATIVE AND COMPARATIVE BIOLOGY). (eg. see Annex 2) Because of the COVID-19 situation, the conference will take place online in January when I will give a talk to present my work.

Motivation for this internship!

My true calling is to become a researcher in biology in the field of birds study, whether it is in morphofunctional biology, in systematics, or in evolutionary ecology. This internship was a perfect occasion for me to discover one of those fields, morphofunctional biology. Indeed, it checked all the boxes! I would be able to learn next to a powerful woman researcher who succeeded in finding funding for her study on birds, who tackles technical challenges by developing emerging techniques, who build collaborative networks to reach a higher level of expertise in an environment where continuous learning, mutual help, and well being are valued. Her work is at the edge of interdisciplinarity, between morpho-biology, the physics of sound, programming, and medical application, all this while promoting open access. It is the perfect setting for a young scientist wannabe researcher who wishes to work and evolve in a pluridisciplinary world using birds as her principal object of study.

Context and Background

“The goal of our lab is to understand the mechanisms by which birds are producing vocalisations. One application of our research is to produce bio-inspired vocal prostheses to improve the voice quality of people with laryngectomies.”, said Pauline Provini to describe her work to her team. When Rachel Olson (a postdoctoral fellow) and I arrived, Pauline Provini’s project was just starting. Despite the COVID-19 situation, she collected and regrouped papers about bird vocalisations to feed her project.

I arrived at the lab at a difficult time. The first COVID-19 lockdown was just over and already signs of a second wave were all over the place. The results I was supposed to work on were not ready, fortunately Pauline Provini and I were ready for this possibility and had prepared a backup plan.

I continued the analysis I started during the SCORE project. SCORE projects are short internships where we allocate a few hours of our time each week to work for a fellow researcher at CRI during 3 weeks. Yakov Uzan and I worked with Pauline Provini during the SCORE project where we mapped bird sounds databases and collected bird recordings for analysis. At the end of SCORE, we had time to start extracting acoustic parameters, with room for improvement due to the short duration of the SCORE project. During this internship I refined the extraction of sound variables started during SCORE and started the analysis of the data. So, I tested the influence of the phylogenetic relationships on the acoustical parameters.

In parallel, I was involved in the development of an experimental protocol to record vocalizing birds. The idea came while observing my myna bird (*Gracula religiosa*) singing. We could see very distinctively the movement of its vocal tract as he swells and retracts his throat as it has already been observed in other species (8,9,28). Because we had no bird specimens yet to study due to COVID-19 complications, Pauline Provini thought it was a good idea to test the experimental protocol on my bird to begin with. So, I was charged with the task of developing a protocol to record vocalising birds to quantify the movements between the syrinx structures and the rest of the vocal tract to identify what motion is associated with what sounds.

Both of these projects are important to better understand the diversity of bird sound and try to understand the relationship between the bird vocal tract structure and the sound they produce. In parallel to my work, Pauline Provini, Rachel Olson and Alireza Kazemi are working on other projects to widen our knowledge of bird vocalisation. To identify what shape is associated with what sounds, Rachel Olson will test for correlations between the vocal system 3D shape using X-ray microtomography (μCT) with contrast agent and sound parameters in order to link the anatomy of the avian vocal system (the syrinx) with the sounds they produce. An aero-acoustic model of bird sound production will be built by Alireza Kazemi to focus on the airflow, which is at the origin of the sound production.

I have to state that during the lockdown my work conditions were very difficult. I did not have a good Internet connection so no easy to access network for the first half of the lockdown. We were two students staying in a 9 meter square room with only a mattress to work on (there was no room for a table). Going to work at CRI was a possible alternative, however it was far from the place I was staying. Moreover, CRI only allows commuting every two weeks. Furthermore, I could not move elsewhere during the lockdown period because I had my bird to take care of and could not leave them behind.

Material and Methods

Project 1 : Gathering and Analysing Data

The SCORE project gave me a sample of preliminary data to analyse and to look for correlations between the sound characteristics and the bird species producing it. However, I realised that those data were probably falsen because the recordings have not been cleansed from noises. So I had to improve the dataset I gathered during the SCORE project.

In this study, I used vocalising birds' recordings coming from open source web pages of bird sound databases. I gathered a total of 130 recordings spread over 22 orders and 69 families of at least one bird species pair existing bird's families. In ideal cases we took multiple recordings of the same species of birds and multiple recordings of species among each bird family to have a sample as representative of the great diversity among the bird phylogenetic tree. We have favored recordings from birds susceptible to be found in France or at least Western Europe to potentially get X-ray microtomography view or dissection of birds of the same species as those of the recordings. Pauline Provini got the bird specimen to analyse from the Museum National d'Histoire Naturelle (MNHN), so most specimens originate from France as citizens collect dead birds and give them to the Museum. The recordings were all taken from the Xeno-Canto database (30), the handiest sound database in the databases Yakov identified during the SCORE project. In this perspective, Pauline Provini provided me the list of bird species currently held by the Museum to match the species recordings with birds held by the Museum. This was done to facilitate the second part of the study, which will require associating the morphology of the vocal tract of birds and the sound they produce.

There are many important things to consider when choosing quality recordings for analysis in the specific case of bird sound study. While working on birds' sound, the available recordings are not always taken in a controlled environment. There is a natural variation between every recording that makes parameters vary a little, essentially when the sound was taken in the wild. We call this natural variation noise. We might not have information on the number of birds singing in the recordings or if they are all from the same species. Maybe other species or sounds are parasitizing the recordings. The sonic texture helps quantify that problem. It is a feature that lists the number of sound sources on a recording. If many birds are singing on the same recordings, the quality of sounds will not be the same as if only one bird is singing (31). Because our subject of interest here is to study bird vocal organs, we need to work on recordings with an individual species of bird singing. We can not work with a recording of multiple bird species, or even a recording with many of the same species of birds all vocalising at the same time. So during the selection process Yakov and I tried to select only recordings where only one bird appears to be singing. There is another way to quantify noises, spatial location. It outlines the placement of the source of a sound in an environmental context (11). It allows quantifying sounds on a scale from "close" to "far" from the recording device. We position ourselves in a 3D plane including a horizontal and vertical axis. The origin represents the source of the sound and we look at the distance separating the sound source and the recording device. We need to consider relevant information like the presence of other noise disturbance or wind that could change the initial sound wave, or the obstacles in the way that could obstruct the quality of the sound wave. The soundwave spectrogram needs to be as clear as possible and as resemblant as possible to the original sound.

During the SCORE project, all sounds were uploaded on mp3 format to our private online database on Notion software. For each recording, we informed the species common name, its family and order, the provenance of the recording, the sound ID number from Xeno Canto, and the type of sound (call/song, adult/juvenile, male/female). We wrote a program on R 3.6.2 to extract the pitch, tone, and rhythm from our recordings using the library Soundgen (a library built to work with bird song) and that append a csv file containing the following informations: name of the species, maximal, minimal, mean and standard deviation of syllable length, syllable rate, harmonics intensity, harmonics to noise ratio (HNR, intensity of the harmonics compared to the background noise), frequency of dominant harmonic, frequency of harmonics 1 to 3, entropy and sound slope. During my internship, I standardised species names by renaming all recordings with the according scientific names, by correcting the language issues, and by correcting the capitalization issues. All my analyses were performed in R v.3.6.3.

SUM UP METHODOLOGY:

- Redefining the variables used to study sound
- Clean the data and remove background noises
- Extraction of acoustic parameters
- Analysis of acoustic parameters

At the end of SCORE project, Yakov and I "concluded that our method for sound analysis does not function well with recordings with too much noise", as we wrote in our final report of SCORE. The problem comes from the fact that "we did not have time to check for the quality of the analysis of each recording", I quote from our final report.

Data Cleansing: I cleaned the data collection from background noises using Audacity® software. I created a profile of the noise and removed this profile from the entire recording to reduce noises. I also trimmed some recordings to reduce their size so that they can be processed with more ease by the program (during the SCORE project Yakov and I noticed that heavy and long recordings tend to make the program crash). To do so, I followed the protocol in Supplementary materials. (eg. see Annex 3)

During the SCORE project, Yakov and I did not have time to learn about sound characteristics and acoustic parameters.

Redefining the variables used to study sound: I conveyed a bibliographic study of sound to identify what variables were scientifically acceptable to study and I concluded that I will extract the following : the mean, maximal, minimal, and standard deviation values of the dominant harmonic frequency (pitch), the harmonics intensity (loudness), and the frequency of the first, second and third harmonics (timbre). The harmonic to noise ratio (HNR) was also taken to make sure all recordings are cleaned.

Part of my findings on the physical parameters of sounds are detailed in the introduction, the subjective parameters are explained in the supplementary materials. (eg. see Annex 1)

As a reminder, Yakov Uzan and I wrote our program in R using soundgen v.1.8.2 package to analyze bird recordings during the SCORE projects. While running the code on the noise-free recordings, the program crashed on some recordings. The errors shown in the terminal were inconclusive and appeared to change at each iteration of the program. I needed to improve the original program to make it work with the new set of data.

Extraction of acoustic parameters: The problem does not come from the code itself but from the noise-free recordings. I noticed that the particularity of all recordings tending to make the program crash was a higher pitch. It appears that removing noises on some recordings brought their average pitch higher than soundgen can support. I searched for other sound analysis packages that can handle high pitch sound to replace soundgen, and did not find any conclusive results as there were not many easy packages to work with. I removed from my dataset the recordings that break the program and in parallel intended to work at making the program user friendly. I improved the ergonomy of the program to allow others to understand my work. To do so I created an R Project that regroups all files and folders needed to make the transition to other computers easier. I pushed the Notebook on the BirdSongTeam GitHub going in the direction of open source science thus allowing Pauline Provini to run the code from her computer. The BirdSongTeam GitHub is unfortunately private, but you can access on this report page (https://github.com/irinade/Internship_Report_L3_GitHub_page) an example of my work. The code creates a CSV file containing information on sound qualities from an MP3 recording, then a function concatenates into a dataframe all CSVs files generated. I learned during the internship how to write the program in RMarkdown, to make it easier to read for others. I worked on making the code both launchable from Rstudio and the Bash terminal to make it easier to work with and with a reproducibility perspective in mind. I used the Make command line and tool in the shell to automatically build an executable program from source code. The Make command enables the program to continue where he left off after having crashed rather than start over from the beginning as it does in R.

I can now focus on analysing acoustic data extracted from the recordings.

Analysis of acoustic parameters: I computed a Principal Component Analysis (PCA) of sound characteristics from the dataset obtained by extracting acoustic parameters. PCA can be used to extract data with many variables, allowing a better visualization of the variation present in the dataset (32). I used the maximal, minimal, mean, and standard deviation of the dominant harmonic frequency, the harmonics intensity, and the frequency of the first harmonic (supposed to be similar to the dominant harmonic frequency) for the PCA. Next, I customized my visualisation using ellipses to regroup birds into categories such as clades, orders, and body mass level. I set other graphical parameters of the plots with the ggbiplot package on R.

I used bird body mass values from (Wilman et al., 2014) (33) as a grouping parameter of the PCA. I grouped each body mass value into 4 categories depending on their quartiles: ‘Xsmall’ (extra small) for every body-value below the first quartile, ‘Small’ for every body-value between the first and second quartile, ‘Medium’ for every body-value between the second and third quartile, and ‘Large’ for every body-value above the third quartile.

I generated 100 trees using Ericson distribution (34) from the Birdtree software (35) and computed the strict consensus tree, using an R code. I used the ‘ggtree’ library version 2.5.0.991 to quantify the correlations between bird sounds and their phylogeny. I used the ‘full_join’ function from the package ‘dplyr’ v.1.0.2 to merge the trees generated by Birdtree and my dataset of bird sounds parameters.

You can access this program here (https://github.com/irinade/Internship_Report_L3_GitHub_page).

Project 2 : Experimental protocol to record vocalizing birds

No bird specimens were available to Pauline Provini yet due to COVID-19 complications. Thus, it was important to start developing an experimental protocol to both figure out where to find birds for the study and how to record vocalizing birds to quantify the movements between the syrinx structures and the rest of the vocal tract. A possibility, to begin with, would be to gather data on my birds. I house four cockatiels (*Nymphicus hollandicus*) and one myna (*Gracula religiosa*). Myna's are known to be one of the best human speech mimicking birds on the planet (36,37). Another possibility is to go for citizen science and let people send recordings of birds that respect certain conditions.

SUM UP METHODOLOGY:

- Recording Cage project:
    - Designing the Recording Cage
    - Building the first prototype of the Recording Cage
    - Getting the birds comfortable with the cage
- Where to get the bird specimens

Recording Cage

<u>The Recording Cage project:</u> The idea behind the recording cage is to be able to film and record birds vocalising anywhere. The birds do not need to be moved and taken to a lab, the lab comes to them. We want the birds to feel safe in an environment that they already know, with people they trust. The birds are put inside a cage that looks like a transportation device, but designed to optimize recordings. So it reduces the trauma caused by taking the birds to a lab. Furthermore, this technique is overall cheaper and easier. Indeed, we do not need to buy the birds, to buy them cages, to find a place to keep them, and to rehome them after the experiment. We just need to build a transportable registering and filming cage, and to find birds to record. Moreover, this allows us to have access to rare specimens of birds, or birds that are trained to mimic human voices. Indeed, buying an African grey parrot or a myna (two of the best-mimicking birds on the planet) and training them to speak in order to study their vocal tract articulation is a long and expensive process. However, finding bird owners' that already trained their pet bird to speak and asking them if we can record their bird for our scientific study is a way easier process. Such a portable device enables the project to take a citizen science path.

<u>The Recording Cage design:</u> The cage needs to look as safe as possible to allow the birds to vocalize in optimal conditions. By looking at transport cages optimized for birds' comfort, I noticed that they all possessed a perch for the bird to stand, and one side of the cage with wired mesh or plexiglas for the bird to see the outside and feel safe. Because we want to reuse the cage for X-ray cineradiography protocol, we avoid metal in the cage to circumvent distortion. The recording cage will be a box the size of a transporting device. It is important that birds have enough space to not feel constrained, but to restrict their movement as much as possible to keep them steady in front of the cameras. We need to consider that birds vary largely in size, which makes the recording cage size change. Let us class birds size into 3 categories, small birds (Zebra finch size to small parakeet), medium birds (parakeet and small parrots), and large birds (parrots, Ara being the largest type of bird we could study). We will need to make 3 different recording cage prototypes, one for each bird size. The box will be made entirely from plexiglas and is designed for laser cutters. We will not be using any glue or nails to build the box, because all pieces fit tightly together using a bolt mechanic. The cage will be disassemblable. The door of the cage will be cut in the plexiglas and will be sliding upward. We will drill holes to allow air to flow through the cage,

and sound to not reverberate inside the cage. A perch will be present on one of the box's sides. We need to be able to move the perch to have the best view of the bird as possible, so we will build a perch using Legos brick ® and a wood stick.

Build a recording cage:

Materials :

- DIY workshop like a MAKERLAB with a laser cutter, computer with Illustrator software
- Transparent plexiglas sheet for laser cutter (the size and width depends on the size of the cage you are building)
- Wood

DIY Build :

1. Think about the size you need for your box:
- What is the size of the bird you are studying?
- Do you want the bird to be able to move in the box?
  IMPORTANT: for the welfare of the bird, the cage must be large enough to allow him to stretch its wings.

2. Design your box on MakerCase website: [MakerCase - Easy Laser Cut Case Design] (https://en.makercase.com/#/)
   a. Choose the Basic box template
   b. Select the Width, Height and Depth of the box, do not forget to mention if those dimensions are from inside the box or from outside the box.
   c. Specify the material thickness, here we are using plexiglas and the thickness depends on the size of the box you are building. If you are building a large cage, you will need thicker plexiglas for better resistance.
   d. Select "Closed box"
   e. You need to choose if you want T-slots or Enlarged Fingered here:
      i. Select T-slots and you will need metallic bolts to attach the box (easier but problematic if you need the box to go through an x-ray scanner) (see Figure 3)

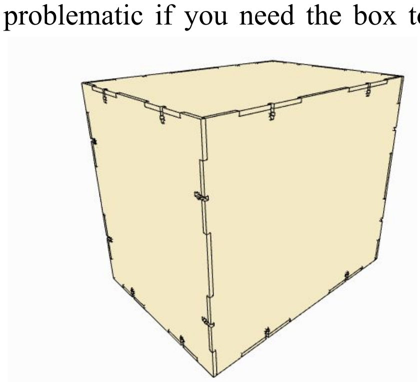

Figure 3: T-slot box that needs bolts to stack together.

      ii. Select Enlarged Fingered and you will need door wedge-like wood to attach the box (harder but no problem if you use your cage to go through an x-ray scanner) (see figure 4.A.) This option requires a closing like the one shown below (see figure 4.B.).

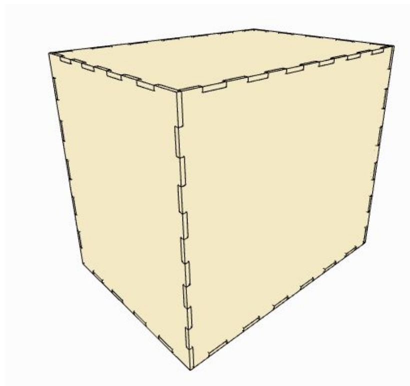 A.  B.

Figure 4: On the right (figure 4.A.), finger box that needs wedge to stack together, on the left (figure 4.B.), wedge example. This is not the method I used to construct the first prototype so I have no example to show. The first prototype was weld together and can not be taken apart like this design can.

f. Download the file
g. Open them into a laser cutter software like Illustrator
h. If you choose option ii., enlarge the finger manually, we need the finger to exceed one another when we put the box together, draw holes to allow closing with wood, like shown in the picture above

ATTENTION: Draw means remove space from inside an existing form. Create means adding new form to the design

i. Draw aeration holes on certain parts of your design (it is best to draw holes at the top) to command the laser cutter to drill holes using Illustrator software.
j. Draw a door in one side of the box using Illustrator software.
k. Create a door on the file 1 cm larger than the door hole you draw.
l. Depending on the size of the door you created, create 6 rectangles (2 x (HeightDoor * h random height) + 2 x (HeightDoor x h/2) + (LengthDoor x h) + (LengthDoor x h/2)) using Illustrator software to allow the building of a sliding door.

3. Laser cut the plexiglas
4. Carve the woods like shown in picture above
5. Build the box by assembling every plexiglas panels together and use the woods part to block each panel with one another
6. Create your door by gluing (use comestible glue to avoid health complications for the bird) the rectangles around the door:
    a. Glue (LengthDoor x h/2) rectangle on the bottom, were you want the doorstep to be, glue it h/2 cm away from the hole
    b. Glue (LengthDoor x h) on top of it
    c. Glue both 2 x (HeightDoor x h/2) on each side of the door, glue them h/2 cm away from the hole
    d. Glue both 2 x (HeightDoor x h) on top of 2 x (HeightDoor x h/2)
    e. You can slide the door panel in, it fits just right!
7. Your recording cage is done! I belt a perch using Legos bricks ® to allow for modulation of the wood perch, and modulation in the height of the perch. (see Figure 5)

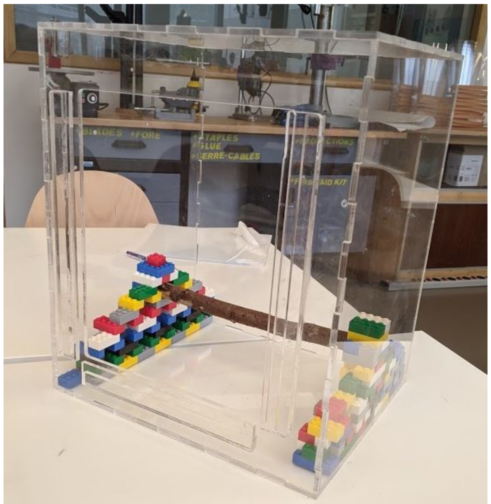

Figure 5: Pictures of the first prototype I belt with Lego perch. I used neither of the techniques presented above in 2.f.. Rather I used a welding method that removed the possibility of taking it apart so that makes it more difficult to transport. It also makes the box more fragile, and maybe toxics for animals in case of extensive usage.

Getting the birds comfortable with the cage: I tested the cage prototype on both my myna and my cockatiels. The myna seems to fit perfectly in the cage. However, the perch was too low for my cockatiels because of their long tail. There were no more Lego bricks ® to adapt the height of the perch for the cockatiels so I removed them from the beta testing program. I used the classic habituation method in experimental biology, with an adaptation phase, to get my myna comfortable with the recording cage. This method consists of observing the specimen, leaving him time to accommodate and adapt to the change, and using positive reinforcement (feeding him grapes) to put him at ease. At no point I forced him inside the recording cage while he seemed scared. It would have wasted the work of getting him confident inside the cage. At one point, I was able to put him inside the cage. I encouraged him and he started vocalizing (see Figure 6 below).

I was able to record with 2 phones (a pixel 3a and a Samsung Galaxy S6). One camera was placed laterally, the other was placed in the front of the bird. The plexiglas can create a resonance effect, however, having aeration holes in the box will help with this matter. I tested the box using my phone as a recording device. The sound was good quality but dampened by the plexiglas box. The lapel microphone needs to be entered in one of the ventilation holes. It will help with the dampening of the sounds from outside. One of the phones was not recording high quality images.
I had no lights directly illuminating the box so there were many unwanted reflections.

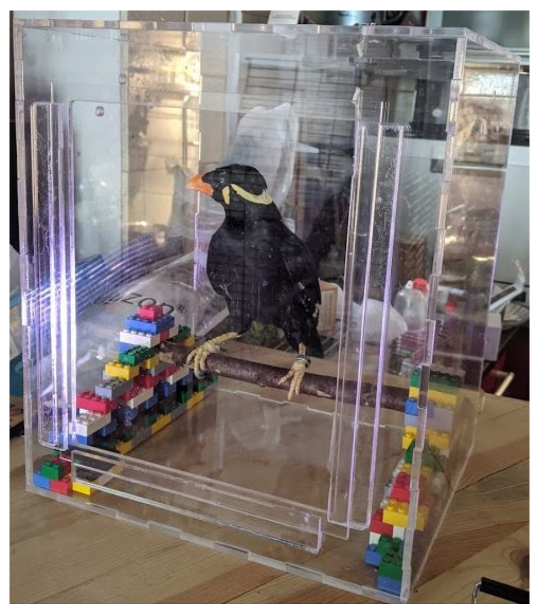

Figure 6: Pictures of a *Gracula religiosa* inside the recording cage prototype.

Acquiring bird specimens for the study

We summarize in Table 1 the different ways to obtain birds.

Options	Details	How to contact them?	Opportunities and Constraints
Institution possessing birds like zoos, breeders, sanctuaries, research foundations	Engage in a partnership with institutions that possess high diversity of birds for our scientific study.	Pauline Provini's personal contacts	Pauline Provini has an agreement with the Ménagerie du Jardin des Plantes for filming the birds when the lockdown will be over. However we will need to manage installing cameras around the cage with no intrusion to the bird's personal space and safety.
Private owners	Bird owners, willing to lend their birds and to welcome our research team at their home to record their birds singing.	Bird owners group and forums on social media.	There are multiple constraint to respect : **Live in france** as our lab is located in Paris, **have multiple birds** so if we make the deplacement it is for gathering multiple data, **know your birds** (if they are not vocal it would be a loss of time for our team, if they are nervous we will stress them and no good will came out of this encounter, on the opposite if they are trained to speak or behave it will be easier for us to work with them)
Citizen science	Publishing online the step to step process explaining how to record correctly birds vocalizing to have people send us the data they gathered.	Publishing all informations online	Needed material: at least one camera, a microphone, and one vocalizing bird. It is best to use multiple cameras in order to construct a 3D model of the bird to calculate the change in volume of the vocal tract. Place every camera at a 90 degree angle from one another around the cage. Prioritise the side(s) and front view of the bird. Using cell phones or GoPros for an easy alternative to replace cameras and microphones. If you only have on camera, you can alternate the front view and the side view. It is important that you use a tripod to maintain the camera's stable! The footage must be send at : pauline.provini@cri-paris.org

Table 1: Table summarizing different ways to get birds for the video and sound recording study

Results and discussion

Project 1 : Gathering and Analysing Data

For this study we used a diverse and balanced sample of birds with at least one representative of each family as you can see on Figure 7. I was able to retrieve data from 113 recordings. Those recordings were picked as they were the ones from the first manual iteration of my program to generate CSV that did not make the code crash. When working with CSV of later iterations of the program, I notice that, in spite of the larger sample of data present on the CSV, on average the values of maximum frequency of the dominant harmonic and maximum frequency of the first harmonic were far from one another while they are supposed to be close. Therefore the data coming from the later iterations were discarded.

I computed a phylogenetic tree of all bird species used in this study, mapped with the magnitude of the maximum pitch extracted from the recordings (see Figure 7). It might not be the higher value the birds can reach but it gives us an average. High pitch values are mainly present in Passeriformes order. We also find them on some other nodes of the tree, such as the Psittaciformes (Parrots) order and the Strisores group (Hummingbirds). Both passerines and parrots are known for the diversity in sound they produce (38,39). Songbirds motions of the vocal tract help modulate the sound (9,28) and parrots use their tongue and beak as an acoustic articulator (8). On the other hand, the vocal organ of hummingbirds shows convergence with songbirds (40). Their upper vocal tract and trachea is shorter than average compared to its body mass (40). Both parrots and songbirds seem to modulate the sound by changing the length of their vocal tract and the opening of their beak (8,9). Therefore we can hypothesize that a correlation might exist between the maximum pitch a bird can produce and the length of the vocal tract.

As passerines, parrots and hummingbirds standing out of the phylogenetic tree (see Figure 7) I computed the Principal Component Analysis (PCA) on sound parameters extracted from the sound recording by grouping separately passerines, hummingbirds, and parrots from the rest of birds. Every species is represented by a number on the plot (see Figure 8) ; species number association can be found on my GitHub page for this internship. We obtained 68.3% of variation explained by the first eigenvalue and 20.4% of variation by the second. The cumulative percentage of PC1 and PC2 explains 88.7% of variations in the dataset, which explains a large amount of the variance. The PCA showcases the principal components responsible for variation on the following variables: maximum harmonics intensity (or maximum loudness of the bird), the maximum dominant harmonic frequency (maximum pitch of the bird), maximum frequency of the first harmonic (supposed to be very close to maxim pitch value as it is well represented in Figure 8), and minimum harmonic intensity (minimum loudness of the bird). There is a clear distinction between passerines and other birds as Passeriformes encompass all the others in terms of maximum pitch and loudness on the PCA (Figure 8). A majority of passerines group at the negative extremity of PC1 below -2 and the other birds mostly gathered above -2 of PC1 (Figure 8). Only three recordings of parrots and hummingbirds, respectively, were added in the study so these data must be taken into account with hindsight. We notice that parrots stand on the negative side of PC1 scattered from 0 to approximately -3.7 and overlapping other birds and passerines. It is interesting to notice the the parrots numbered as 23 that scatters away from the other Psittaciformes responsible for the oval ellipse shape is the African grey parrot (*Psittacus erithacus*), considered as the best mimicking parrots (41). Hummingbirds, on the other hand, are all gathered in one specific spot at -1.5 of PC1 and 1 of PC2 just above the groupment of other birds. They stand out from other birds groupement and overlap passerines which makes sense as hummingbird vocal organs show convergence with songbirds despite their phylogenetic distance (40). The other parrots species still showcase high maximum pitch and loudness (Figure 7) but overlap with other birds on the PCA (Figure 8). The repartition on Figure 8 seems to be linked with the MinHarmIntensity, which can be seen as a proxy for loudness. The groups seem to be distributed along this axis, meaning that this variable explains the differences between the groups. A correlation might exist between the mimicking capacities and the maximum pitch and intensity produced. We also find data standing out for the low maximum pitch on the phylogenetic tree on the passerines branch that correspond to those standing out in the PCA (see Figure 7). PCA confirms that passerines, hummingbirds and some parrots have higher maximum pitch value than other birds. Passeriformes and Psittaciformes are sister groups of the Australavians subclade. Strisores, the clade containing hummingbirds, is not part of the Australavians subclade. One could convey that the high maximum pitch in both Psittaciformes and Passeriformes is due to the close phylogenetic relationship between both orders. Hummingbirds are not closely related to either order, however their vocal organ shows convergence with the one of songbirds form the Passeriformes clade (40).

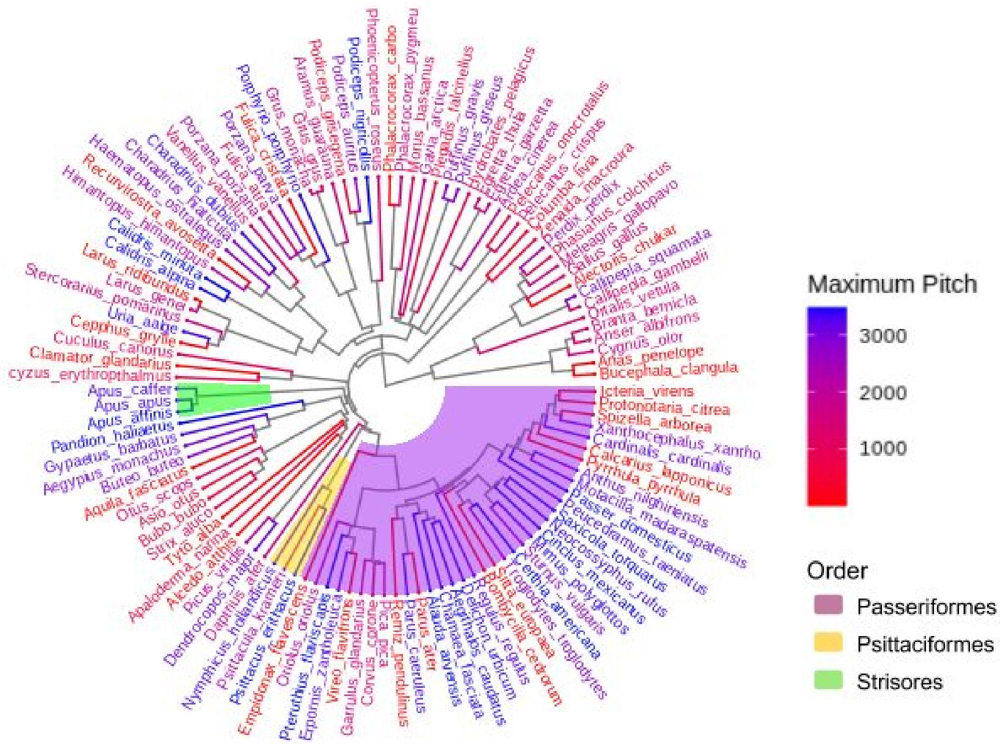

Figure 7: Phylogenetic tree of birds used in this study highlighting maximum pitch in hertz (Hz). Higher pitches are colored in blue.

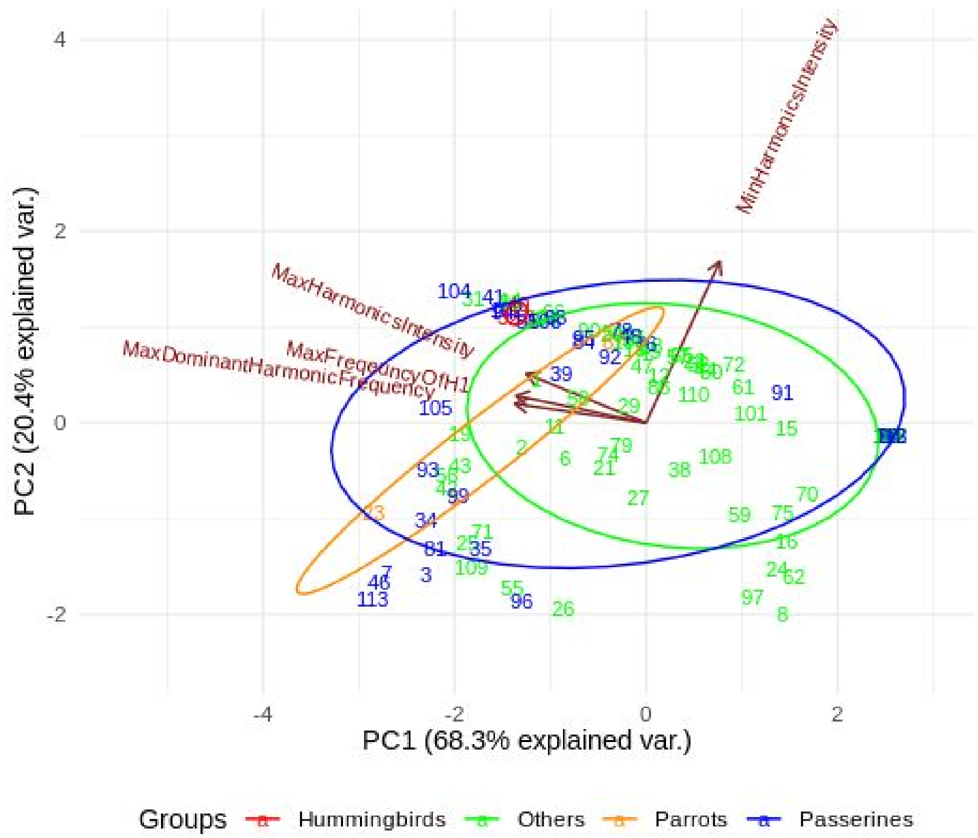

Figure 8: PCA of birds sound parameters grouping by passerines, hummingbirds and parrots. The variables' axes are seen as red arrows contributing to PC1 and PC2. Ellipse's around each groupement and different colors help regroup each specimen by the clade they belong to. The eigenvalue of PC1 and PC2 are both elevated which explains most of the total variance. Others regroups all birds apart from passerines, hummingbirds and parrots.

I computed a second phylogenetic tree to highlight the maximum loudness from each recording (see Figure 9). There does not appear to be any phylogenetic correlation between loudness and bird species as no clades stand out from others. However, when interpreting results by PCA using body mass as a grouping parameter, it becomes clear that size rather than phylogenetic closeness and vocal organ morphology explains loudness repartitions in birds. In Figure 10, body mass seems to discriminate 'minHarmonicIntensity' variable. There seems to be a negative correlation between 'small' body mass and the minimum loudness reached by a bird. There is a positive correlation between 'small' and 'extra small' body mass and the maximum loudness and pitch reached. Small and extra small birds will tend to be louder and have higher pitch than medium and large birds. Larger birds will have, regardless of their phylogeny, different loudness from smaller birds even if they belong in the same family.

To go further, it would be useful to gather more sounds to have a wieder sample. It would be especially useful for Passeriformes and Strisores as in this study we did not have many recordings to analyse from those clades.

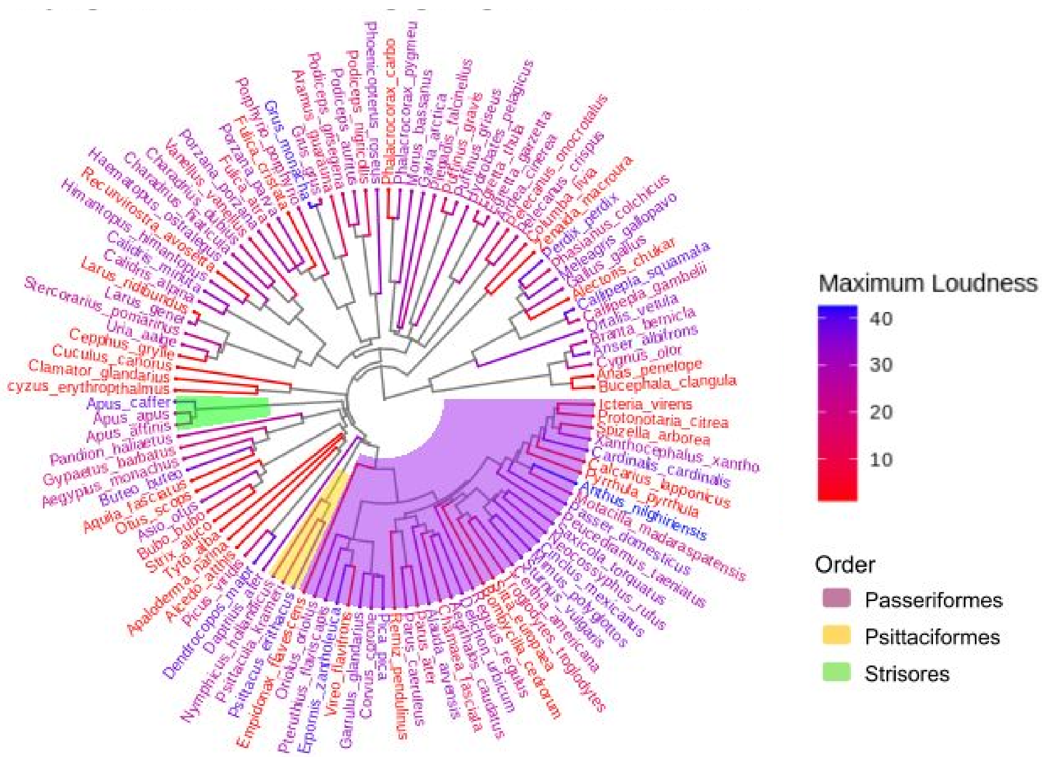

Figure 9: Phylogenetic tree of birds used in this study highlighting maximum loudness in decibel (dB). Loud sounds are colored in blue. The previous orders studied are highlighted for comparison with figure 7

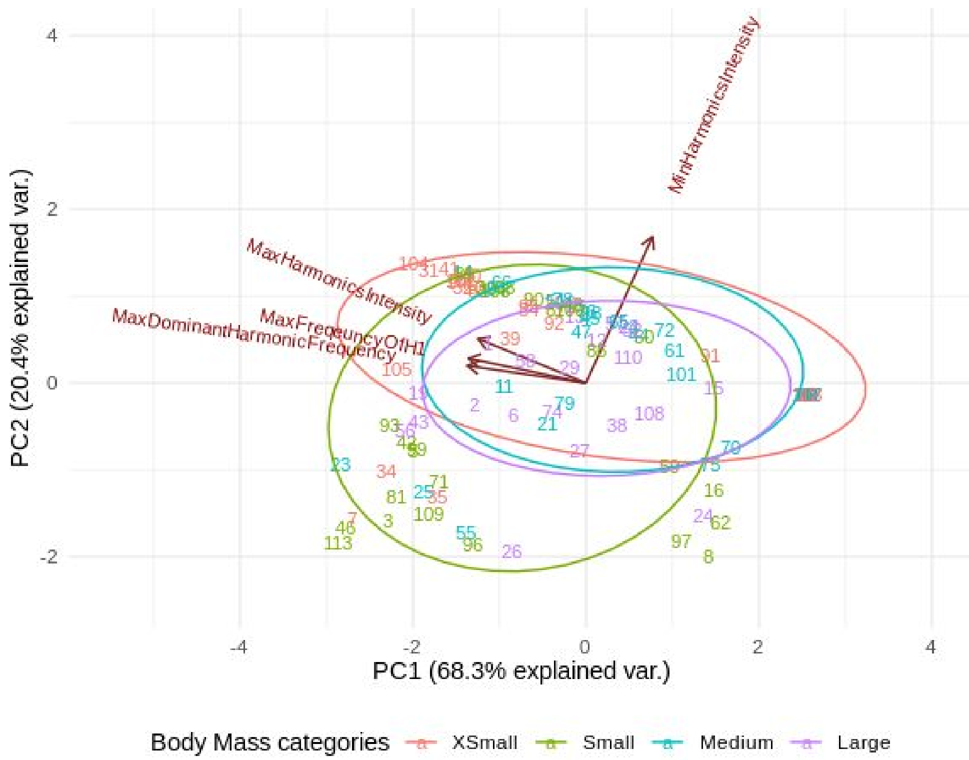

Figure 10: PCA of birds sound parameters grouping by body mass.

I computed another PCA on bird sound dataset by grouping birds by their vision type (diurnal or nocturnal) (see Figure 11). I only have a small sample of nocturnal species so this result must be taken with hindsight. There is a negative correlation between the nocturnal vision type and both the maximum pitch and maximum loudness. It might be interesting to see if there is a phylogenetic relationship between vision type and produced sound. This negative correlation might be explained by different communication mechanisms between day and night. “As avian nocturnal vocalizations are understudied” (42), it would be interesting to look at the correlation between the maximum pitch and loudness produced and the nocturnal lifestyle in birds.

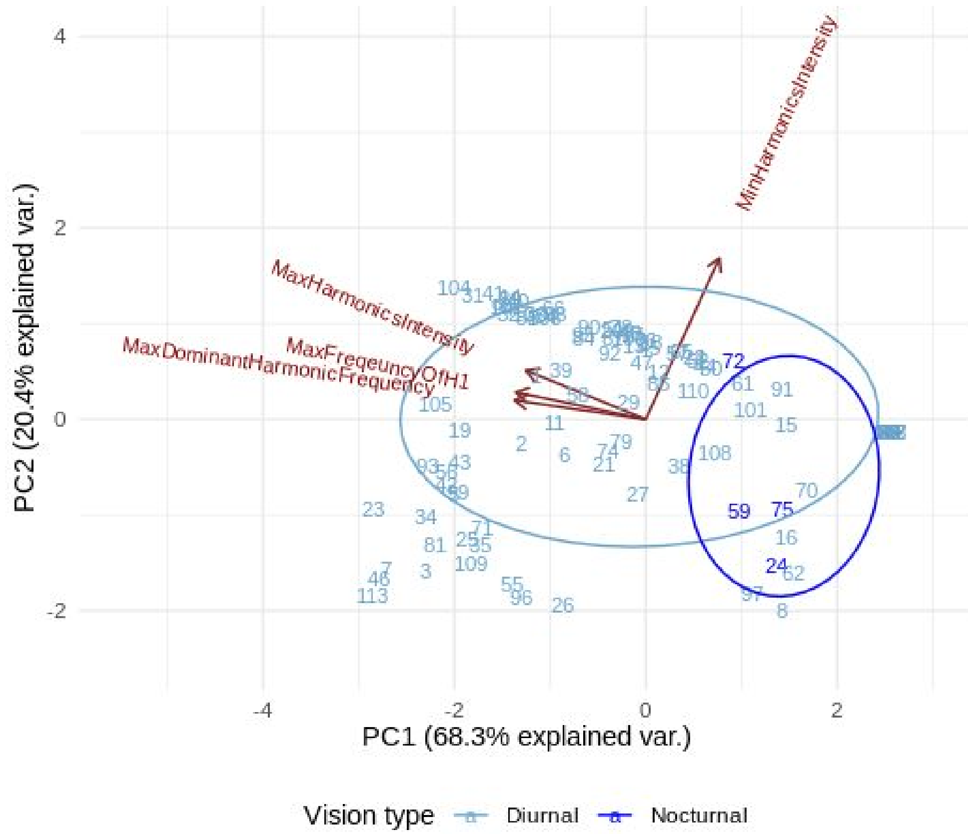

Figure 11: PCA of birds sound parameters grouping by vision type.

There are some limitations of using sounds from online open source databases. First of all, there is no certitude on the species that was recorded. The recordings are usually taken outside, where other sounds might parasite the recording. Despite the sound cleaning technique used before processing the recording, there is no assurance that all unwanted sounds were removed. There is no indication of the distance from where the recording was taken, for a study on loudness this is inconvenient because these parameters vary a lot with the distance. The PCA showed that most of the variance in our sample came from the maximum pitch and the maximum loudness reached by birds. However, there is no way to be sure that all birds were singing at their maximum pitch and at the louder they could get at one point during the recording.

Project 2 : Experimental protocol to record vocalizing birds

I developed an experimental protocol to record vocalizing birds using a recording cage. I tested the recording cage protype with a *Gracula religiosa* and a *Nymphicus hollandicus.* I filmed the front and lateral view of the bird using 2 phones. The recording was good quality, however the sound from the bird was dampened by the plexiglas box and most of what we could hear was from outside noises. To improve the quality of the recording we will need to add a microphone to the setup. The lapel microphone is a good choice as his small size allows it to be slid inside the recording cage by the ventilation holes. It will resolve the sound issues and dampened noises from outside the recording cage. The Samsung Galaxy S6 phone camera I used is outdated and the quality of the obtained recording is unsatisfactory. Many unwanted reflections are present on the videos as ambient lights were on in different parts of the room. When turn off, there was not enough light for the cameras and the videos were of poor quality. We could improve this by bringing special light to control this parameter or use a camera filter that removes reflections. It is important to consider that the plexiglas can create a resonance effect that changes the bird sound. It would be useful to measure the exact impact on sounds that a plexiglas box has. In addition, I filmed using a side or front view *Nymphicus hollandicus* vocalizing freely in an open room. The recording was good quality, however we could hear noises (from outside the room, from inside the room and from other *Nymphicus hollandicus* present in the room but not being recorded). A microphone could resolve this problem. Some recordings were taken from far away and we could not distinguish easily the bird from the surrounding. A uniform background might help with this problem. The videos are available on my GitHub account (https://github.com/irinade/Internship_Report_L3_GitHub_page).

The hypothesis was that a recording cage would resolve many unwanted issues and facilitates the study. The recording cage is supposed to help the birds to feel safe in an environment that they already know, with people they trust. However, it will stress them out if they are not used to it. Furthermore, the recording cage is indeed overall cheaper than keeping birds in a lab, however, it is more restrictive than placing cameras directly inside birdcages (bird cages that can be present in a zoo or pet store, not necessarily in a lab). In continuation of this project I would advise to leave aside the recording cage idea as it is not the best option both for our research project (restrictive) and for bird subjects (stressing).

Pauline Provini had planned to buy a set of 3 professional cameras. However, after seeing some test recordings of *Nymphicus hollandicus* vocalizing freely in an open room, she is considering buying GoPros as a replacement. There is a need for more powerful recording devices than smartphones on this project, however there might not be an extensive need for excellent quality cameras (see Table 2) and the sampling rate of gopros (up to 240 frames per second) would be sufficient to record bird vocalisations We can summarize the pros and cons of the recording material in Table 2.

Recording material	Pros	Cons
Phones or gopros	Relatively cheap, easy to procure, open for citizen science possibility	Lower qualities than professional cameras
Professional cameras	Excellent quality, possibility to create a 3D model to calculate change in vocal tract volume, many different options allowing excellent quality slow motion for example (is there really a need for it?)	Expensive, encombrant

Table 2: Recording materials pros and cons comparison table

Conclusion and perspectives

I have acquired preliminary data helping to understand the links between the sounds produced and the evolution of birds. The trends show that maximum loudness seems to be related to body mass and maximum pitch seems to depend on phylogenetic proximity and vocal organ morphology. Those results, although very promising, need to be taken cautiously. To help validate the trend showcased in our results it is important to do further statistical analyses. For this we would need to increase the dataset of our study to encompass more diversity between species. It would be important to also increase the diversity within the species, to confirm our results. In addition, it would be important to have a better control on the quality of the data extracted from the online sound database, to prevent potential artefacts due to problems (noise or several species vocalising at the same time) in the recordings.

Overall, little is known about the evolution of birds' syrinx and vocal tract (Clarke et al., 2016). Many detailed morphological descriptions of bird vocal organs have been made in the past on a relatively large number of birds (12,29,43). However, the questions of the relationship between the vocal organs shapes and acoustic characteristics has never been tackled. It would be interesting, as a continuation of this study, to look at the relationship between the syrinx morphology and the produced sounds.

Another trend of our results suggest that nocturnal birds produce lower maximum pitched sound and are overall quitter than diurnal birds. Vocalisation of nocturnal bird species have been left unnoticed for a long time (42). It would be interesting to look for a phylogenetic relationship between sound and vision type as there might be a correlation between the maximum pitch and loudness produced and the nocturnal lifestyle in birds.

Concerning the experimental protocol developed to record vocalising birds, the recording cage appears not to be the best option and should not be used in further light video recordings. The protocol development helped Pauline Provini realise that she could downgrade the quality of the materials she had planned to buy as less advanced materials seemed to be as accurate, cheaper and more handy. My internships continue until mid January and I plan to analyse the video recordings I took to test the recording cage. Those data will help me prepare for the SICB talk I will give at the beginning of 2021.

All the data I gathered during this internship brings a better understanding of the biomechanical principle behind bird vocalization that will be useful for the team in the coming years.

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Supplementary material

Annex 1

Humans tend to associate sound with subjective quality as pitch, loudness, duration, and timbre.

Pitch

Pitch is the subjective perception of the fundamental frequency (44). Pitch is a quality that enables qualifying a sound as "high" or "low". A high pitch emanates from a quick vibration. A low sound on the opposite originates from a slow vibration. Pitch is proportional to frequency, so a low sound will have a low frequency, and a high sound a high frequency like you can see in Figure 1.1 below (45).

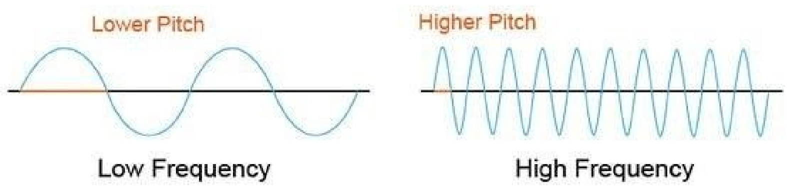

Figure 1.1 : Scheme representing the difference between a low and high pitch
https://quizlet.com/379299977/sound-waves-test-312-diagram/

The fundamental harmonic wave has the slowest vibration compared to the other harmonics that are known as higher. When looking at the pitch of a sound, we tend to look at the fundamental harmonic because it resembles the original sound the most, and it is easier to study because it is a sinusoidal signal. For simple sounds, the pitch is the frequency of the slowest vibration, the fundamental harmonic.
A soundwave can be decomposed into multiple sinusoidal waves (see below). The sinusoidal wave that resembles the most the original wave, also called the composite wave, is called the fundamental frequency or the 1st harmonic (see Figure 1.2 below).

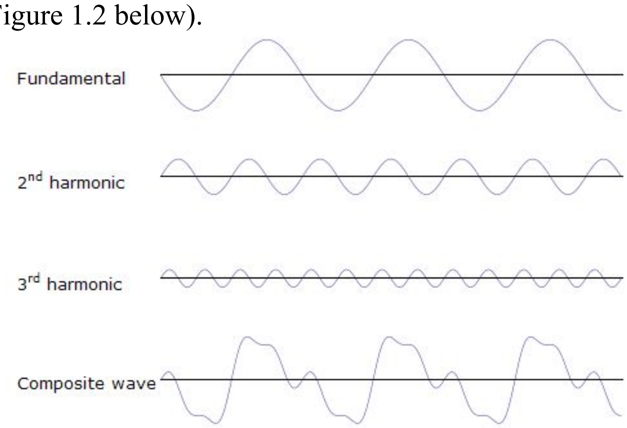

Figure 1.2 : Scheme representing a soundwave (composite wave) and its decomposition into harmonics
https://webassign.net/sample/unc/lab_8/manual.html

However, if the sound is complex (too many harmonics) or has too much noise (unwanted sound), it becomes more and more fallacious to associate the original wave to the first harmonic to identify the pitch. In that case, the pitch can be determined by mixing low harmonics together.

Loudness

Loudness is the subjective perception of sound pressure (44). Loudness is an attribute that allows ordering sounds on a scale from "quiet" to "loud". A sound is considered quiet if its pressure level is low. On the contrary, a sound is loud if its pressure level is high. Amplitude is proportional to pressure level, so a quiet sound has a low amplitude, and a loud sound has a high amplitude (see figure 1.3 below). Sound pressure is defined by the pressure deviation (caused by a sound wave) from the atmospheric pressure (46).

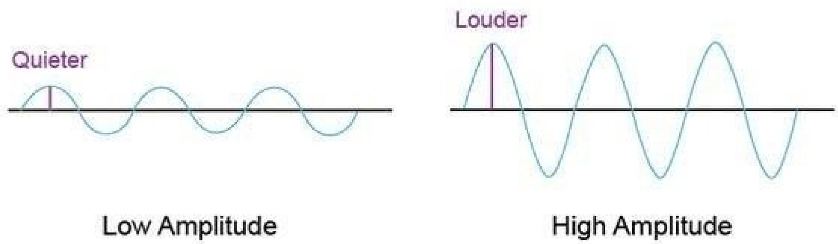

Figure 1.3 : Scheme representing the difference between a quiet and loud sound
https://quizlet.com/379299977/sound-waves-test-312-diagram/

Duration

Duration is the subjective perception of the length of time Duration is a trait that permits the classification of sounds on a scale from "short" to "long". A short sound will be produced for a small amount of time, and so on... Time is proportional to duration (47).

Timbre

Timbre is the subjective perception of the quality of a sound (48). If you consider two sounds with the exact same pitch (the sounds have the same frequency), loudness (they have the same amplitude), and duration, you might end up with two recordings that will sound different from one another. This is due to the difference in timbre.
Timbre defines the quality of a sound, it is sometimes said to be the sound "color"! In Figure 1.4 below, we can clearly see that the notes have the same frequency and amplitude, and last for the same amount of time. The contrast between these two notes resides in their different timbre. We can notice that their spectrograms are different. They have the same pitch, so the same fundamental frequency (first harmonics), but their higher harmonics diverge.

Timbre is a trait that permits the grading of sounds from "clear" or "simple" to "mixed" or "complex". A clear sound will have a soundwave that resembles a sinusoidal wave. On the opposite, a mixed sound will have a more complex soundwave.

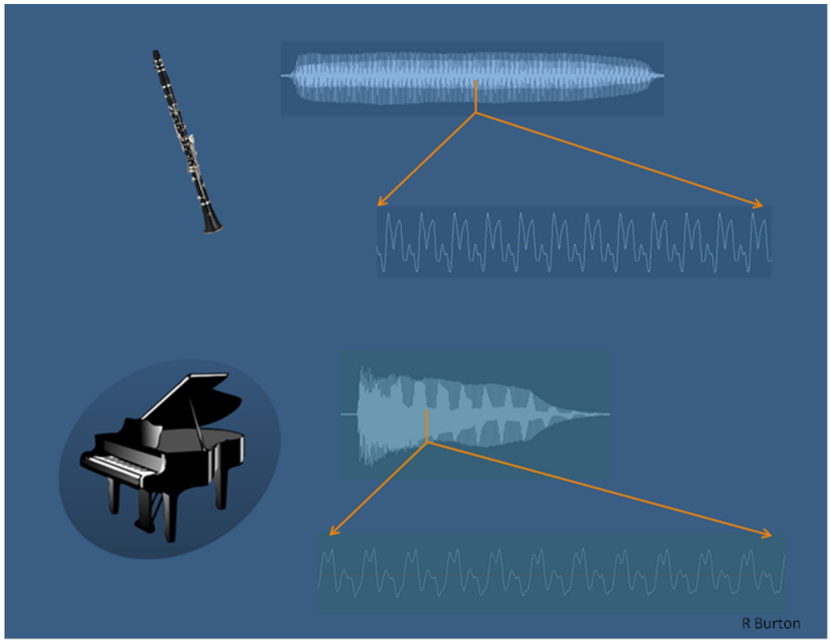

Figure 1.4 : Scheme representing the difference in timbre of a music note played by two different instruments. https://en.wikipedia.org/wiki/Sound#/media/File:Timbre_perception.png

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Annex 2

How do birds modulate sound with their vocal tract?

Birds are able to produce a great diversity of sounds. They vocalize with a specific vocal organ, the syrinx, producing the primary vibration. However, it is not fully understood how the produced sound is filtered afterward by the vocal tract (trachea, upper part of the esophagus, oral cavity, and beak). We know that in songbirds, motions of the vocal tract help modulate the sound. When producing low pitch sounds, the throat and breast area expend visually, and the beak stays fairly closed. Conversely, while making high pitch sounds, the neck elongates and the beak opens widely. We hypothesize that all birds can filter the sound by changing the length and volume of their vocal tract. To determine how the different parts of the vocal tract modulate sound in songbird and non-songbird species, we filmed birds vocalizing with light cameras and synchronously recorded their sound with microphones to quantify the motions of the beak, neck, and pouch, while birds produced different sounds. Our results suggest a correlation between vocal pitch and the motions of the beak, neck, and pouch, supporting previous observations. This work shows that the complexity of bird vocalizations is not only related to the syrinx, but also to the entire upper vocal tract, which provides elements to better understand birdsong functional morphology.

Annex 3

Cleaning Recordings Protocol

1. Open Audacity software version 2.4.2
2. Pre Download the recordings you want to clean
3. Open the recordings using *File/Open... and* by selecting it
4. Now that the recording is open, you can see its spectrogram, see Figure 3.1

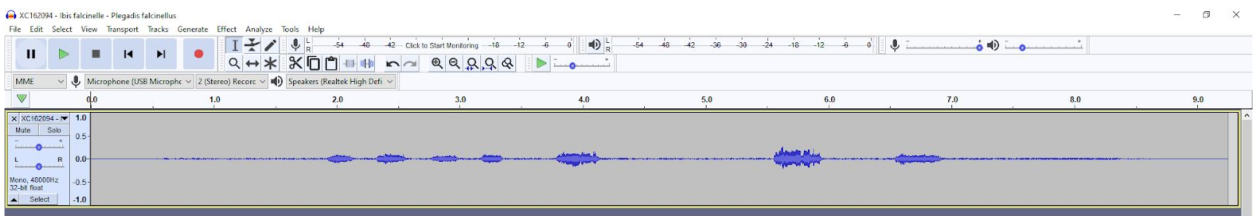

Figure 3.1: Spectrogram of Plegadis falcinellus. We can identify noises and spaces with no sound. I will first reduce the noise on the recordings, and then remove the part of the recordings with no sounds.

5. Select a part of the noise and then go-to *Effect/Noise Reduction* and select *Get Noise Profile*. This will create a profile of the noise and apply it to the entire recording to reduce noises. See Figure 3.2

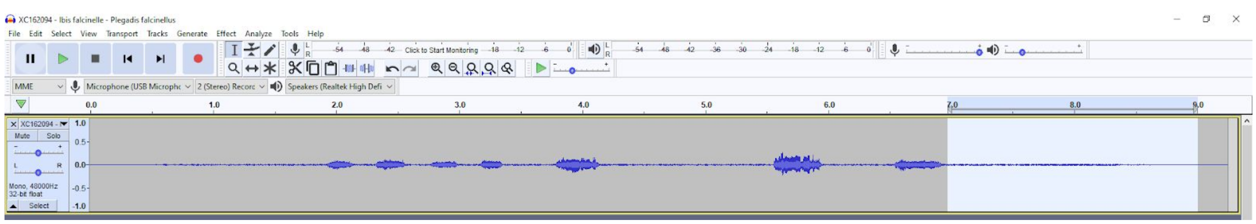

Figure 3.2: Spectrogram of Plegadis falcinellus with noise selection and processed by the software.

6. Select the entire recording by going to *Select/All*
7. Apply the reduction of the noise to the entire recording by *Effect/Repeat Noise Reduction*. Then you obtain a clean recording with fewer noises. See Figure 3.3

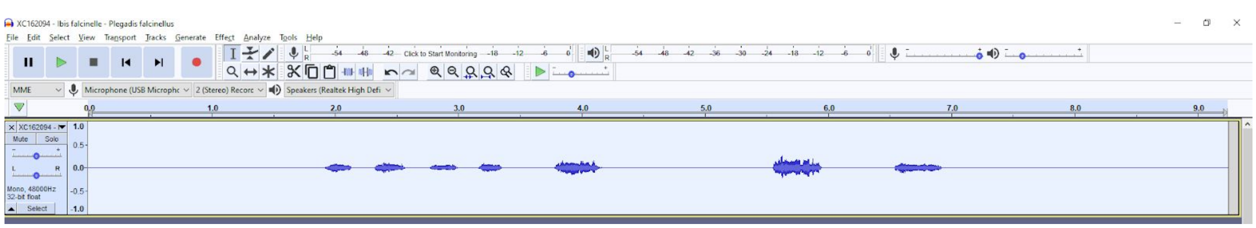

Figure 3.3: Spectrogram of Plegadis falcinellus with noise reduction.

8. Remove the empty spaces in the recording by selecting the part of the recording you want to remove, and by *Edit/Remove Special/Splete Delete*. This is useful in case some recordings have noises that were not removed using the Noise reduction method. There is also an option to silence a part of the recording rather than delete it. Getting rid of extra blank space between two notes can also be useful to shorten your recording in order to make the file leighter in case you are working with a large amount of sounds.
9. Use the *Time Shift Tool* to move the recording to match time t=0
10. Voila !! A clean and shorter recording. See Figure 3.4

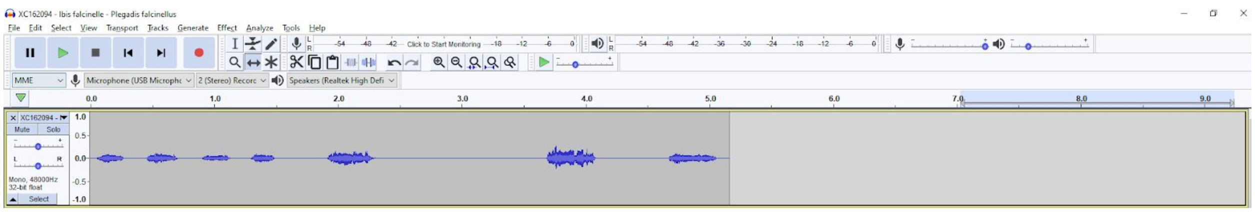

Figure 3.4: Spectrogram of Plegadis falcinellus with noise reduction and time reduction.

Sometimes recordings won't open with Audacity and the software will show this error : *MP3 Decoding failed: Lost synchronization.* In those cases, I converted the MP3 recordings into WAV recordings and Audacity could open those files. It also happened in some cases that there was no pause between syllables so I was not able to isolate noise to remove it from the recording.