Loon Syringes: Do Anatomical Differences Underpin Gender-Specific Vocalizations?

A Major Qualifying Project
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Abstract

Avians possess a distinctive sound-producing organ, the syrinx, located at the tracheal bifurcation. The Common Loon has a unique set of vocalizations used for communication, including a male-specific call. This project aimed to explore anatomical differences in male and female syringes that account for differences in vocalizations. Micro-CT imaging was used to examine syrinx morphology, creating 3D models of loon syringes. Results revealed a tracheobronchial syrinx type in loons, yet no anatomical differences between male and female loon syringes were observed.

Acknowledgments

We would like to thank Professor Karen Troy for her generosity in letting us utilize the micro-CT scanner. Additionally, we would like to thank all the graduate students that we consulted with, especially Andrew Wilzman, for teaching us how to create 3D models and how to analyze samples. Finally, thank you to all of our advisors for their guidance and support throughout the entire project.

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1.0 - Introduction

Within the animal kingdom, reptiles, amphibians, and mammals have a larynx, a sound-producing organ located cranially to the trachea. While the avian larynx helps to regulate airflow during respiration, they have a unique sound-producing organ called the syrinx. The syrinx is located at the bifurcation of the trachea and consists of cartilage rings, flexible membranes, and syringeal muscles (King, 1989). Among avian species, the syrinx is morphologically diverse, with three types of syringes recognized. Morphological differences in the syrinx can also be seen within species, such as between different sexes of species (Miller et al., 2008, Ballintijn & Cate, 1997). This suggests that morphology can account for differences in vocalizations between the sexes of species.

The Common Loon (*Gavia immer*) has a unique set of vocalizations used for communication, with one call specific to male loons (McIntyre, 1988). Yodels are a territorial call made specifically by male loons that indicate the body mass and condition of the loon (Mager et al., 2007). This is of particular research interest since it is currently unknown if morphological differences in their syringes allow males to produce yodels. Currently, the anatomy of the Common Loon syrinx has not been documented, as well as how the anatomical structure of the Loon syrinx produces their unique vocalizations. In addition, there is currently no research that has investigated if there are anatomical differences between male and female loon syringes. The goal of this project was to determine if there are anatomical differences between the male and female loon syrinx, and it was hypothesized that male loons can produce yodels due to morphological differences in syrinx anatomy. To examine the anatomical structure of loon syringes, micro-CT imaging was used to scan and create 3D models of loon syringes for analysis.

1.1 - Anatomy and Classification of Syringes

According to the *"Functional Anatomy of the Syrinx"* by King (1989), the syrinx is the part of the airway located at the division of the trachea into the left and right primary bronchi of the lung. It lies in front of the heart, surrounded by the interclavicular air sac. Different species of birds have different numbers of syringeal muscles around the syrinx, with some bird species having five pairs and others having none (King & McLelland, 1984). The syrinx is made up of a

cartilaginous framework of rings that are flexible enough to stretch and are capable of vibrating. There are three different types of syringes which include the tracheobronchial syrinx (Figure 1a), tracheal syrinx (Figure 1b), and bronchial syrinx (Figure 1c). The tracheobronchial syrinx is a type of syrinx that includes both tracheal and bronchial elements, where the tracheosyringeal cartilages form a box-like tympanum. The tracheal syrinx is a type of syrinx where tracheal elements are the dominant features. The tracheosyringeal cartilages in the tracheal syrinx are reduced to thin circlets that are either embedded in the membrane or absent altogether. Finally, the bronchial syrinx mainly shows modifications of the bronchial elements whereas the tracheal elements show little to no specialization. This type of syrinx has cartilages which are called bronchosyringeal cartilages, which can be either complete or incomplete C-shaped cartilage rings.

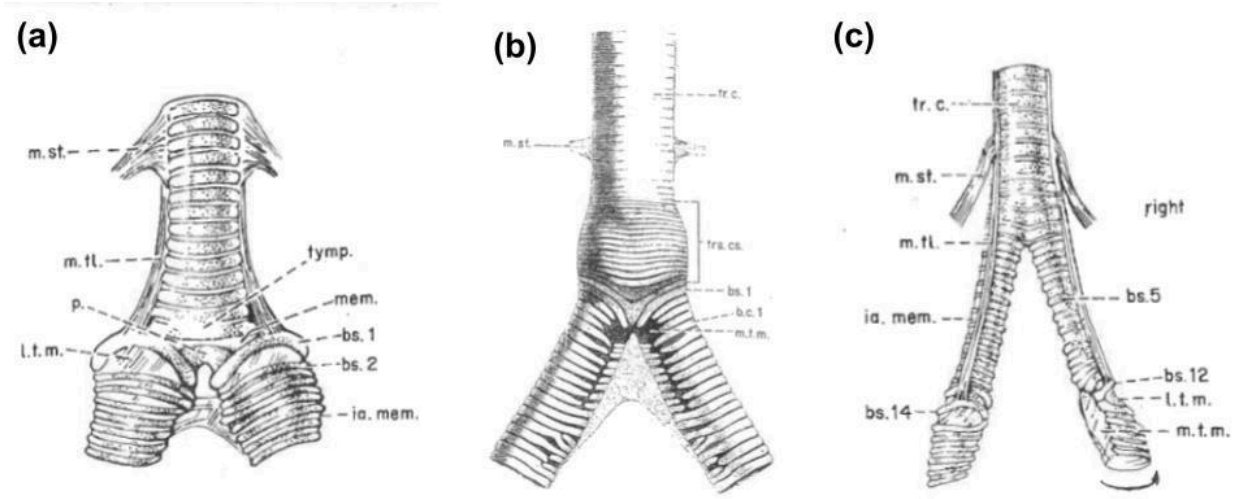

Figure 1: *The three different types of syringes, (a) tracheobronchial, (b) tracheal, (c) bronchial. Abbreviations: tr.c. = tracheal cartilage, trs.cs. = tracheosyringeal cartilages, tymp. = tympanum, L.t.m = lateral tympaniform membrane, P. = Pessulus, bs. = bronchosyringeal cartilage, m.t.m. = medial tympaniform membrane, m.st = m. sternotrachealis (King, 1989).*

The syrinx is important in avians given that it functions as the sound-producing organ. The sounds are typically generated by the vibration of the tympaniform membranes, particularly the lateral tympaniform membrane (Larsen et al., 2006). The vibration of the tympaniform membranes is thought to be caused by changing pressure gradients between the interclavicular air sacs and the lumen of the syrinx. During expiration, increasing pressure in the interclavicular

air sac causes the tympaniform membranes to move into the lumen, where air then flows past the membranes causing them to vibrate (King & McLelland, 1984).

1.2 - Loon Calls and Types

The Common Loon uses four distinct types of calls to communicate with one another: hoots, wails, tremolos, and yodels (McIntyre, 1988). Hoot consists of short, single notes, and are used as contact calls. Hoots are usually used when loons are close to one another, such as in social gatherings. The most commonly heard Common Loon vocalization are wails, which consist of one, two, or three-note calls. Wails are used to locate and communicate with other loons over longer distances. In response to a perceived threat, loons will use tremolos to indicate danger. There are three types of tremolos (Type I, Type II, and Type III), with each type moving to a higher frequency to indicate the intensity of the threat (McIntyre, 1988). Finally, yodels are calls made only by male loons and are the interest of this project. Yodels are used to mark territory and are also used for territorial defense. Yodels also indicate the body mass and condition of the loon (Mager et al., 2007). Only adult male loons yodel, and it is currently unknown at what age young male loons are capable of yodeling (McIntryre, 1988).

1.3 - Micro-CT

Micro-computed tomography (micro-CT) is an imaging tool that can produce 3D images of a specimen using 2D image scans, or slices (Boerckel et al., 2014). A micro-CT consists of an X-ray source that produces X-rays, which pass through a collimator before passing through the specimen. As the X-rays pass through the sample, they are recorded by an X-ray detector. This produces a 2D image or a slice of the specimen. The specimen is then rotated, and the process described above is repeated to obtain a series of 2D slices of the entire specimen (Boerckel et al., 2014). The 2D image slices can be used to create a 3D image or model of the specimen. Previous studies have used micro-CT to examine the syrinx structure of other avians, such as the zebra finch (Düring et al, 2013). In 2023, WPI undergraduate student Kaylee Gladu developed a micro-CT procedure specific for loon syringes, in addition to creating 3D models of syringes (Gladu, 2023). This procedure was used to investigate the anatomical differences between male and female loon syringes using micro-CT.

2.0 - Materials and Methods

2.1 - Loon Syrinx Preparation and Storage

The loon syringes used for this project were obtained from loons that had been found dead by wildlife rehabilitators. The syringes were removed from the loons during necropsies, and samples were obtained after the completion of the loon necropsies. No loons were euthanized or killed specifically for this project. The syringes were stored in vials containing formalin, and samples were removed prior to imaging.

2.2 - Micro-CT Imaging

The loon syringes were scanned following the "µCT Imaging" protocol in the *Investigating the Common Loon Syrinx* report by Kaylee Gladu. The loon syringes were scanned using the SCANCO Medical XtremeCT scanner. Before scanning the samples, the scanner must be pre-calibrated, and it must be re-calibrated every 20 minutes. Prior to each scan, the control file to be used was selected. The control file indicates parameters such as the number of slices, size, and resolution of the scan. For all scans, control file 28 was used (Table 1). After pre-calibrating the scanner, the sample can be loaded onto the tray. To separate the sample from the tray, the syrinx was either placed on a slightly inflated ziploc bag (Figure 2a) or on a disposable pillowcase (Figure 2b). While the first few scans used a ziploc bag, a disposable pillowcase was used for the remaining scans to better prevent the sample from shifting inside the scanner. After placing the sample into the scanner, "scout view" was used to view the sample and ensure that it was within the scanning range. Using the scout view, the reference line was drawn to select the final area to be included in the scan. After drawing the reference line, the sample was scanned.

***Table 1**: Control file 28 parameters*

Control File	E (kVp)	I (µA)	Voxel Size	Slices
28	60	900	82.000	880

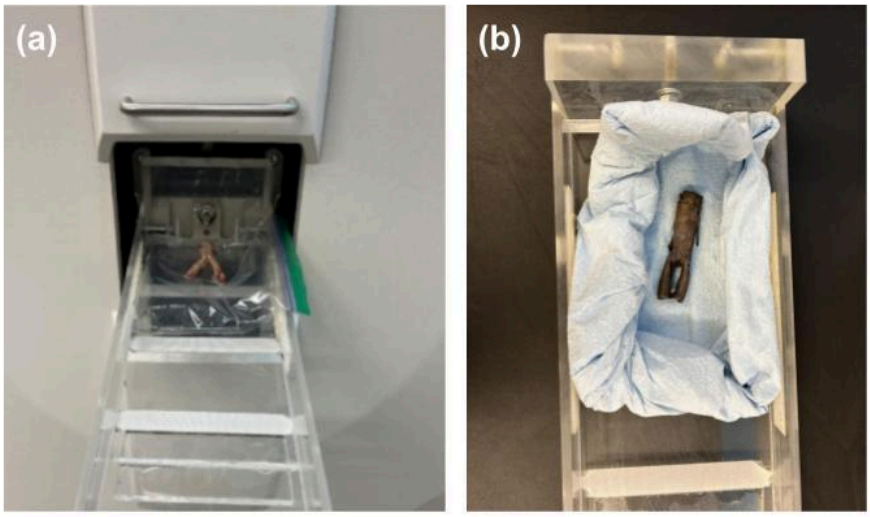

Figure 2*: Samples were loaded into the scanner either on a ziploc bag (Figure 2a - Image taken by Kaylee Gladu) or on a disposable pillowcase "nest" (Figure 2b).*

2.2 - Constructing 3D Models of Loon Syringes

After scanning a sample, the μCT Evaluation Program converts and then exports the image slices as DICOM files. After changing the task to "Convert to DICOM", the x, y, and z coordinates of the sample being converted were adjusted. First, the z coordinate was adjusted, with the first value representing the first slice to be converted, and the second value representing the total number of slices to be converted. Next, the x and y coordinates were adjusted to ensure that the entire sample fit within the range of the box (Figure 3). After converting the image slices to DICOM files, a program developed by a PhD student was used to sort and transfer the DICOM files to a personal hard drive.

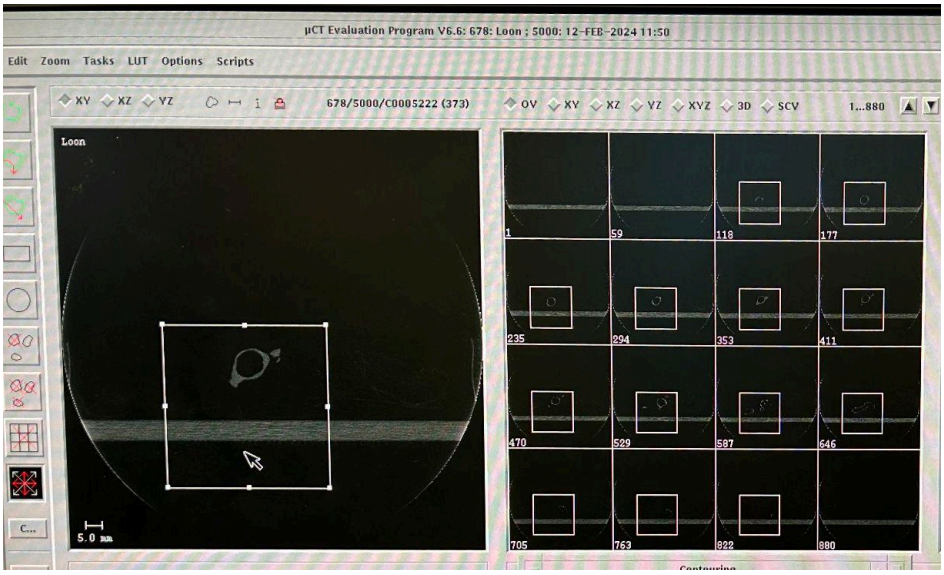

***Figure 3**: µCT Evaluation Program used to convert image slices to DICOM files.*

After extracting the DICOM files, 3D models of the syringes were created using Materialize Mimics 24.0 (Mimics) and InVesalius 3.1. Both programs can be used for 3D medical image processing, and InVesalius offers similar features to Mimics but is free to download. When using Mimics, the DICOM files were copied from a personal hard drive to the desktop with Mimics. After opening Mimics, a new file can be uploaded by selecting “new project”. Once the file has been uploaded, the scan can be viewed from the coronal plane (Figure 4: top left), transverse plane (Figure 4: top right), and sagittal plane (Figure 4: bottom left). To create a 3D model of the sample, select “segment”, and then select “new mask”. To apply a new mask, the threshold must be adjusted so that all parts of the sample are highlighted. The threshold includes a range of intensity, and any pixels that fall into the selected range will be highlighted. For each scan, a custom threshold was set to ensure that the entire sample was highlighted, while also minimizing any background noise around the sample (Figure 5).

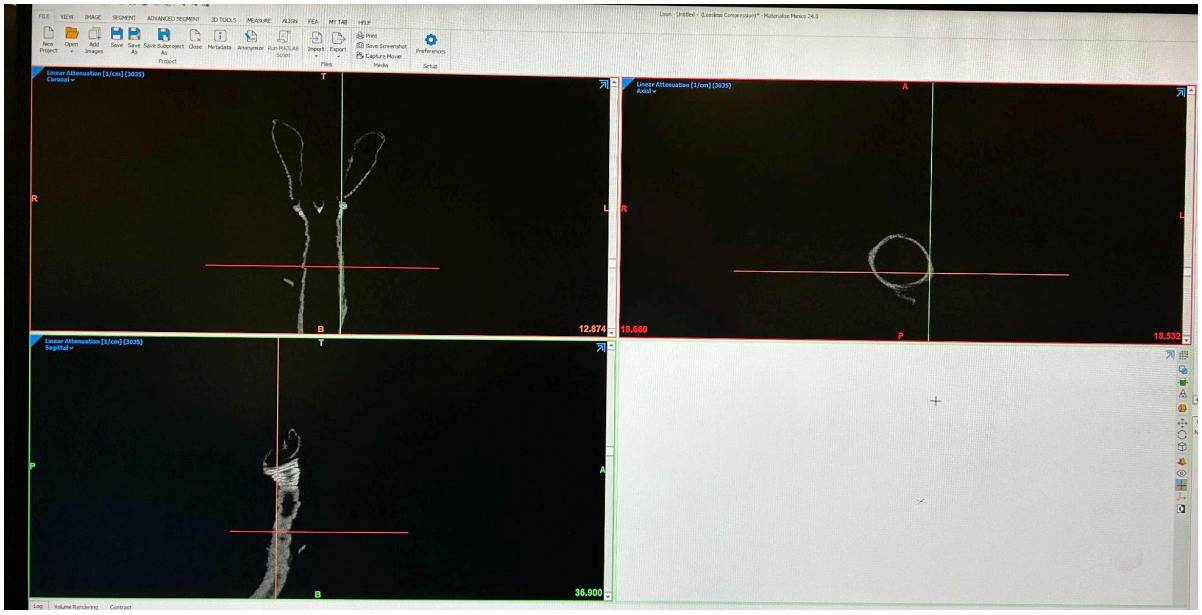

***Figure 4**: Syrinx scan viewed from the coronal, transverse, and sagittal plane.*

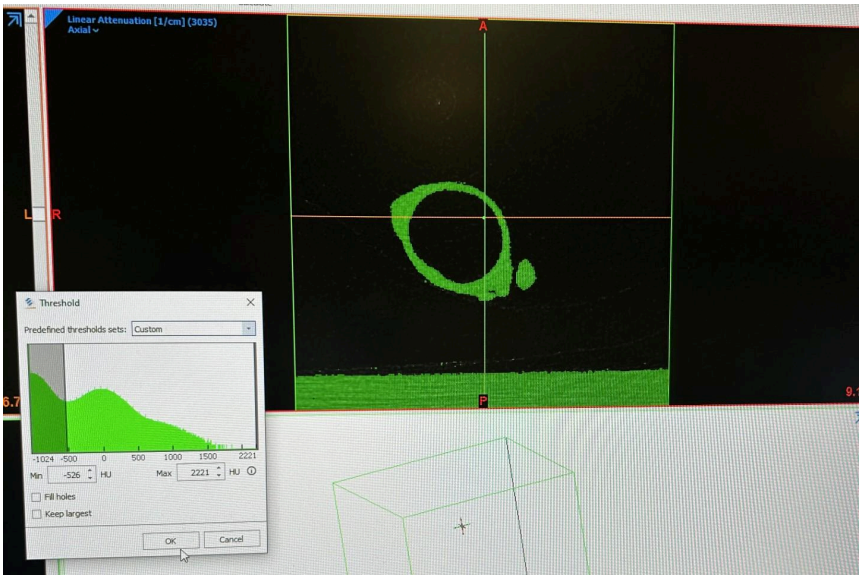

***Figure 5**: Setting threshold in Mimics. A slice of the sample is shown on the black screen, and the threshold range is shown in the green graph in the bottom left. The stage can be seen at the bottom of the image.*

After selecting the threshold, the stage must be removed from the mask. To remove the stage, select “multiple slice edit” in the project management bar. Multiple slice edit can be used to make fine adjustments to the mask, including removing or adding pixels to the mask. In multiple slice edit, select “remove” and go to the first and last image slice and select the area to be removed. After selecting the area to be removed, select “interpolation” to remove the stage from all slices. If interpolation is not selected, then the stage will only be removed from the individual slices where the stage was selected. Finally, to generate the 3D model of the mask, right click on the mask and select “calculate part” and apply. The 3D model will then appear on the lower right side of the screen. To make the mask smoother, right click on the mask and select “wrap”.

InVesalius can also be used to create 3D models with a similar procedure to Mimics. After opening InVesalius, select the “Import Medical Images” folder and then the file to be uploaded. Once uploaded, an initial preview of the image slices will be displayed along with the option to “keep all image slices” or to skip a select number of slices. After selecting the number of slices, Invesalius may recommend reducing the original resolution. To keep the original resolution, type “100%” as the percentage of the original resolution and select ok. The image scans can then be viewed from an axial, coronal, or sagittal view. Similar to Mimics, a mask must be selected before creating a 3D model. First, select “Create a new mask”, and then adjust the desired threshold for the mask (Similar to Figure 5: Depicting setting threshold in Mimics). Before creating the 3D model, the stage also has to be removed. The “crop” feature or “remove parts” features can be used to remove the stage from the image. After creating a mask and selecting the desired threshold, select “Create surface” and InVesalius will generate a 3D image.

2.2 - Analyzing 3D Models of Loon Syringes

After constructing the 3D models, the samples were analyzed through observation and by measuring anatomical features of loon syringes. The measurements were taken within Mimics and Invesalius using the “measure” feature. Measurements can be taken directly on the 3D models or from a select image slice. First, the external and internal width of the syrinx was measured using an axial image slice, and was measured at the caudal end of the syrinx prior to the start of the lateral tympaniform membrane (Figure 6).

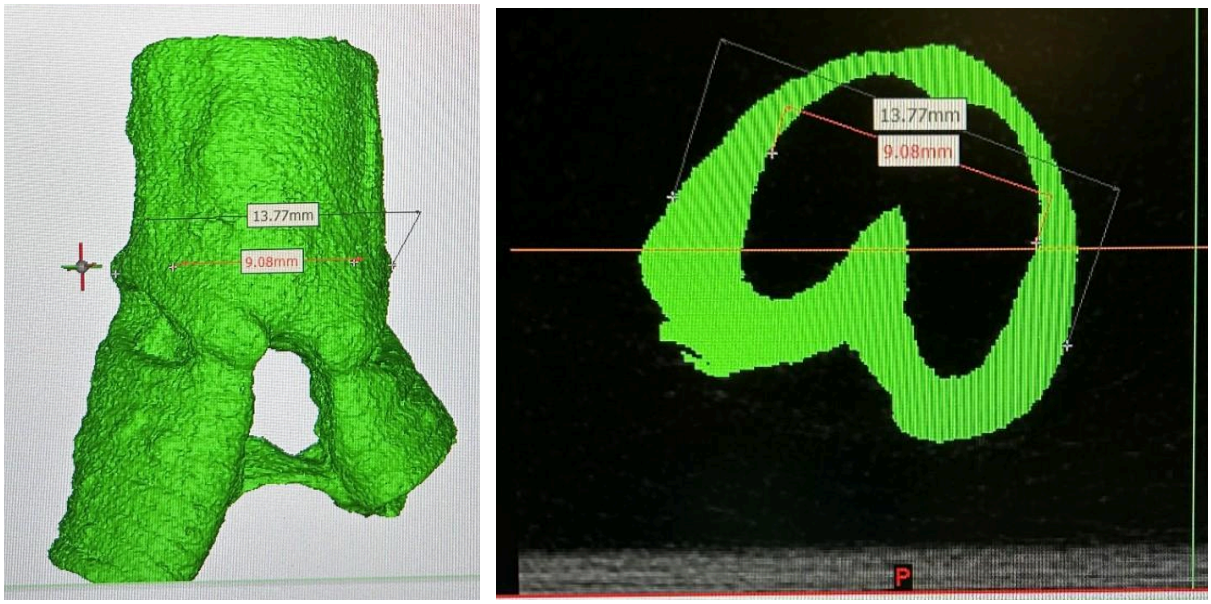

Figure 6: *Measuring the internal and external width of the syrinx. The internal width is shown in red and the external width is shown in green, and the location of the measurement is also shown on the 3D model.*

In addition, the number of complete tracheal and bronchial rings were measured and counted. For the loon syringes, the length of the tracheal and bronchial portions of the samples varied due to the samples being extracted with varying amounts of the tracheal and bronchial elements attached. When measuring the number of complete tracheal rings, up to 6 rings were counted for all samples. For bronchial rings, all incomplete rings were counted. Additionally, the length of the lateral tympaniform membrane (LTM) was measured, which occurs between the second and third bronchial rings. Finally, the last set of measurements included the length of bronchial rings 4 through 6 (Figure 7).

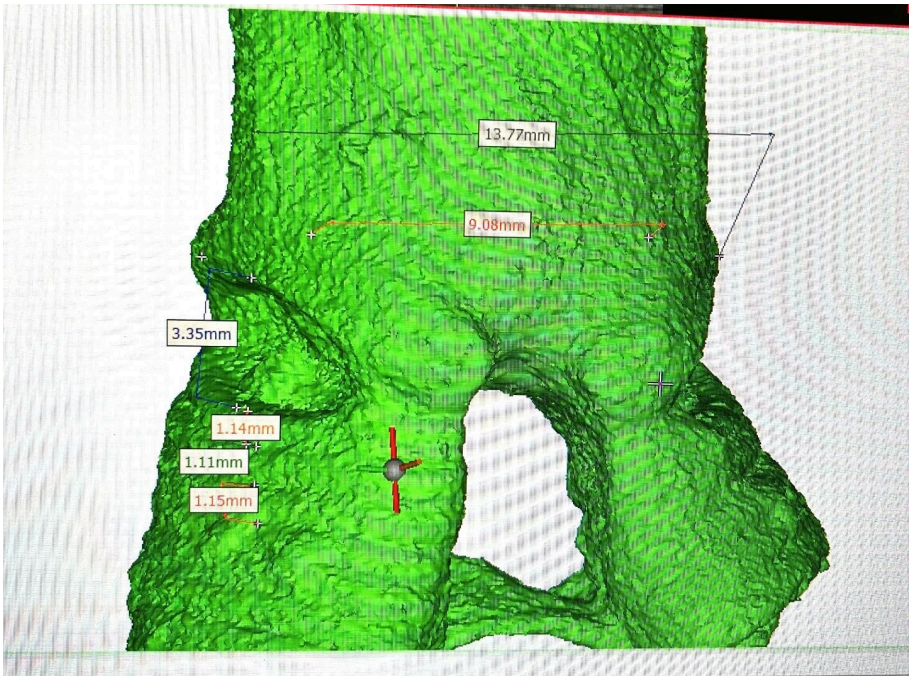

Figure 7: *Measuring the length of the LTM and bronchial rings 4 through 6. The LTM looks like a gap between bronchial rings 2 and 3. Below the LTM, the length of bronchial rings 4 through 6 were measured.*

3.0 - Results and Discussion

3.1 - Type of Syrinx

Due to a lack of research on vocalizations and syrinx anatomy in the Common Loon, there was no clear explanation as to what type of syrinx loons possess. By reviewing background research on the different syrinx types and examining the 3D models that were made to obtain measurements, it was identified that the Common Loon has a tracheobronchial syrinx. A tracheobronchial syrinx has both tracheal and bronchial aspects shared in the formation of the syrinx. As shown in Figure 8, the tracheal elements usually consist of a tympanum, which contains closely attached and ossified cartilage rings, and the bronchial rings are usually C-shaped half rings. A median cartilage, also known as the pessulus, separates the left and right medial tympaniform membranes from each other (Figure 8). Moreover, it was also noted that the medial tympaniform membrane stretches across the open end of the C-shaped bronchial rings (King, 1989). After observing the 3D models (Figure 9), the number of complete cartilage rings was noted. For all samples, all tracheal cartilage rings were complete. In addition, most caudal tracheal cartilage rings appear to be more ossified and closely attached, indicating the presence of a tympanum. Similarly, most observed samples had a number of incomplete C-shaped bronchial rings, therefore indicating the presence of a tracheobronchial syrinx type in the Common Loon.

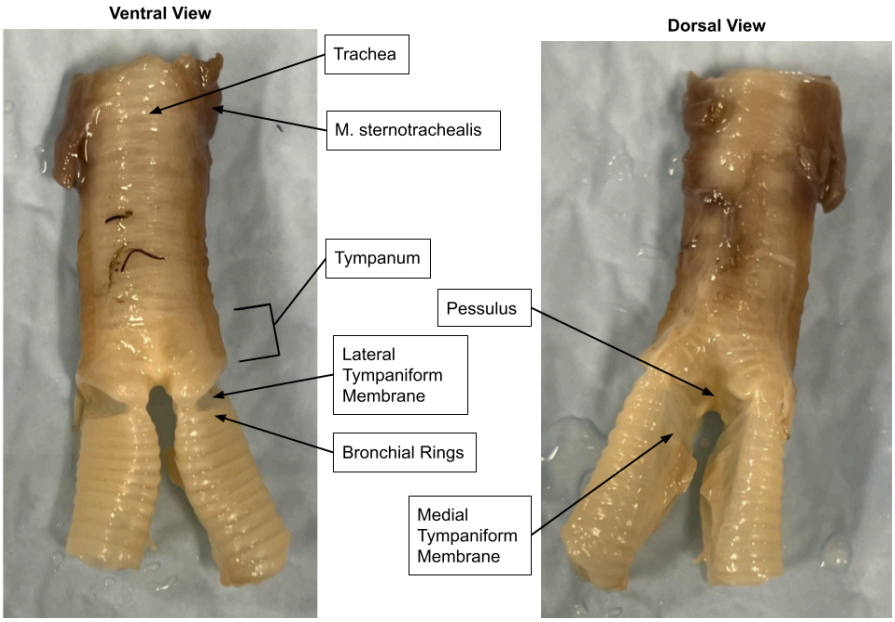

Figure 8: *Labeled ventral (left) and dorsal (right) view of a Common Loon tracheobronchial syrinx*

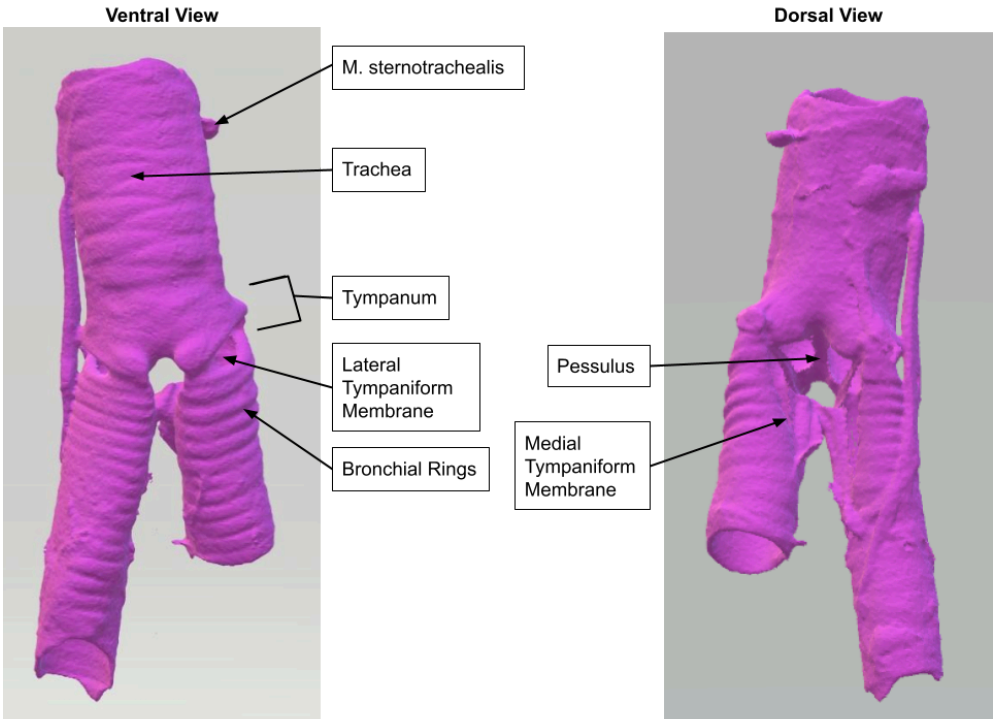

Figure 9: *Ventral (left) and dorsal (right) view of a Common Loon tracheobronchial syrinx displayed as a 3D model*

3.2 - Anatomical Measurements of Loon Syringes

In total, 13 loon syringes were scanned, with 8 male syringes and 5 female syringes (Figure 10). While some of the loon syrinx specimens were confirmed to be from adults, the age of the loon was not known for all samples used in this project. Of those samples, measurements were completed for 4 male syringes and 3 female syringes due to time constraints and issues with a couple of 3D models (Table 2). When scanning, some of the syringes placed on a plastic ziplock bag had accidentally shifted during the scan, making some of the 3D models hard to use for analysis.

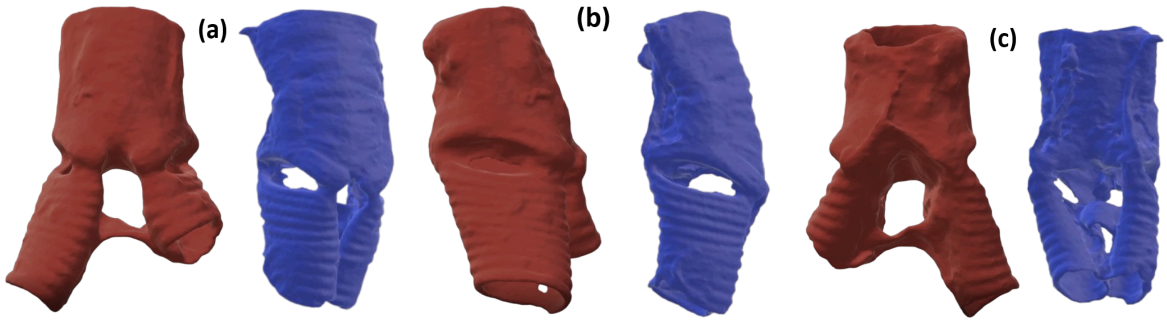

Figure 10: *3D models of female (red) and male (blue) loon syringes. (a) Frontal view, (b) Lateral view, and (c) Ventral view.*

Table 2: *Anatomical measurements of female loon syringes (red, N=3) and male loon syringes (blue, N=4). All measurements are in millimeters.*

Sample ID	Sex	Internal Width	External Width	Length of LTM	B4 Length	B5 Length	B6 Length
TV220102	F	9.95	13.22	2.73	0.88	1.01	1.14
TV230132	F	9.40	13.16	2.78	1.05	1.04	1.04
TV230136	F	9.08	13.77	3.35	1.14	1.11	1.15
TV230130	M	9.09	13.28	3.12	1.03	1.08	1.11
TV230121	M	9.13	13.39	1.70	0.79	0.91	0.99
TV230071	M	9.97	15.41	2.98	1.10	1.19	1.33
TV220101	M	10.07	14.21	1.68	0.83	1.03	1.29

The measurements were used to compare the anatomical features of male and female loon syringes. Overall, males had a larger internal and external width of the syrinx; however, some of the female loons had larger internal and external widths compared to the smaller males. Males had an average internal width of 9.5 mm while females had an average internal width of 9.4 mm (Table 3). Similarly, males had an average external width of 14.0 while females had an average external width of 13.3 mm (Table 3). This difference in internal and external width could be potentially attributed to larger body size, as male loons are generally about 25% larger than female loons (Loon Preservation Committee, 2024).

Table 3: *Averaged measurements for male (N=4) and female (N=3) loons. All measurements are in millimeters.*

	Internal Width	**External Width**	**Length of LTM**	**B4 Length**	**B5 Length**	**B6 Length**
Male Loons (N=4)	9.5 ± 0.52	14.0 ± 0.98	2.3 ± 0.78	0.93 ± 0.15	1.05 ± 0.11	1.18 ± 0.15
Female Loons (N=3)	9.4 ± 0.44	13.3 ± 0.33	2.9 ± 0.34	1.02 ± 0.13	1.05 ± 0.05	1.11 ± 0.06

In contrast, the length of the LTM was found to be larger in females than in males. For females, the average length of the LTM was 2.9 while for males the average was 2.3 (Table 3). The length of the LTM was measured as the syrinx produces sound through vibration of the tympaniform membranes, including the medial and lateral tympaniform membranes (King and McLelland, 1984). While the results indicate a difference in the length of the LTM, more measurements are needed to confirm if this result is significant and to rule out individual variation as the cause. Overall, the length of bronchial rings four through six were similar in size for both female and male loons. For bronchial ring four (B4), the average for male loons was 0.93 compared to 1.02 for female loons. For B5, both male and female loons had the same average of 1.05. Finally, males had a slightly larger B6 bronchial ring with an average of 1.18 compared to 1.11 (Table 3). Based on these results, there appears to be minimal differences between the length of bronchial rings in male and female loon syringes.

4. 0 - Conclusions and Recommendations

The goal of this project was to investigate if there are anatomical differences between the syringes of male and female Common Loons using micro-CT imaging. It was hypothesized that male loons are able to produce yodels due to anatomical differences in the syrinx. In total, 13 loon syringes were scanned, but only four male syringes and three female syringes were measured due to time limitations and issues with some of the models. After examining the 3D models, the Common Loon syrinx was determined to be a tracheobronchial syrinx. All tracheal cartilage rings were complete, and most caudal tracheal rings appeared to be ossified and closely attached, indicating the presence of a tympanum. The majority of bronchial rings were incomplete and had a modified C-shape, as the medial tympaniform membrane stretches across the open end of the C-shape. From the measurements, anatomical differences were observed in the internal length, external length, and length of the LTM. For all measurements, more samples are needed to rule out individual variation as the cause of these differences, as only a small number of samples were measured for males and females. Based on the results, it remains unclear whether male loons are able to produce yodels due to differences in syrinx anatomy.

Although this study provided insights into the general anatomy of the Common Loon syrinx and began the process of investigating anatomical variations in loon syrinx anatomy, more research is needed to fully understand the process of sound production in Common Loons. For a continuation of this project, future studies could focus on increasing the sample size and measuring more loon syringes to rule out individual variation as the cause of variation in the internal length, external length, and length of the LTM. Additionally, variations in the internal and external width of the syrinx could be attributed to body size, and future studies could investigate if the size of the syrinx is correlated with body size. Another area for future research could focus on investigating developmental differences in syrinx anatomy, as only adult male loons yodel (McIntyre, 1988). The results of the micro-CT analysis can also be expanded upon using histology to better understand the structure of tissues present in the syrinx. While micro-CT can be useful to model and measure anatomical structures, histology can provide further insights into the characteristics and type of tissues present in the syrinx. Lastly, future studies can also consider the influence of the surrounding musculature on sound production. Different species of birds have different numbers of syringeal muscles around the syrinx, and the

musculature surrounding the loon syrinx has not been investigated, as well as how the musculature might influence sound production. Hopefully, the work of this project can be expanded upon in future research to shed light on how the Common Loon produces its unique vocalizations.

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