2020-04-08

Aerodynamic Measurement Stability During Rabbit Versus Pig Benchtop Phonation

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

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Combination corticosteroid inhalers are the primary treatment option for long-term pulmonary disorders including asthma, persistent bronchitis, and chronic obstructive pulmonary disease. Common side effects of these medications are xerostomia in the mouth and throat, hoarseness, and soreness in the oropharynx. Research indicates that a large percentage of the inhaler particles are deposited onto laryngeal tissue, leaving an alteration of laryngeal mucosal properties. As the first stage in a long-term project, this thesis addresses the need for baseline phonatory data that will lay groundwork for quantifying inhaler-induced phonatory changes. Excised larynx research is a powerful tool for assessing aerodynamic alterations that accompany laryngeal pathology. Porcine (pig) larynges are a traditional species employed in voice disorder research, though leporine (rabbit) larynges are an emerging species that lends itself to histologic vocal fold studies as they have the most similar vocal fold cover structure to humans compared to any other animal to date. The purpose of this study was to examine the measurement stability of six aerodynamic parameters in a traditional excised larynx benchtop model. Specifically, the current author assessed measurement stability of leporine larynges compared to porcine larynges with the following aerodynamic metrics: phonation onset pressure (PTP; cmH_2O), phonation onset flow (PTF; L/m), sustained pressure (cmH_2O), sustained flow (L/m), onset laryngeal resistance (cmH_2O/L/m), and sustained laryngeal resistance (cmH_2O/L/m). A total of 30 larynges—15 leporine and 15 porcine—were mounted on a benchtop setup; phonation was sampled over 15 trials for each larynx. Measurement stability for the above six tokens was examined using coefficient of variation (%) analyses. Leporine larynges demonstrated significantly less variation across all six aerodynamic parameters when compared to porcine larynges. The leporine PTP values were most stable as compared to leporine and porcine pressure and airflow values. Leporine airflow values were also more stable than porcine PTP and PTF values. These results indicate that leporine larynges might be a preferred excised larynx specimen for certain benchtop phonation studies. These findings are important for establishing expected measurement variability in porcine and leporine larynges, particularly when translating benchtop research to laryngeal pathology.

Keywords: phonation pressure, phonation flow, laryngeal resistance, leporine, porcine, benchtop model, excised larynx

ACKNOWLEDGMENTS

Primarily, I thank Dr. Kristine Tanner for her devoted support and inspiring mentorship throughout my research experience in her lab and my academic journey in her courses. I appreciate the time she has sacrificed to support my success within the data collection, data analysis, and thesis writing stages of this project. I additionally thank my comanager of our lab, Amber Prigmore, who became a dear friend as we worked many long hours together establishing protocols, troubleshooting technology, collecting and analyzing data, researching advanced lab supplies, and training new lab members. I thank Dr. Christopher Dromey and Dr. Scott Thomson for their professional insight and generous time sacrificed to help perfect lab setup and protocol establishment. I thank Miriam Bake, Heidi Robison, and Christina Weist who aided Amber and me in data collection. I thank my patient husband, Trevor, who has continually encouraged and supported me throughout my entire graduate school experience. I thank my parents and family for their motivation and interest in my research, as well as their examples of diligence and perseverance. Additionally, I thank Circle V Meats, located in Spanish Fork, Utah, for their continuous pig larynx donations, and the National Institute of Health for their generous funding which enabled this important research to take place. Finally, I acknowledge God's enabling and guiding hand in my life during all stages of this thesis and my graduate work.

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DESCRIPTION OF THESIS STRUCTURE AND CONTENT

This thesis entitled *Aerodynamic Measurement Stability During Rabbit Versus Pig Benchtop Phonation* received financial support from the David O. McKay School of Education at Brigham Young University and the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (1R01DC016269-01A1). These grants were awarded to support a five-year project with Dr. Kristine Tanner as principal investigator. This thesis was part of the larger project being conducted in Dr. Tanner's laboratory. It is written in a journal-style format including a corresponding annotated bibliography in Appendix A. Appendix B includes dissection and tissue preparation protocols. Appendix C contains detailed descriptions of data acquisition. Appendix D includes images of tracheal adapters for rabbit benchtop work. Appendix E contains protocols for calibrating air pressure and flow measurements. Appendix F consists of information regarding trial and error methods for eliciting phonation in pig and rabbit larynges. In Appendix G, information about acquiring and training LabChart™ software is documented. Finally, Appendix H contains a thesis timeline expanding from August of 2018 through February 2020. Data from this thesis were part of a poster presented in Orlando, Florida on November 22, 2019 at the annual convention of the American Speech-Language-Hearing Association. The data will also be included in a peer-reviewed journal publication based on the larger project, with the thesis author contributing as one of several multidisciplinary authors.

Introduction

Voice disorders exist in approximately 6% of the population in the Unites States at any given time (Roy et al., 2004). They can occur at any age across the lifespan, although certain disorders are associated more frequently with particular age ranges, such as neurological vocal tremor in the geriatric population or congenital vocal webbing found in children. It has also been suggested that voice disorders are underidentified and undertreated, particularly in specific groups such as children and those over age 65 (Roy, Stemple, Merrill, & Thomas, 2007). Certain patient populations are also underserved, such as those with reduced physical or financial access to health care, as well as specific disease categories, such as those with autoimmune disorders (Sanz et al., 2012; Tanner et al., 2015). People with asthma who use inhalers are another population that are prone to voice disorders but are not always referred to multidisciplinary clinics for assessment and management (Erickson & Sivasankar, 2010; Hassen & Hasseba, 2016).

This fact can prove problematic as voice changes associated with inhalers can be irreversible and little is known about the mechanics of how precisely these drugs affect the voice. The little research in this area is seemingly equivocal as some researchers conclude that the voice should not be affected by inhaler medications (Barnes, 2001), while more recent studies find that up to 58% of the population who use inhalers experience voice disorders (Sahrawat, Robb, Kirk, & Lutz, 2014; Torre & Barlow, 2009). Voice disorders have significant negative effects on communication, including limitations on activities of daily living, occupational performance, and social and emotional health (Roy, Merrill, Gray, & Smith, 2005). Therefore, population-specific research is needed to treat and prevent voice disorders related to these illnesses, injuries, or

disease processes. Related to the current thesis, research is needed to address voice disorders in people with asthma who experience inhaler-induced voice disorders.

One of the challenges in patient-based voice research is the inherent complexity of speech and voice production. Expressive communication is often divided into physiologic and psycholinguistic subsystems, such as respiration, phonation, resonance, articulation, speech-motor control, language, and cognition, to name a few (Hoit & Weismer, 2018; Seikel, Drumright, & King, 2010). Phonation occurs within the context of these other speech subsystems and varies due to a number of subsystem interactions. For example, public school teachers are the highest occupational risk group for developing voice problems, with a 60% lifetime prevalence of at least one voice-related work absence (Roy et al., 2004). But so many factors influence the development and perpetuation of voice disorders in teachers, including voice demands, voice dosage, vocal loudness, environmental noise, and laryngeal health factors specific to the individual. Manipulating only one of these factors is nearly impossible without influencing others. Similarly, in patients with asthma, it is very difficult to examine cause and effect relationships when one cannot, for example, withhold inhaler treatment from a control group. As a result, alternative research designs and models have been developed to examine experimental questions that may then be translated to human populations.

Translational Nonhuman Research Models

To investigate cause and effect variables related to voice disorder onset and treatment, a variety of nonhuman experimental methodologies have been employed. Generally, these include in vivo animal, ex vivo, and in vitro studies. In vivo animal models may include those wherein the animal is exposed to an independent variable, which might be studied later using in vivo or ex vivo methodologies; for example, an animal could receive a previously determined dose of a

treatment, which could later be studied through videostroboscopy or tissue analysis. It is also quite common to obtain tissue from animal vocal folds and surrounding structures to be compared with the respective human tissues (Mau, Muhlestein, Callahan, Weinheimer, & Chan, 2011). Various mechanical models exist in voice research, such as synthetic self-oscillating (Thomson, Mongeau, & Frankel, 2005) or two-layer physical vocal fold models (Zhang, 2009) that have been designed to test different physical properties of the vocal folds, such as effects of epithelium thickness (Titze, Schmidt, & Titze, 1995) or flow-structure interactions (Becker et al., 2009). Ultimately, these lay the foundation for other types of advanced studies involving computational modeling and voice simulation (Bailly et al., 2018; Murray, Thomson, & Smith, 2014). The use of a mechanical model provides the benefit of more standardized data collection methodologies and is important to consider in research design. Additional work has been done to better standardize data collection methods when using excised animal larynges (Alipour & Jaiswal, 2008; Birk et al., 2017), as this type of model adds an additional benefit of utilizing biological tissue.

Among the continuum of nonhuman voice research models, excised larynx studies have emerged as a gold standard for examining specific independent variables while minimizing covariates that exist in vivo. Specifically, excised larynx benchtop experiments involving simulated exhalation to vibrate the vocal folds are commonly used to study voice function. The use of an excised larynx benchtop apparatus allows for researchers to control for variables such as elongation or adduction of the vocal folds, which cannot be controlled using an in vivo animal model, as animals cannot follow directions or maintain established parameters. Benchtop studies are particularly common in voice research involving aerodynamic and environmental (e.g., external hydration) variables (Khosla, Murugappan, Lakhamraju, & Gutmark, 2008; Stevens,

2017; Witt et al., 2009). Likewise, some of the most common outcome measures involve aerodynamic parameters of voice production.

Phonation threshold pressure (PTP, cmH_2O) is the minimum amount of air pressure needed to initiate and sustain vibration of the vocal folds (Titze, 1992). Since the early 1990s, PTP has been widely scrutinized in both theoretical and methodological research. The PTP values appear to provide direct insight on the health of the vocal fold mucosa, including vocal fold viscosity, velocity of the mucosal wave, prephonatory glottal width, and vocal fold thickness (Plexico, Sandage, & Faver, 2011; Titze, 1988). PTP is one of the most commonly used aerodynamic outcome measures in voice research (Plexico et al., 2011), although direct measurement of PTP in the clinical setting is impractical because it would require tracheal puncture. A protocol for making indirect estimates of PTP from oral air pressure during /p/ occlusion has been established as valid. The PTP values are used to assess voice rehabilitation clinically by upwards of 44% of speech-language pathologists who specialize in voice (Plexico et al., 2011).

Phonation threshold flow (PTF, L/min) is a separate but corresponding aerodynamic value defined as the observed airflow rate at the onset of phonation, or the minimum glottal airflow required to initiate phonation (Jiang & Tao, 2007). This parameter appears to be equally sensitive, if not more sensitive, to vocal fold mucosa health. Hottinger, Tao, and Jiang (2007) compared the sensitivity of PTP and PTF for varying prephonatory glottal widths. They found that PTF was significantly more sensitive than PTP to posterior glottal width changes on an excised benchtop model. Using a one-mass model, Tao and Jiang (2008) further mathematically confirmed that PTF should be more sensitive than PTP in abducted vocal fold pathologies because of the pressure recovery experienced by PTF at the glottal exit. Further, PTF appears to

be more reliably measured as it can be quantified extraorally by use of a pneumotachograph mask, rather than estimated with PTP acquisition. This additional parameter is relatively new; its research and clinical viability is promising, although it awaits further scrutiny prior to being accepted as a standard for voice assessment.

Laryngeal resistance is a separate aerodynamic measurement that describes the relationship between pressure and flow within the boundaries of the larynx. Unlike onset and sustained pressure and flow, it is not a direct measurement acquired during phonation, but is rather a calculated quotient of subglottic pressure divided by translaryngeal airflow (Huth, Scholp, & Jiang, 2020; Plant & Hillel, 1998). This measurement presumably provides quantifiable data about vocal effort and adds to the information initially provided by individual PTF and PTP data about mechanical functioning of holistic laryngeal tissue (Rieves, Hoffman, & Jiang, 2009). Smitheran and Hixon (1981) were among the first to calculate onset and sustained laryngeal resistance clinically. They argued that pressure and flow are correlated and maintain a constant ratio in healthy vocal functioning. Controlling for sex, Netsell, Lotz, Duchane, and Barlow (1991) found that female larynges have a higher laryngeal resistance when compared to males, likely because of their geometrically smaller larynges and airways. A smaller laryngeal diameter, influenced by geometry and degree of adduction of the vocal folds, lead to increased resistance values (Scherer & Guo, 1990; Titze, 2000; Wang & Huang, 2005). Other factors such as sharpness and shape of glottal entry and exit and speed of air particles affect the laryngeal resistance (Berke, Moore, Monkewitz, Hanson, & Gerratt, 1989; Huth et al., 2020).

Other outcome measures such as those derived from high-speed video imaging have been valuable in quantifying phonatory changes in excised larynx studies (Döllinger et al., 2011; Maytag et al., 2013). These studies have led to insights into voice features such as vocal health,

vocal effort, and vocal efficiency, among others (Oren, Khosla, Dembinski, Ying, & Gutmark, 2015; Witt et al., 2009).

Within the scope of excised larynx studies, a variety of animals have been examined, including canine, sheep, pig, cow, deer, lion, tiger, and rabbit species (Alipour & Jaiswal, 2008; Jiang, Raviv, & Hanson, 2001; Klemuk, Riede, Walsh, & Titze, 2011; Maytag et al., 2013). The laryngeal mechanism in each species has a unique geometry, vibratory pattern, recruitment of extralaryngeal structures, or histology that may provide further insight about human vocal folds. Although lion and tiger larynges provide insight into the relationship between vocal fold morphology and function, feasibility purposes dismiss these species from being studied extensively in research (Klemuk et al., 2011). Conversely, the white-tailed deer is more ubiquitous and accessible in the United States and has a similar sized larynx when compared to that of a human. The deer larynx, however, has a much more limited range of mobility at the cricothyroid joint, negatively influencing its potential for pitch control (Jiang et al., 2001). The sheep larynx has been found to have laryngeal muscles that compare well to human musculature in fiber type, while maintaining soft and malleable vocal folds. Similar to the deer larynges however, sheep larynges have a small cricothyroid rotation angle, resulting in a smaller fundamental frequency (F_0) range (Alipour & Jaiswal, 2008).

In cows, the vocal folds are triple the thickness of sheep folds and are also longer and stiffer. Neither the sheep nor the cow vocal folds have a distinct ventricle or distinction between the vocal folds and surrounding tissue, making them less similar structurally to a human larynx; however, both have large mucosal waves that have a visible horizontal and vertical movement pattern. The canine larynx has historically been the most common model because of their geometrical similarities to the human larynx and similar oscillation pattern. But limitations of

the canine larynx in benchtop studies are both scientific and ethical; canines have a poorly defined vocal ligament and are increasingly difficult to secure for research purposes since they are considered domestic animals (Jiang et al., 2001; Maytag et al., 2013).

In recent years, the pig, or porcine larynx has gained popularity in research labs because of its similar F_0 range to the human larynx. The pig larynx additionally has a vocal fold thickness more comparable to the human larynx than any animal model previously discussed and also has a distinct ventricle differentiating the true and false folds. Larynges are often easier to obtain from pigs than other previously mentioned animals, depending on researchers' proximity to a butcher shop. Pigs, however, can prove challenging for treatment or pre-post research designs due to size and housing considerations. Traditionally, pig larynges are obtained from a butcher shop; this limitation is similar to canines, whose larynges are usually retrieved from an animal involved in nonvoice or combined area research.

Rabbit, or leporine larynges, are relatively new in the voice literature and may present some solutions to the challenges associated with the abovementioned canine and pig models. Rabbit larynges are more histologically similar to human larynges than other animal models, thus providing more insight to human immunological responses to tissue change (Maytag et al., 2013; Mills, Dodd, Ablavsky, Devine, & Jiang, 2016). Because of these histological similarities, studies involving vocal fold epithelial changes such as edema and erythema might be ideal for the rabbit model. Additionally, smaller animals are more appropriate for longitudinal or pre-post experimental designs, making rabbit larynges a viable option for translational benchtop and tissue studies. Larger groups of rabbits can be housed and maintained at a time because of the small size and relatively low living cost, particularly when compared to pigs. Rabbits have shown particular potential in high-speed in vivo experiments as researchers have explored the

resilience of the rabbits' epithelial layer of the vocal folds in recovering following episodes of phonotrauma (Rousseau et al., 2017). Methodological studies have adapted the traditional benchtop apparatus to accommodate the small size of the rabbit larynx (Maytag et al., 2013; Mills et al., 2016) and indicate that rabbit phonatory parameters may be quantified reliably using both aerodynamic and acoustic measures. Despite the developing literature base for rabbit larynx studies, further data need to be acquired in order to better understand how rabbit excised larynx phonation compares with other traditional animal models.

Context for the Current Study

This thesis is an initial study of porcine phonation as compared to leporine phonation. It is part of a larger project being conducted in Dr. Kristine Tanner's laboratory related to the adverse effects of inhaled corticosteroids on vocal fold tissue and its phonatory consequences. Over 10% of people in the United States suffer from chronic pulmonary diseases, such as asthma, chronic bronchitis, and chronic obstructive pulmonary disease for which inhaled corticosteroids are a common treatment approach. These inhalers typically fall under two classifications: preventive medications and acute quick-relief medications. Long-term preventive medications, such as inhaled corticosteroids, are intended to be taken in small daily doses in order to decrease swelling in the bronchioles. Although the inhaled corticosteroids are designed to reduce inflammation at the bronchioles, previous research has indicated that large amounts of inhaled treatments are deposited onto the larynx due to drug particle size, delivery device, spacer use, and administration technique (Erickson & Sivasankar, 2010; Perkins et al., 2017; Rau, 2005). Additional research has revealed an alteration of laryngeal mucosal properties in 50% of patients, and supraglottic hyperfunction in more than 40% of patients (Lavy, Wood, Rubin, & Harris, 2000). This thesis addresses the need for baseline phonatory data that will lay

groundwork for quantifying inhaler-induced phonatory changes in the larger project during the next several years.

Statement of Purpose

Because human studies involve a number of research design complexities such as voice use covariates, animal models offer a strong option for translational studies of inhaler-related voice problems. Excised larynx studies permit certain types of experimental controls, including individual manipulation of independent variables and untreated control groups. Therefore, excised larynx benchtop studies are often used to examine mechanical vibratory features that ultimately inform human voice treatment and prevention studies. The purpose of the current thesis is to collect and examine phonatory data from healthy, normal porcine and leporine excised larynges, which will serve as a normative data for future comparisons with inhaler-induced voice changes in the larger asthma project.

Research Questions

This study was undertaken to answer the following research questions:

1. How stable are PTP, PTF, and phonation onset laryngeal resistance measurements during repeated pig versus rabbit benchtop phonation trials?
2. How stable are sustained pressure, sustained airflow, and sustained laryngeal resistance measurements during repeated pig versus rabbit benchtop phonation trials?

Method

All methods pertaining to the current study were carefully structured to fit the guidelines prescribed by Risk Management and the Institutional Animal Care and Use Committee at Brigham Young University. The animal larynges used in this thesis were sacrificed for purposes unrelated to this study. For this study, excised rabbit larynges were obtained from the University of Utah and excised pig larynges were donated by a local butcher shop (Circle V Meats, Spanish Fork, UT). All of the following procedures were completed on the campus of Brigham Young University. Dissection, storage, and data collection were performed in the John Taylor Building Annex (rooms 105 and 106), while preservation techniques were performed both in the John Taylor Building Annex (rooms 105 and 106) and in the Joseph K. Nicholes Building (room 126, Chemistry Central Stockroom).

Research Design

The design of the present study was a prospective between-groups design. The two groups involved in the study were determined by animal-type. The independent variable was group, including an experimental cohort of 15 white rabbit (leporine) larynges and a control cohort of 15 pig (porcine) larynges. Each rabbit larynx was from an adult rabbit approximately 4 to 6 months of age. For the pig larynges, animals were food-grade and less than 2 years old. The dependent variables for this study were onset and sustained pressure in cmH_2O, onset and sustained flow in L/m, and onset and sustained laryngeal resistance in cmH_2O/L/m.

Larynges

Rabbit (Leporine) larynges. The 15 leporine larynges were extracted immediately upon animal sacrifice by investigators at the University of Utah. A vertical incision was made along the anterior portion of the neck to expose the larynx and trachea. Metal hemostats were used to

maintain neck opening while the neck musculature was dissected using a fine scalpel. Once the neck musculature was sufficiently open, the superior border of the thyroid cartilage and inferior border of the trachea were exposed. The thyroid cartilage, including the epiglottis, arytenoid cartilages, false vocal folds, and the full length of the trachea were extracted. The laryngeal and tracheal tissue were placed in small vertical plastic containers filled with phosphate-buffered solution (PBS). These containers were placed in a tray of isopropyl alcohol to prevent ice crystal formation during the flash freezing process. The larynges were then flash frozen in liquid nitrogen and stored in a -80-degree Celsius freezer at the University of Utah. Subsequently, these larynges were transported to Brigham Young University in an ice-filled cooler to the -80 °C freezer prior to data collection and stored in a ThermoScientific freezer in room 105 of the John Taylor Building Annex. Larynges were thawed in a lukewarm water bath immediately before each data collection session.

Pig (Porcine) larynges. The 15 porcine larynges were obtained from a local butcher shop within six hours after sacrifice. The butcher was instructed to preserve the cartilage of the larynx, including the epiglottis, as well as the majority of the trachea. When the larynges were retrieved, they were immediately driven back to the lab and underwent a gross dissection, depicted in Figure 1. This process entailed removing all extrinsic muscles of the larynx, the esophagus, and other external tissue using a disposable gross dissection scalpel. The larynges were additionally qualitatively inspected and any larynx that appeared to have superior damage to the trachea or cartilage perforations from the butcher shop process were discarded. After this stage of dissection, each larynx was placed in its own cylindrical container of freezer bag and immersed in PBS solution. Within an hour of gross dissection, these larynges were transported to the Chemistry Central Stockroom and flash frozen in liquid nitrogen, as depicted in Figure 2.

Larynges were stored in a ThermoScientific -80 degrees Celsius freezer and thawed in a warm water bath before any remaining fine dissection and data collection.

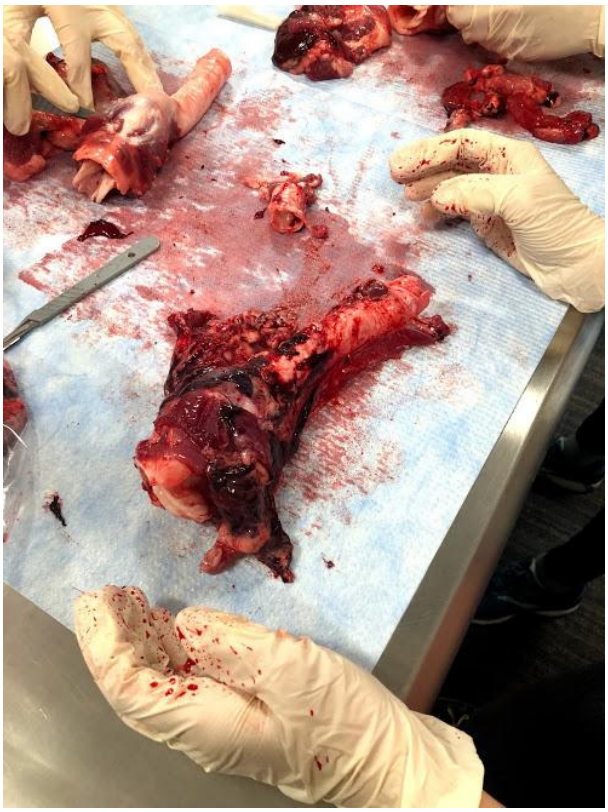

Figure 1. Gross dissection of pig larynges.

Figure 2. Flash freezing of pig larynges.

Operational Procedures

Phonatory bench model. The benchtop apparatus experimental setup that was used for this study was based on the design established by Jiang and Titze (1993). Similar to the setup prescribed by Jiang and Titze, in the current study a mechanical model of the larynx was studied on a benchtop apparatus as the larynges were mounted vertically on plastic tubing. The plastic tubing protruded through a space in the benchtop and was suspended via benchtop pins, and strings as shown in Figure 3 (Model 1460, Kopf Industries, Tujunga, CA). Beneath the bench, the tubing connected to a 20 cm aluminum foam-insulated custom pseudolung, which was used to reduce reverberation, as shown in Figure 4. Before the pseudolung, the air was humidified by

a TheraHeat heated humidifier, shown in Figure 5. (Model RC70000, Smiths Medical, Dublin, OH).

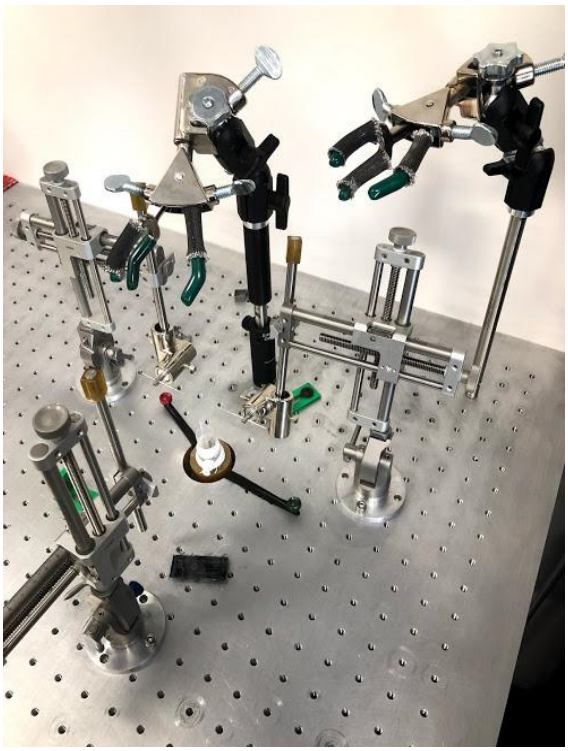

Figure 3. Vertical tubing and micropositioners in rabbit setup.

Figure 4. Pseudolung with suspension.

The airflow source originated from a medical-grade air tank containing compressed, low-humidity air (50 psi, <1% relative humidity). These tanks were securely chained to the laboratory wall with pressure regulators per Joint Commission on Accreditation of Healthcare Organizations and the Occupational Safety and Health Administration standards. Along the tubing connecting the air tanks and humidifier was an interchangeable respiratory flow head (Model MLT300L, AD Instruments, Sydney, Australia), shown in Figure 5. The flow head was zeroed and calibrated before each set of data collection for purposes of airflow sampling.

Figure 5. Benchtop setup, humidifier, flow meter, air tanks, and computer system.

On the superior surface of the benchtop, the larynges were mounted on top of the previously described vertical tubing. To prevent any air leakage subtracheally, two methods were used. For smaller rabbit larynges, Teflon™ tape and cable ties secured the larynges to a custom-made piece which adjusted the diameter of the larger tubing to the significantly smaller rabbit-sized trachea, seen in Figure 6. For larger pig larynges, a circular hose clamp was tightened with a screwdriver, seen in Figure 7. A subtracheal outlet was drilled into the PVC tube where a physiological pressure transducer (Model MLT844, AD Instruments, Sydney, Australia) was attached perpendicular to the upward vertical flow. This pressure transducer was additionally zeroed and calibrated before each set of data collection and reported PTP

aerodynamic values to the LabChart™ data acquisition system version 8 (PowerLab™, AD Instruments, Colorado Springs, CO).

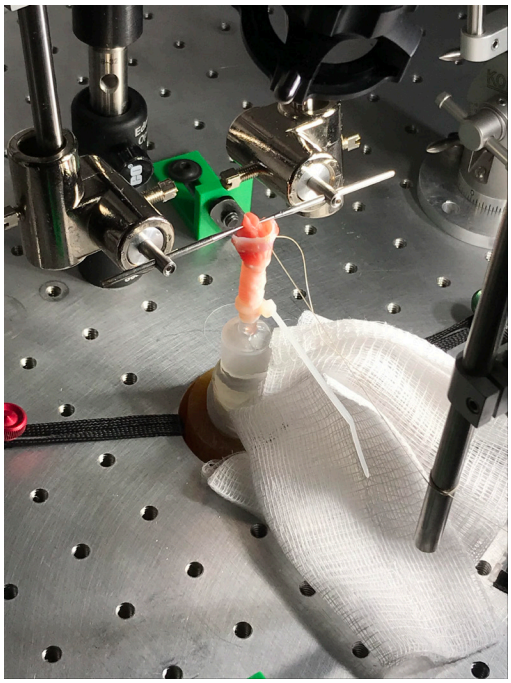

Figure 6. Rabbit larynx, mounted.

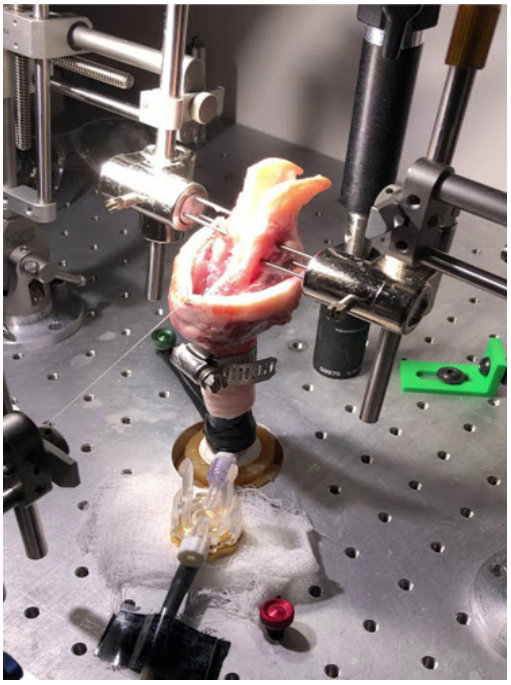

Figure 7. Pig larynx, mounted.

The micropositioners were secured into the benchtop with ¼-20 headless screws via custom bases. Two of the micropositioners were placed laterally to the larynx mount. These micropositioners additionally had interchangeable prong attachments: for the rabbit larynges, the attachment contained only one prong, see Figure 6; for the pig larynges, the attachment contained three prongs, see Figure 7. These micropositioners were adjustable and were manually situated at the level of the arytenoid cartilage of each larynx. These prongs gently pierced the lateral surface of the arytenoid cartilage, which applied sufficient pressure to adduct the vocal folds. The third micropositioner was located near the anterior commissure to allow for further stabilization of the larynx as the suture string could be tied to and secured by the micropositioner.

Phonatory trials. After successfully securing and mounting the larynx to the benchtop apparatus, each larynx underwent 15 phonatory trials. Compressed air was released from the air tank, measured by the flow meter, passed through the humidifier and pseudolung, and was measured by the pressure transducer before reaching the level of the vocal folds. In order to elicit phonation, the laterally-placed micropositioners were tightened until the vocal folds began to phonate. Once phonation was initiated, this degree of adduction remained constant for the remainder of the 15 phonatory trials for the given larynx. Once sustained phonation was achieved, air supply was stopped until the next trial.

Data acquisition, segmentation, and analysis. Data were collected for the calibrated acoustic signal, air pressure, and airflow signals. These three signals were direct inputs into the PowerLab™ data acquisition software (AD Instruments, Sydney, Australia) and were subsequently displayed on the LabChart™ data analysis software, version 8 (AD Instruments, Sydney, Australia). In order to acquire acoustic data, a dynamic microphone (Model SM-48, Shure, Niles, IL) was placed superior to the larynx, approximately 6 inches above the vocal folds, as depicted in Figure 8.

All phonation onset trials were marked with preset comments in LabChart™. Per previously established methodology (Stevens, 2017), the 10 ms before and after phonation onset, was averaged for pressure and flow examination and PTP quantification. Phonation onset was determined through analyzation of repeating cyclic oscillations in the acoustic signal, which is depicted in Figure 9 in red. Postprocessing analyses were performed using a custom Matlab program (MathWorks, Natick, MA) written by Dr. Christopher Dromey. Fundamental

frequencies for each phonatory trial were analyzed using Praat software, version 6.0.49 (Boersma & Weenink, 2020).

Figure 8. Mounting of pig larynx, with microphone placed superiorly.

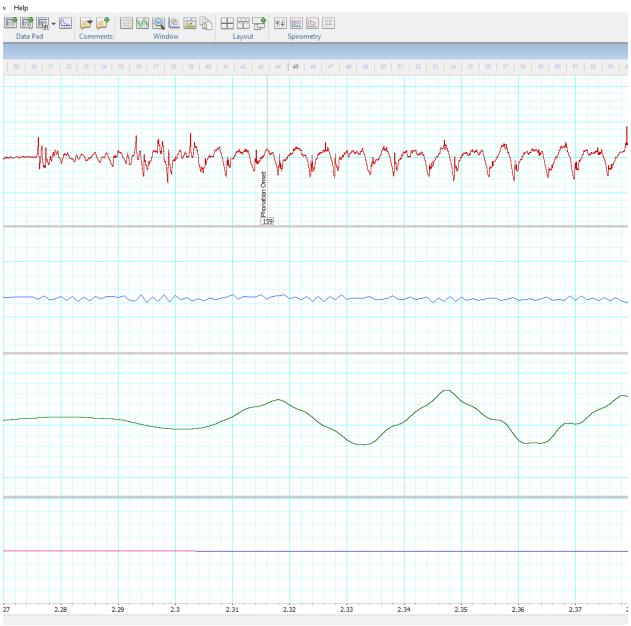

Figure 9. Sample of acoustic (Volts), pressure (mmHg), and flow (L/m) signal at phonation onset. Pressure units were later converted to cmH_2O.

Statistical analysis. For the pig and rabbit groups, summary statistics were generated, including mean, standard deviation, median and range. Intrajudge and interjudge reliability were estimated using 15% repeated measurement by the same examiner and another examiner, respectively. To examine measurement stability, the coefficient of variation was calculated for the 15 repeated phonation trials for each larynx in the rabbit and pig group. Variation was examined for six metrics: PTP, PTF, onset laryngeal resistance, sustained pressure, sustained airflow, and sustained laryngeal resistance. The sustained values were based on the data analysis procedure above using the midpoint of each individual phonation trial. Statistical procedures were performed using SPSS, version 23 (IBM Corp., Armonk, NY) and Microsoft Excel, version 16.33 (2019; Microsoft Corp., Redmond, WA).

Results

Pearson Product Moment Correlations were calculated to estimate both interjudge and intrajudge reliability for token identification and segmentation prior to the automated Matlab analysis. Interjudge reliability was calculated as .99 and intrajudge reliability was .99. These correlations indicated acceptable levels of reliability for purposes of the present study. During pig phonatory trials, the average relative humidity in the laboratory was 32.8% ± 5.6% while the average temperature was 74.1 °F ± 1.04 °F. During rabbit phonatory trials, the average relative humidity in the laboratory was 51% ± 6.3% while the average temperature was 73.03 °F ± 0.89 °F. Minor fluctuations in relative humidity and temperature were observed between groups.

Table 1 contains descriptive group data for the pig group, including group mean, standard deviation (SD), median, minimum, maximum, and range; corresponding rabbit group data are in Table 2. Tokens included onset pressure (i.e., PTP), sustained pressure taken at the midpoint of phonation, onset airflow (i.e., PTF), sustained airflow at the midpoint of phonation, onset laryngeal resistance (i.e., PTP/PTF) and sustained laryngeal resistance (i.e., sustained pressure/sustained airflow).

Table 1

Pig Group Descriptive Statistics (n = 15 larynges)

Statistic	Onset Pressure (cmH_2O)	Sustained Pressure (cmH_2O)	Onset Airflow (L/m)	Sustained Airflow (L/m)	Onset Resistance (cmH_2O/L/m)	Sustained Resistance (cmH_2O/L/m)
Mean	19.98	28.28	0.27	0.43	100.74	83.06
SD	13.06	16.51	0.12	0.22	58.46	47.83
Median	17.84	22.59	0.30	0.42	95.31	77.88
Minimum	5.76	6.92	0.07	0.12	15.12	14.01
Maximum	60.59	68.43	0.43	0.79	193.04	185.99
Range	54.83	61.51	0.36	0.67	177.92	171.98

Note. SD = group standard deviation.

Table 2

Rabbit Group Descriptive Statistics (n = 15 larynges)

Statistic	Onset Pressure (cmH_2O)	Sustained Pressure (cmH_2O)	Onset Airflow (L/m)	Sustained Airflow (L/m)	Onset Resistance (cmH_2O/L/m)	Sustained Resistance (cmH_2O/L/m)
Mean	8.70	12.62	0.08	0.09	135.94	142.63
SD	2.64	5.72	0.04	0.04	47.03	44.52
Median	8.52	11.66	0.07	0.08	115.99	128.06
Minimum	5.20	6.64	0.03	0.05	64.35	74.96
Maximum	15.16	28.70	0.14	0.19	238.27	245.51
Range	9.96	22.06	0.11	0.14	173.92	170.55

Note. SD = group standard deviation.

Table 3 includes individual anatomical measurements for the rabbit and pig tracheas. Individual anatomical measurements for rabbit and pig thyroid cartilages are provided in Table 4. Table 5 includes individual vocal fold anatomical data for both groups. Lastly, Table 6 provides the average aerodynamic data from 15 phonation trials for each rabbit and pig larynx.

Table 3

Vocal Folds Anatomical Size and Dimensions

Group	Session Date	Length (mm)	Width (mm)	Width from Vocal Folds to Thyroid Cartilage (mm)
Pig				
Pig 01	07/16/19	26.7	2.5	12.9
Pig 02	07/17/19	22.7	3.1	12.9
Pig 03	07/17/19	19.6	2.2	13.8
Pig 04	07/17/19	19.6	2.1	13.9
Pig 05	07/17/19	21.5	2.3	12.0
Pig 06	07/18/19	18.1	2.7	11.1
Pig 07	07/18/19	21.5	2.4	10.7
Pig 08	07/18/19	21.5	2.3	10.7
Pig 09	07/18/19	25.6	2.8	12.6
Pig 10	07/19/19	17.5	2.0	15.5
Pig 11	07/19/19	24.0	2.0	13.4
Pig 12	07/19/19	17.1	2.5	11.9
Pig 13	07/19/19	17.3	2.1	10.5
Pig 14	07/25/19	20.7	1.9	11.9
Pig 15	07/25/19	16.5	1.8	13.3
Rabbit				
Rabbit 01	07/26/19	5.5	0.9	2.8

Rabbit 02	07/29/19	7.5	1.4	3.9
Rabbit 03	07/29/19	4.3	1.0	2.2
Rabbit 04	07/29/19	4.6	2.0	3.0
Rabbit 05	07/31/19	7.1	1.9	4.0
Rabbit 06	07/31/19	5.6	1.9	4.6
Rabbit 07	07/31/19	6.1	2.0	4.0
Rabbit 08	07/31/19	4.8	1.8	3.5
Rabbit 09	08/01/19	5.4	2.5	4.0
Rabbit 10	08/01/19	5.8	2.7	4.7
Rabbit 11	08/01/19	5.8	2.4	4.6
Rabbit 12	08/01/19	6.2	1.9	3.6
Rabbit 13	08/01/19	4.2	1.2	3.8
Rabbit 14	08/01/19	4.4	1.8	3.5
Rabbit 15	08/01/19	6.7	2.3	4.4

A digital caliper was utilized to calculate measurements for Table 3. To measure the vocal fold length, the caliper measured the vocal fold attachment at the anterior commissure to the attachment at the vocal process of the arytenoid cartilage. For vocal fold width, the caliper measured the lateral edges of the vocal folds when adducted. The final measurement was computed as the caliper expanded from the medial edge of a vocal fold at the depth of the vocal process, across the thyroarytenoid muscle, to the medial surface of the thyroid cartilage.

Table 4

Thyroid Cartilage Anatomical Dimensions

Group	Session Date	Height (Protuberance to Top; mm)	Height (Protuberance to Bottom; mm)	Width (mm)
Pig				
Pig 01	07/16/19	49.3	9.3	46.4
Pig 02	07/17/19	47.8	13.5	45.5
Pig 03	07/17/19	52.9	12.9	52.8
Pig 04	07/17/19	46.9	11.7	51.0
Pig 05	07/17/19	44.9	11.7	48.5
Pig 06	07/18/19	48.6	12.6	46.6
Pig 07	07/18/19	59.4	9.5	49.5
Pig 08	07/18/19	51.9	13.0	46.7
Pig 09	07/18/19	49.0	8.7	47.9
Pig 10	07/19/19	48.2	12.0	47.5
Pig 11	07/19/19	53.4	8.6	48.9
Pig 12	07/19/19	53.3	12.8	47.4
Pig 13	07/19/19	51.4	8.7	43.8
Pig 14	07/25/19	55.8	7.0	43.5
Pig 15	07/25/19	47.0	12.25	50.6
Rabbit				
Rabbit 01	07/26/19	5.9	2.0	14.2
Rabbit 02	07/29/19	6.9	3.2	12.7

Rabbit 03	07/29/19	6.5	3.1	8.8
Rabbit 04	07/29/19	6.5	4.8	11.2
Rabbit 05	07/31/19	5.4	5.1	14.9
Rabbit 06	07/31/19	5.8	3.8	14.2
Rabbit 07	07/31/19	6.1	3.7	13.1
Rabbit 08	07/31/19	3.9	3.6	10.6
Rabbit 09	08/01/19	6.0	4.6	14.0
Rabbit 10	08/01/19	5.6	6.0	14.5
Rabbit 11	08/01/19	6.1	4.0	11.6
Rabbit 12	08/01/19	4.9	4.3	12.6
Rabbit 13	08/01/19	6.8	3.7	10.6
Rabbit 14	08/01/19	6.4	2.5	11.0
Rabbit 15	08/01/19	6.5	3.3	14.4

A digital caliper was additionally utilized to calculate measurements for Table 4. Once the laryngeal prominence or protuberance was identified, the distance from this point to the superior and inferior borders of the thyroid cartilage were measured and reported. Thyroid cartilage width was measured as the caliper spanned from the widest point at one lateral edge of thyroid lamina to the other.

Table 5

Trachea Anatomical Dimensions

Group	Session Date	Trachea Length (mm)	Trachea Width (mm)
Pig			
Pig 01	07/16/19	23.5	18.7
Pig 02	07/17/19	21.9	23.0
Pig 03	07/17/19	20.6	19.0
Pig 04	07/17/19	43.2	21.5
Pig 05	07/17/19	35.5	18.7
Pig 06	07/18/19	20.1	17.3
Pig 07	07/18/19	20.1	19.0
Pig 08	07/18/19	43.1	16.5
Pig 09	07/18/19	38.7	16.2
Pig 10	07/19/19	31.8	17.0
Pig 11	07/19/19	41.1	18.9
Pig 12	07/19/19	51.3	21.6
Pig 13	07/19/19	46.4	18.2
Pig 14	07/25/19	44.1	21.3
Pig 15	07/25/19	53.9	18.7
Rabbit			
Rabbit 01	07/26/19	23.1	5.7
Rabbit 02	07/29/19	22.3	5.5
Rabbit 03	07/29/19	17.5	3.5

Rabbit 04	07/29/19	21.4	4.8
Rabbit 05	07/31/19	30.8	6.7
Rabbit 06	07/31/19	21.3	5.7
Rabbit 07	07/31/19	20.3	5.4
Rabbit 08	07/31/19	26.2	4.5
Rabbit 09	08/01/19	28.5	5.6
Rabbit 10	08/01/19	28.1	5.2
Rabbit 11	08/01/19	22.2	5.6
Rabbit 12	08/01/19	22.1	5.9
Rabbit 13	08/01/19	21.4	3.7
Rabbit 14	08/01/19	19.0	3.8
Rabbit 15	08/01/19	25.9	4.9

Finally, the digital caliper was additionally utilized to calculate measurements for Table 5. Tracheal length was measured from the inferior edge of the cricoid cartilage to the inferior edge of the trachea. Trachea width was identified as the digital caliper measured the inner diameter of the widest point of the trachea.

When information provided by pig and rabbit larynx anatomical dimensions are compared to aerodynamic measurements, no apparent association was observed with gross vocal fold structure and ease of phonation, indicated by low onset pressure and flow values. When groups are compared, the following pattern was observed: consistently more pressure and flow were required at onset and sustained phonation to initiate pig vocal fold vibration compared to rabbits.

Table 6

Average Aerodynamic Data from 15 Phonation Trials for Each Larynx

Group	Onset Pressure (cmH_2O)	Sustained Pressure (cmH_2O)	Onset Airflow (L/m)	Sustained Airflow (L/m)	Onset Resistance (cmH_2O/L/m)	Sustained Resistance (cmH_2O/L/m)
Pig						
Pig 01	5.76	6.92	0.39	0.49	15.12	14.01
Pig 02	18.19	26.56	0.35	0.49	52.66	54.01
Pig 03	7.44	16.24	0.34	0.79	22.56	20.67
Pig 04	18.80	34.98	0.19	0.44	102.45	80.85
Pig 05	31.37	45.20	0.43	0.72	80.58	64.57
Pig 06	60.59	68.43	0.34	0.38	185.72	185.99
Pig 07	32.00	41.35	0.30	0.42	145.76	124.13
Pig 08	17.84	19.22	0.09	0.12	193.04	170.01
Pig 09	15.01	14.22	0.10	0.15	159.05	97.68
Pig 10	21.46	46.46	0.27	0.60	95.31	77.88
Pig 11	9.23	9.33	0.06	0.12	149.38	81.52
Pig 12	12.13	38.41	0.43	0.74	28.70	51.80
Pig 13	17.25	19.82	0.11	0.17	154.38	116.82
Pig 14	12.66	14.44	0.32	0.42	45.24	37.41
Pig 15	20.01	22.59	0.29	0.34	81.10	68.48
Rabbit						
Rabbit 01	8.75	12.79	0.14	0.17	64.35	74.96
Rabbit 02	8.52	11.47	0.07	0.08	115.99	141.72

Rabbit 03	15.16	28.70	0.13	0.19	114.81	148.06
Rabbit 04	9.13	13.99	0.09	0.10	114.57	144.05
Rabbit 05	12.19	14.98	0.10	0.10	120.17	155.76
Rabbit 06	6.26	7.88	0.04	0.06	191.14	123.63
Rabbit 07	6.45	8.20	0.03	0.05	238.27	166.94
Rabbit 08	8.99	11.45	0.08	0.09	115.63	128.06
Rabbit 09	7.15	7.56	0.04	0.05	175.56	165.75
Rabbit 10	6.41	6.64	0.04	0.05	169.10	126.81
Rabbit 11	6.72	11.87	0.08	0.11	84.64	106.75
Rabbit 12	11.07	13.54	0.13	0.15	87.93	96.41
Rabbit 13	8.13	11.66	0.05	0.05	172.73	219.47
Rabbit 14	10.37	20.53	0.06	0.08	163.57	245.51
Rabbit 15	5.20	7.95	0.05	0.06	110.63	125.55

Data from 15 tokens, or phonatory trials, were collected for each of the 15 rabbit and pig larynges involved in the study. For each token, onset pressure (i.e., PTP), onset flow (i.e., PTF), sustained pressure, and sustained flow measurements were recorded. Onset and sustained laryngeal resistance measurements were calculated post hoc. Figures 10 and 11 depict all data points measured from each larynx across 15 trials, indicated by black dots, for each aerodynamic metric. Red dots signify averaged values for each respective larynx, which are also reported in Table 6.

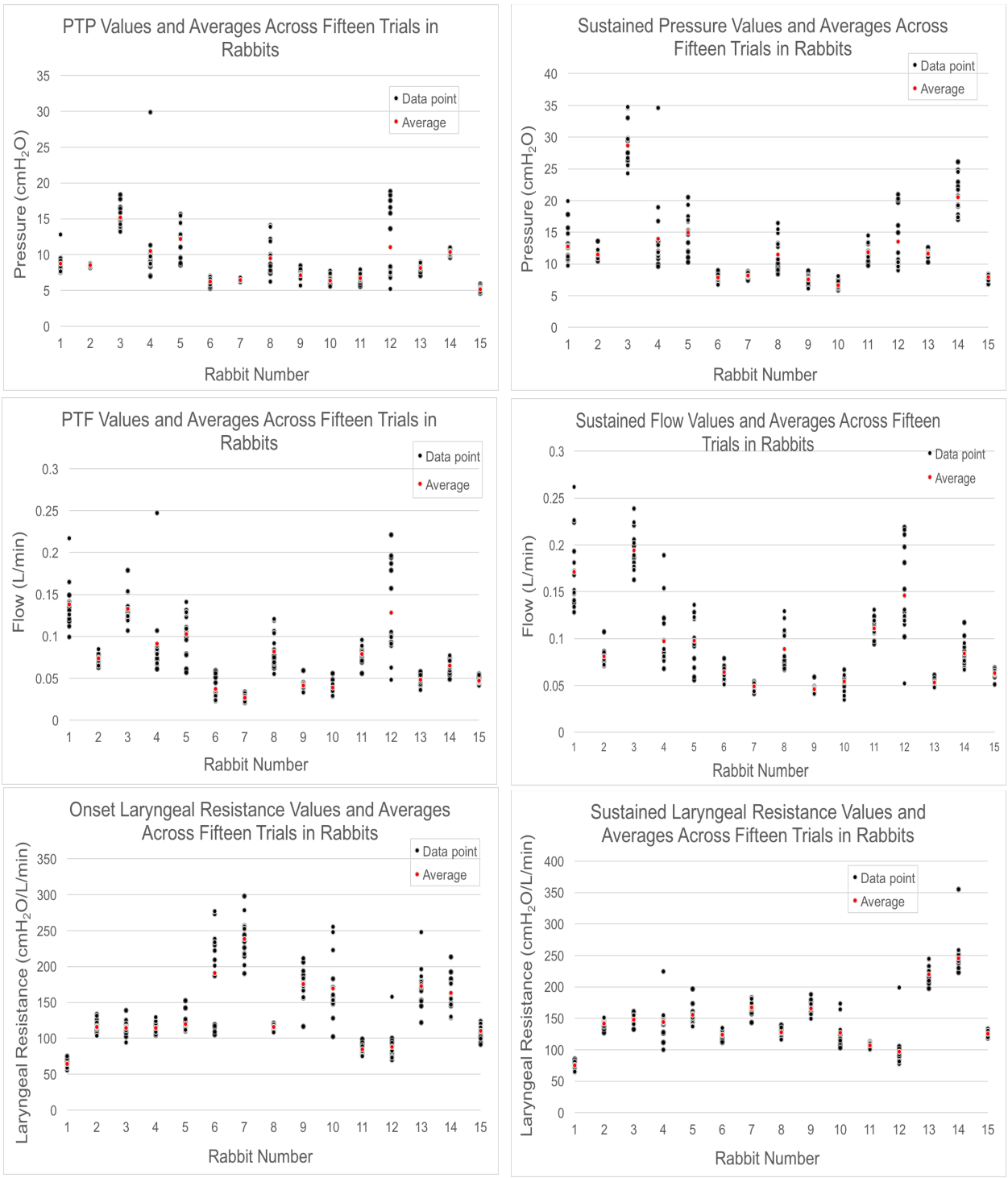

Figure 10. Spread of 15 Tokens Per Rabbit Larynx Across All Metrics.

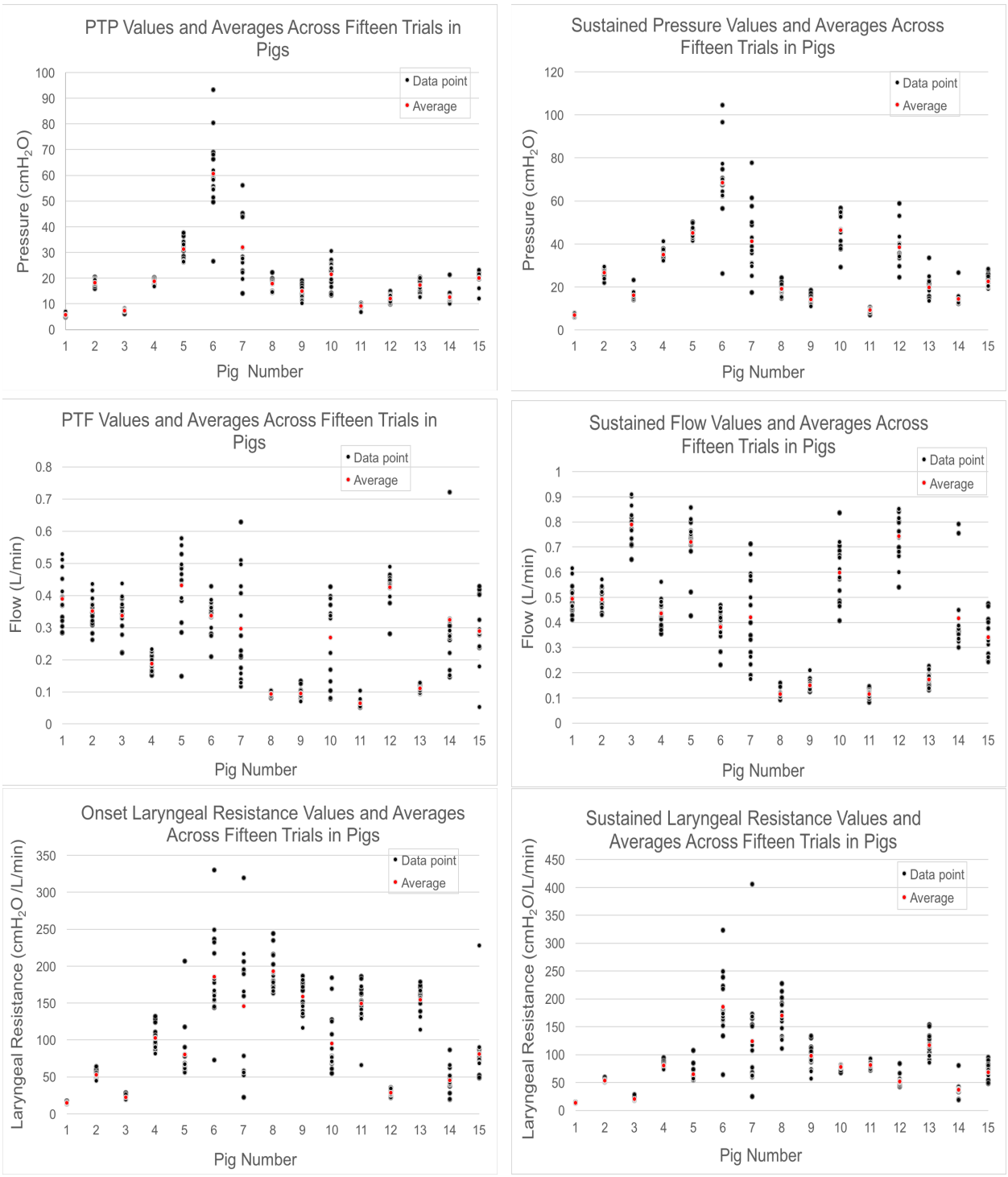

Figure 11. Spread of 15 Tokens Per Pig Larynx Across All Metrics.

The coefficient of variation, defined as the standard deviation divided by the mean and expressed as a percentage, was calculated for each of the 15 larynges in both the rabbit and pig groups. Aerodynamic tokens for phonation onset, sustained phonation, and laryngeal resistance served as the basis for coefficient of variation calculations. Figure 12 illustrates onset and sustained pressure for each of the 15 individual rabbits; Figure 13 shows onset and sustained airflow.

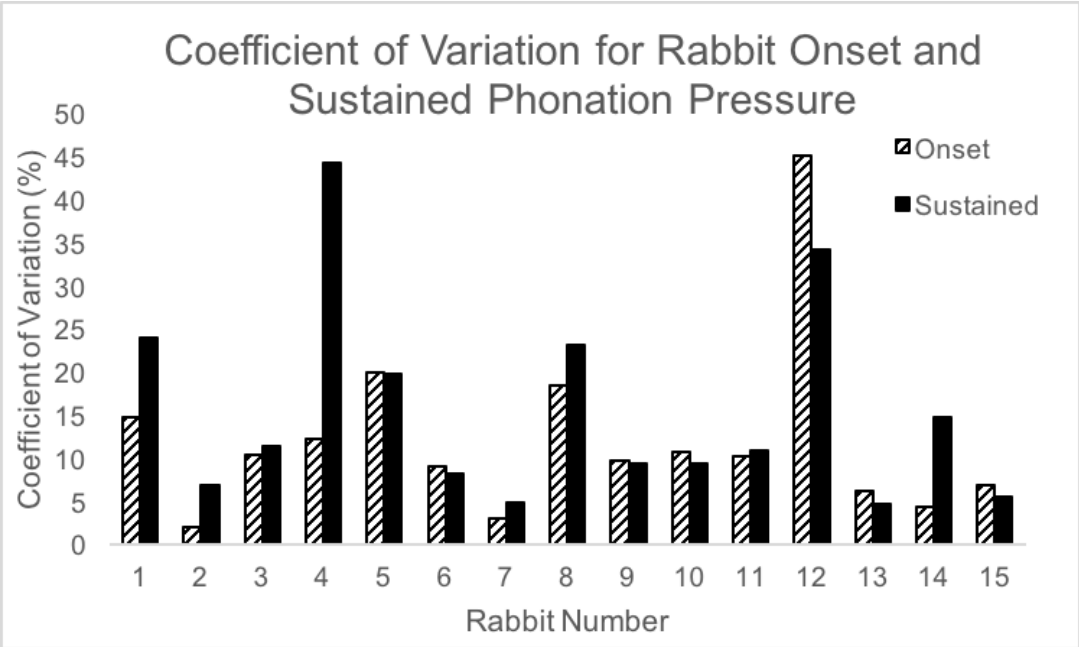

Figure 12. The coefficient of variation (%) for onset pressure and sustained pressure across 15 phonation trials for each larynx in the rabbit group.

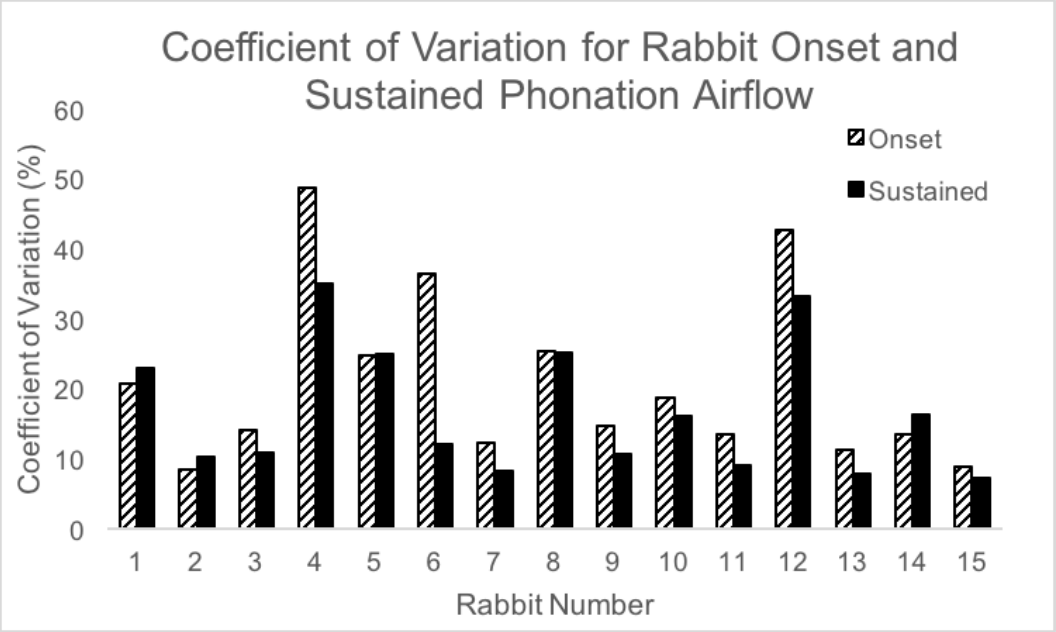

Figure 13. The coefficient of variation (%) for onset airflow and sustained airflow across 15 phonation trials for each larynx in the rabbit group.

Figure 14 illustrates laryngeal resistance –or pressure divided by flow—for each rabbit at the onset of phonation and at the midpoint of phonation. Figures 15, 16, and 17 show corresponding data for each of the 15 pig larynges for pressure, airflow, and laryngeal resistance.

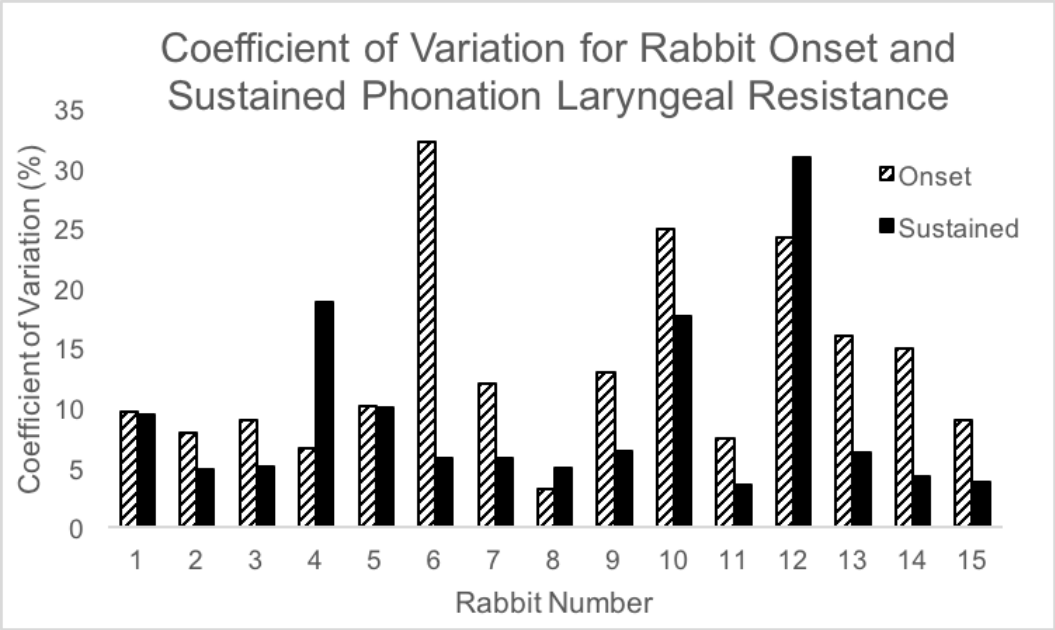

Figure 14. The coefficient of variation (%) for onset laryngeal resistance and sustained laryngeal resistance across 15 phonation trials for each larynx in the rabbit group.

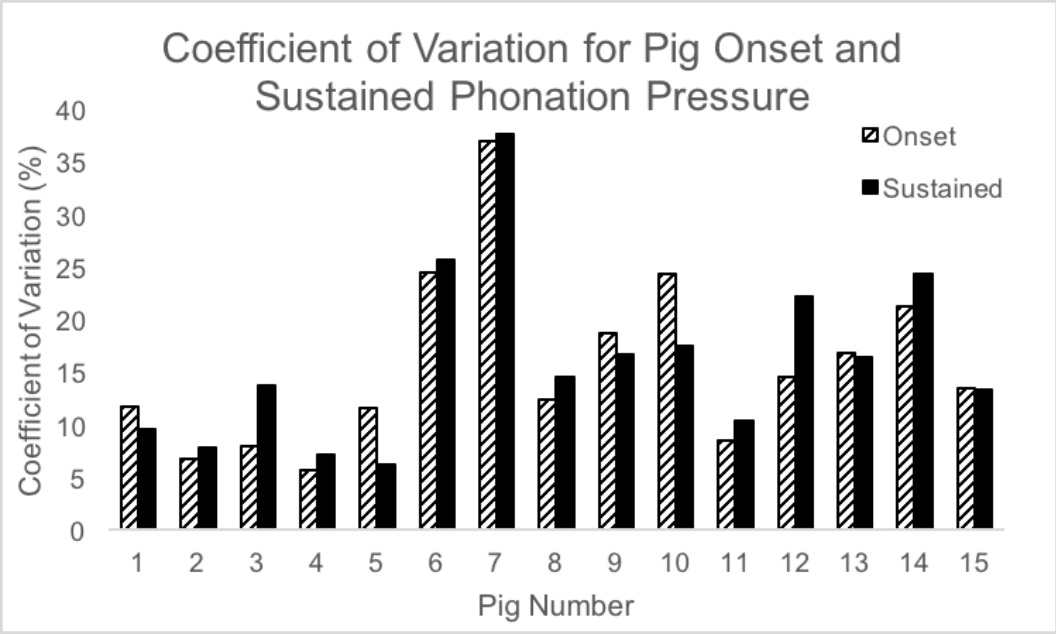

Figure 15. The coefficient of variation (%) for onset pressure and sustained pressure across 15 phonation trials for each larynx in the pig group.

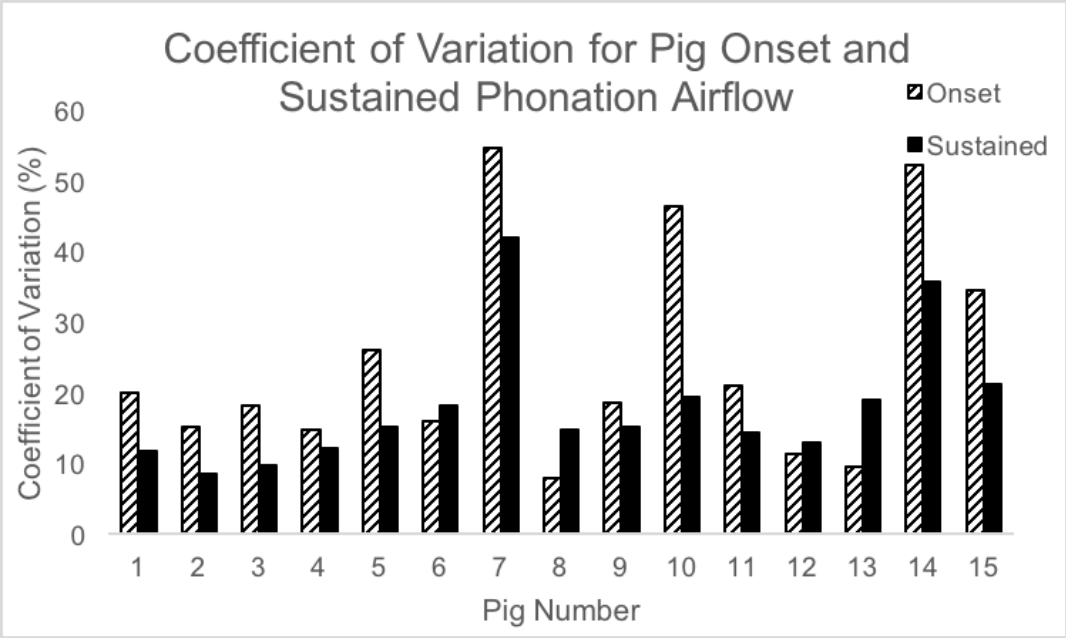

Figure 16. The coefficient of variation (%) for onset airflow and sustained airflow across 15 phonation trials for each larynx in the pig group.

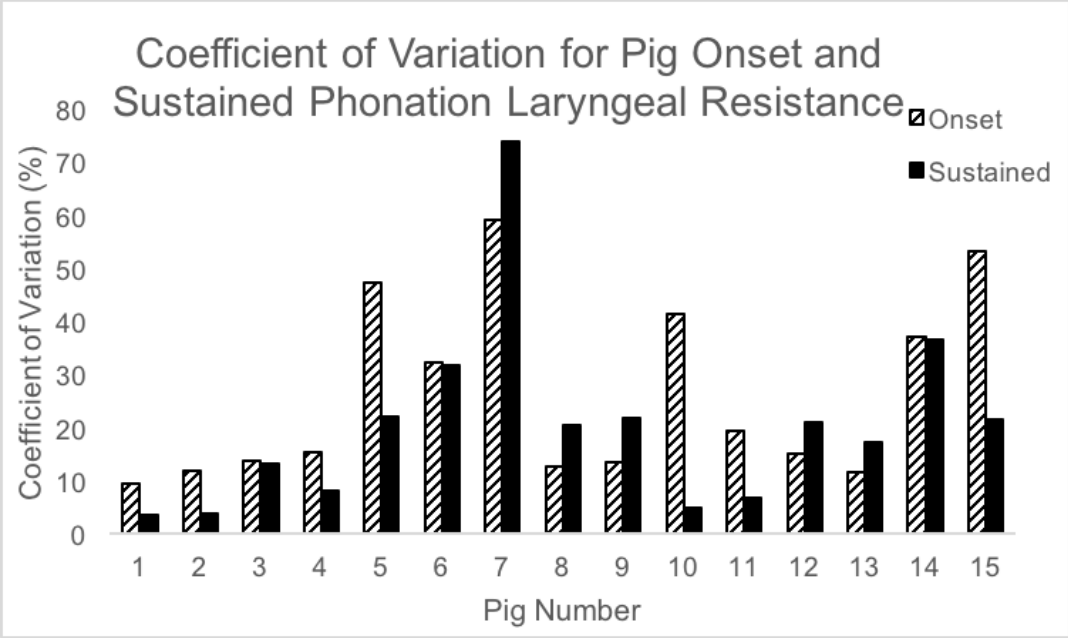

Figure 17. The coefficient of variation (%) for onset laryngeal resistance and sustained laryngeal resistance across 15 phonation trials for each larynx in the pig group.

Discussion

The current research aimed to address two primary questions. First, this study included the examination of measurement stability for aerodynamic parameters in a traditional excised larynx benchtop model. Second, the measurement stability for a more novel benchtop species (i.e., the rabbit) was compared to a more traditional species employed in voice disorders research (i.e., the pig). Aerodynamic measurement variability was anticipated based on previous human (Plexico et al., 2011; Smitheran & Hixon, 1981; Titze, 2000), animal (Mau et al., 2011; Mills et al., 2016; Rousseau et al., 2017) and other mechanical modeling research (Khosla et al., 2008; Thomson et al., 2005; Titze et al., 1995; Zhang, 2009). Therefore, it was essential to determine the extent of aerodynamic measurement instability that may be expected in both traditional and emerging animal species used in benchtop phonation research.

Measurement stability has been a topic of interest for decades, yet data are lacking. This is perhaps due to the relatively rapid advancements in models of human phonation and the need for baseline stability of the measurements employed within these models. For example, Scherer and Vail (1995) studied acoustic measurement stability as applied to clinical voice samples. The study included several acoustic voice perturbation measures as well as harmonic-to-noise ratio. The authors examined coefficients of variation to identify the minimum number of repeated sample tokens needed to be reliable. The findings suggested that 15 tokens are ideal for accurate phonation sampling. The number of tokens correlated with the quality of the voice sample, with more aperiodic signals requiring the greatest number of tokens and very periodic voices requiring the fewest. Similar observations have been made by researchers examining measurement stability in normal and disordered voices (Pierce, 2017; Tanner et al., 2015). Andrews, Shrivasatav, and Yamaguchi (2000) also examined typical and dysphonic voices

during repeated voice sampling. The results supported the conclusion that voice measurements—like any measurement—are susceptible to instability and improve with repeated sampling, particularly if the voices are not highly periodic (i.e., not normal). Because excised larynx benchtop research involves phonation sampling in the absence of a vocal tract and with artificially simulated muscular control, these models do not always result in normal phonation and merit repeated sampling to ensure that measurement instability does not obscure experimental results. Thus, the current study addresses a critical gap in the literature regarding the measurement instability in random samples of an emerging species employed in excised larynx models and the comparison of that to more traditionally used species.

Rabbit (Leporine) Findings

As illustrated in Figure 10, for the case of onset pressure (i.e., PTP) and sustained pressure, some rabbit larynges (e.g., rabbits 2 and 7) produced more consistent values across 15 repeated trials compared to others (e.g., rabbit 12). These results are reported as low and high coefficient of variation values in Figure 12, respectively. For PTP, the rabbit larynges demonstrated some variability based on coefficient of variation calculations from the 15 phonation trials for each of the 15 specimens. The PTP values varied less than 20% in all but one rabbit larynx; this larynx, rabbit 12, demonstrates a wide spread of reported PTP data across the measured 15 trials, as observed in Figure 10, and appears to be an outlier in its sample group. Twelve of the 15 rabbit larynges varied less than 15% across repeated trials. Regarding pressure during sustained phonation, defined as the midpoint of each 4 to 5-second phonation trial, sustained pressure was generally commensurate with PTP values; sustained pressure varied less than 20% in 11 of the 15 larynges. It has been theorized that PTP measurement might be less stable than PTF due to aerodynamic perturbation that occurs during the initiation of phonation

(Hottinger et al., 2007; Jiang & Tao, 2007). But in this study, rabbit larynges demonstrated more measurement stability for both onset and sustained pressure as compared with onset and sustained flow. That said, the coefficients of variation for rabbit airflow were also fairly stable, with only five specimens with greater than 20% variability at flow onset and five above 20% during sustained airflow for phonation.

Pig (Porcine) Findings

As indicated in Figure 11, more individual pig larynges, compared to rabbit larynges, demonstrated a wider spread of values for a single measurement across 15 repeated trials, particularly within onset airflow (i.e., PTF) and sustained flow measurements. This data is displayed in figures 15 through 16; the pig larynges demonstrated consistently higher variability compared to the rabbit larynges. Calculations for coefficient of variation were computed in the same manner as the rabbits, across 15 trials for each of the 15 pig specimens. The PTP values varied less than 20% with the exception of four specimens. Measurement stability was nearly analogous for sustained pressure: four specimens varied more than 20%. Airflow onset (i.e., PTF), appeared to be the parameter with the least amount of measurement stability in pigs, as five specimen demonstrated variability greater than 20%. Sustained airflow exceeded 20% variation in two larynges.

Laryngeal Resistance Comparisons

Figure 10 displays all reported data per individual rabbit larynx for onset and sustained laryngeal resistance values while Figure 11 displays the same distribution of data for pig larynges. Figure 14 displays the coefficient of variation for rabbit onset and sustained laryngeal resistance. Onset laryngeal resistance values varied less than 20% with the exception of three

specimens. Commensurate with onset pressure, only three rabbit specimens varied more than 15% for sustained laryngeal resistance values and one outlier varying more than 20%.

Finally, Figure 17 documents laryngeal resistance for pig specimens. Following the variability patterns of other metrics, pig specimens demonstrated variance in onset laryngeal resistance values larger than 20% in six larynges. Sustained laryngeal resistance in pig specimen exceeded 20% variance in eight larynges.

Species Comparisons

Based on these measurement stability data, it is apparent that within-larynx measurement stability is not only greater for rabbits versus pigs, the magnitude of variability is nearly equivalent for pressure and flow in rabbits. This finding strengthens the argument for the inclusion of rabbits as a viable species for voice aerodynamic research. In a canine model, onset flow has previously been reported to be less variable than onset pressure (Hottinger et al., 2007; Jiang & Tao, 2007; Regner, Tao, Jiang, & Zhuang, 2008)—a finding which appears to be consistent with the current research when only group descriptive statistics are observed. But rabbits as a group demonstrate significantly smaller standard deviations within flow measurements compared to pressure, or either measurement for the pig group. When coefficients of variation are analyzed for within-subject variability in rabbits, airflow variability appears to be greater than pressure variability. Sustained pressure measurements within individual rabbit larynges appeared to be more consistent than sustained flow measurements as well. Only 27% of rabbit larynges had coefficients of variation higher than 20% for sustained pressure, while 33% of the same larynges had coefficients of variation higher than 20% for sustained flow.

It is interesting that in this particular study when 15 rabbits are observed across 15 phonatory trials, onset airflow seems to be more variable than onset pressure. It is possible that airflow data are more variable in the rabbit species than previously hypothesized when individual stability is observed rather than group descriptive statistics. This supports the current research hypothesis that rabbit within-subject designs are best for excised larynx studies; although rabbit group statistics appear reliable, more variability may exist at the individual larynx level.

When actual calculated values for laryngeal resistance were compared between species, the rabbit group produced significantly higher laryngeal resistance values at onset and sustained phonation conditions, despite having drastically smaller values for both pressure and flow. This pattern is likely replicating the previously identified relationship that as airflow measurements decrease—the denominator in the resistance equation—the resulting quotient—the laryngeal resistance value—will increase. Comparable to Netsell et al. (1991), the rabbit larynx, having significantly smaller geometrical tracheal and laryngeal measurements when compared to the pig larynx, will result in lower airflow and higher laryngeal resistance measures.

Relationship Between Pressure and Flow

The relationship between pressure and flow has been theoretically scrutinized within the study of fluid mechanics and dynamics. The following electrical and physical laws describe typical and atypical vocal functioning: Ohm's law, the Bernoulli effect, and Poiseuille's Law. Ohm's law states that as pressure increases, flow also increases for a constant resistance. Additionally, when pressure is kept constant, resistance and flow are inversely related (Collins Dictionary, n.d.). The linear relationship between pressure and flow has been replicated in numerous excised larynx and in vivo animal studies (Bielamowicz et al., 1993; Koyama, Kawasaki, & Ogura, 1969; Smith, Green, & Berke, 1991). Muta and Fukunda (1988) further

found that the rate of pressure increase slows at high flow levels. Sercarz et al. (1994) proposed that the decrease in laryngeal resistance during increased airflow was linked to an increased glottal area.

Laryngeal resistance values may indicate disorder if either pressure or flow are influenced, resulting in fluctuating ratios. In a study by Zheng et al. (2012), they assessed the viability of using laryngeal resistance among other aerodynamic measurements to aid in the diagnosis of muscle tension dysphonia (MTD). In this mode of phonation, the larynx exerts excessive amounts of pressure, leading to "pressed" or aperiodic phonation, in which little airflow is possible. An excessive range of 1,087.14 $cmH_2O/L/m$ was reported among a 26-person sample of individuals with MTD. Due to the extreme fluctuation of resistance values, it was not recommended as a diagnostic indicator for identifying MTD. With the introduction of covariates, the linear pressure-flow relationship becomes skewed and interrupts the Ohm's model. In the case of MTD, a covariate of extreme pressure has been introduced which consequently alters the linear pressure/flow relationship. When the relationship between pressure and flow is disturbed, it can indicate atypical phonation or pathology (Jiang, Regner, Tao, & Pauls, 2008). Atypical phonation may occur without the presence of a vocal tract, or if the overall quality of phonation is decreased. Animal models can be limited as phonation among rabbits appears to be breathier, pig phonation is reminiscent of squealing, and the dog larynges lack a vocal ligament.

The inverse relationship between airflow and laryngeal resistance, also identified in Ohm's Law, does not always indicate disorder. In 2013, Awan, Novaleski, and Yingling reported the coefficient of variation for laryngeal resistance to be 28% in a population of 60 healthy adults, and proposed that observing intrasubject change and differentiating between

normal and disordered vocal functioning becomes less precise as laryngeal resistance may have increased sensitivity for outliers.

The Bernoulli Effect states that in order to maintain constant volume rate across a constriction, incompressible fluids need to increase in velocity. When applied to the larynx, regions of a pressure drop, such as separation of vocal fold covers, will allow for high velocity and airflow (*Bernoulli's equation,* n.d.). The covers of the vocal fold will subsequently snap back due to relatively decreased pressure experienced within the pressure drop. The application of this principle was apparent as the excised larynges produced periodic phonation with measurable fundamental frequencies.

Poiseuille's Law states that fluid flow through a tube is affected by the pressure of the current, the tube radius and length dimensions, and the viscosity of fluid. When applied to the larynx, Poiseuille's Law dictates that decreases in tube diameter create increased resistance that reduces flow (*Viscosity and Poiseuille flow,* n.d.). This phenomenon was observed in the current study as increased resistance values were recorded with lower flow rates in rabbit larynges.

Limitations

This study may be subject to limitations. In order to adjust the benchtop apparatus to accommodate significantly smaller rabbit larynges, a prototype adaptor piece was constructed, although the material could not withstand repeated mounting and unmounting of rabbit larynges. The upper third portion of a syringe replaced the prototype because the diameter of the syringe barrel matched the pig larynx tubing previously set in place, and the diameter of the syringe tip accommodated tracheal mounting of the smaller rabbit larynges. One drawback to this syringe was its 90° angle which created a sudden contraction and sharp-edged minor head loss. This shape may lead to recirculating separation zones at the 90° angles where decrease in tube

diameter is present, which likely affects the stream of airflow. In benchtop research, it is unclear how much this tubing shape may affect pressure and flow recordings. Additionally, occasional reverberation was observed in pressure and flow signals without any clear source. It is possible that the reverberation is secondary to nonlinear circulating airflow from the minor head loss. Finally, while all rabbit larynges were obtained from male rabbits, the sex of each porcine larynx was not specifically controlled. While geometric size and angle of the vocal folds may have provided some indication of sex, the variability in the pig aerodynamic data may in part due to significant phonatory differences between male and female pigs.

Implications for Future Research

Future studies could optimize the benchtop setup so that the tubing conversion to accommodate rabbit-sized tracheas would have a more gradual narrowing, thus encouraging continuous airflow and eliminating recirculated airflow through the tubing. Further investigation into the source of reverberation in the aerodynamic signals could produce more reliable data. Additionally, future research could collect data controlling for the sex of pig and rabbit larynges to see if a significant difference in phonatory characteristics when considering sex exists. All rabbits have bilateral fat pads superior to the ventricular folds; however, female fat pads have a larger mass. In the current work, female rabbits were excluded from the study because their enlarged fat pats partially obstruct videostroboscopic images. Although the current study did not observe in vivo rabbit phonation, future stages of the larger asthma project will, and so baseline phonatory data were only collected for male rabbit larynges. Since a majority of the human voice disordered population includes females, the potential translational value of female rabbit laryngeal functioning would be of interest. In the current study, percent elongation and degree of glottal closure were controlled for; however, future studies might experiment with manipulation

of elongation and glottal closure and observe whether these independent variables produce results with higher variability.

Conclusion

The results from the present study indicate that rabbit larynges demonstrate significantly less variability across PTP, PTF, sustained pressure and flow, and onset and sustained laryngeal resistance values when compared to pig larynges. Pig larynges appear to produce similar degrees of within-larynx variability across onset and sustained pressure and flow aerodynamic metrics. Rabbit larynges demonstrate very little within-larynx variability with PTP when compared to PTF measurements, although PTF, sustained pressure, and sustained airflow within-larynx variability are generally less than 20% variability. This study provided information regarding intralarynx variability in a population of healthy New Zealand white rabbits. Identifying the normal degrees of variability to be expected in a healthy larynx can aid in diagnosis sensitivity and specificity as values that exceed the normal limits of normal measurement fluctuation may be indicative of laryngeal pathology.

Certain animal species lend themselves better to certain studies. Depending on the independent variables of interest, aerodynamic measurements, and study design, one particular animal may provide better results and greater translational value. Excised pig larynges appear to be more variable than canine and rabbit larynges for pressure, flow, and resistance measurements, although larynx size may prove beneficial for vocal fold elongation studies. In canine larynges, flow may be less variable than pressure (Hottinger et al., 2007; Jiang & Tao, 2007; Regner et al., 2008); thus PTF in canines may be a better model for studying voice disorders characterized by insufficient vocal fold adduction. Phonation threshold pressure may be a better metric for disorders characterized by scarring and increased stiffness (Zhuang et al.,

2013). Excised rabbit larynges generally have fairly low levels of variability in both pressure and flow, although breathy phonation was frequently identified based on examiner perception and acoustic analysis. It is possible that this breathiness led to less variability. For purposes of this study, rabbit larynges have great advantages due to previously established sensitivity to translational voice parameters, generally less variability compared to pig larynges, and measurement stability within larynges.

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APPENDIX A

Annotated Bibliography

Alipour, F., & Jaiswal, S. (2008). Phonatory characteristics of excised pig, sheep, and cow larynges. *Acoustical Society of America, 6,* 4572-4581. doi:10.1121/1.2908289

Purpose of the study. This study evaluated the phonatory characteristics of pig, sheep, and cow larynges in order to better understand the resemblance of these species to human vocal fold phonation.
Method. A group of 22 larynges (eight pig, eight sheep, and six cow) were utilized in his study. After following a freezing protocol, the larynges were dissected and sutures were added to the arytenoid cartilages to simulate adductor movements of the lateral cricoarytenoid muscle. A variety of graded weights were added to the sutures in order to provide an objective measurement of adduction. An electroglottograph was placed the thyroid cartilage in order to identify the fundamental frequency (F_0, Hz) during phonation. Three pressure-flow sweeps were conducted, which included a 20 second phonatory period, indicating the full range of pressure, flow, and F_0. These helped identify individual laryngeal differences and then various adduction levels. Sustained phonation was accomplished to view oscillation. Subglottal pressure and flow were recorded. Vocal fold oscillations were observed and recorded using videostroboscopy.
Results. The pig larynx has well-defined ventricular and vocal folds, which angle upwards at an approximate 40 ° angle. The sheep larynx houses flexible vocal folds that are double the thickness of the pig folds. Cow vocal folds are triple the thickness of sheep folds and are longer and stiffer. Neither the sheep nor cow vocal folds have a distinct separation between the vocal folds and surrounding tissue, but both have large mucosal waves. Average oscillation frequencies were 220 +/-57 Hz (pig), 102+/- 33 Hz (sheep), and 73+/- 10 Hz (cow). Average phonation threshold pressure (PTP, cmH_2O) was 7.4 +/- 2.0 cmH_2O (pig), 6.9 +/- 2.9 cmH_2O (sheep), and 4.4 +/- 2.3 cmH_2O (cow).
Conclusion. Pig, sheep, and cow larynges can all serve as models for phonatory and aerodynamic insights into human laryngeal functioning. When substituting pig for canine larynges, angle of the folds, and relative size differences of the folds and cricothyroid muscle should be considered.
Relevance to the current work. This study validated the use of pig larynges and provided a comparison for the PTP values that were identified in the current work.

Birk, V., Döllinger, M., Sutor, A., Berry, D., Gedeon, D., Traxdorf, M., Wendler, O., Bohr, C., & Kniesburges, S. (2017). Automated setup for ex vivo larynx experiments. *Journal of the Acoustical Society of America, 141,* 1349-1359. doi:10.1121/1.4976085

Purpose of the study. There are multiple inherent confounding variables that accompany excised laryngeal benchtop work that can affect the frequency and amplitude of phonation within and across larynges. This study attempted to automatize adduction and elongation variables in order to standardize and expedite the experimental process.
Method. A customized computerized setup was created to control for the following three variables: vocal fold adduction and elongation and P_s, or subglottal pressure. Mounting and posturing the larynx were completed using an electro-mechanical device. A simulation for

cricothyroid contraction was also electro-mechanically controlled. A force sensor regulated the amount of force applied to the thyroid cartilage for elongation. Using a prong with three needles, torque was applied to the arytenoid cartilages to simulate adduction. A torque sensor similarly measured this force. An ultrasound nebulizer prevented dehydration of the tissue by humidifying and heating the air which coursed through the larynx. Air flow and pressure were controlled by an interface. Technology was developed to detect onset phonation based on the P_s. Four pig larynges were used to validate methodological functioning.
Results. Subglottal pressure and F_0 both increased as a result of the elongation and adduction procedures, which has also been illustrated in other works.
Conclusion. The proposed setup was able to control for vocal fold elongation and adduction, as well as glottal airflow. Measurements for each larynx were able to be completed during a maximum of 4 minutes, which helped minimize tissue degradation.
Relevance to the current work. This study highlights factors that need to stay consistent across larynges during data collection. It also describes how phonation onset was detected which may be helpful in the current work as onset and offset data are analyzed.

Chan, R., & Tayama, N. (2002). Biomechanical effects of hydration in vocal fold tissues. *Otolaryngology—Head and Neck Surgery, 126,* 528-536. doi:10.1067/mhn.2002.124936

Purpose of the study. The purpose of this study was to understand the effects of dehydration and rehydration on sheer properties of in vitro canine vocal fold epithelial tissue.
Method. Five excised canine larynges were dissected within 30-minutes post-mortem, from which biomechanical data was obtained. One vocal fold mucosa (epithelium and lamina propria) was dissected away from each larynx. The vocal fold tissue samples were then systematically immersed in each of the three following conditions: isotonic, hypertonic, or hypotonic solutions. Following 30-minute immersion in each of the fluids, a rheometer was used to measure viscoelastic shear properties of the tissue. In order to measure rheology, the tissue was subject to sinusoidal shear stress and strain (torque and deformation, respectively).
Results. Across all five in vitro subjects, aversive biomechanical effects caused by dehydration of mucosal tissue was only partially reversed by rehydration. Increased tissue dampening was observed with dehydration, while the opposite was observed with rehydration attempts. Despite these general patterns, neither value approached statistical significance. Hyaluronic acid appears to be responsible for hydration regulation in the extracellular matrix of the mucosa.
Conclusion. From a rheological standpoint, the degree of hydration directly affects the ease of phonation onset and efficiency of vocal fold oscillation. Hydration treatments that target hydration at the level of the lamina propria extracellular matrix would theoretically help approximate vocal functioning to baseline, though it may not completely return to its original health, based on observations of canine mucosa in vitro.
Relevance to the current work. In the current research, phonatory trials with rabbit larynges are trying to eliminate a confounding variable of dehydration, as rheological and biomechanical data suggest that dehydration inflates PTP values.

Erickson, E., & Sivasankar, M. (2010). Evidence for adverse phonatory change following an inhaled combination treatment. *Journal of Speech, Language, and Hearing Research, 53,* 75-83. doi:10.1044/1092-4388(2009/09-0024).

Purpose of the study. The purpose of this study was to observe whether participation in inhaled corticosteroid (ICS) treatment affected PTP and perceived phonatory effort (PPE) of human subjects.
Method. Fourteen human patients with typical laryngeal functioning participated in the study (nine females, five males). Inclusion criteria included daily (n = 6) or twice daily (n = 8) treatment of Advair diskus® for at least 4 months. In each session, prior to administration of a treatment, pitch range and /pi/ productions at specified semitones were initially calculated, followed by baseline measures of PTP, PPE, and forced vital capacity (FVC). These measures were collected again immediately following an IC or sham treatment, and at 1 and 2 hours post treatment. PTP was measured per Fisher and Swank (1997) procedures using a circumferentially vented pneumotachograph face mask. The PPE was measured using a visual analog scale.
Results. The ICS treatment was correlated with increased PTP values over time across all measured semitones. When treatment-by-time interaction effects were measured, only PTP values at 80th percent pitch were considered significant. These observed changes lasted upwards of two hours. The FVC values were consistently within normal limits throughout the experimental sessions. The PTP values did not increase with the sham treatment. PPE values were not significantly affected by either IC or sham treatment, though participants typically did indicate increased PPE effort values with ICS treatment when compared to the sham. Weak correlations were found between PTP and PPE following treatments across each observed timeframe.
Conclusion. Clinical PTP tasks at the 80th percent pitch, or "high" pitch, may be the most indicative of phonatory changes occurring due to ICS medication. Increased viscosity in the mucosa of the vocal folds may lead to phonatory changes secondary to ICS treatment. Chloride ion transport may be critical in regulating hydration of vocal fold tissue and consequently vocal fold viscosity. This should be indicated by increased PTP values.
Relevance to the current work. This study indicates that statistically significant increased PTP values are observable with higher pitches in healthy tissue exposed to IC treatment in humans, which future studies under the current grant will attempt to replicate in leporine larynges. The current study is compiling baseline data which PTP values will be used as a comparison.

Faver, K., Plexico, L., & Sandage, M. (2012). Influence of syllable train length and performance end effects on estimation of phonation threshold pressure. *Journal of Voice, 26*, 18-23. doi:10.1016/j.jvoice.2010.10.021

Purpose of the study. The purpose of this study was to investigate whether using five instead of seven syllable trains, would, produce significantly different PTP outcomes. Specifically, the authors investigated whether a performance end effect existed in the first and last syllables of the train sequence.
Method. Data was reported for ten healthy female participants. The participants' pitch ranges were collected and converted into differences in semitones. From this semitone range, each participant's 10th, 20th, and 80th percentiles were obtained and appointed as the participant's low, modal, and high pitches, respectively. While holding a Phonatory Aerodynamic System (PAS)

mask over her nose and mouth, each participant produced five smooth and connected repetitions of /pi/ at each participant's adjusted low, modal, and high pitches. The same task was repeated, with the exception of seven productions of /pi/ at low sound intensity levels. Maximum pressure points at each peak were measured and used as PTP estimates.
Results. The PTP values were significantly increased with the high pitch compared to low and modal pitches. The PTP values significantly decreased for the first syllable compared to middle and final syllables. A statistically significant interaction effect between pitch and syllable position was identified: PTP for the first syllable was lower than middle and final syllables when calculated in the high pitch.
Conclusion. No significant differences in PTP measurements were found when values were estimated using a five-syllable train compared to a seven-syllable train. Additionally, no significant differences in PTP were found in low and modal pitches between the combined first and last syllables compared to the middle three syllables. The PTP values were significantly lower in the first syllable compared to the middle and final syllables in the high pitch analysis. The PTP values at high pitches were greater than PTP at low and modal pitches.
Relevance to the current work. This study argues that PTP data estimated at modal pitches are consistent whether the value is collected in the first, middle, or last syllable in a train. When analyzing PTP data in the current work, the first and last phonatory trials will not be discarded as the data is collected at modal pitches and no performance end effect should exist.

Hassen, H., & Hasseba, A. (2016). Voice evaluation in asthma patients using inhaled corticosteroids. *Turkish Journal of Nose and Throat, 26,* 101-108. doi:10.5606/kbbihtisas.2016.79740

Purpose of the study. The purpose of this study was to evaluate the effects of ICS medication from a laryngeal-phonatory perspective in order to better understand the practical effects that the asthma treatment has on voice disorders.
Method. Thirty patients participated in this study (15 females and 15 males, ranging in ages from 16 to 27). Inclusive criteria included a diagnosis of bronchial asthma and usage of beclomethasone dipropionate aerosol solution (400 mcg, two times per day) or budesonide inhaler (200 to 400 mcg, two times per daily). Exclusive criteria included additional use of systemic steroids or history of a voice disorder. Speech samples and laryngoscopic videos were obtained and analyzed by two phoniatricians. Degree of dysphonia was measured using a modified GRBAS (Grade, Roughness, Breathiness, Asthenia, and Strain) scale, while video images were analyzed for the following laryngeal characteristics: edema, erythema, bowing, atrophy, irregular edges, interarytenoid thickening, and supraglottic hyperfunction. Acoustic analyses (including mean F_0, noise-to-harmonic ratio, and phonatory frequency range in semitones) were completed using a sustained /a/ vowel at normal pitch and loudness. A case history was also collected for each participant.
Results. Among the 30 participants, 53% had dysphonia (36.7% mild, 16.7% moderate), 46.7% had phonasthenia, or a combination of globus sensation, throat dryness, and frequent throat clearing, and 36.7% had laryngopharyngeal reflux and cough. There were no significant differences in duration of inhaler use between the two sexes, as well as time of onset of dysphonic symptoms and start of inhaler use between the two sexes. Although a positive correlation between inhaler use and dysphonia existed, the relationship was statistically insignificant ($p = 0.12$, $r = 0.49$). Of the seven visual vocal fold characteristics analyzed from

laryngoscopy videos, the most common conditions were interarytenoid thickening and vocal fold erythema (occurred in 56.7% of cases), while supraglottic hyperfunction and irregularity of vocal fold edges occurred in 53.3% of cases, and vocal fold edema was present in 36.7%. Bowing and atrophy were the least common conditions (5.8% and 3.5%, respectively). There were significant differences in percent jitter and shimmer, and a moderate positive correlation between ICS duration.
Conclusion. Most acoustic parameters correlated with higher-than-standard values, though not all parameters were considered significant. Dysphonia existed in over 50% of participants, and may be related to increased cough or reflux secondary to inhalers.
Relevance to the current work. This study highlights acoustic and visual changes that correlate with inhaler use. Normative values for rabbit vocal fold phonation are collected in the current work, which will later be compared to disordered rabbit acoustic and visual changes after inhaler exposure.

Hoffman, M., Rieves, A., Budde, A., Ketan, S., Zhang, Y., & Jiang, J. (2012). Phonation instability flow in excised canine larynges. *Journal of Voice, 26,* 280-284. doi:10.1016/j.jvoice.2011.03.007

Purpose of the study. The purpose of this study was to measure both phonation instability flow (PIF) and phonation flow range (PFR) in an attempt to quantify the aerodynamic parameter of flow within irregular voice production. The PIF value is defined as the minimum airflow that precedes chaotic voice production, and PFR is the magnitude of the difference between PIF and phonation threshold flow (PTF, L/m).
Method. Seven canine larynges were dissected and mounted on an excised larynx set-up. Resting vocal fold length was measured and designated as 0% elongation. The PTP and PTF data were recorded at phonation onset. Airflow was then increased to the instability threshold, which was determined by aperiodic phonation and noise-like broadband spectra. Pressure and flow values were recorded and labeled phonation instability pressure (PIP) and PIF. The PFR was determined by subtracting PTF from PIF. Five of these trials ensued at each of the following conditions: 0% elongation, 20% elongation without posterior glottal chink, and 20% elongation with a 3mm posterior glottal chink.
Results. The PIF values displayed a statistically significant difference between 20% elongation with no posterior glottal chink, and 20% elongation with a 3 mm posterior glottal chink. The PIP did not display a significant difference between elongation or abduction (glottis size) differences. No statistical significance was noted with increased elongation of the vocal folds and PTP or PTF values, though both PTP and PTF values did notably increase.
Conclusion. The PIF was able to identify changes in glottis size during phonation, but was not sensitive to differences in vocal fold elongation. The PIP did demonstrate increased values when the vocal folds were elongated, but not to a statistically significant degree.
Relevance to the current work. The research affiliated with the current work utilizes a similar excised larynx benchtop set-up. Pig and rabbit larynges were sutured anteriorly and fastened to a micropositioner, though elongation was not performed to avoid an added variable that could increase PTP and PTF values.

Hottinger, D., Tao, C., & Jiang, J. (2007) Comparing phonation threshold flow and pressure by abducting excised larynges. *Laryngoscope, 117,* 1695-1699. doi:10.1097/MLG.0b013e3180959e38

Purpose of the study. The purpose of this study is to compare the sensitivity of PTP and PTF values as changes in glottal width increase.
Method. Ten canine larynges were dissected and mounted on an excised larynx set-up including an artificial lung, flow and pressure transducers, and a high-pressure air source. Acoustic and aerodynamic data were recorded, including PTF and PTP values in five trials at five different abduction settings utilizing differing metal shim thicknesses. Baseline 0.0 mm data were recorded without a shim, and then shims of increasing thickness were utilized: 1.0, 2.0, 3.0, and 4.0 mm thickness. Data were recorded in voltages and converted to mL/m for flow and cmH_2O for pressure.
Results. Reported average air flow data for 0.0, 1.0, 2.0, 3.0, and 4.0 mm abduction were 376, 415, 580, 567, and 1,199 mL/m, respectively. The difference of each of these average values were found to be statistically significant. Additionally, PTF was found to be sensitive to differing degrees of vocal fold abduction. Reported pressure data for 0.0, 1.0, 2.0, 3.0, and 4.0 mm were 9.89, 9.26, 8.75, 9.02, and 9.71 cmH_2O, respectively. Statistical significance was not indicated for average PTP data, indicating that PTF measurements were more sensitive than PTP measurements to glottal width changes.
Conclusion. Unlike PTP, PTF measurements are linearly sensitive to glottal width variations, which can be helpful in identifying many laryngeal pathologies where incomplete vocal fold adduction occurs. Other works illustrate usefulness of PTP measurements in identifying vocal fold pathology, though it is clinically difficult to measure. The PTF may prove more accurate in identifying pathology and is more clinically feasible.
Relevance to the current work. This study identifies PTF as an important aerodynamic measurement tool, particularly when identifying vocal fold pathologies that are defined by incomplete vocal fold adduction. The current work additionally measures and analyzes PTF values alongside PTP values as both have useful research and clinical implications.

Howard, N., Mendelsohn, A., & Berke, G. (2015). Development of the ex vivo laryngeal model of phonation. *Laryngoscope, 125,* 1414-1419. doi:10.1002/lary.25149

Purpose of the study. The purpose of this study was to develop a methodology for utilizing ex vivo phonation methods in animals as a foundation for future possible ex vivo phonation methods with human larynges.
Method. Nineteen male mongrel canines between the ages of 2-6 served as research participants. The canines were administered with general anesthesia and were supported by mechanical ventilation following tracheostomy. Temperature and hydration were controlled externally. The larynx was gradually detached from the body. A cuffed endoctracheal tube was placed at the level of the sternum to provide controlled airflow. Varying perfusion solutions and techniques were utilized to simulate oxygenated blood flow to laryngeal tissue. Electrodes were attached to the extant recurrent and superior laryngeal nerves (RLN, SLN). Phonation was attempted every five minutes as researchers heated and humidified 500 to 700 mL/min of airflow, stimulated the RLN and SLN, and adducted vocal folds.

Results. Through many experimental phonatory trial attempts, the most consistent longterm ex vivo phonation was obtained by reperfusing the excised larynx with whole (noncitrated) blood delivered in a pulsatile flow manner. Strong phonation resulted after physiologic cardiac output was provided with a flow switch. Strong, replicated neuromuscular stimulation and phonation was observed following reimplantation of the larynx to the femoral artery of the same animal.
Conclusion. Ex vivo larynx phonation is viable and replicable, which opens another experimental methodology. Ex vivo larynx phonation could be adapted to aid in organ preservation for larynx transplantation or reinnervation procedures.
Relevance to the current work. The current work compares various phonatory methodologies, including ex vivo, in vivo, and in vitro phonation studies, and acknowledges viable outlets of application for each.

Jiang, J., O'Mara, T., Conley, D., & Hanson, D. (1999). Phonation threshold pressure measurements during phonation by airflow interruption. *The Laryngoscope, 109*, 425-432. doi:10.1097/00005537-199903000-00016

Purpose of the study. Research methods to quantify (PTP) pose challenges that include variability across participants and relying on either an estimate or invasive tracheal puncture. This study attempted to more accurately estimate PTP in humans at different dB levels of vowel production. The PTP values were compared between humans with and without dysphonia to note patterns of change.
Method. A total of 24 patients were included in this study, 11 in the control group and 13 individuals with dysphonia secondary to vocal polyp. Prior to the human experiment, researchers studied pressure and flow values in a laryngeal model with and without a vocal tract. Constant values of pressure and flow were sent through a benchtop apparatus, and both subglottal and supraglottal pressures were measured around a flow interruption device, serving as a model for the glottis. Human subjects were required to hold a tone while wearing a custom mask which covered the nose and mouth. This mask recorded pressure, flow, and intensity. The PTP was estimated by taking a difference between an approximate subglottal pressure value and pressure at the level of the vocal tract after the airflow was interrupted. These estimated values were compared to one of the patients with normal laryngeal functioning, but also had a tracheotomy for other reasons.
Results. The PTP values for participants from the control group were averaged at 2.38, 2.67, and 2.98 cmH_2O at intensity levels of 75, 80, and 85 dB. The PTP values for the dysphonic group were averaged at 4.79, 5.85, and 7.37 cmH_2O at 75, 80 and 85 dB. After statistical analysis, these differences were found to be significantly different, and representative of theoretical disordered PTP levels.
Conclusions. In patients with vocal fold polyps, significant increase in pressure is required in order to sustain high intensity phonation at all three intensity levels measured. The new airflow valve, which interrupted airflow in the face mask, was reasonably accurate and more clinically functional when estimating PTP values in both healthy and disordered larynges.
Relevance to the current work. The PTP parameter is a prognostic indicator of vocal fold disorder, which is why the current work and this study both use it as the primary aerodynamic value.

Jiang, J., Raviv, J., & Hanson, D. (2001). Comparison of the phonation-related structures among pig, dog, white-tailed deer, and human larynges. *Annals of Otology, Rhinology & Laryngology, 110,* 1120-1125. doi:10.1177/000348940111001207

Purpose of the study. The purpose of this study was to compare canine, pig, and deer larynges to human larynges in an attempt to find the most comparable model for human phonation. Prior to the study, little was known about deer larynx phonation, and pig models were beginning to gain evidence for its similarities in humans regarding mucosal thickness and histology.
Method. Excised larynges from human (n = 2), pig (n = 3), dog (n = 3) and white-tailed deer (n = 3) species were included as participants. Vocal fold length, height and stiffness, as well as range of thyroid cartilage rotation were observed. Length of membranous vocal fold and vocal fold height were measured in a resting state. Vocal fold height was observed laterally as a long needle was placed parallel to the vocal fold body. Stiffness was measured by noting the displacement of the vocal fold when a 98-gram weight was placed perpendicularly along the length of a vocal fold. Stiffness was measured in three conditions: elongated, neutral, and shortened. Angular range of the cricothyroid joint was measured with a 1,500 mg force across the joint in three conditions: toward, and against the natural pull of the cricothyroid joint, and neutral position. Prephonatory glottal configurations were also noted.
Results. Geometrical structures including thyroid lamina and cricothyroid muscle were compared via imaging. Cricothyroid joint rotation is significantly limited in deer larynges. Vocal fold lengths were comparable across species, but canine larynges were most similar to humans. Vocal fold heights and cricothyroid joint angles or rotation were comparable across humans, canines, and pigs. Lateral displacement was inversely related to vocal fold length in all larynges. Deer larynges were cost similar to human larynges in degree of lateral displacement in all three positions, while pig larynges demonstrated the smallest degree of lateral displacement in all positions. Audio recordings from animals at the San Diego Zoo were analyzed for frequency range: pig grunts and squeals occur between 100 and 450 Hz, while the measured dog bark was 150 Hz, and deer guttural grunts were 61 Hz.
Conclusions. Cricothyroid musculature and rotational mobility are comparable across human, pig, and canine larynges. Pig larynges are most comparable across the measured species to human larynges in measures of vocal fold thickness, structure of vocal fold cover and stiffness. Fundamental frequency and phonation range are additionally more comparable in pigs to humans. The pig larynx may prove to be superior to canine larynges as an excised phonatory model.
Relevance to the current work. The current work compares pig and rabbit phonation to other animal models. This article provides fundamental frequency ranges for pig phonation in live animals which can be compared to fundamental frequency values measured in the current work's research.

Jiang, J., & Tao, C. (2007) The minimum glottal airflow to initiate vocal fold oscillation. *Acoustical Society of America, 121,* 2873-2881. doi:10.1121/1.2710961

Purpose of the study. The purpose of this study is to introduce a new aerodynamic parameter, PTF, which is the minimum value of airflow where phonation is initiated. Authors additionally analyzed the utility of PTF within the body-cover model of vocal fold structure using a stability analysis.

Method. The authors utilized the body-cover model of vocal fold structure (Titze, 2000) to format a mathematical equation for vocal fold functioning during phonation. To account for vocal fold viscosity and elasticity, a mass-spring model with damping was applied. To account for mucosal wave, a linearized glottal area with a time delay and inferior to superior movement within the vocal folds was applied. A modified Bernoulli equation was utilized to account for glottal aerodynamics. Finally, a resistance constant and inertial constant were utilized to account for vocal tract loading.
Results. The PTF parameter is influenced by glottal shape and size. The PTF values will decrease with a small glottis or a long and narrow glottis. Tissue properties, including viscosity and mucosal wave velocity, additionally influence PTF values. Small PTF values correlate with low tissue viscosity. The PTF values increase with increased tissue damping. Sufficient tension needs to exist in larynx musculature to initiate phonation.
Conclusions. Phonation threshold flow can be mathematically estimated within the boundary between regions IV and V: $B^{*}=0$ and $K^{*}>0$. The PTF values can be reduced with decreased glottal area, reduced tissue viscosity, reduced mucosal wave velocity, increased vertical length of glottis, reduced prephonatory convergent angle, increased prephonatory divergent angle, or decreased vocal tract resistance. In excised larynges, authors have observed that PTF increases with increased elongation of vocal folds. PTF can be more readily measured in a clinical setting than PTP, and can arguably identify vocal fold pathologies more reliably than PTP.
Relevance to the current work. The current work measures and analyzes both PTF and PTP values in both pig and rabbit larynges. An understanding of variables that effect PTF from this article will aid interpretation of PTF values in the current work.

Kojima, T., Valenzuela, C., Novaleski, C., Van Deusen, M., Mitchell, J., Garrett, C., Sivasankar, M., Rousseau, B. (2014). Effects of phonation time and magnitude dose on vocal fold epithelial genes, barrier integrity, and function. *Laryngoscope, 124,* 2770-2778. doi:10.1002/lary.24827

Purpose of the study. The purpose of this study was to determine the effects of increased time and dosage of tissue vibration exposure produced during phonation on microbial parameters of the epithelial layer of the vocal folds. These microbial parameters include transcription of the vocal fold's junctional proteins, structural alterations, and functional tissue changes.
Method. One-Hundred New Zealand White breeder rabbits participated in the study. Rabbits were anesthetized and randomly assigned to 10 per group, nine experimental groups and a control group. The control group experienced adducted vocal folds with no induced airflow or phonation. Other groups experienced modal- intensity or raised-intensity phonation for 30, 60, or 120 minutes. One group served as normative data for transepithelial resistance of normal vocal fold epithelium tissue. Modal-intensity phonation included phonation with 59.60 dB intensity at baseline, 782 Hz, and 59.74 dB sustained phonation intensity. Raised-intensity phonation included phonation with 69.98 dB at baseline, 735 Hz, and 68.97 dB sustained phonation intensity. Thirty minutes following phonation, larynges were harvested. Half of the larynges from each group were analyzed for gene expression and functional tissue outcomes. One vocal fold was analyzed with quantitative real-time polymerase chain reaction experiments, while the other was used to identify transepithelial resistance immediately following the larynx extraction.

Results. Gene transcript level change was absent for 30- and 60-minute time doses of phonation. The 120-minute time dose during raised-intensity phonation resulted in downregulation of the occludin gene and E-cadherin gene when compared to the control and the modal-intensity phonation. The ZO-1 gene was significantly upregulated with a 120-minute time dose with modal-intensity and downregulated following a 120-minute time dose with raised-intensity phonation. Transmission electron microscopy analysis demonstrated significant desquamation within stratified squamous epithelial cells in the vocal folds, which increased in intensity as time and magnitude dose of phonation increased. Though insignificant, transepithelial resistance generally decreased within raised-intensity phonation groups.
Conclusions. The 120-minute time dose during raised- intensity phonation resulted in the most significant microbial changes to epithelial tissue. Tight junction proteins (occludin and ZO-1), which are important for protecting deep tissue from harmful pathogens, were downregulated in this condition. Advanced desquamation was observed earlier in the epithelium with raised-intensity phonation compared to modal-intensity phonation. Similar advanced desquamation was observed in modal-intensity phonation with the 120-minute time dose.
Relevance to the current work. The current work elicits periods of phonation in excised rabbit and pig larynges. This article elucidated that increased periods of phonation with rabbit vocal folds can affect genes and proteins found within the epithelial layer. Short windows of phonation will be implemented in the current research to avoid aversive tissue changes. In future stages of this research, changes in gene expression in the epithelium following chronic exposure to inhaled combination corticosteroid medications will be assessed.

Lucero, J., & Koenig, L. (2005) Phonation thresholds as a function of laryngeal size in a two-mass model of the vocal folds. *Journal of the Acoustical Society of America, 118,* 2798—2801. doi: 10.1121/1.2074987

Purpose of the study. The purpose of this study was to understand how parameters of phonation, namely onset, offset, and the relationship between vocal folds and airflow, may be affected by geometrical laryngeal size.
Method. A mathematical model for the larynx was used based on previous work by Lucero & Koenig. The model was scaled so that adult male configuration is β; adult female configuration is β= 0.72, and 5-year-old child configuration is β= 0.64. Masses and volumes of the larynx were scaled accordingly. Tissue stiffness, oscillation frequency, and a damping ratio were constant across all sizes. Oral airflow was simulated for the adduction-abduction pattern of the vowel-consonant-vowel cluster /aha/. Oscillation onset values were determined when flow amplitude exceeded 1 cm^3/s. Oscillation offset values were determined when flow amplitude decreased below 1 cm^3/s.
Results. Male flow demonstrated larger amplitude and lower fundamental frequency. As the size was reduced, flow amplitude additionally decreased as the glottal resistance increased. Oscillation frequency also increased as larynx size was reduced. A hysteresis phenomenon was noted in female and child models as the oscillation region became increasingly restricted with smaller size. With decreased β sizes, the subglottal pressure required to produce phonation onset (PTP) must be higher, unless the vocal folds are relatively more adducted or the vocal folds are more relaxed. Both phonation onset and offset values increase with decreased larynx sizes.
Conclusions. Smaller larynges have a more restricted phonation region due to a decreased glottal surface and decreased energy transference from airflow to vocal fold oscillation.

Relevance to the current work. The current work is establishing normative acoustic and aerodynamic values for excised rabbit phonation compared to other animal models (i.e., canine, porcine). The findings in this article that smaller larynges theoretically result in increased PTP values may be useful in interpreting data from the current work.

Mau, T., Muhlestein, J., Callahan, S., Weinheimer, K., & Chan, R. (2011). Phonation threshold pressure and flow in excised human larynges. *The Laryngoscope, 121,* 1743-1751. doi:10.1002/lary.21880

Purpose of the Study. This study evaluated whether PTP and PTF correlated with posterior glottal width changes, and to confirm the existence of hysteresis in human vocal fold tissue, noted by differing onset and offset PTP and PTF values.
Method. Nine human larynges were harvested within 24 hours' post-mortem, and were preserved in a sealed beaker immersed in phosphate buffered saline. Larynges were dissected to expose vocal folds and allow for adduction of arytenoid cartilages. Phonation experiments were additionally completed within 24 hours' post-mortem. Alternating current subglottal pressure, alternating current flow, acoustic, sound pressure level, and electroglottograph data were acquired at 5000 samples/second using DATAQ and WINDAQ software. Posterior glottal width measurements were controlled using plastic shims of varying thickness (0.5, 1, 2, 3 mm), and were compared to a non-shim condition. Five phonatory trials using each posterior glottal width condition was observed in each larynx. For analysis purposes, an algorithm was created to determine a 20-cycle window at 60% on the standard deviation curve.
Results. Ninety-eight percent of trial PTP and PTF onset/offset data were consistent with hysteresis modeling. Significant variability with onset PTP and PTF measurements within each larynx. The PTF offset measurements were significantly more stable than PTF onset data. A significant within-group variance in PTP and PTF values was observed when the glottal width was consistent. Positive correlation between PTF onset and posterior glottal width was indicated. The PTF onset and offset values were greater in male larynges than female larynges, secondary to an increased glottal area. The PTP values were not significant across genders.
Conclusions. The theory of hysteresis was validated in human larynx models when PTP and PTF onset and offset values were compared. Offset PTP and PTF values were more reliable than onset values. Canine larynges may be better than human larynges for studies observing the degree of adduction on PTP and PTF values because of smaller inter-subject variability and less patient-specific factors which would need to be controlled for in humans.
Relevance to the current work. Hysteresis is observable in human and canine vocal fold tissue, which will be avoided in the current work by selecting midpoint of phonation rather than phonation offset.

Maytag, A., Robitaille, M., Rieves, A., Madsen, J., Smith, B., & Jiang, J. (2013). Use of the rabbit larynx in an excised larynx setup. *Journal of Voice*, *27*, 24-28. doi:10.1016/j.jvoice.2012.08.004

Purpose of the study. The purpose of this study was to establish a methodology for eliciting reliable rabbit phonation, including dissection and excision of the larynx, mounting of the larynx on a modified bench apparatus, and collecting acoustic, aerodynamic, videokymographic, and electroglottographic data.

Method. Five excised adult New Zealand white rabbit larynges were used as specimens. Each larynx was dissected to expose the vocal folds, trachea, and arytenoid cartilages. In order to secure this view, extralaryngeal muscles and tissues, including the epiglottis, were also removed. Following dissection, the larynges were frozen and stored for a period ranging from 2 days to 9 weeks before they were thawed and used for data collection. These larynges were thawed by means of a room-temperature water bath. Larynges were mounted onto a custom-made apparatus, which was mounted onto a bench and attached to an air source. This apparatus included structures such as bilateral smooth rods, which adducted of the vocal folds. An elongation apparatus was also included, onto which a string was tied. This string was also sutured into the anterior commissure of the larynges. A luer lock helped secure the trachea onto the tubing. Five phonatory trials were performed for each larynx. Prior to reaching the larynx, air was passed through a humidifying system. Acoustic data was taken using a microphone, placed posterior to the arytenoid cartilages. Aerodynamic data was recorded by using a pressure meter and an Omega airflow meter, placed below the larynx. Videokymographic data of the mucosal wave was recorded by a superiorly-located high-speed digital camera, electroglottographic measurements were acquired using silver electrodes bilaterally at the level of the vocal folds.
Results. The PTP values were recorded at 16.50 +/- 1.24 cmH_2O and PTF values were recorded at 4.61 +/-0.41 L/min. Coefficients of variation for each parameter between canine and rabbit larynges were comparable in size, despite being different mean values.
Conclusions. This study demonstrates that rabbit larynges are a reliable specimen for excised laryngeal studies. Because rabbit larynges are histologically similar to the human larynx, vocal fold tissue change in the ex vivo rabbit larynx can provide useful information about the tissue change in the human larynx.
Relevance to the current work. This study established the general setup parameters that were included in the current work. It served as a feasibility foundation for the novel benchtop model used in this thesis.

Mills, R., Dodd, K., Ablavsky, A., Devine, E., & Jiang, J. (2016). Parameters from the complete phonatory range of an excised rabbit larynx. *Journal of Voice, 31,* 517.e9-517.e17. doi:10.1016/j.jvoice.2016.12.018

Purpose of the study. The purpose of this study was to adapt a previously used canine setup for rabbit phonatory range data collection utilization. The authors were additionally interested in the effect of various degrees of elongation on subsequent phonatory ranges.
Method. Seven white New Zealand rabbit larynges were used as participants, which were dissected and preserved. For data collection, larynges were mounted on an excised larynx benchtop set-up. An adapter was utilized to convert canine trachea diameter size to rabbit trachea diameter. Data were collected to include the complete phonatory pressure range (PPR), or the range from PTP to PIP for each larynx. Corresponding measurements were made for flow: PFR, PTF, and PIF. Following phonation onset, airflow was gradually increased in 0.25 L/min increments until PIP and PIF values were accomplished, which was determined by a lack of stable harmonic frequency structure and noise-like broadband spectra. Five seconds of phonation was sustained at each air flow increment. The PPR and PFR ranges were collected at five different elongation conditions: 0%, 5%, 10%, 15%, and 20% elongation conditions.

Results. The following parameters were determined to be statistically significant across elongation conditions: PFR, PTP, PPR, F_0 at PTP, F_0 at PIP, F_0 range, sound pressure level (SPL) at PTP, SPL range, vibratory amplitude (VA) at PTP, VA at PIP, and VA range. Differences in PTF values did not qualify for statistical significance across elongation conditions. Both PIF and PFR values decreased as elongation conditions increased. PTP increased with increased elongation conditions.
Conclusions. Phonatory range decreases with increased elongation of the vocal folds during phonation. The F_0 fluctuated across 330 and 810 Hz and PTP varied between 5 and 21 cmH_2O. One variable when collecting rabbit larynx data is the increased sensitivity involved when mounting the larynx, such as pressure imposed on arytenoid cartilages. Increased airflow and elongation of the vocal folds affects PTP, F_0, SPL, and VA.
Relevance to the current work. The current study is establishing normative PTP and PTF data within rabbit larynges to serve as a comparison when later studies analyzing histological changes in vocal folds will be performed. This article validates the efficacy of rabbit larynges for histological research.

Plexico, L., Sandage, M., & Faver, K. (2011). Assessment of phonation threshold pressure: a critical review and clinical implications. *American Journal of Speech-Language Pathology, 20,* 348-366. doi:10.1044/1058-0360

Purpose of the study. The purpose of this study was to review the available research on PTP, describe current clinical task elicitation procedures, and identify clinical variability from research-prescribed methodology.
Method. A literature search was performed on the FirstSearch and EBSCO search engines in August 2009. In order for the article to be reviewed, it needed to meet the following inclusion criteria: contain one of the following search terms—PTP, P_s and phonation, pressure measurement and phonation, lung pressure and phonation, and/or vocal fold oscillation onset; published between 1980 and 2009; written in English; and contained in a peer-reviewed journal. Articles that included indirect assessment of PTP were targeted. Exclusion criteria consisted of studies of animals, computational or physical models, alaryngeal speakers, laryngeal airway resistance, or mechanical airflow interruption techniques. Following this search, 24 articles met inclusion and exclusion criteria. An online survey was additionally conducted among 59 practicing speech-language pathologists (SLPs) and voice researchers to identify the utilization of PTP measurement in the clinic and research settings, as well as identify variables that are considered when assessing PTP.
Results. Across the 24 studies, a large variety of data acquisition equipment was used. Most study topics included hydration, PPE, and/or vocal fatigue. No study reported normative PTP values for English-speaking human subjects. Populations most often contained young adult women. No study evaluated children. The prescribed consonant-vowel sequence /pi/ was utilized in 67% of studies. Total number of syllables in a measured syllable train ranged between five and seven. Of the studies, 75% followed the prescribed 1.5 syllables/s rate. Various environmental and patient variables were considered inconsistently across studies. Of survey respondents, 59% reported that they did not collect PTP data for research or clinic purposes. Of those who did collect PTP data, 50% reported clinical utilization, while 75% reported research utilization. When collecting PTP data, 38% of respondents used a five-train sample, and 28.6% used a seven-train sample, while others varied between six-, nine-, ten- train

samples, or did not control the number of syllables. When asked about test stimuli, 57.1% of respondents used the syllable /pi/, while others used /pæ/, /pa/, or did not respond. When asked about syllable rate, 61.9% reported a rate of 1.5 syllables/s, while others estimated a rate of 3 syllables/s, did not control, or did not respond. When asked about accounting for F_0, 61.5% collected data at a high F_0. Of respondents, 19% controlled for room humidity when collecting PTP data. Of SLPs, 57% consider hydration; 57% take vocal training into consideration; 33.3% consider hormonal changes; 71.4% consider age; 61.9% consider presence of vocal fold pathology; 52.4% consider voice use prior to session; 47.6 consider vocal fold function, sex, and medication use; 42.9% consider history of smoking, laryngopharyngeal reflux (LPR), and time of day; 38.1% consider alcohol consumption, history of respiratory infections, and client profession; 33.3% consider history of asthma, and 23.8% consider native language.
Conclusions. The /pi/ stimuli has the strongest rationale. Research indicates that syllable-train length does not matter as long as three adjacent pressure peaks are used to calculate PTP. The parameter F_0 is strongly associated with PTP values and should be considered in PTP data collection. The nares could be occluded if the patient is unable to create a velopharyngeal seal when articulating the phoneme /p/. Hydration affects PTP values. Medications, ambient humidity, oral breathing, and menstrual cycle can affect hydration of vocal fold mucosa. Increased age may increase vocal fold viscosity. Vocal training can influence PTP values as trained singers may produce greater peak flow for particular lung pressures. Edematous pathologies (LPR, asthma, chronic obstructive pulmonary disease), smoking, and hormonal fluctuations affect F_0 and PTP values as mass and vibratory characteristics change. Drier relative humidity may increase PTP values.
Relevance to the current work. The PTP parameter is an important outcome measure in the current work that is indicative of vocal fold mucosal health. This article articulates the history of PTP values, summarizes evidence-based research that supports PTP theory, and attempts to establish a standardized protocol for clinically estimating PTP.

Regner, M., & Jiang, J. (2011) Phonation threshold power in ex vivo laryngeal models. *Journal of Voice, 25,* 519-525. doi:10.1016/j.jvoice.2010.04.001

Purpose of the study. The purpose of this study was to observe whether phonation threshold power (P_{th}), equated as PTF x PTP, was sensitive to physiological differences in the vocal folds that indicate the presence of a pathology. These pathologically sensitive parameters that were evaluated were increased posterior glottal gap, bilateral vocal fold elongation, and vocal fold lesioning.
Method. Participants included 30 canine larynges were harvested and preserved. The 30 larynges were divided into three groups: 10 were assigned to the posterior glottal width changes group, while 10 were assigned to a bilateral vocal fold elongation group, and 10 were assigned to have unilateral and/or bilateral vocal fold lesions. Larynges were dissected to expose the true vocal folds and mounted onto the benchtop apparatus. An anterior suture was secured to an anteriorly-placed micropositioner in order to provide tension to the vocal folds during phonatory trials. Within the posterior glottal gap sub-study, small metal shims were placed between the arytenoid cartilages. The width of these shims ranged between 0.0 mm to 4.0 mm and increased in size by 0.5 mm. Five phonatory trials were completed at each adducted width per larynx. For the vocal fold elongation sub-study, five elongations were created relative to each larynx: 0%, 5%, 10%, 15%, and 20%. Five phonatory trials were completed at each elongation condition per

larynx. For the vocal fold lesioning sub-study, 10 phonatory trials were completed as baseline functioning for each larynx. A soldering iron (850 degrees Fahrenheit) was used to burn a small area on the medial midpoint of one vocal fold, after which ten more phonatory trials ensued. A second, bilateral lesion was then formed on the second vocal fold for each larynx which was followed by ten more phonatory trials.

Results. For the posterior glottal width sub-study, a positive statistically significant relationship was found between P_{th} and posterior glottal width (p=0.005). For the elongation sub-study, a weak positive relationship was found between P_{th} and vocal fold elongation (p=0.003). For the lesion sub-study, statistically significant differences were found between averages of each treatment group and P_{th} (p<0.001).

Conclusions. The parameter P_{th} was identified as being sensitive to posterior glottal width. A floor effect was noted with posterior glottal widths below 1.5 mm, while a ceiling effect was observed above 3.0 mm, indicating that P_{th} is most sensitive to adductory changes between 1.5 and 3.0 mm. The P_{th} value was not strongly sensitive to changes in vocal fold length. Clinical measurement of P_{th} may prove helpful in evaluating the ability of the larynx to transduce subglottal energy, especially in laryngeal disorders where this energy conduction may be inhibited.

Relevance to the current work. This study explores a potentially helpful aerodynamic parameter, P_{th}, in which the ability of the larynx to transduce energy is quantified. The current work is evaluating laryngeal functioning using similar aerodynamic parameters, and it appears that the functionality of this parameter is not appropriate for the current work.

Regner, M., Tao, C., Jiang, J., & Zhuang, P. (2008). Onset and offset phonation threshold flow in excised canine larynges. *Laryngoscope, 118,* 1313-1317. doi:10.1097/MLG.0b013e31816e2ec7

Purpose of the study. The purpose of this study was to test the hysteresis hypothesis that values for onset PTF are consistently greater than values for offset PTF in an excised canine larynx model.

Method. Ten canine larynges were harvested and preserved. Dissection of the larynx exposed muscular process of the arytenoid cartilages and the true vocal folds. The larynx was mounted and secured onto a benchtop apparatus. An anterior suture was secured to an anteriorly-placed micropositioner in order to provide tension to the vocal folds during phonatory trials. Acoustic and aerodynamic data were recorded and analyzed. Phonatory trials included gradual and slow increase of subglottal airflow until phonation commenced. After phonation was observed, the airflow was gradually decreased until cessation of phonation. Three trials were completed without elongation of the vocal folds. Elongated phonation was then observed in three trials for each of the following elongation conditions: 5%, 10%, and 15%. This was repeated for every larynx. Both PTP and PTF onset and offset values were recorded and analyzed. Average offset and onset values for PTP and PTF were recorded for each larynx at each elongation condition. An onset-offset ratio was calculated for each trial.

Results. The mean PTF onset-offset hysteresis ratio for each phonatory trial was 0.795 +/- 0.116. All data were bound by a [0.515, 0.972] domain. Approximately 80% of the data fell within the hypothesized domain [0.707,1]. The mean PTP onset-offset hysteresis ratio was 0.876 +/- 0.0752. Both PTP and PTF onset values were higher than each trial's PTP and PTF offset values in every trial.

Conclusions. PTF onset and offset values are significantly affected by increased vocal fold tension secondary to elongation conditions, indicating that both values could help identify laryngeal pathology. Together, PTP and PTF can provide a greater description of laryngeal health. Other parameters, such as laryngeal resistance (pressure/airflow) and aerodynamic power (pressure x airflow) can further our understanding about laryngeal functioning.
Relevance to the current work. This article verifies the hysteresis theory within both PTP and PTF values, which phenomenon was avoided in the current research and work as the midpoint in phonation was analyzed instead of phonation offset.

Rousseau, B., Kojima, T., Novaleski, C., Kimball, E., Valenzuela, C., Mizuta, M., …& Sivasankar, M. (2017). Recovery of vocal fold epithelium after acute phonotrauma. *Cells Tissues Organs, 204,* 93-104. doi:10.1159/000472251

Purpose of the study. Based on previous work, this study is investigating the timing of epithelial barrier recovery of the vocal folds after being subject to phonotrauma through modal or raised intensity phonation.
Method. The participant population included 65 adult male New Zealand white rabbits. The rabbits were anesthetized throughout data collection. Participants were randomly divided into treatment groups of modal (59.5 dB) or raised intensity phonation (66.97 dB), or the control group. All larynges were harvested at 0 hours, 4 hours, 8 hours, 24 hours, 3 days, or 7 days after the in vivo experimentation. The larynx and trachea were exposed during dissection, and a tracheostomy was inserted to provide continuous airflow through the larynx. Electrodes were bilaterally placed on each cricothyroid muscle and cricothyroid membrane, which provided three seconds of electrical stimulation to the larynx, followed by seven seconds of rest. This elicited phonation continued for 120 minutes. A rigid endoscope allowed the vocal folds to be visualized and recorded during phonation. Acoustic data were also collected every 15 minutes. Upon excision of the larynges, the two vocal folds underwent an analysis using RT-qPCR to measure gene expression particularly of inflammation indicators.
Results. This study confirmed that epithelial responses in other body functions cannot be directly compared to those of the vocal folds because the vocal folds endure repeated trauma during phonation. Additionally, the COX-2 protein plays an important role in epithelial restoration after phonotrauma. There are significant differences between cell damage between the no-treatment, modal intensity phonation, and raised intensity phonation treatments.
Conclusions. The epithelial layer of the vocal folds displayed a dynamic response to environmental shearing and impact stresses during phonation. A recovery period of approximately 6 days was crucial to the ability of the epithelial layer to regenerate.
Relevance to the current work. Rabbits prove very useful in studies where an independent variable is manipulated prior to extracting the larynx. The rabbits were chosen for the current work as future research will contain a similar transitional structure from in vivo experimentation to excised benchtop work to microbiological tissue analysis.

Sahrawat, R., Robb, M., Kirk, R., & Beckert, L. (2014). Effects of inhaled corticosteroids on voice production in healthy adults. *Logopedics Phoniatrics Vocology, 39,* 108-116. doi:10.3109/14015439.2013.777110

Purpose of the study. The purpose of this study was to observe whether short-term repeated use of ICS affected the acoustic output in the voices of healthy adults, and whether there was a sex difference in the overall effects of ICS on voice functioning.
Method. Study participants included 30 healthy young adults between the ages of 18 and 30 (15 males, 15 females). All participants were screened for normal voice functioning and a negative history for smoking, asthma, and/or other respiratory disorders. A baseline of voice functioning was collected prior to ICS administration. Voice samples included eliciting three samples of three different vowels (/i/, /a/, /u/) for a period of 5 seconds. A connected speech sample was then recorded. Administration of ICS consisted of the patient inhaling a 500 µg of fluticasone propionate provided with a spacer attached to a metered-dose inhaler. One hour following administration of the ICS, another voice recording commenced. Over the span of 6 days, ICS medications were administered six times, and five voice recordings took place, including the baseline recording. Acoustic analyses were performed, observing the following acoustic measurements: F_0, formant frequency (F_1 and F_2), formant bandwidth (BW_1 and BW_2), long-time spectral analysis (LTAS), first spectral peak (FSP), and spectral tilt (ST).
Results. Significant main effect for F_0 and gender, with females having a higher F_0 than males. Inhalation of ICS treatment had no significant effect on F_0. Analyses of F_1 and F_2 demonstrated minimal changes of vocal tract resonance during vowel production. Analyses of BW_1 and BW_2 additionally displayed no statistically significant changes. Additionally, FSP values were sensitive to ICS data collection intervals, indicating that effects of ICS were more significant during running speech compared to isolated vowels. Finally, ST values were significantly lower with ICS treatment indicating hyper-adductory phonatory behavior. Changes in ST and FSP reversed within 24 hours post-ICS administration.
Conclusions. When controlling for comorbid effects by using healthy adults as participants, effects of ICS on voice production were significant within 6 days of ICS treatment initiation. Effects were mostly evident with running speech compared to sustained vowels. Negative changes were reversed with discontinuation of the ICS treatment within 24 hours.
Relevance to the current work. This article provides evidence that small changes in voice functioning are correlated with ICS usage alone. The effect of ICS usage on vocal folds of those with asthma is the long-term goal of the current research and work.

Swanson, E., Abdollahian, D., Ohno, T., Ge, P., Zealear, D., & Rousseau, B. (2009). Characterization of raised phonation in an evoked rabbit phonation model. *Laryngoscope*, *119*, 1439-1443. doi:10.1002/lary.20532

Purpose of the study. The purpose of this study was to observe the effects of phonation on the extracellular matrix of the lamina propria. The authors were interested in variables that would affect said phonation, including vocal fold closure and phonation intensity. This study specifically analyzes how airflow rate affects vocal fold closure and intensity in raised vs modal phonation *in vivo* rabbit models.
Method. Six New Zealand white breeder rabbits served as participants in the study. The rabbits were anesthetized, vitals were monitored, and rabbits were tracheotomized for the study.

Electrodes were placed bilaterally onto the cricothyroid muscles and cricothyroid membranes, to serve as cathodes and anodes for electrical stimulation. Compressed air was sent through a flowmeter and humidifier prior to reaching the rabbit larynx. A rigid endoscope was placed to secure video data of glottal closure and vocal fold positioning during phonation.
Results. Average phonation intensity for modal phonation was 54.19 dB SPL, while average phonation intensity for raised phonation was 60.31 dB SPL. Phonation intensity significantly increased during raised phonation. Average F_0 was 672.02 Hz during modal phonation, and 616.27 Hz for raised phonation; within subject differences were not statistically significant for F_0. Endoscopic images displayed a convergent glottis shape for both modal and raised phonation, though greater separation of the vocal folds was apparent during raised compared to modal phonation.
Conclusions. An increase in airflow rate resulted in increased phonation intensity. Endoscopic images confirmed an increase in amplitude during vibration in raised phonation models. The F_0 values did not differ significantly between modal and raised intensity groups.
Relevance to the current work. This study analyzed the effect of increased airflow on phonation intensity and F_0 levels in an in vivo evoked phonation model. The current work additionally utilizes rabbit larynges to simulate phonation, though in an excised model. The results from this article will aid in interpretation of data results in the current work as differing levels of airflow were provided in an attempt to elicit phonation.

Witt, R., Regner, M., Tao, C., Rieves, A., Zhuang, P., & Jiang, J. (2009). Effect of dehydration on phonation threshold flow in excised canine larynges. *Annals of Otology, Rhinology & Laryngology, 118*, 154-159. doi:10.1177/000348940911800212

Purpose of the study. The purpose of this study was to understand the effect of dehydration on vocal fold phonation by specifically evaluating the aerodynamic parameter of PTF.
Method. A total of 11 canine larynges were studied and split into experimental and control groups: eight larynges were placed in the dehydration-only experimental group, while two larynges served as control, and one larynx participated in a control and a dehydration phonatory trial. During dehydration trials, the humidifier was omitted from the benchtop setup and saline solution was withheld between phonatory trials. Within the dehydration-only phonatory trials, non-humidified air set to 25% relative humidity was directed through the larynges for a period of 10 seconds, followed by 3-second period of rest. Both PTP and PTF were recorded during the 10-second phonatory trial periods. A total of 23 cycles of 10-second phonation + 3-second rest periods were performed on each of the eight larynges. Following the 23 cycles, two of the larynges were maintained on the dry air until phonation ceased. For the control group, the same 23- cycle protocol with 10-second phonation followed by 3-second rest periods occurred with the addition of 100% relative humidified air during trials and saline sprays during resting periods.
Results. Significant differences were reported between initial and final PTF values in all dehydrated larynges across the 23 trials, but not in hydrated larynges. An average critical threshold of 543 seconds (approximately 9 minutes) was observed in the two larynges that maintained phonation on dry air. Additionally, PTF values significantly increased, by an average 373.4 mL/m, with time as each larynx was exposed to dry air.
Conclusions. Abnormally high PTF values may be indicative of desiccated surface epithelial tissue. Significant dehydration can lead to dysphonia, while hydration treatments may lead to easier onset of phonation.

Relevance to the current work. In the current research, we will be mindful of unintentional desiccation during data collection by noting the time elapsed during mounted larynx trials in order to eliminate variables that would consequently increase PTF measurements.

Witt, R., Taylor, L., Regner, M., & Jiang, J. (2011). Effects of surface dehydration on mucosal wave amplitude and frequency on excised canine larynges. *Otolaryngology—Head and Neck Surgery, 144*, 108-113. doi:10.1177/01945998101390893

Purpose of the study. The purpose of this study was to understand the effect of dehydration on vocal fold phonation and to quantify the mucosal wave differences using high-speed video technology (HDSI).
Method. Ten canine larynges served as participants in the study. They were dissected and preserved, then methodically defrosted immediately prior to data collection. The larynx was dissected to expose the true vocal folds and allow for ease in mounting. Vocal fold elongation was constant throughout the study. Two control and eight dehydration trials were conducted for each larynx. During dehydration trials, phonation was induced using non-humidified air set between 36-38 °C and 25% relative humidity. This air was directed through the benchtop apparatus at a constant pressure gradient of 20 cmH_2O until the larynges stopped phonating. Saline solution was withheld from the larynges during these phonatory trials. During regular phonation, the high speed camera collected 768 frames every 60 seconds. When phonation became relatively more irregular, 768 frames were taken every 10 seconds. During control trials, warm, humidified air set between 36-38 °C and 100% relative humidity was directed through the benchtop and larynges with the same pressure gradient for a period of 30 minutes. Saline solution was spritzed every 30 seconds onto the phonating larynx. During control trials, the high-speed video camera recorded 800 frames every 60 seconds for the 30-minute phonation period. During analysis, HDSI images of the larynx were split into quadrants, and mucosal wave characteristics of each quadrant were quantified using digital videokymography.
Results. Increased dehydration was correlated with decreased mucosal wave amplitude and frequency in most larynges. Dehydration was also strongly correlated with increased stiffness and viscosity measurements in the vocal fold tissue and mucosa. Measurements of amplitude over time while the larynx was phonating showed a significant decrease in the dehydrated phonatory trials when compared to the control. With increased dehydration PTP values were noted to increase. A significant negative correlation between level of dehydration and mucosal wave frequency was observed in all larynges in the experimental (dehydrated) group.
Conclusions. Dehydration has an inverse relationship with mucosal wave amplitude and frequency, as normal fluid balance in the vocal folds is altered.
Relevance to the current work. In the current research, we will be mindful of unintentional desiccation during data collection to eliminate variables that would consequently increase PTP and PTF measurements.

APPENDIX B

Dissection and Tissue Preparation

Materials for Dissection and Tissue Preparation: Pig and Rabbit Groups

- Stainless steel disposable scalpels (sizes 10, 11, and 15)
- Disposable plastic aprons
- Gloves (black nitrile powder free examination gloves)
- Dissection mat
- Gallon-sized plastic bags
- ThermoScientific Nalgene™ bottles (1000 mL, closure diameter 63 mm, low density poly-ethylene)
- Hemostats (4)
- Sutures (silk black braided 45 cm suture, 24 mm needle)
- Protective goggles
- Dissection table
- Phosphate-Buffered Saline (PBS) solution
- Red hazardous waste box (for scalpel and suture needle disposal)
- Sani-Cloth™ germicidal disposable wipes
- Lab sink
- Room temperature water
- Digital Caliper (UltraTECH™ no. 1433)
- Digital scale (Ozeri Model ZK14-S™)
- ThermoScientific™ Freezer
- Food-grade Refrigerator
- Styrofoam boxes
- Permanent Marker
- Cryogenic gloves
- Liquid nitrogen (provided by BYU Chemistry Store)
- Timer

Dissection and Tissue Preparation Protocols: Pig Group

Gross Dissection Protocol

1. Pick pig larynges up from Circle V Meat (609 Arrowhead Trail, Spanish Fork, Utah, 84660)
2. Deliver larynges to room 106 Taylor Building Annex (106 TLRA)
3. Put on protective goggles, a disposable plastic apron, and dissection gloves.
4. Lay two sheets of dissection mat on the dissection table.
5. Using a No. 10 stainless steel disposable scalpel, carefully remove all extralaryngeal tissue and structures, including the esophagus, extrinsic laryngeal

muscles, tendons, lungs, vascularization, innervation, glands, and fat. Leave approximately 60 mm of trachea, if possible.
6. Check the larynx for any damage to the superior 30 mm of the trachea, or thyroid, cricoid, and arytenoid cartilages.
7. Discard any larynx that appeared to have superior damage to the trachea or perforations in the thyroid, cricoid or arytenoid cartilages.
8. Briefly rinse the larynges under room temperature water to remove excess blood clots or tissue.
9. Place each larynx in its own gallon-sized plastic bag, or Nalgene™ bottle (if the larynx width is smaller than 63 mm).
10. Pour PBS solution in the plastic bag or Nalgene™ bottle until the entire larynx is immersed.
11. Dispose of the scalpels by rinsing them and placing them in the red hazardous waste box.
12. Using a permanent marker, write the date of dissection on the plastic bag or Nalgene™ bottle.
13. Wipe down all surfaces in the lab with disinfectant wipes.
14. Transport the larynges over to the BYU Chemistry Store to undergo flash freezing process with liquid nitrogen.

Flash Freezing Protocol

1. Immediately following gross dissection, transport the larynges in two Styrofoam boxes, timer, and extra plastic bags to the BYU Chemistry Store.
2. At the Chemistry Store, ask an employee fill both Styrofoam boxes with liquid nitrogen.
3. Place cryogenic gloves over hands and lower arms.
4. While wearing the cryogenic gloves, carefully place the closed Nalgene bottles into one of the Styrofoam boxes and begin a 10-minute timer.
5. In the other Styrofoam box, carefully place the plastic bags in the liquid nitrogen, and begin a 10-minute timer. The plastic bags should be open and suspended in the liquid nitrogen. If the plastic bags break, place the ripped bag in a new bag and continue to freeze.
6. When the timer ends, check each of the bottles and plastic bags for remaining PBS solution that has not frozen completely. If there is still liquid, submerge the bags for another minute. Continue to check each minute until the larynx and solution are completely frozen. Do not leave the bottles in the liquid nitrogen for longer than necessary as the plastic has been known to explode.
7. Pay for liquid nitrogen using McKay School of Education grant.
8. Once all of the larynges are frozen, ask a BYU Chemistry Story employee to discard the remaining liquid nitrogen. Place the larynges in the Styrofoam boxes, and transport them back to the lab.
9. Place all of the larynges in the ThermoScientific™ Freezer at -80°C until they are to be used for data collection. Larynges should be frozen for at least 24 hours before data collection.

**Note: Zip-ties were used in the bagging process to decrease the amount of PBS solution needed to fill the bag and fully submerge the larynx. The bag was folded around the larynx, and a zip-tie was placed around the bag. It was then tightened until the bag was securely folded around the larynx. Excess solution was then poured from the bag. After being zip-tied, the larynx was then put into a separate bag to facilitate suspension in liquid nitrogen during the flash freezing process. This method was only used once and abandoned shortly thereafter due to the difficulty in zip-*

tying the bags, as well as tissue damage occasionally induced from the zip-ties being too tight. When this method failed, we decided to use temperature-resistant plastic Nalgene bottles to immerse the larynges in PBS solution and freeze them. We also used Ziploc bags to immerse the larynx in saline, seal the bag, put it in another bag, and freeze the larynx this way. However, this way was not as efficient. Lab assistants would have to suspend the larynx in the liquid nitrogen by holding the bag, and although they used cryogenic gloves to protect their skin from being freezer burned, the process was uncomfortable and inefficient.

Fine Dissection Protocol

1. Remove frozen larynges from the ThermoScientific™ freezer and place in a room temperature water bath in the lab sink.
2. Leave the larynges in the water until fully thawed (approximately 60 minutes when in the plastic bags and 90 minutes in the Nalgene™ bottles).
3. Lay two sheets of dissection mat on the dissection table.
4. Put on protective goggles, a disposable plastic apron, and dissection gloves.
5. Using a No.10 stainless steel disposable scalpel, remove the superior portion of the epiglottis.
6. Using the same scalpel, make an incision approximately .5 cm above the thyroid prominence into the thyroid cartilage.
7. Continue to cut the thyroid cartilage bilaterally at a slight upward angle posteriorly.
8. Carefully cut away the muscles and fascia that connect the thyroid cartilage to the ventricular folds.
9. Leaving the arytenoid cartilage and true vocal folds intact, dissect away the ventricular folds using a hemostat and No. 11 stainless steel scalpel.
10. Carefully separate the fascia on the superior surface of the thyroarytenoid muscle from the true vocal folds and dissect away the fascia.
11. Remove any leftover tissue superior and posterior to the vocal folds to prevent vibration of tissue outside of the vocal folds.
12. Trim the inferior end of the trachea, leaving at least 30 mm of length for ease in mounting the larynx to the benchtop.
13. Tie a large knot to the end of the suture thread. Secure the suture needle with a hemostat and while providing digital pressure to the thyroid cartilage, insert the suture needle just above the anterior commissure of the thyroid cartilage.
14. Repeat the suture four times to secure attachment: the suture should not be able to rip through the cartilage.
15. Using the scale and the measuring device, measure the following of each larynx: weight (in ounces) before and after fine dissection, length and width of trachea, width of the thyroid cartilage, length of the thyroid cartilage from the prominence to the top and from the prominence to the bottom, length and width of the vocal folds, and the width of the thyroarytenoid muscle (width of the vocal fold to the inner surface of the thyroid cartilage). The sex of the pig or rabbit should also be noted if possible.
16. Immerse the larynx in a plastic bag filled with fresh PBS solution, and place in the refrigerator until mounted on benchtop. The larynx number should be written on the bag with a permanent marker.

Dissection and Tissue Preparation Protocols: Rabbit Group

Gross Dissection Protocol

1. Researchers at the University of Utah harvested the rabbit larynges used in this study from rabbits that were sacrificed for other research purposes.

Flash Freezing Protocol

1. Researchers at the University of Utah flash froze the rabbit larynges in PBS solution at the University of Utah facility.
2. The researchers then transported the larynges in a Styrofoam container with ice from the University of Utah to the Brigham Young University laboratory.
3. Researchers at Brigham Young University placed frozen larynges in ThermoScientific™ Freezer at -80 °C.

Fine Dissection

1. Remove frozen larynges from the ThermoScientific™ freezer and place in a room temperature water bath in the lab sink.
2. Leave the larynges in the water until fully thawed (approximately 30 minutes).
3. Using No. 15 stainless steel scalpel, remove upper thyroid cartilage to just above the false vocal folds..
4. Attach a hemostat to each of the two facia of false vocal folds. Spread the false folds apart using the hemostats.
5. Using No. 15 stainless steel scalpel, remove bilateral fat pads superior from false vocal folds.
6. Using a No. 15 stainless steel scalpel, make an incision at the anterior commissure of the false vocal folds.
7. Follow cut posteriorly toward arytenoid cartilages, cutting false fold from the facia of the true vocal folds.
8. Carefully cut away facia along the thyroarytenoid muscle and the vocal ligament.
9. Create an attachment mechanism through the thyroid cartilage using a surgical suture. Tie a knot at the end of the string, use a hemostat to guide the needle through the cartilage, and sew four loops through the cartilage.
10. Cut the needle off the end of the string, and place in red hazardous waste basket.
11. Place larynx in small cylindrical container and immerse in fresh PBS solution. Store in refrigerator until mounted on benchtop. The larynx number should be written on the container.

APPENDIX C

Data Acquisition

Materials for Acquisition:

1. Dell computer
2. Dell computer monitor
3. PowerLab™ data acquisition hardware (AD Instruments)
4. LabChart™ data acquisition software (AD Instruments)
5. Custom benchtop apparatus
    a. Tubing (Vinyl: 1½" outer diameter (OD), 1" inner diameter (ID); Clear Vinyl: 1⅛" OD, ⅞" ID; 1" OD, ¾" ID; ¾" OD, ½"ID; ⅞" OD, ⅝" ID; ⅝" OD, ½"ID; ½" OD, ⅜" ID; ⅜" OD, ¼" ID; 5/16" OD, 3/16" ID; 3/16" OD, ⅛" ID)
    b. Metal hose clamps (22 mm diameter, 25 mm diameter, 30 mm diameter)
    c. Screwdriver for tightening hose clamps
    d. Micropositioners, three (Model 1460, Kopf Industries)
    e. Micropositioner prong attachments, single prong and three prong (Kopf Industries)
    f. 20 cm aluminum foam-insulated custom pseudolung
    g. TheraHeat™ humidifier (Model RC70000, Smiths Medical)
    h. Distilled water
    i. Medical-grade air tanks (2) containing compressed, low-humidity air (30 psi, <1% relative humidity)
    j. Flow head meters (Model MLT300L, AD Instruments)
6. Physiological pressure transducer (Model MLT844, AD Instruments)
7. Sphygmomanometer (AD Instruments)
8. Syringe (25 cc/ml)
9. Pressure calibration block
10. Gauze (decreasing reverberation signals under the pressure transducer)
11. Teflon™ tape for sealing air leaks
12. Cable ties
13. Velcro™ for securing transducers during calibration and data collection
14. Custom tray for securing airflow transducer during data collection
15. Pneumotach Calibration Unit (MCU-4, Glottal Enterprises)
16. Audio Output Extension
17. Dynamic microphone (Model SM-48, Shure)
18. Bose™ Amplifier
19. Pulse transducer (AD Instruments)
20. Polygrip™ (sealing up air leaks in the supraglottis)
21. Sani-Cloth™ germicidal disposable wipes
22. Permanent Marker
23. AcuRite™ Hygrometer (Model 01083M)

LabChart™ Signal Acquisition Protocol

1. Power on the computer, (Dell™, bottom shelf of black storage unit), the monitor (Dell™), and PowerLab™ unit.

2. Open LabChart™ 8 Application; open the template entitled "Official Pig Template" or "Official Rabbit Template" depending on which species is participating in data collection.
3. Allow the application to run for 15 minutes before calibrating or collecting data.
4. Confirm settings under "Channel Settings." Three channels should be used: 1. Microphone, 2. Pressure, 3. Flow.
    a. For pig data, the microphone should be set at a sampling rate of 20k/s, with a range of 10mV. The mains filter should be activated. Pressure should have a sampling rate of 1k/s and a range of 50mV, and should be measured in millimeters of Mercury (mmHg). Flow should have a sampling rate of 1k/s and a range of 200mV with the unit of measurement being liters per minute (L/min).
    b. For rabbit data, the microphone should be set at a sampling rate of 20k/s, with a range of 10mV. The mains filter should be activated. Pressure should have a sampling rate of 1k/s and a range of 20mV, and should be measured in millimeters of Mercury (mmHg). Flow should have a sampling rate of 1k/s and a range of 200mV with the unit of measurement being liters per second (L/min).
5. Add a comment noting that channel settings have been set.
6. To begin data acquisition, press "start." At the onset of phonation, select the predetermined key for phonation onset. Once phonation has ceased, select "stop." Continue this pattern for all 15 trials of each larynx.

Microphone Signal:

- SHURE™ SM-48 microphone is placed posteriorly and superiorly to the larynx and is secured approximately 15 cm away from the glottis.
- The microphone signal can be played back if the signal in channel one is selected, the "Setup" drop down bar is clicked, "Audio Output" is clicked, and the source is identified as channel one. The "Play Selection" button may then be pressed and an audio signal is produced from the Bose™ amplifier.

Measuring Humidity:

- Record percentage of humidity at the beginning and end of the 15 trial period for each larynx.
- Hygrometer is located 50 cm above each larynx.
- AcuRite™ Hygrometer will show degree of accurate calibration.

Humidifier:

- Thera-Heat™ Heated Humidifier-Portex, by Smiths Medical™ sits below the benchtop apparatus.
- The level of distilled water within the humidifier should fall within the set maximum and minimum range.
- The humidifier should be powered on at least 15 minutes before data acquisition. This allows it to warm up the water.
- Extra noise from the humidifier can be muted by pressing the red mute button in the upper left- hand corner.

APPENDIX D

Tracheal Adapters for Rabbit

Below is the prototype for subglottal and tracheal adapters for small animal models. Image A depicts the tracheal adapter that was ultimately used for data collection. The white silicone prototype (Image B) includes a domed subtracheal space and tracheal mount that might better account for fluid dynamic principles in the benchtop model versus the current tracheal mount.

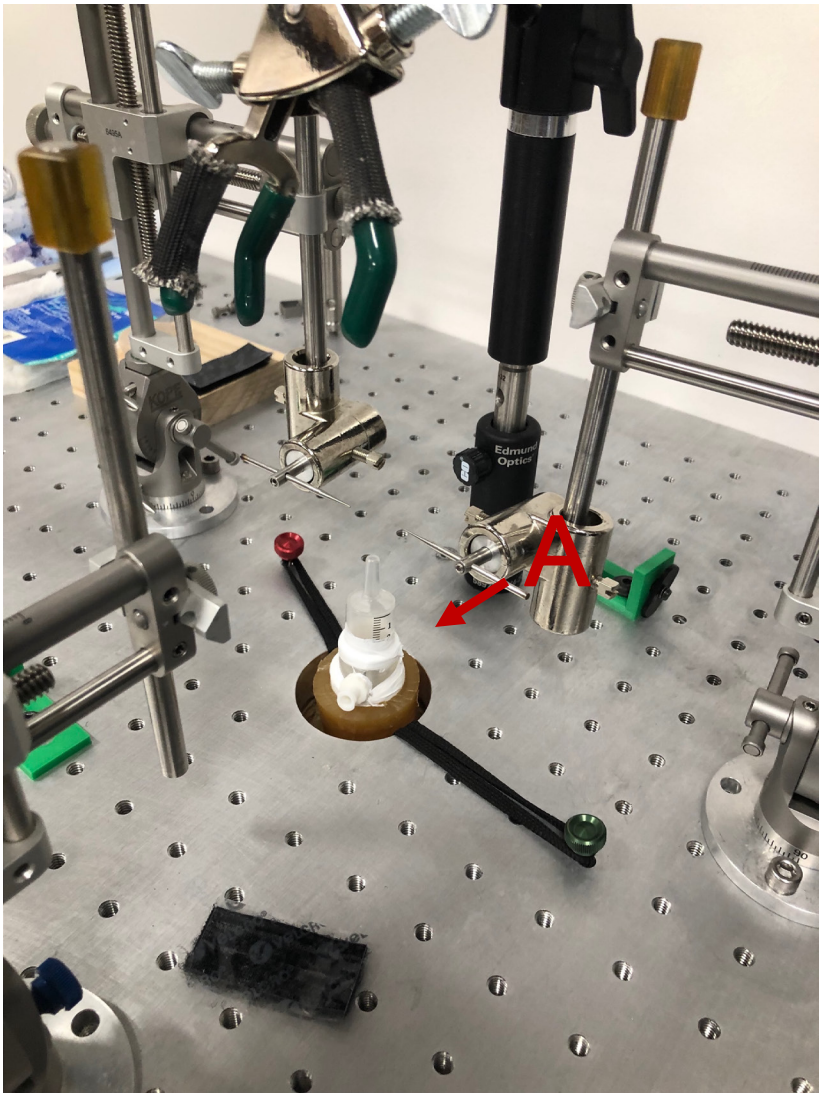

Image A. Tracheal adaptor mount used during data collection.

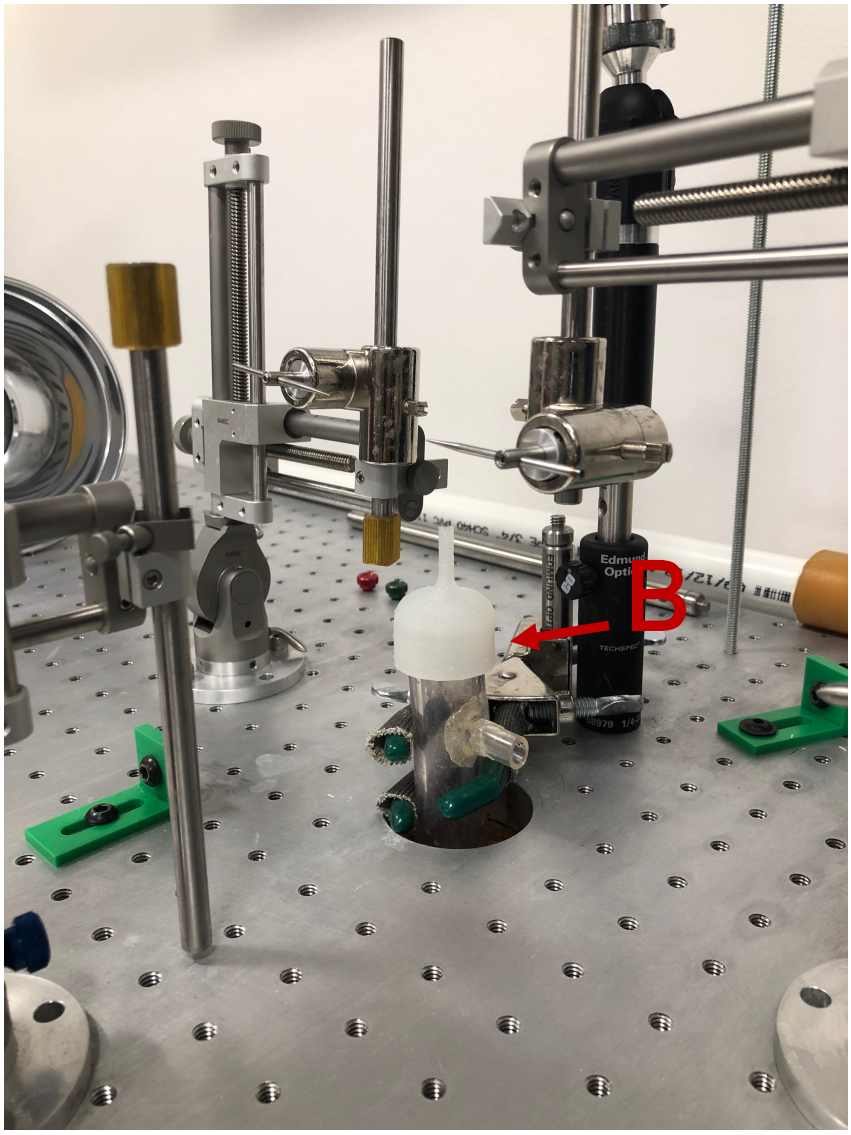

Image B. Tracheal adaptor prototype not used in data collection.

APPENDIX E

Pressure and Flow Calibration

Pressure Calibration

1. With the transducer being securely attached to the pressure calibration block and sphygmomanometer, unscrew the white stop cock (allowing the transducer to be open to atmospheric air pressure) and run data acquisition software for five seconds.
2. Stop the acquisition. Select the pressure signal; under the "Pressure" drop down box, select "Bridge Amp"; zero the signal; add a comment "Zero."
3. Pull the plunger to the 25 cc/mL limit; attach the syringe to the open input on the sphygmomanometer's stop cock.
4. Start the acquisition. While watching the sphygmomanometer dial, push the plunger into the syringe until the dial has reached 40 mmHg; hold this for five seconds.
5. Select the wavelength from the "zero" plateau up to half-way through the 40 mmHg plateau; under the "Pressure" drop down box, select "Units Conversion."
6. For "Point 1," click on a point of data that resembles zero pressure; press the right arrow. Manually insert "0" in the following text box and click the mmHg unit option.
7. For "Point 2," click on a point of data that resembles 40 mmHg of pressure; press the right arrow. Manually insert "40" in the following text box. Click "OK;" add a comment "40 mmHg."
8. Attach pressure transducer to the plug on the trachea mount on benchtop apparatus; make sure there is an airtight seal.
9. System is ready to record pressure data. Press "start" and "stop" to begin and pause data collection.

Flow Calibration

1. Remove tubes from either side of the flow head meter. While maintaining its position on the flow calibration tray, start the acquisition and run for five seconds.
2. Stop the acquisition. Select the flow signal; under the "Flow" drop down box, select "Spirometer"; zero the signal; add a comment "Zero."
3. Power on the pneumotach calibration unit; attach the flow head meter to the input on top of the unit via the blue seal piece.
4. Make sure that the flow rate is switched to "½" and the liter volume is at "1." Start the acquisition on LabChart™. Flip up the "Start" switch on the pneumotach unit while data is being collected on LabChart™. Allow pneumotach unit to run airflow for several seconds. It will automatically stop when complete.
5. Stop the acquisition when pneumotach turns itself off. Select the middle "exhalation" signal (up); click the "Flow" drop down box, select "Spirometry Flow".
6. Next to "Flow Head", find the MLT 300 L option; select "Calibrate"; insert 1 L as the injected volume; click "OK"; add a comment "1L."
7. Attach tubing from pressurized air tank to the right side of the flow head with the arrow pointing toward the humidifier, and then attach the tubing that runs to the humidifier on the left side of the flow head.
8. System is ready to record flow data. Press "start" and "stop" to begin and pause data collection.

APPENDIX F

Pig and Rabbit Phonation Trial and Error

Various methods were taken to achieve phonation. Both pig and rabbit phonation was best achieved with larynges whose vocal folds were adducted at rest, and whose vocal folds were on a level plane. A clean dissection with few punctures to the TA muscle also seemed to facilitate vibration in the pig larynx, however, this method was abandoned due to its invasive nature. Micropositioners were used to approximate the arytenoid cartilages and help with vocal fold adduction in both species. When adduction was maximized but air leakage through the posterior glottis (between the arytenoid cartilages) persisted, dental cream (poly-grip) was used to seal the posterior glottis off, prevent air leakage posteriorly, and facilitate directed airflow through the vocal folds exclusively. Stabilization of the larynx was in part achieved via a suture string tied to a positioner which moved posteriorly.

Air leakage at other areas of the set-up proved to be a common problem for each larynx. In addition to air leakage out of the posterior glottis, air could potentially leak out the bottom of the trachea, a hole in the trachea, or a loose tube in the airflow pathway. Air leakage at the base of the trachea was reduced by using a hose clamp to secure the edge of the trachea to the benchtop. If air leakage persisted, then Teflon™ tape was wrapped around the base of the trachea to the air flow tube. Some pig larynges had cuts in the trachea due to prior excision. These were either taped shut or sutured to provide an adequate seal for airflow.

Additionally, if a larynx was not phonating properly, all the tubes through the airflow pathway were checked for possible air leaks.

APPENDIX G

LabChart™ Installation and Training

LabChart™ with LabView™, a state-of-the-art data viewing and acquisition system was used in the current study. Since this system was new to the BYU voice research lab and few voice research labs around the country used the system, regular and intensive training and troubleshooting of the equipment set-up and software was required. An application and technical representative of the company was sent to the lab to provide a brief introduction to the system and demonstrate the many uses of LabChart™. However, increased training and education were necessary to properly understand the system and adapt it to the benchtop setup. Each member of the research team read and understood the detailed user's manual, and it was referred to frequently when adapting the system to the unique needs of the lab. Additionally, a PhD student at Purdue University who used a version of LabChart™ with similar equipment was also consulted when setting up the system for the benchtop. The research team frequently held video conferences with a software engineer of ADInstruments to ask questions about equipment purposes, parameters, the meaning and interpretation of signals seen in LabView™, as well as data analysis features in the system. The entire training, education, implementation, and completion of LabChart™ and the benchtop setup took approximately one year to finish.

APPENDIX H

Thesis Timeline

- 8/18 supervise construction and renovation of laboratory
  - Oversee shelf and cabinet carpentry, construction, and placement
    - Worked with BYU Carpentry to design/assemble
  - Move lab equipment
    - Worked with BYU Moving to transport equipment to the lab
  - High speed camera and computer, stroboscopy equipment
  - Experimental bench-top: assemble pseudolung, humidifier, flow head, high speed camera connection, pressure transducer connection
  - Move and set up data collection computer
  - Move dissection table
    - Scalpels, sharps container, sanitation aprons, sutures, scale, calipers, dissection mats, gloves, hemostats, plastic bags
  - Move craftsmen storage container
    - Tools: drills, drill bits, wrenches, screwdrivers, protective eyewear, Teflon™ tape, duct tape, vinyl, PVC, and plastic tubing, scissors, nebulizers, cable ties, superglue
  - Move other lab materials
    - Saline solution, nebulizers, dissection mats, tubing, pressure transducer
- 9/18 Oversee delivery of freezer
  - Coordinate delivery of ThermoScientific™ freezer
  - Set up freezer with alarm backups in place to notify of significant temperature drops, movement of freezer, history of temperature changes, door openings
- 10/18 Install LabChart™ Data Acquisition Hardware and Software
  - Bridge amp, spirometer, PowerLab™ connections
  - Conference calls with software and technical engineers of ADInstruments to understand data acquisition signals, calibration procedures, options for various flow heads
  - Adaptation of benchtop set-up for ADInstruments equipment
  - Adapt air flow tubing to secure pressure transducer to the unit
    - Collaborate with mechanical engineering professor to develop sound set-up without air leaks
    - Custom build pieces from materials in lab and at hardware stores
  - Develop custom silicone pieces for pig and rabbit attachment to the air flow system
    - 3-D printing
  - Adapt flow head to attach to spirometer, humidifier, air tank, and pseudolung
    - Order new flow heads for pig, rabbit, and human flow values

    - Attach microphone to PowerLab™
- Develop XLR-BNC cord connection
    - Learn how to read signals in LabChart™ software
    - Conference calls with software engineers to understand signals coming in
- 1/19
    - Oversee installation of computers from Education Computing Services (ECS)
        - Crashplan™ installation in 4 computers
        - Computer clean-up (deletion of old files and freeing up space on computers)
    - Set-up external back-up systems for each computer
- 2/19
    - further consultation with technical engineers of ADInstruments to understand signal acquisition, troubleshoot pressure transducer, adapt system to record airflow instead of fluid flow as the default
- 4/19
    - make plans for data collection
    - obtain pig larynges from slaughterhouse and prepare them for fine dissection and flash freezing
    - obtain rabbit larynges used for further study of the larynx itself and prepare for fine dissection of the specimen
- 5/19
    - continued consultation with technical engineers of ADInstruments to answer questions, establish firm understanding of system
    - read user manual to understand fine details of signal acquisition system
- 7/19
    - complete data collection for both pig and rabbit larynges
- 8/19
    - consultation with thesis committee member Dr. Christopher Dromey for audio signal understanding backflow, sinusoidal wave found in audio signal, filtering of the audio signal to decrease noise effect, and overall understanding of signal to aid in future analysis
- 9/19
    - complete Prospectus meeting in which optimal benchtop set-up as well as air flow system was discussed.
    - Segment data, clean data, and prepare all data for future analysis
- 10/19
    - collaborate with thesis committee member Dr. Christopher Dromey to develop MatLab program to analyze data
    - develop 3 different modifications to the program due to trial and error of data analysis (including introducing another analyzation system, audacity)

    - began data analysis
- 11/19
    - complete data analysis of PTP and PTF using modified MatLab and Audacity programs
- 12/19
    - use Praat to find true F_0 in each pig and rabbit trial
    - submit all data analysis for statistical analysis
- 1/20
    - complete first written draft of thesis
- 2/20
    - schedule and complete oral defense