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Brittany Tiffany Jones
Brigham Young University

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A thesis submitted to the faculty of
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Master of Science

Kristine Tanner, Chair
Christopher Dromey
Scott L. Thomson

Department of Communication Disorders

Brigham Young University

ABSTRACT

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Department of Communication Disorders, BYU
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The purpose of the present study was to examine the effects of external laryngeal vibration on voice function. The current study was based on a recent pilot study using silicone vocal folds that demonstrated a decrease in phonation threshold pressure (PTP; cmH_2O) when an external oscillation was applied to the vocal folds. Using a within-subjects experimental design, a custom external oscillatory device was fitted to the posterior portion of 12 excised pig larynges using a traditional benchtop phonation setup. For each larynx, phonation was elicited during 30 repeated trials, including 15 with and 15 without external oscillation. During the phonation trials, aerodynamic measures were collected. The outcome measure for this study was PTP, which has been established in the literature as being correlated with physiologic and self-perceived vocal effort. Furthermore, PTP is used routinely as an aerodynamic indicator of voice function, vocal efficiency, and the nature and severity of voice disorders. Although the aim was to quantify either positive (i.e., PTP decrease) or negative (i.e., PTP increase) effects of external oscillation on PTP, it was hypothesized that external oscillation would result in a reduction in average PTP values. The results of the study indicate that application of an external oscillatory device results in significantly lower PTP. These findings have important clinical implications for PTP signal acquisition and the potential use of external oscillation as a therapeutic tool to improve voice function.

Keywords: excised larynx, pig larynx, phonation threshold pressure (PTP), benchtop model

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Introduction

Voice disorders have significant adverse impacts on many individuals during all stages of life. Research indicates that approximately 30% of individuals in the U.S. experience a voice disorder during their lifetime (Roy et al., 2005). Furthermore, at any given time, approximately 6–12% of individuals have a current voice disorder. It is also believed that pediatric voice disorders are under identified (Theis, 2010). Voice disorders are associated with a negative impact on psychosocial wellbeing, physical health, and financial stability. There are many risk factors that can contribute to the presence of a voice disorder, including voice use patterns, high occupational demands, chemical exposures, frequent sinus infections, and esophageal reflux, to name a few (Roy et al., 2005; Roy et al., 2004; Roy et al., 2007; Thibeault et al., 2004). Voice disorders can be structural, functional, or neurological in origin, or might present as a combination of these (Stemple et al., 2020). Given the significant adverse impact voice disorders have on individuals, it is essential to be able to accurately diagnose and provide treatment for this population.

The thorough assessment of voice necessitates both quantitative and qualitative measures (American Speech-Language-Hearing Association, n.d.; American Speech-Language-Hearing Association, 2009; Rosen et al., 2004). The minimum standards for instrumental voice assessment were recently the subject of a subcommittee initiative by the American Speech-Language-Hearing Association (Patel et al., 2018). Among the committee's recommendations, aerodynamic measures were included as an essential component of all voice assessment protocols. In particular, onset pressure measures were identified as important contributors to the diagnostic process. For both clinical and research purposes, phonation threshold pressure (PTP, cmH_2O) has been the single most widely used aerodynamic estimate of vocal effort. Titze (1988)

has defined PTP as the minimum lung pressure required to initiate and sustain vocal fold oscillation. In humans, PTP values traditionally represent an estimate of subglottic pressure at the onset of phonation, acquired through a sequence of consonant-vowel productions (Smitheran & Hixon, 1981). Unfortunately, PTP poses some challenges in the acquisition of reliable signals, including those related to patient instruction and compliance, as well as those intrinsic to the measure itself (Plexico et al., 2011; Sundarrajan et al., 2015). Specifically, PTP, by definition relies on perturbation of an air stream and as such can vary naturally, all other variables being equal. An expanded discussion of these factors that influence PTP is included below.

Certain aerodynamic measures are valuable in diagnosing voice disorders. PTP, in particular, is not only valuable in the diagnosis of voice disorders but is also useful to track changes throughout treatment. A number of factors influence PTP, including fluid viscosity, velocity of the mucosal wave, vocal fold thickness, glottal configuration, and tissue properties (Jiang & Tao, 2007; Plant et al., 2004; Titze, 1988). PTP decreases as the fluid viscosity of the vocal folds decreases because less energy is needed to oscillate the vocal folds (Chan et al., 1997; Plexico et al., 2011). PTP is also directly related to mucosal wave velocity. A decrease in the mucosal wave velocity will result in a decrease in PTP. Additionally, PTP can be increased by increasing the glottal width before phonation (Titze et al., 1995). PTP is inversely related to the thickness of the vocal folds. PTP increases as the vocal folds become thinner and tension and stiffness increase. PTP is also higher in individuals with polyps, nodules, edema, vocal fatigue, vocal fold dehydration, and benign mass lesions (Jiang et al., 1999; Lamb et al., 2020; Tanner et al., 2016; Zhuang et al., 2013). Due to the increased mass of the vocal folds, more pressure is required to initiate and maintain phonation. It is also believed that PTP overlaps to some extent

with vocal effort (Sivasankar & Fisher, 2002; Tanner et al., 2007). In laryngeal desiccation studies, an increase in vocal effort was observed along with an increase in PTP measures.

PTP, often quantified in cmH_2O units, can be measured directly and estimated indirectly. There are three primary ways that PTP is measured directly: a translaryngeal catheter can be inserted through the trachea via the nasal passages and through anesthetized folds; a percutaneous catheter can be inserted into the trachea; or a small balloon can be swallowed to use an intraesophageal catheter (Lofqvist et al., 1982; Plant et al., 2004; Plant & Hillel, 1998; Plant & Younger 2000; Plexico et al., 2011). Although direct measures of PTP do produce accurate measurements, these techniques are invasive. Typically, direct measures are only used in research and are not feasible clinically.

Based on the theory of fluid dynamics, Smitheran and Hixon (1981) developed an indirect technique to estimate PTP. They theorized that if the velopharyngeal, oral, and laryngeal valves were adjusted correctly, it would be possible to use oral pressure measurements to estimate subglottal pressure. When a voiceless stop-plosive vowel sequence is produced, just prior to the initial point of lip separation, the pressure in the oral cavity is equal to the subglottal pressure during the stop-plosive production (Plant, 2005). Traditionally, the syllable /pi/ is produced by the individual at a rate of 1.5 syllables/second in a seven-syllable train.

Research regarding phonation has been conducted using both in vivo and ex vivo models. In vivo research has used anesthetized mammals to study vocal fold dynamics. There are benefits and disadvantages to using an in vivo model. With the in vivo model, the larynx is provided with blood. Both neuromuscular and sensory components are functional and can be stimulated during research (Döllinger et al., 2011). When studying onset pressure in living animals, it is possible for the researcher to control for subglottal pressure, physiological

variables, and motion of the vocal fold tissues. Muscles are engaged and involved during the study, allowing for observation of their roles and effects on phonation. Electrical stimulation can be used to study neuromuscular function. Based on the above, in vivo animal studies offer additional unique information that may be translated to human voice function and clinical scenarios. However, there are also disadvantages associated with the in vivo model (Durkes & Sivasankar, 2015; Mizuta et al., 2017). In order to elicit phonation, an invasive surgical procedure requiring a tracheal puncture must be completed. In vivo research is very expensive, making it a less practical option for many researchers. Additionally, it is difficult to control other factors including hydration, adduction, stiffness, and lengthening of the vocal folds. Another inherent challenge associated with in vivo research is that animal subjects involved may experience mortality. It is common to only be able to complete the procedure once for each subject because mortality rates associated with tracheoesophageal puncture are high. Because of this, it is difficult to collect multiple data points. In vivo research has not been completed in larger animals. Animals involved in in vivo research must also be housed and cared for, which is even more difficult for larger animals. These extra requirements lead to increased costs and work requirements. Many research facilities do not have the means to house animals for in vivo research. Alternatively, ex vivo studies only require storage of the larynges. Even though there have been great advances in using in vivo models, the associated problems discussed previously still persist. As a result, this creates a need for a variety of models, including mathematical, silicone, and cellular and tissue work. Collectively, these models help us understand the biomechanical and physiological processes of the larynx (Alipour & Jaiswal, 2008).

An ex vivo research model was first used by Leonardo da Vinci, who found that by blowing air through the vocal folds of a cadaver, voice could be produced (Döllinger et al.,

2011). Ex vivo models provide easy access to the larynx, both physically and visually. Muscles, cartilages, and ligaments can be included in the ex vivo model to create more life-like surroundings. Research utilizing ex vivo larynges can provide valuable findings regarding phonation parameters and fluid dynamics. However, just like the in vivo model, there are disadvantages that accompany the ex vivo model. When using an ex vivo larynx, it is not possible to stimulate muscle contraction. However, it is possible to simulate muscle contractions using micropositioners on a benchtop setup to adduct the vocal folds. Another disadvantage of utilizing ex vivo larynges is that tissues will experience decay and atrophy over time. It is essential to provide frequent and consistent hydration to the tissues using a saline solution.

Due to the difficulty of acquiring human larynges, animal larynges are frequently used to study phonation. A variety of species have been utilized including dogs, pigs, sheep, rabbits, cows, tigers, and elephants (Alipour & Jaiswal, 2008; Herbst et al., 2012; Mills et al., 2017; Stevens et al., 2016). Numerous studies have been conducted to determine which species are most like human larynges to serve as a model when researching human phonation. Pigs, sheep, and cows have a larger epiglottis and arytenoids than humans (Luo et al., 2018). Their lateral walls are also higher. Both dogs and pigs have a similar oscillation frequency range to that of humans. However, the oscillation range of pigs, at 100-300 Hz, is higher than the canine range, making pig larynges closer to those of humans. Dogs, sheep, and pigs all have a two-layered lamina propria, whereas humans have a three-layered structure (Alipour et al., 2011). Although the pig larynx only has a two-layered structure, the composition of the superficial and deep layers of the vocal folds is extremely similar to the human larynx. Within the superficial layer of the lamina propria, the density of elastic fibers decreases and the density of the collagenous fibers increases near the vocalis muscle, which is similar to the makeup of the human vocal folds

(Hirano et al., 1983). The stiffness of pig larynges is also close to that of human larynges, in that the stiffness of the vocal folds increases as it nears the vocalis muscle. In addition, the stiffness is the greatest in the intermediate layer of the lamina propria and decreases deeper into the folds. It has been hypothesized that the similar composition of the superficial and deep layers of the vocal folds accounts for the large dynamic range exhibited by both pigs and humans. In addition to having a similar oscillation frequency range and stiffness to humans, the geometry of pig vocal folds is also comparable to human vocal folds (Stevens et al., 2016).

Although pigs have been determined to be the model of choice when researching phonation, they still exhibit differences when compared to human larynges (Alipour & Jaiswal, 2008; Alipour et al., 2013). For example, the anterior portion of the pig vocal folds is lower than the posterior portion, sloping downwards at a 45˚ angle (Stevens et al., 2016). The superior vocal folds in pigs vibrate. There are also differences in sound pressure level, PTP, and subglottal pressure. It is important to be aware of and consider these differences when applying findings to human phonation. Despite these differences, pigs are still the preferred model to study phonation due to how closely they resemble humans in fundamental frequency (F_0), vocal fold shape, vocal fold stiffness, and range of phonation (Jiang et al., 2001).

While there is general agreement regarding the value of PTP, it has its own limitations and is not always consistent. One of the biggest limitations is that PTP is an estimate. Without tracheal puncture it is not possible to know if measurements are accurate. In order to ensure estimates are as reliable as possible, researchers must rely on strict protocols and eliminate sources of bias. Furthermore, there is difficulty in acquiring the measure of PTP itself. In the collection of PTP in human subjects, variability can arise from clinician error, differences in following instructions, number of practice trials, and physical differences in individuals such as

lung capacity and oral mechanism differences. Measures of PTP will also not be accurate if there is any nasal airflow during the production or if the pressure is not held constant during the production. Research has found ways to address some of these specific concerns by training participants for phonation tasks, identifying the appropriate target pitches for the individual, and eliminating bias in signal acquisition and analysis. However, even the most highly trained researchers have documented inherent variability in the measure of PTP. Although ex vivo models eliminate the variables involved with human subjects, there are different extraneous variables. Examples of these variables include examiner training error and bias. Additionally, differences in larynx dissection, preparation, mounting, and storage can result in variability in PTP measures. PTP can show variability from larynx to larynx and even from phonation trial to phonation trial. Even if all extraneous variables are controlled for, there is still variability of PTP within each individual or model. Plant and Hillel (1998) found that PTP measurements can vary up to 30-50% during production of a single phonation. Although PTP is accompanied by difficulties, it is still valuable in clinical and research implementation.

A final important consideration relevant to the current work are the limitations of any model of human phonation. By definition, PTP is always greater than phonation offset pressure; however, this difference can be exaggerated in modeling work. Variables related to phonation elicitation, signal acquisition, and the medium itself can result in inflated PTP values relative to phonation offset. These factors could include the rate of airflow (i.e., if the supply of airflow is abruptly or gradually adjusted), the shape of subglottic tubing, and the mechanical adduction of the arytenoids without actual thyroarytenoid engagement, to name a few. These issues can cause non-linearities in the relationship between onset pressure and offset pressure (Titze, 1988; Titze et al., 1995). In a study by Jackson and Thomson (2021), it was found that PTP could be reduced

and so that it more closely approximated offset pressure. In this study, external oscillation was applied to a silicone vocal fold model. The frequency and amplitude of oscillation was manipulated to determine the effects on PTP. Analysis of the results indicated that an external oscillation device could lower PTP in a silicone model of phonation, causing PTP to be closer to offset pressure. Extending this work to an excised larynx benchtop tissue study is the logical next step in determining if external oscillation can reduce PTP. If successful, it is possible that an external oscillation might be used to reduce PTP, and thereby vocal effort, in humans with and without voice disorders. That said, differences in either direction (i.e., PTP increase or decrease) are both of interest to this line of research.

Current Problem and Purpose

As described previously, when the vocal folds begin to vibrate, they create a perturbation in the air stream moving from the lungs through the vocal tract and into the atmosphere. Several cycles of vibration are required before the acoustic signal becomes periodic, even in those with normal voices. The entrainment of periodic vibration is even more challenging in those with voice disorders. In the normal larynx, vibration quickly becomes periodic. This phenomenon has been likened to the vocal folds "warming up," which can be similar to, for example, the practice effect that can influence PTP acquisition in humans (Conroy et al., 2014; Plexico et al., 2011). The theory behind this study is that if an external vibration is applied to the vocal folds prior to voicing, there will be a mechanical transfer of vibratory energy to the vocal folds. This energy has the potential to (a) lower the pressure required to initiate and sustain vocal fold vibration, and (b) influence the random effects of airstream perturbation during the measurement of PTP. A pilot study completed by Jackson and Thomson (2021) tested this theory using silicone vocal folds. These synthetic vocal folds underwent a series of phonation trials. During each trial,

compressed air was routed through the vocal folds and a mechanical shaker was attached to the vocal folds. The mechanical shaker then vibrated cyclically at a selected frequency. During each phonation trial, aerodynamic measures were collected, including PTP. Based on the data that were collected, Jackson and Thomson concluded that as the frequency applied increased, the average onset pressure decreased. The data showed correlation values above 0.8 for the average onset pressure vs input frequency for low and medium amplitude settings. These fairly good correlation values led to the conclusion that onset pressure decreases toward the offset pressure with increased external oscillation frequency. The current study applies this same theory, as well as an external oscillation device, in order to reduce the amount of energy required to initiate phonatory vibration, therefore reducing measurements of PTP. By applying the external oscillatory device, there will already be energy and movement within the vocal folds. Thus, the impact of airflow may cause less perturbation, resulting in more consistent and decreased measures of PTP. Therefore, the purpose of this study was to examine the effects of an external oscillatory device on PTP in an excised larynx benchtop model.

Research Question

This study aimed to answer the following question: does external oscillation affect PTP (cmH_2O) in benchtop pig excised larynx phonation?

Method

All procedures involved in this study were completed in compliance with the Risk Management and the Institutional Animal Care and Use Committee at Brigham Young University (BYU). The food grade excised pig larynges used in the study were donated by a local butcher shop (Circle V Meats, Spanish Fork, UT). All larynges for this project were obtained from animals sacrificed for purposes unrelated to this study. All preparatory procedures, storage, and data collection relating to this thesis were completed in the John Taylor Building Annex (rooms 105 and 106) and the Chemistry Central Stockroom, room 126 in the Joseph K. Nicholes building, located at BYU.

Research Design

This thesis was conducted as part of a larger ongoing project that is being conducted by Kristine Tanner, PhD, associate professor in the Department of Communication Disorders at BYU. The design of the current study was a within-subject experimental design. The independent variable for this study had two levels: the presence or absence of external oscillation of the thyroid cartilage. The dependent variable for this study was PTP (cmH_2O).

Pig Larynges

Rough Dissection and Flash Freezing

The fifteen pig larynges used in this study were collected from a local butcher shop. The butchers preserved the cartilage of the larynx, the epiglottis, and the majority of the trachea to facilitate inclusion in the study. The larynges were obtained within 3 hours postmortem and immediately transported to the Taylor Building Annex lab at BYU. The larynges were kept in a Styrofoam™ cooler. Immediately upon arrival at the lab, the larynges underwent gross (i.e., rough) dissection. Figure 1 shows pig larynges prior to the gross dissection procedure. Using a

disposable scalpel, all extrinsic muscles were removed, in addition to the esophagus, and other external tissue. All larynges underwent inspection to ensure that the tissues, muscles, and cartilages involved in phonation did not appear damaged. Any larynges that appeared to have cartilage perforations, muscle damage, or superior damage to the trachea were discarded. After gross dissection was completed, each cartilage was individually placed in a plastic, cylindrical container or freezer bag. Each larynx was immersed in saline solution. Within an hour of gross dissection, the larynges were transported in a Styrofoam™ cooler to the Chemistry Central Stockroom on BYU campus. Once at the lab, the larynges underwent flash freezing, using liquid nitrogen. Flash freezing took between 7-15 minutes, depending on the size of the larynx. The larynges remained in the liquid nitrogen until all saline solution within the container or bag was frozen, indicating complete freezing of the encased larynx. The larynges were then transported in the Styrofoam™ cooler back to the Taylor Building Annex lab. There, they were stored in a ThermoScientific -80 degrees Celsius freezer until they were needed for data collection.

Fine Dissection

The larynges underwent fine dissection immediately prior to data collection. Two hours before initiation of dissection, the larynges were thawed by immersing the container or bag they were in within a warm water bath. Anatomical and weight measurements were taken for each larynx. Dissection was completed using hemostats, tweezers, dissecting scissors, and disposable scalpels. The thyroid cartilage was cut superior to the thyroid notch. The epiglottis and false vocal folds were removed to expose the true vocal folds. The trachea was cut below the cricoid cartilage, leaving 2-5 cm of trachea for purposes of mounting onto the benchtop setup. Figure 2 shows a larynx after completion of fine dissection. Each larynx was sutured on the thyroid cartilage, superior to the thyroid notch. This suture was used during data collection to aid in

supporting the larynx and to maintain a constant length of the vocal folds. Throughout the above process, each larynx was regularly sprayed with PBS solution (normal, isotonic saline with an antibacterial agent) to ensure tissue hydration and preservation. After completion of dissection, each larynx was immersed in PBS solution within a plastic bag and stored in a refrigerator until it was mounted on the benchtop for phonation.

Figure 1

Pig Larynges Prior to Gross Dissection

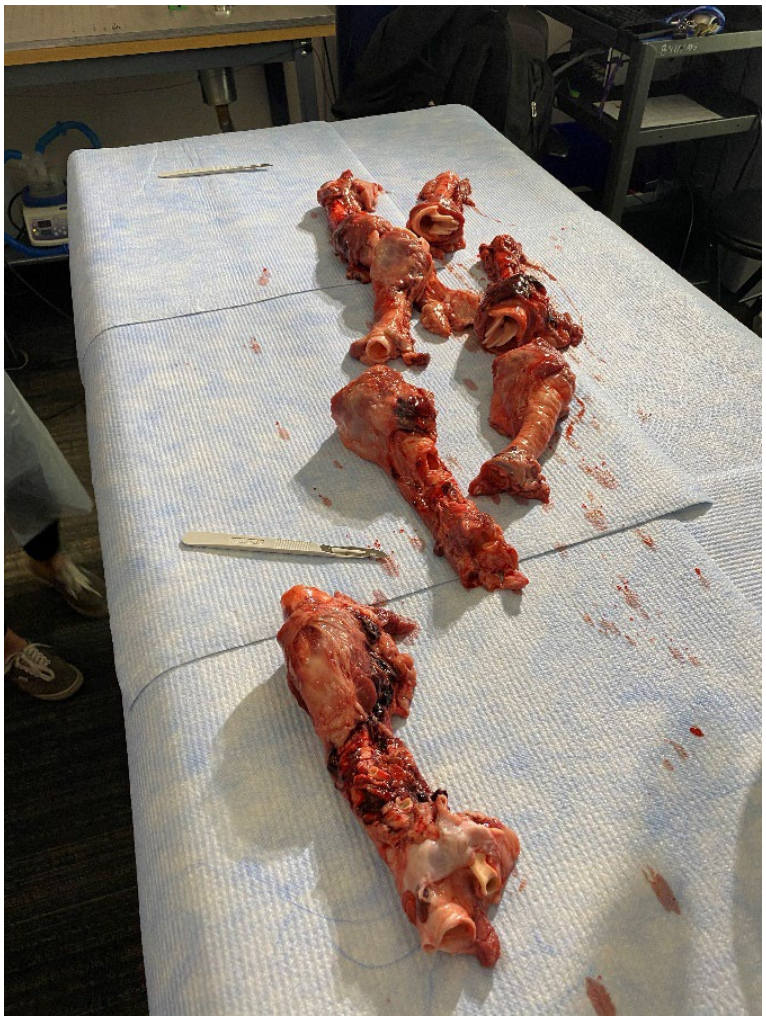
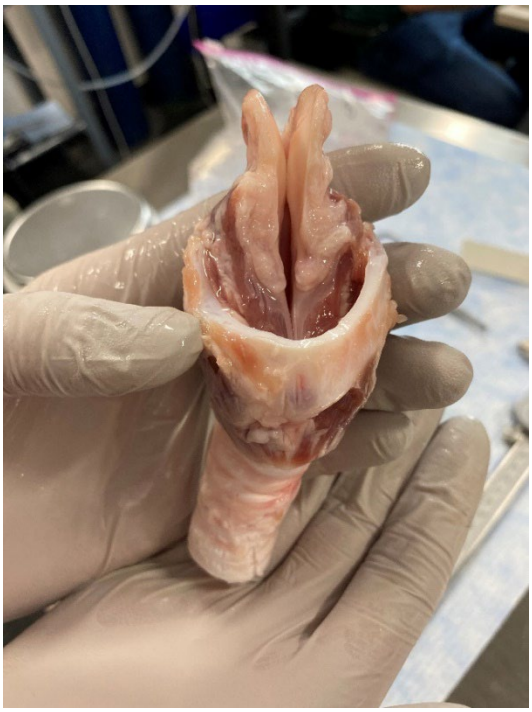


Figure 2

Anterior View of Pig Larynx After Fine Dissection



Operational Procedures

Benchtop Setup

This thesis used a benchtop model based on the one described in Jiang and Titze (1993). The setup consists of a medical-grade air tank containing compressed, low-humidity air (50 psi, <1% relative humidity). The air tanks used had pressure regulators and were securely chained to the wall in accordance with the Joint Commission on Accreditation of Healthcare Organizations and the Occupational Safety and Health Administration standards. Air flowed out of the tank through an adjustable flow meter, standardized at 50 psi, before passing through a series of tubes and flowing through a respiratory flow head (Model MLT300L, AD Instruments, Sydney, Australia). Graduated tubing was used to avoid any sharp angles that might impact airflow. Air was then directed through a TheraHeat Humidifier (Model RC70000, Smith Medical, Dublin,

OH) filled with distilled water to heat and humidify the air. Air flowed through suspended tubing into a 20 cm, aluminum, foam-insulated pseudolung. The pseudolung was custom made to reduce acoustic reverberation. Finally, the air was directed through plastic tubing that protruded from the top of the benchtop. This protruding tubing was where the larynges were mounted. A pressure transducer (Model MLT844, AD Instruments, Sydney, Australia) was attached into a subtracheal outlet that was drilled immediately above the benchtop surface. Three adjustable micropositioners surrounded the larynx, both laterally and anteriorly. The two lateral micropositioners consisted of three prongs used to support the larynx and assist in adduction of the vocal folds to produce phonation. The third micropositioner, located anteriorly to the larynx, was used to secure the suture running from the anterior portion of the thyroid cartilage. This suture was used to support the larynx and prevent it from tipping posteriorly. Figure 3 shows the benchtop setup and computer system.

Figure 1

Benchtop Set Up, Air Tanks, Humidifier, Power Source, and Computer System

External Oscillatory Device

In conjunction with BYU's Mechanical Engineering department, an external oscillatory device was designed to apply a constant vibration to the larynx during half of the phonation trials. An initial prototype was tried before making revisions, resulting in the current device. The prototype utilized a plastic clamp that was placed around the trachea and tightened using bolts to hold it in place. After initial trials, it was decided that the external oscillatory device should be applied to the larynx at the level of the vocal folds, instead of the trachea, in order to have the largest impact. It was also discovered that the plastic clamp did not wrap tightly around the larynx at the level of the vocal folds and would slip down during phonation trials. In the revised

design, this clamp was replaced with an adjustable arm and Velcro strap. When first using the prototype of the device, the motor and device were mounted to the benchtop surface anteriorly and laterally to the larynx. This placement of the device interfered with the mounting of the larynx. When the revised device was used, the placement was changed to sit posteriorly and laterally to the mounted larynx. This placement allowed for the adjustable arm to better access the mounted larynx at the level of the vocal folds. An additional modification that was made to the prototype device was the addition of a rubber dampening sheet between the device's motor and the benchtop surface. During initial trials, the vibrations of the motor vibrated the whole benchtop and resulted in excessive noise interfering with the audio signal. The rubber dampening sheet absorbed much of the motor's vibrations and resulted in a quieter setting for data collection.

As discussed, a revised version of the external oscillatory device was created after testing the prototype. The revised device was mounted to the benchtop posteriorly and laterally compared to the larynx. The device was adhered to a vibrationally dampening sheet of rubber that was then bolted to the table in order to minimize vibratory noise. A power source (Atten Instruments PPS3005S Program DC Power Supply) was used to power the device. Leads connected the power source to the control panel for the device. A 4-Pin Molex connector then connected the controller to the device. The device consists of an adjustable arm that extends to the larynx. A Velcro™ strap was attached to the end of the arm and then securely wrapped around the larynx at the level of the vocal folds, as shown in Figure 3. The switch on the control panel turned the device on and off. The knob on the control panel adjusted the speed of the motor within the device. A mini-USB cable connected the control panel of the device to a laptop. Arduino software (IDE) was used on the laptop to open the code NanoCode. The Serial Monitor

was also used on the laptop to monitor RPM, Hz, and % power of the device. While running phonatory trials, the knob on the control panel was used to adjust the speed of the motor until it was consistently running at 100 Hz. Oscillation at 100 Hz was within the range of application based on Jackson and Thomson (2021) and selected as the maximum F_0 that was feasible without an excess of noise that might influence acoustic signal acquisition using the current device prototype. When phonatory trials were completed for the mounted larynx, the device was then turned off using the switch on the control panel. The external oscillatory device and code were developed by Adam Jackson in the Scott L. Thomson, PhD lab in the Mechanical Engineering department at BYU. Figure 4 shows the control panel for the external oscillatory device. Figure 5 shows the external oscillatory device attached to a mounted pig larynx.

Figure 4

Control Panel for the External Oscillatory Device

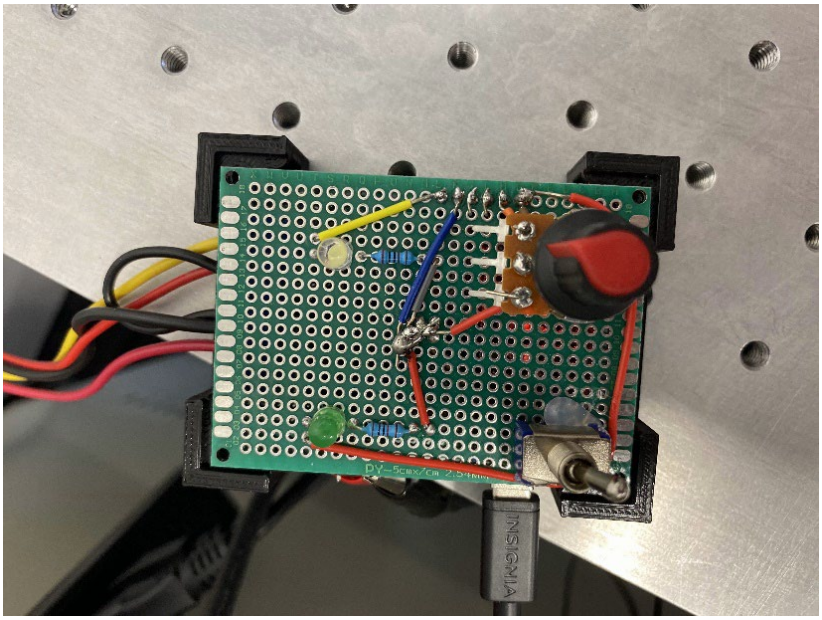
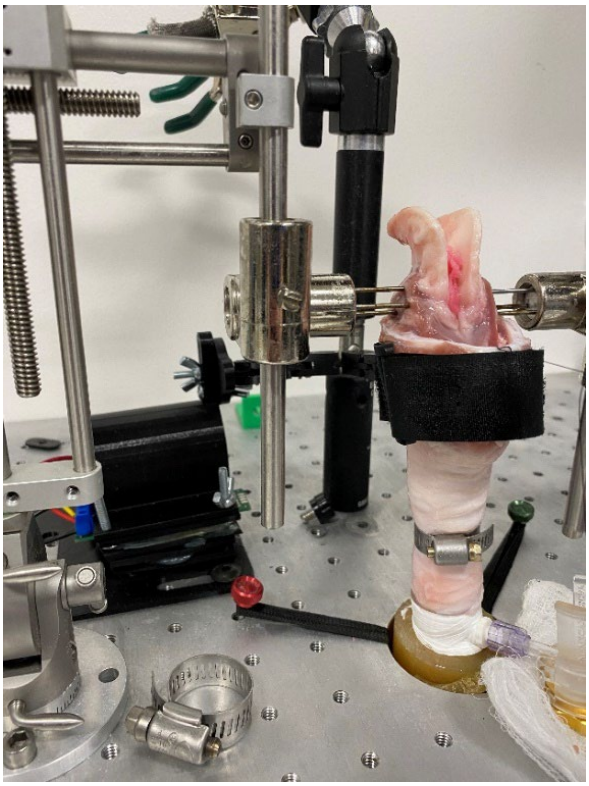


Figure 5

Anterior View of the External Oscillatory Device Attached to the Pig Larynx



Data Collection

Prior to data collection, the respiratory flow head and pressure transducer were calibrated, following standardized instructions. When it was time to run phonatory trials on a larynx, it was removed from the refrigerator and the bag of PBS solution. The larynx was then mounted securely on tubing extending out of the pseudo lung through the benchtop surface. A metal hose clamp was tightened securely around the trachea to ensure that no air would escape through the base of the trachea. As needed, denture cream (Poligrip Super Strong, All-Day Hold Denture Adhesive Cream) was applied between the arytenoid cartilages to ensure that air would not escape through that opening but would be directed through the vocal folds during phonation (see Figure 6). The lateral micropositioners were adjusted so that the three prongs were inserted into

the arytenoid cartilages (see Figure 7). They were tightened medially until the vocal folds were adducted and even with one another. The suture attached through the anterior thyroid cartilage was tied around the anterior micropositioner, which was then adjusted until the suture was taut. Each larynx underwent a total of 30 trials, 15 with the external oscillatory device running and 15 without the device running. Random assignment was used to determine whether the larynx would be phonated with the device running for the first 15 trials or the second 15 trials. The Velcro™ strap from the external oscillatory device was attached to the larynx for both the trials with the device running and without the device running.

Figure 6

Denture Cream Sealing Gap Between Arytenoid Cartilages

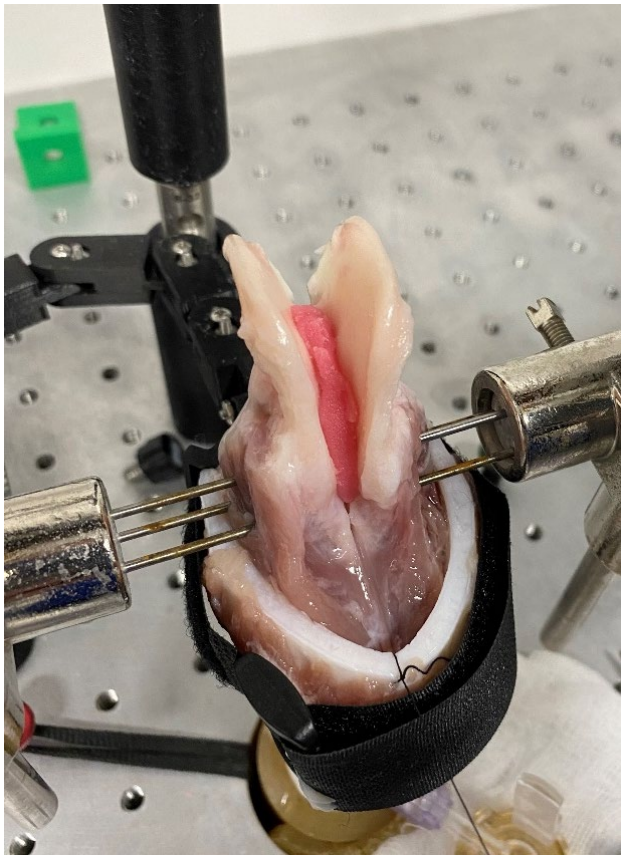
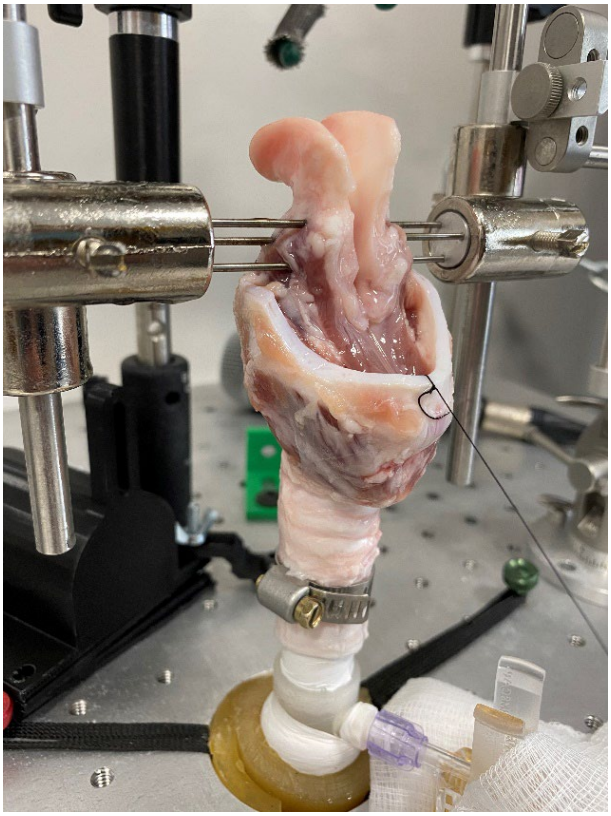


Figure 7

Mounted Pig Larynx

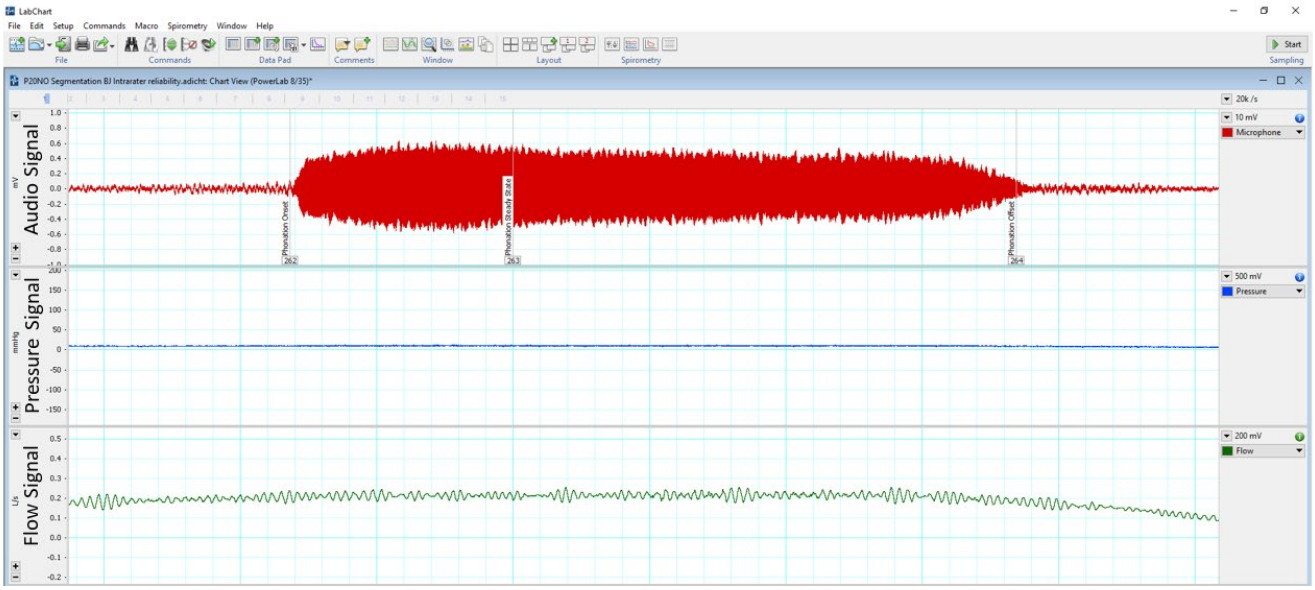


During data collection, air was supplied from the air tank by gradually twisting a knob until phonation started. Once periodic phonation began, the airflow was held constant. Phonation was sustained for 3 seconds before airflow was gradually decreased by turning the same knob. This was repeated 15 times sequentially while the external oscillatory device was running and then 15 times sequentially while the device was not running, or vice versa. The larynx was periodically misted with PBS solution after every three trials to ensure tissue preservation and eliminate dehydration effects. During each trial, pressure measurements were collected using a pressure transducer placed immediately inferior to the vocal folds. Flow measures were collected using the flow meter head. Figure 8 shows an example of pressure, flow, and audio data

collected during phonatory trials. Immediately after phonation trials, the remainder of the physical measurements were collected from each larynx.

Figure 2

Sample of LabChart™ Data for Phonatory Trials.



Data Segmentation and Analysis

After phonation trials, data for each pig larynx were segmented. For each trial, markers were placed to signify phonation onset, sustained phonation, and phonation offset using a method previously established (Prigmore, 2020). Phonation onset was determined acoustically and visually by listening to the audio signal of phonation and viewing the recurring oscillations in the acoustic signal. Data analysis was conducted using a custom MatLab program (MathWorks, Natick, MA) designed by Christopher Dromey, PhD. Analysis produced measures for onset pressure, onset flow, and onset resistance. For both data segmentation and analysis, inter-rater and intra-rater reliability measures were conducted for 20% of the pig larynges. The

larynges that were included in the interrater and intra-rater reliability measures were randomly selected.

Statistical Analysis

Raw PTP data were analyzed first using summary statistics, including mean, median, range, as well as visual inspection of the data distribution using box plots. A repeated measures analysis of variance (RM ANOVA) was performed using IBM SPSS Statistics for Windows, version 27 (IBM, Corp., Armonk, NY). Tukey's HSD was used for post hoc comparisons. The alpha level for statistical analyses was .05. Although data segmentation was a relatively automatic process, 20% of samples were resegmented by the initial examiner and a second examiner for purposes of intra-judge and inter-judge reliability. Average Pearson correlations of .96 and .85 indicated acceptable levels of inter-judge and intra-judge reliability, respectively.

Results

Average PTP for the 12 larynges when the device was running was 9.03 cmH_2O (SD = 3.29; range = 5.32 to 13.54). For those same larynges when the device was not running, average PTP was 8.09 cmH_2O (SD = 2.64; range = 4.07 to 11.90). Paired-samples *t*-tests were performed for PTP with and without the laryngeal device placement. Significant differences were observed for PTP between the two conditions, t (df = 11) = 2.421, p = .034. The effect size for these differences was calculated using Cohen's d, with a point estimate of .699, with a 95% CI of .051 to 1.322. Table 1 lists PTP measurements for each pig larynx. Figure 9 shows measures of PTP for both experimental conditions.

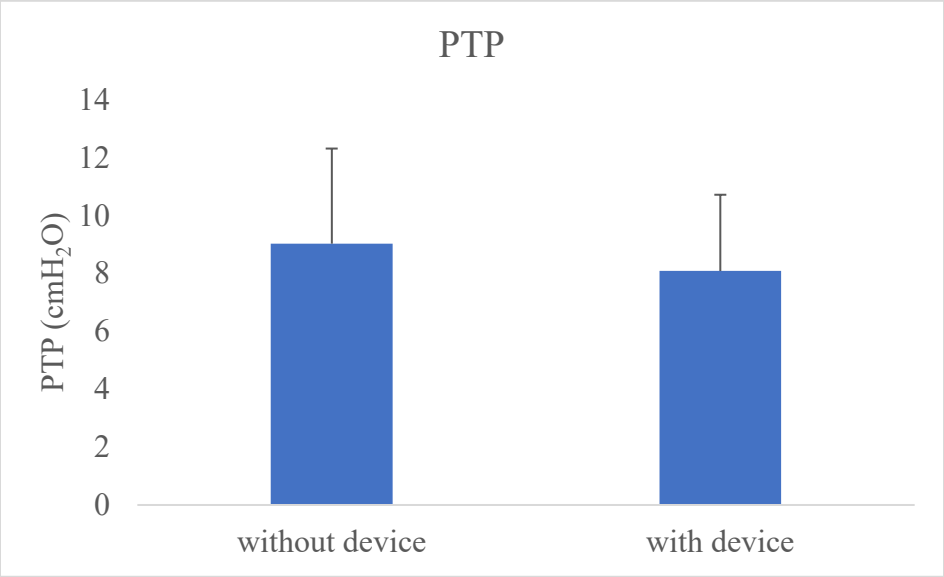
Table 1

PTP Measurements for Each Pig

Pig #	PTP with External Oscillatory Device Applied (cmH_2O) (SD)	PTP without External Oscillatory Device Applied (cmH_2O) (SD)	Order (Device applied for 1st 15 trials or 2nd 15 trials)
1	9.477 (SD = 0.541)	12.883 (SD = 0.871)	Device 2nd
2	8.145 (SD = 0.472)	7.397 (SD = 0.575)	Device 2nd
3	6.180 (SD = 1.300)	9.603 (SD = 1.353)	Device 2nd
4	10.664 (SD = 1.762)	11.666 (SD = 2.355)	Device 1st
5	11.734 (SD = 1.436)	13.541 (SD = 0.881)	Device 1st
6	5.717 (SD = 0.356)	5.896 (SD = 0.385)	Device 1st
7	7.039 (SD = 0.333)	8.183 (SD = 0.320)	Device 2nd
8	4.066 (SD = 0.974)	3.672 (SD = 0.477)	Device 1st
9	5.202 (SD = 0.256)	5.316 (SD = 0.619)	Device 2nd
10	6.898 (SD = 0.842)	7.056 (SD = 0.531)	Device 1st
11	11.902 (SD = 0.478)	12.957 (SD = 0.347)	Device 1st
12	10.026 (SD = 0.707)	10.225 (SD = 0.578)	Device 2nd

Figure 9

Measures of PTP for Both Experimental Conditions



Discussion

The purpose of the present study was to examine the effects of external laryngeal vibration on voice function. More specifically, a custom device (Jackson & Thomson, 2021) was fitted to the posterior portion of 12 excised pig larynges using a traditional benchtop mount setup (Jiang & Titze, 1993); phonation was elicited across 30 repeated trials, 15 with and 15 without applying external oscillation. The outcome measure for this study was PTP, which has been established in the literature as being correlated with physiologic and self-perceived vocal effort (Titze, 1988; Verdolini et al., 1994). Furthermore, PTP is used routinely as an aerodynamic measure of voice function, vocal efficiency, and the nature and severity of voice disorders (Plexico et al., 2011). Although the aim was to quantify either positive (i.e., PTP decrease) or negative (i.e., PTP increase) effects of external oscillation on PTP, it was hypothesized that external oscillation would result in a reduction in average PTP values. This hypothesis was based

on previous preliminary work by Jackson and Thomson (2021) and pilot trials in our own laboratory. A discussion of these observed effects, including their potential future applications in clinical settings, is provided below.

PTP is one of the critical components of voice assessment protocols (Patel et al., 2018). Abnormal measures of PTP can indicate vocal pathology. The measure of PTP is an important tool used to track changes in the voice throughout voice therapy (Jiang & Tao, 2007; Plant et al., 2004; Titze, 1988). Changes in PTP can reflect improvement or deterioration in the voice. PTP is also widely used to estimate vocal effort. It has been observed that an increase in PTP measures has been accompanied by an increase in vocal effort (Sivasankar & Fisher, 2002; Tanner et al., 2007). However, there is not a perfect overlap between PTP and vocal effort (Baldner et al., 2015). Although PTP and vocal effort are not synonymous, there is still substantial overlap in the physiologic constructs upon which both are measured (Sivasankar & Fisher, 2002; Sivasankar & Fisher, 2003; Tanner et al., 2010; Verdolini et al., 2002). PTP is still often used today in clinical diagnosis and treatment (Faver et al., 2012; Hoffman et al., 2019). It is also still used routinely as a measure in excised larynx studies (Liu et al. 2021; Palaparthi et al., 2019).

As previously discussed, this study was based on a theory developed and piloted in an earlier study (Jackson & Thomson, 2021). The theory is that applying an external vibration to the vocal folds will cause PTP to decrease. The pilot study was conducted using synthetic vocal folds and a benchtop setup. The synthetic vocal folds underwent a series of phonation trials during which compressed air was directed through the vocal folds. A mechanical shaker was attached to one of the vocal folds and vibrated cyclically at a set frequency. During the trials, aerodynamic measures were collected, including PTP. Based on the data, it was concluded that as the frequency applied increased, the average onset pressure decreased. The data reflected a

fairly good correlation between the average onset pressure vs. input frequency for low and medium amplitude settings. Both correlation values were above 0.8, allowing the researchers to conclude that onset pressure decreases toward the offset pressure with increased frequency. By applying this theory and external oscillatory device, the current study aimed to reduce the amount of energy required to start phonatory vibration, thus reducing PTP. When applying an external oscillatory device, there will already be energy and movement in the folds. Thus, the impact of airflow will not cause such an abrupt vocal fold disturbance, resulting in more consistent and decreased measures of PTP.

In the present study, average PTP was lower for the oscillation device group (M = 8.09 cmH_2O; SD = 2.64; range = 4.07 to 11.90) versus the control group (M = 9.03 cmH_2O; SD = 3.29; range = 5.32 to 13.54). We conclude from these results, given the conditions and parameters in this study, that device vibration reduced PTP by approximately 1 cmH_2O. These findings support previous pilot work in silicone models (Jackson & Thomson, 2021). It is essential to note that this was from an excised larynx study, where phonation was aerodynamically induced. In these models, there is no muscle activation and vocal folds are approximated mechanically to study voice function. Nevertheless, there is a strong literature base for the translation of excised larynx studies to human voice production. The effect size observed in this study would be clinically significant in both normal and dysphonic voices. It has been established that effect sizes in this range indicate substantial differences in the pulmonary effort required to initiate and sustain vocal fold oscillation. For example, only small reductions in PTP result in large reductions in the amount of effort needed to maintain intensity levels during speech. Each doubling of the subglottal pressure that exceeds threshold causes glottal source power to increase by 6 dB. Therefore, small reductions in subglottal pressure will result in

significant vocal effort reductions for the speaker (Titze, 1994). The results from this study demonstrate the potential for external oscillation to produce important physiological voice production changes during speech.

The main purpose of this project was to mechanically lower PTP. The hope for future application would be that it could be used therapeutically to train more efficient, less effortful voice production. For example, external oscillation might also be useful during assessment and trial therapy to quantify potential improvements in the behavioral aspects of voice production. It is possible that an external oscillatory device could also be applied therapeutically. A small, quiet, portable external oscillatory device would be used to facilitate voice, similar to other voice facilitating techniques such as a semi-occluded vocal tract, resonance, and humming. During voice therapy, the speech therapist helps the patient use a variety of techniques to find a better voice in particular contexts. As the patient progresses, cues and strategies are lessened, leading to generalization of the better voice to a wider variety of situations, including functional scenarios (American Speech-Language-Hearing Association, n.d.). Application of this external oscillatory device could potentially be used to stimulate a better voice. With practice, the patient would then be able to sustain during voice therapy, and eventually, more functional scenarios. This therapeutic treatment would ultimately result in an improved voice. There are multiple therapeutic applications of this device due to its potential portability and ease of use. This external oscillatory device could also be applied therapeutically during voice assessment. Use of this device could result in PTP measurements that are more representative of the voice since it may result in more stable measurement during the measurement process. This would be ideal for assessing more severe voices as it can be more difficult to quantify them using other assessment

measures. For example, it is difficult to use strobe tracking in assessing severe dysphonias due to their irregular vibratory patterns.

Despite the best efforts in research, this study is certainly subject to limitations. This preliminary investigation was limited to a small sample size of 12 pig larynges. A larger sample size may have resulted in more consistent and accurate data. Although the process of data collection was highly standardized, human error may also account for some limitations. Human error may have impacted larynx dissection, placement of the external vibratory device, and data collection. Another limitation of this study is that the external oscillatory device was only applied to ex vivo pig larynges. Therefore, it is unknown if the results of this study can be extrapolated to human larynges. Different species exhibit differences in laryngeal properties, which may impact the effect of the external vibratory device. Future research can be conducted using in vivo human larynges to determine if the effects of an external vibratory device would be the same as when applied to ex vivo pig larynges. Additionally, the device used in this study was a prototype based on several pilot devices and configurations in collaboration with Dr. Scott Thomson's laboratory at BYU. In future, a smaller, quieter device with a range of frequencies and amplitudes would be more suitable for human application. Although there are some possible limitations, this study was the first to document the positive effects of focal external oscillation on PTP in an excised larynx benchtop model.

Conclusion

The results from this study indicate that focal laryngeal oscillation has a positive effect on PTP, specifically lowering the pressure and thereby the effort required to initiate and sustain phonation. The current work was an important next step in extending the results from Jackson and Thomson (2021) to a tissue model. The current findings also suggest that external oscillation

might be useful as a portable therapeutic tool to improve voice production in clinical and nonclinical environments. Future research should examine the effects of external oscillation on different measures of voice function, including the optimization of the device itself and its application to human phonation.

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APPENDIX A

Annotated Bibliography

Alipour, A., & Jaiswal, S. (2008). Phonatory characteristics of excised pig, sheep, and cow larynges. *The Journal of the Acoustical Society of America, 123*(6), 4572-4581. https://doi.org/10.1121/1.2908289

Purpose of the study: The purpose of this study was to investigate the phonatory characteristics of pigs, cows, and sheep in order to determine which most closely resembles human phonation. They also explored the relationship between the F_0 and subglottal pressure of vocal fold vibration.

Method: This study utilized eight pig larynges, eight sheep larynges, and six cow larynges. After the larynges were collected from a local butcher shop, they underwent dissection to remove excess tissue and muscles. They were slow frozen and then stored in a freezer. Before data collection, the larynges were thawed overnight in a saline solution. The larynx was mounted on a benchtop setup. Sutures were used both to stabilize the larynx and to aid in adduction of the vocal folds. Electrode plates were placed on both sides of the thyroid laminae to obtain the EGG signal during phonation, which would later be used to determine the F_0. Air was provided to the system. It was filtered and then traveled through a flow meter before being heated and humidified. It then entered the larynx via tapered tubing. Subglottal pressure was monitored with a manometer. During trials, the researchers had control over the level of adduction of the vocal folds and the subglottal pressure. The trials for each larynx started with two pressure-flow sweeps for low, medium, and high adduction levels. Then the larynx underwent sustained oscillation trials to view the oscillation of the vocal folds in slow motion using a strobe light. For

each trial, data was collected on subglottal pressure, pressure amplitude, F_0, and flow rate.

Results: The pig larynx consists of a well-defined boundary separating the ventricular folds from the true vocal folds. The vocal folds within the pig larynx slant upwards posteriorly at a 40˚ angle. Pig vocal folds are distinct, whereas both sheep and cow vocal folds lack distinct boundaries. Cow vocal folds are longer (37+/-0.7 mm) and stiffer (18mm). The average oscillation frequency was 220+/-57 Hz for pigs, 102+/-33Hz for sheep, and 73+/-10Hz for cows. The average phonation threshold pressure was 7.4+/-2.0 cmH_2O for pigs, 6.9+/-2.9 cmH_2O for sheep, and 4.4+/-2.3 cmH_2O for cows.

Conclusions: Pig, sheep, and cow larynges can all be used as valid phonatory and aerodynamic models when researching human laryngeal functioning. However, it is important to consider the differences in size of the folds, size of the cricothyroid muscle, and the angle of the vocal folds.

Relevance to the current work: This article validated the use of pig larynges in research work comparing to human phonation. The current work will utilize pig larynges in preparatory research that will eventually be applied to human subjects.

Alipour, F., Finnegan, E. M., & Jaiswal, S. (2013). Phonatory characteristics of the excised human larynx in comparison to other species. *Journal of Voice*, *27*(4), 441-447. https://doi.org/10.1016/j.jvoice.2013.03.013

Purpose of the study: The purpose of this study was to better understand how pitch is controlled through vocal fold tissue vibrations and elasticity in different species.

Method: This study utilized six excised larynges from individuals between the ages of 70 and 89 years. Larynges were thawed and underwent dissection to remove

extraneous tissues and supraglottic structures. The larynx was mounted, and electrode plates were placed on both sides of the thyroid lamina. Airflow was humidified and heated before flowing through the larynx. Sutures pulled on the muscular processes of each arytenoid to control adduction and simulate lateral cricoarytenoid and thyroartyenoid muscle activity. Adduction levels varied between low, medium, and high. Each larynx underwent two pressure flow sweeps for low, medium, and high adduction. During the sweeps, flow rate gradually decreased or increased. Each larynx also underwent sustained phonations for low, medium, and high adduction. During trials, data was collected from the microphone, EGG, pressure transducer, and flow transducer.

Results: The average frequency of the human larynges was most similar to the frequency of canine larynges; however, the frequency range of human phonation was more comparable to pig larynges. This similarity between human and pig larynges is likely due to the comparable collagen content of the vocal folds.

Conclusions: The data collected in this study was comparable to other studies. Based on the similarities in frequency range, the pig larynx is a suitable comparison for the human larynx.

Relevance to the current work: This article provides evidence of the similarities between human and pig larynges. The current work will use pig larynges to research aerodynamic measures of human phonation.

Awan, S. N., Novaleski, C. K., & Yingling, J. R. (2013). Test-retest reliability for aerodynamic measures of voice. *Journal of Voice*, *27*(6), 674-684. https://doi.org/10.1016/j.jvoice.2013.07.002

Purpose of the study: The purpose of this study was to examine the intrasubject reliability of aerodynamic measures in typical subjects.

Method: The study consisted of 60 adults between the ages of 18 and 31 years. All participants were healthy and had a perceptually typical voice. Each participant was tested using the Phonatory Aerodynamic System (PAS). Testing took place at about the same time on two different days about a week apart. PAS is a hardware and software system. It measures and records aerodynamic measures including airflow, air pressure, expiratory volume, vocal intensity, maximum sustained phonation, and F_0. Participants held the PAS facemask tightly against their face to cover their mouth and nose. An intraoral tube was inserted into the participant's mouth. Each participant completed four PAS protocols in a randomized order. During the vital capacity protocol, participants took the maximum inhalation, held their breath for a moment, and then exhaled maximally. They repeated this task three times and measures of expiratory volume were collected. In the maximum sustained phonation protocol, the participants took a deep breath and held the vowel /a/ for as long as possible while keeping a constant loudness and pitch. They completed this task three times and phonation time was measured. During the comfortable sustained phonation protocol, each individual held the vowel /a/ for five seconds at a comfortable loudness and pitch. They completed this task three times and measurements of mean expiratory airflow were collected. In the voicing efficiency protocol, each participant took a deep breath and produced the syllable /pa/ seven times on a single breath at a rate of 1.5-2 syllables/second. During this task, measures of peak air pressure, aerodynamic power, aerodynamic resistance, target

airflow, vocal intensity, and frequency were collected. All tasks were completed at a comfortable loudness and pitch while the participant was standing.

Results: The data showed a high level of mean test-retest reliability for aerodynamic measures when comparing the two testing periods. However, there were weaker ICCs for glottal power, MEA, and glottal efficiency. There were large intersubject standard deviations for glottal power, resistance, and efficiency. Intersubject variation was responsible for the majority of test-retest variations in aerodynamic measures.

Conclusions: Comfortable pitch and loudness can be used to acquire reliable aerodynamic and associated measures. Intersubject variability in aerodynamic measures make it difficult to differentiate between normal and disordered voices.

Relevance to the current work: This article addressed the variability experienced in intersubject aerodynamic measures and the need for more stable measures. The current work aims to develop a more consistent means of collecting PTP.

Barsties, V. & Latoszek, B. (2020). Treatment effectiveness of Novafon local vibration voice therapy for dysphonia treatment. *Journal of Voice*, *43*(1), 160.e7-160.e14. https://doi.org/10.1016/j.jvoice.2018.05.009

Purpose of the study: The purpose of this study was to determine the effects of a Novafon sound wave appliance on aerodynamics, acoustics, multiparametric indices, and self-evaluation measurements and its effectiveness in treating dysphonia.

Method: This study consisted of 22 subjects with an organic or nonorganic laryngeal pathology. Participants were divided into two groups. One group was the treatment group and followed the initial NLVVT program. The other group was the

control group and only used the voice exercises from the NLVVT program. Each participant completed the NLVVT program over the course of five weeks. The NLVVT program consisted of voice exercises including humming, chewing, lip-trill, tongue-trill, combination of tongue-trill and hand over mouth approach, and resonant voice. Each participant had a 45-minute session each week, during which they would complete the next step in the program. Each participant also practiced at home twice a day, for 10 minutes each. The voice exercises varied each week. The participants in the treatment group used the Novafon classic sound wave appliance during voice exercises. The intensity level varied based on their level of comfort. The device was placed on the thyroid lamina. Objective voice measurements were collected for multiparametric indices, aerodynamic measurement, self-evaluation, and acoustic measures.

Results: Based on the findings, the treatment group exhibited clinically significant changes after the NLVTT treatment in almost all outcome measures, including VHI, spectrogram analysis, and DSI. Although VHI did not exhibit significant improvements, it did reach normal values.

Conclusions: When comparing vibration therapy with voice exercises to voice exercises alone, voice therapy with voice exercises results in superior outcomes. Vibrational therapy could be a viable option for treating voice disorders.

Relevance to the current work: This article reports the benefits of using vibrational therapy on voice outcomes. The current study will implement vibrational therapy to determine its impact on aerodynamic voice measures, specifically PTP.

Birk, V., Döllinger, M., Sutor, A., Berry, D. A., Gedeon, D., Traxdorf, M., Wendler, O., Bohr, C., & Kniesburges, S. (2017). Automated setup for ex vivo larynx experiments. *The*

Journal of the Acoustical Society of America, 141(3), 1349-1359.

https://doi.org/10.1121/1.4976085

Purpose of the study: This study sought to automatize the experimental procedure when running phonatory trials on ex vivo larynges in order to decrease the procedural time and in turn minimize the effects of dehydration on laryngeal tissues. The study also aimed to modify procedural parameters to decrease the effects of manually modifying vocal fold adduction.

Method: An electro-mechanical device was used to mount and posture an ex vivo larynx on a stainless steel tube. A customized, computer controlled setup was developed and used to control for vocal fold adduction, vocal fold elongation, and P_s. Subglottal pressure was measured. A high-speed camera was used to record vocal fold motion. To simulate contraction of the cricothyroid muscle, an electro-mechanical device applied a predetermined force to the thyroid cartilage, resulting in tilting of the cartilage. Using a three-pronged applicator, the arytenoid cartilages were rotated to adduct the vocal folds. An interface-controlled air flow and pressure. Air was heated and humidified in order to prevent dehydration of tissues. Phonation onset was determined using technology. Methodological functionality was validated using four pig larynges.

Results: The study showed that the computer-controlled setup was functional. Both the subglottal pressure and F_0 increased as a result of the adduction and elongation procedures.

Conclusions: The customized computer-controlled setup was able to control vocal fold elongation, vocal fold adduction, and glottal airflow. Using this system,

complete measurements for each larynx were completed in an average of four minutes, reducing dehydration effects on the tissues.

Relevance to the current work: As discussed in the article, the current study will also keep vocal fold elongation, vocal fold adduction, and glottal airflow consistent during data collection. This article also outlined methodology for precise identification of phonation onset, which may be used in the current study.

Chapin, W. J., Hoffman, M. R., Rieves, A. L., & Jiang, J. J. (2011). Comparison of labial and mechanical interruption for measurement of aerodynamic parameters. *Journal of Voice*, *25*(3), 337-341. https://doi.org/10.1016/j.jvoice.2020.01.004

Purpose of the study: The purpose of this study was to investigate the intrasubject precision and repeatability over time of mechanical interruption and labial interruption techniques.

Method: This study included 35 individuals, all of whom did not have a history of laryngeal or respiratory disease. Half of the participants completed the labial interruption task first and the mechanical interruption task second. The other half of participants completed the mechanical interruption task first and the labial interruption task second. Before each task, participants had a practice trial to become more comfortable with the device and maintain the appropriate SPL. In the labial interruption task, a PAS mask was held tightly to the face. The individual inhaled and then produced /pa/ 5-7 times. The participant was instructed to produce the syllables with equal emphasis on a single breath at a rate of 1.5-2 syllables/sec. The participants were given instruction and visual feedback to keep their SPL at 71 +/-2 dB. After the practice trial, the participant performed 5-7 labial plosives in 10 trials. In the mechanical interruption

trials, each participant produced the sound /a/ into a tube connected to the PAS. They held the phonation for 3-5 seconds and were given visual feedback to help them maintain a SPL of 72 +/-2 dB and a comfortable F_0. During each trial, the researcher manually caused inflation of the balloon valve five times to interrupt subject airflow.

Results: The coefficient of variation for mechanical interruption was 0.0995 for P_S, 0.127 for MFR, and 0.129 for R_L. The coefficient of variation for labial interruption was 0.102 for P_S, 0.147 for MFR, and 0.159 for R_L. The 95% confidence intervals of measurement repeatability for mechanical interruption were (-0.050, 0.072) for MFR, (-0.543, 1.832) for P_S, (-2.498, 10.528) for R_L. The 95% confidence intervals for measurement repeatability for labial interruption were (-0.018, 0.031) for MFR, (0.057, 2.442) for P_S, and (-3.267, 10.595) for R_L.

Conclusions: Although procedures eliminated all compounding variables, there were still variations in PS, MFR, and RL measurements for both mechanical and labial interruption techniques. Repeatability was comparable for mechanical and labial interruption. The coefficient of variation for RL was lower using mechanical interruption.

Relevance to the current work: This article provided evidence that even when all extraneous variables are eliminated, PTP measurements are still variable. The goal of the current study is to determine if implementing an external vibrating device will result in more consistent measurements of PTP.

Faver, K. Y., Plexico, L. W., & Sandage, M. J. (2012). Influence of syllable train length and performance end effects on estimation of phonation threshold pressure. *Journal of Voice*, *26*(1), 18-23. https://doi.org/10.1016/j.jvoice.2010.10.021

Purpose of the study: The purpose of this study was to examine if the number of syllables in the train impacts PTP measurements and determine if the initial and final syllables in the train are significantly different compared to middle syllables.

Method: Ten women participated in this study. Participants demonstrated their pitch range in order to determine their low, modal, and high pitches. A stroboscopic examination was completed to ensure that participants did not have any laryngeal abnormalities. A urine sample was collected to test for dehydration and to determine if the participant was ovulating. Each participant underwent a training to perform the PTP task that consisted of listening to prerecorded directions and practicing. Data was collected on frequency, loudness, air pressure, and airflow. The participant wore a PAS face mask and produced /pi/ five times in a smooth and connected manner. They repeated the task at low, modal, and high pitches. They repeated the same procedure with a seven-syllable train of /pi/.

Results: PTP measures differed significantly across both pitch and syllable position. PTP was significantly greater at the high pitch than the low and modal pitches. PTP was significantly lower for the first syllable than for the middle and final syllables.

Conclusions: There is no significant difference in PTP measurements in a five-syllable train versus a seven-syllable train. Performance end effects do not cause a significant impact on PTP estimates for low and modal pitches. For low and modal pitches, there is no difference in PTP estimates between the first syllable of the train, middle syllable, or final syllable. However, these findings do not apply to PTP estimates for the high pitch.

Relevance to the current work: The data presented in this article shows that PTP values collected at low and modal pitches are consistent regardless of if they are collected on the first, middle, or final syllable. In the current work, pig larynges will be phonated at a modal pitch. Based on the findings of this article, the current work will analyze PTP measures collected on all syllable trials.

Hertegård, S., Gauffin, J., & Lindestad, P. (1995) A comparison of subglottal and intraoral pressure measurements during phonation. *Journal of Voice*, *9*(2), 149-155. https://doi.org/10.1016/s0892-1997(05)80248-6

Purpose of the study: The goal of this study was to investigate sources of error relating to varying speech rate and modes of phonation that impact the estimation of subglottal pressure from intraoral pressure.

Method: The participant for this study was a 37-year-old male. He had a normal larynx and no voice problems. A local anesthesia was applied before inserting a cannula into the cricothyroid space. The cannula connected to a pressure transducer to collect measures of subglottal pressure. The subject was instructed to produce a variety of phonations. He repeated /pa/, without interruption, between the syllables at a slow rate (1.3 syllables/sec) and then at a faster rate (2.3 syllables/sec). He produced three /pa/ syllables within a carrier phrase at a rate of 2.3 syllables second. He also produced chopped phonation, inserting interruptions between /pa/ syllables, at a rate of 1.3 syllables/sec. For all of the tasks, the subject was not given instruction concerning the pitch of the phonation. The vocal effort varied between loud, soft, and normal phonation for each task.

Results: During the last 20 ms of vocal fold contact, there was a slight decrease in EGG amplitude. For fast syllables, slow syllables, and sentences, there was a high correlation between subglottal pressure and peak intraoral pressure during /p/ occlusions. The difference between absolute pressure values was less than 5% on average. There was a high correlation between subglottal pressure and the interpolated intraoral pressure. The correlation for the peak subglottal pressure and intraoral pressure was high for the chopped mode of phonation. The intraoral pressure was not stable during the /p/ occlusion.

Conclusions: Estimating subglottal pressure during chopped phonation by interpolating the intraoral pressure is not consistent. During glottal vibratory cycles, mean subglottal pressure decreased during the open phase and increased during the closed phase.

Relevance to the current work: This article discussed possible sources of error when measuring PTP. It also discussed the ideal type of phonation for collecting PTP measurements. PTP is the primary measure in the current study. The current study will avoid sources of error when collecting PTP and will not utilize phonation types that result in unstable PTP measurements.

Howard, N. S., Mendelsohn, A. H., & Berke, G. S. (2015) Development of the ex vivo laryngeal model of phonation. *The Laryngoscope*, *125*(6), 1414-1419. https://doi.org/10.1002/lary.25149

Purpose of the study: The purpose of this study was to revise and enhance the procedures of animal ex vivo phonation in hopes of being able to apply the same procedures to ex vivo human larynges in the future.

Method: This study utilized nine mongrel dogs between the ages of two and six years. General anesthesia was administered to the canine while a tracheostomy was completed. The canine's temperature and hydration were monitored and maintained externally. After the larynx was completely removed, the canine was humanely killed using IV Eutha-6. Researchers placed a cuffed endotracheal tube at the level of the sternum to provide controlled airflow. They used a variety of perfusion techniques and solutions to increase oxygenated blood flow to laryngeal tissue. Electrodes were placed on the surviving superior and recurrent laryngeal nerves (SLN, RLN). Phonation trials were run every five minutes with a heated and humidified airflow of 500 to 700 mL/min. During phonation attempts, researchers adducted the vocal folds and stimulated the SLN and RLN.

Results: Ex vivo phonation was produced by a number of larynges using a variety of reperfusion techniques and solutions. The most consistent and long-lasting phonation was produced by reperfusing the excised larynx with whole blood, delivered using a pulsatile flow.

Conclusions: Excised larynges can be used to produce and study viable and replicable phonation. Ex vivo larynx phonation can be a platform to study effects of organ ischemia, neuromuscular reinnervation capabilities, and organ transplant preservation techniques.

Relevance to the current work: This article demonstrated that excised larynges can be utilized to study phonation. Based on these findings, the current work will run phonatory trials on excised pig larynges to collect measures of PTP and PTF.

Jiang, J., O'Mara, T., Conley, D., & Hanson, D. (1999). Phonation threshold pressure measurements during phonation by airflow interruption. *The Laryngoscope*, *109*(3), 425-432. https://doi.org/10.1097/00005537-199903000-00016

Purpose of the study: The purpose of this study was to test a new device to obtain PTP measurements and determine the impact that vocal fold pathology has on PTP compared to normal vocal folds.

Method: This study consisted of 11 participants with normal vocal folds and 13 participants with vocal fold polyps. Each participant wore a fitted anesthesia mask. A flow meter and pressure transducer were used to collect flow and pressure measurements. The participant produced /a/ at soft, medium, and loud intensity levels. During phonation, flow was randomly interrupted using a valve controller. PTP was calculated for each intensity level.

Results: For normal human subjects, the mean PTP was 2.38 cmH_2O for 75 dB, 2.67 cmH_2O for 80 dB, and 2.98 cmH_2O for 85 dB. For individuals with vocal polyps, the mean PTP was 3.73 cmH_2O for 65 dB, 4.20 cmH_2O for 70 dB, 4.79 cmH_2O for 75 dB, 5.85 cmH_2O for 80 dB, and 7.37 cmH_2O for 85 dB. There is a statistically significant difference between the mean PTPs for normal human subjects and subjects with vocal polyps.

Conclusions: The presence of vocal polyps results in higher measurements of PTP. Measurements of PTP that were collected for normal subjects using the new device were consistent with PTP measures from other studies, demonstrating the device's reliability.

Relevance to the current work: PTP is one of the primary measures being investigated in the current study. This article demonstrates that PTP is an indicator of vocal fold health, which is why the current study is evaluating a more consistent way of measuring PTP.

Jiang, J. J., Raviv, J. R., & Hanson, D. G. (2001). Comparison of the phonation-related structures among pig, dog, white-tailed deer, and human larynges. *Annals of Otology, Rhinology & Laryngology*, *110*(12), 1120-1125. https://doi.org/10.1177/000348940111001207

Purpose of the study: The purpose of this study was to compare the anatomical and biomechanical features essential to phonation in humans, pigs, dogs, and white-tailed deer.

Method: This study utilized excised larynges from two humans, three pigs, three dogs, and three white-tailed deer. The cuneiform cartilage and epiglottis were removed and the strap muscles were trimmed. Measures were taken of the resting length of the membranous vocal fold, height of the vocal fold, and angular range of motion of the cricothyroid joint. A 98-g weight applied a constant force along the axis of the vocal fold at three different positions of vocal fold elongation. They divided the original vocal fold length by its lateral displacement to determine the vocal fold stiffness.

Results: The human, dog, and pig larynges all had similarly sized cricothyroid muscles. When compared to the deer larynx, the human, dog, and pig larynges all exhibited greater rotational mobility at the cricothyroid joint, which results in more vocal fold tension and pitch control. The vocal fold stiffness, as well as the thickness and structure of the vocal fold cover in pig larynges was most similar to human larynges. Pigs exhibited the largest frequency range, from 100 to 450 Hz.

Conclusions: When comparing the four species, pig larynges are the most similar to human larynges. Like the human larynx, the pig larynx has a large vocal range and a similar range of motion of the cricothyroid joint for vocal fold elongation. Based on these findings, pig larynges are the best model for studying phonation.

Relevance to the current work: This article provides rationale for utilizing pig larynges as a phonatory model. The current work will use pig larynges in phonation trials to collect measures of PTP.

Jiang, J., Regner, M. F., Tao, C., & Pauls, S. (2008). Phonation threshold flow in elongated excised larynges. *Annals of Otology, Rhinology, & Laryngology*, *117*(7), 548-553. https://doi.org/10.1177/000348940811700714

Purpose of the study: The purpose of this study is to investigate the reliability and validity of the new parameter, PTF.

Method: This study utilized 10 excised canine larynges. Once dissected, the larynx was mounted on a pipe that was attached to a pseudolung. The larynx was stabilized using two three-pronged devices that were inserted into the arytenoid cartilages. The anterior edge of the thyroid cartilage was sutured, and a thread ran to a micrometer positioned anteriorly. A pressurized air source provided airflow. The air ran through a humidifier. Each larynx underwent five trials in which the airflow was gradually increased until the larynx phonated. Using an airflow meter and a pressure meter, measures of pressure and flow were collected. Five trials were also performed in which the elongation of the vocal folds was varied using the suture attached to the anterior thyroid cartilage.

Results: Based on the data collected, no significant correlation between larynx size and PTF was found. However, it was observed that there were statistically significant differences in PTF between each elongation level. When the vocal folds were at physiological length, the mean PTFs ranged from 101 mL/s to 217 mL/s. When compared to the magnitude of elongation, the average percentage change of PTF was statistically significant. PTF is proportional to the percent of the vocal fold elongation.

Conclusions: PTF will vary depending on the tension of the vocal folds. It may be advantageous to use PTF as a clinical diagnostic tool when conducting general assessment of laryngeal functioning.

Relevance to the current work: The aim of the current study is to develop a device to obtain more precise measures of PTP. This article discusses the importance of accurate measures of PTP in diagnosing vocal pathologies. The current study will also utilize a similar benchtop setup to the one outlined in this article. This article found that varying the lengths of the vocal folds will result in changes in PTF. Based on this finding, the current study will aim to keep the vocal folds a consistent length throughout testing.

Jiang, J. J., & Titze, I. R. (1993). A methodological study of hemilaryngeal phonation. *The Laryngoscope*, *103*(8), 872-882. https://doi.org/10.1288/00005537-199308000-00008

Purpose of the study: The purpose of this study was to develop a methodology to investigate how phonatory measures change when one vocal fold is removed and replaced with a plexiglass plate compared to a full larynx.

Method: This study utilized nine excised canine larynges. Each larynx underwent dissection to remove excess muscles, tissue, esophagus, etc. The larynx was mounted on the benchtop by inserting a 3-pronged micrometer into the arytenoid cartilages. An air

compressor provided the airflow that was controlled by a valve. The air ran through a humidifier and pseudolung before entering the larynx. For each larynx, phonatory measures were collected, including sound pressure level, phonation threshold pressure, average glottal flow, F_0, and amplitude of the vocal fold vibration. After the trial, each larynx underwent a secondary dissection in which the left vocal fold was removed and replaced with a vertical plexiglass plate. The larynx was then phonated again, and the same measures were collected.

Results: The data showed that when comparing the full larynges and hemilarynges, there was no detectable statistical difference in terms of a mean value or standard deviation. The differences for phonation threshold pressure, phonation instability pressure, vibrational amplitude, and F_0 were all less than 10%. The data also showed that the average airflow was scaled 2:1 and the acoustic power was scaled 4:1.

Conclusions: An excised hemilarynx can be used in future research instead of a full larynx when researchers need to access the medial plane of the vocal folds.

Relevance to the current work: The current study will utilize a benchtop setup based on the setup outlined in this article.

Lamb, J. R., Schultz, S. A., Scholp, A. J., Wendel, E. R., & Jiang, J. J. (2020). Retest reliability for complete airway interruption systems of aerodynamic measurement. *Journal of Voice*, *36*(1), 27-33. https://doi.org/10.1016/j.jvoice.2020.02.024

Purpose of the study: The purpose of this study was to compare intra-subject variation and test-retest reliability of Ps, PTP, MFR, and LR using labial and mechanical interruption methods in adult participants.

Method: This study included 55 participants who had no history of dysphonia. Each participant completed both labial and mechanical interruption tasks. In the labial interruption task, an intraoral tube was inserted into the subject's lips and sat on top of the tongue. The participant placed their face in a silicone mask. They were instructed to say /pa/ at a comfortable volume to measure Ps and /pa/ as quietly as possible without whispering to measure PTP. They completed each task 10 times, producing the syllable five times during each trial. During the mechanical interruption tasks, each participant was fitted with a silicone mouthpiece. They plugged their nose to prevent air leakage. Each participant was instructed to sustain /a/ at a comfortable volume for 10 seconds. During that interval, the balloon valve inflated five times for 250 milliseconds, cutting off the airflow. This task was completed 10 times. Two weeks after initial data collection, 30 participants returned and completed the same labial interruption and mechanical interruption tasks.

Results: For mechanical interruption, the percent difference in measurement results for test-retest was 12.88% +/- 10.15 for Ps and 21.46% +/- 16.01 for PTP. For labial interruption, the percent difference in measurement results for test-retest was 27.56%+/-17.14 for Ps and 17.04%+/-14.62 for PTP. The intra-subject coefficients of variation for mechanical interruption were 0.086+/-0.046 for Ps and 0.177 +/-0.083 for PTP. The intra-subject coefficients of variation for labial interruption were 0.161+/-0.078 for Ps and 0.186+/-0.091 for PTP.

Conclusions: Test-retest measures of PTP and Ps exhibited variability of these measures when using mechanical and labial interruptions.

Relevance to the current work: This article provides evidence of the variability of PTP measurements within an individual, despite researchers' best efforts to control for extraneous variables. The aim of the current study is to test the efficacy of an external oscillatory device in collecting more consistent measures of PTP.

Murray, P. R., Thomson, S. L., & Smith, M. E. (2014). A synthetic, self-oscillating vocal fold model platform for studying augmentation injection. *Journal of Voice*, *28*(2), 133-143. https://doi.org/10.1016/j.jvoice.2013.10.014

Purpose of the study: The purpose of this study is to examine the pre- and post-injection vibratory responses when using a multilayer, self-oscillating, synthetic vocal fold model.

Method: This study utilized a multilayer, synthetic, self-oscillating vocal fold model with different layers of stiffness to represent the unique layers of the vocal folds. Two configurations were used in this experiment, the first being a full larynx, which consisted of a normal model and a bowed model. This setup was used to collect pre- and post-injection onset pressure, flow rate, open quotient, frequency, and glottal gap high-speed imaging data. A hemilarynx configuration that consisted of a single bowed model was also used. This setup was used to track pre- and post-injection medial surface motion. After pre-injection measurements were collected, various amounts of silicone were injected into the bowed model to achieve "sufficient," "insufficient," and "excess" glottal closure. After injection, the previously mentioned metrics were taken again and then compared.

Results: Initially the models successfully displayed bowing, but the bowing improved when injected with filler material. The mucosal wave-like motion of vibration, onset pressures, and frequencies that were exhibited by the models are typical of human

phonation. After injection, the models exhibited a decrease in glottal flow rate, onset pressure, and open quotient and an increase in vibration frequency.

Conclusions: It can be concluded that the injections resulted in a decreased mechanical "effort" that was required to vibrate the vocal folds. This decrease in required effort makes up for the negative consequences experienced because of bowing. The methodology used in this study can be used in future studies researching how augmentation injections result in mechanical consequences.

Relevance to the current work: The current study is also investigating the change in onset pressure when a mechanical variable is altered.

Nascimento, A., Korn, G. P., Sacaloski, M., & Azevdo, R. R. (2021). Effects of mechanical vibration stimulation of the larynx on voice production. *Journal of Voice*, *S0892-1997*(20), 30483-30485. https://doi.org/10.1016/j.jvoice.2020.12.024

Purpose of the study: The purpose of this study was to determine the impact that mechanical stimulation on the larynx has on vocal exercises, both over an immediate and mid-term time range.

Method: This study consisted of 14 subjects, all of whom were professional voice users. Each subject was randomly assigned to one of two groups. One group was the experimental group who completed humming exercises while applying a local mechanical vibration stimulation. The other group was the control group who only completed the humming exercises. All subjects completed the humming exercises once a day for 30 days. The daily routine consisted of four humming exercises. During the first exercise, the participant sustained humming at maximum phonation time at a comfortable loudness and pitch in the modal register. In exercise two, the subject performed an

ascending glissando while humming for the effortless highest pitch. Exercise three consisted of the subject sustaining the humming at the maximum phonation time and a comfortable intensity and a high pitch. During the fourth exercise, the subject started at a comfortable pitch and then performed three ascending tones and two descending tones while humming. While performing the exercises, the experimental group applied the localized, mechanical, vibrational device to the angle of the thyroid cartilage and applied a gentle pressure during voice production. Subjects completed each exercise four times, pausing five to ten seconds between each repetition. During the first treatment session, participants performed the exercises individually to ensure standardization of the exercises. Throughout the 30 days, subjects were able to receive guidance virtually. Evaluations were completed three times throughout the study. Prior to beginning the study, evaluations were collected to establish baseline data. Evaluations were also completed after the first session to determine immediate effects and after the 30 days to analyze mid-term effects. Evaluation measures collected included the Voice Signs and Symptoms Questionnaire, auditory-perceptual analysis of the voice using the GRBASI scale, maximum phonation time, and acoustic analysis. Acoustic parameters consisted of average F_0, noise-to-harmonics ratio, harmonics-to-noise ratio, spectrogram analysis, and perturbation amplitude jitter and shimmer.

Results: The experimental group using the local mechanical vibration stimulation experienced significant improvement in the post-immediate evaluation and post-treatment evaluation for jitter, shimmer, maximum phonation time, and noise-to-harmonics ratio. In the post-treatment evaluation, they also showed improvements in Grade, Roughness,

Breathiness, and Instability based on the GRBASI scale. The experimental group also exhibited better voice quality and easier phonation than the control group.

Conclusions: Application of a mechanical vibration to the larynx during humming voice production results in both immediate and mid-term improvements in vocal patterns and voice quality.

Relevance to the current work: This article discusses how application of vibrational stimulation impacts phonation within humans. It is important to understand these effects, as the current study will apply a vibrational force to the larynx in order to determine its impact on aerodynamic measures, specifically PTP.

Plant, R. L. (2005). Aerodynamics of the human larynx during vocal fold vibration. *The Laryngoscope*, *115*(12), 2087-2100. https://doi.org/10.1097/01.mlg.0000184324.45040.17

Purpose of the study: The purpose of this study was to investigate the relationship of aerodynamics and laryngeal function, including subglottic pressure, sound intensity, and F_0.

Method: Six healthy individuals with no history of voice disorder participated in this study; four men and two women. While in the sitting position, a Rothenburg-type mask was placed over the face of the participant. Each participant was instructed to produce the sound /i/ during five different tasks. The first task included repeating /i/ three times with a breathy onset. The second task was to say /i/ three times at a low pitch, with the first utterance at a low intensity and the next two increasing in intensity. The third task consisted of producing /i/ three times at mid-pitch, with the first utterance at a low intensity and the next two increasing in intensity. The fourth tasks included producing /i/

three times at a high pitch, with the first utterance at a low intensity and the next two increasing in intensity. The final task consisted of producing /i/ three times on a pitch glide from low to high. If possible, the series of tasks was repeated. During each task, airflow, electroglottography, subglottic pressure, and the sound signal were all collected.

Results: Most of the participants exhibited a linear relationship between sound intensity and subglottic pressure. Opening of the vocal folds at the initiation of each cycle resulted in rapid variations in subglottic pressure. Threshold pressure is primarily dependent on sound intensity and F_0. Onset PTP was higher than offset PTP.

Conclusions: Despite variability in specific parameters, the larynges responded predictably to aerodynamic forces. Physical properties of air and the anatomy of the vocal tract govern the behavior of the larynx, however, variability among human subjects may be due to differences in neuromuscular control.

Relevance to the current work: This article discussed the variation in PTP due to the opening of the vocal folds at the beginning of the cycle. The aim of the current work is to test the efficiency of a device in reducing PTP variability.

Plant, R. L., Freed, G. L., & Plant, R. E. (2004). Direct measurement of onset and offset phonation threshold pressure in normal subjects. *The Journal of the Acoustical Society of America*, *116*(6), 3640-3646. https://doi.org/10.1121/1.1812309

Purpose of the study: This study sought to identify which laryngeal factors impacted phonation threshold pressure. They also investigated if there was a hysteresis effect for phonation threshold pressure, causing onset phonation threshold pressure to be different from offset phonation threshold pressure.

Method: Five normal subjects participated in this study; four men and one woman. The participants were instructed to complete a series of five tasks. The first task included repeating /i/ three times with a breathy onset. The second task was to say /i/ three times at a low pitch, with the first utterance at a low intensity and the next two increasing in intensity. The third task consisted of producing /i/ three times at mid-pitch, with the first utterance at a low intensity and the next two increasing in intensity. The fourth tasks included producing /i/ three times at a high pitch, with the first utterance at a low intensity and the next two increasing in intensity. The final task consisted of producing /i/ three times on a pitch glide from low to high. If possible, the series of tasks was repeated. During the tasks, each participant wore a Rothenberg-type mask over their face. EGG electrodes were placed on both sides of the neck on the thyroid cartilage. A 21-guage needle was inserted through the cricothyroid membrane and attached to a pressure transducer to measure subglottic pressure.

Results: Researchers performed univariate and multiple linear regression analysis of the relationship between variables and threshold pressure. Based on the data, it was concluded that there was the largest number of significant relationships between intensity and F_0. There were also significant relationships between F_0 and onset or offset pressure. It was also found that offset phonation threshold pressure was lower than onset phonation threshold pressure.

Conclusions: Phonation threshold pressure can be influenced by F_0, vocal intensity, and laryngeal airway resistance. A hysteresis effect impacts phonation threshold pressure, causing offset phonation threshold pressure to be lower than onset phonation threshold pressure.

Relevance to the current work: This article discusses the variability associated with direct and indirect measurements of PTP. The aim of the current work is to develop a more consistent way to measure PTP.

Plexico, L. W., Sandage, M. J., & Faver, K. Y. (2011). Assessment of phonation threshold pressure: A critical review and clinical implications. *American Journal of Speech-Language Pathology*, *20*(4), 348-366. https://doi.org/10.1044/1058-0360(2011/10-0066)

Purpose of the study: The purpose of this article is to culminate a historical account of PTP using published evidence that supports its clinical application.

Method: First, a critical review was conducted. Using search engines, they completed a literature search for articles that included one of the following words: phonation threshold pressure, vocal fold oscillation onset, subglottal pressure and phonation, lung pressure and phonation, pressure measurement and phonation. All articles used were in English, in a peer-reviewed journal, and published between 1980 and 2009. Based on the literate search that was completed, an online survey was distributed to 59 practicing speech-language pathologists and voice researchers. The purpose of this survey was to investigate the use of PTP measurement within the clinic and research settings and to identify variables taken into consideration in these settings when assessing PTP.

Results: A variety of data acquisition equipment was used in the articles reviewed. 62.5% of the studies covered more than one topic area. Topics covered include hydration, vocal fatigue, and perceived phonatory effort. The studies primarily included women participants and the mean age of participants ranged from 20 to 30 years. There were no studies that evaluated children. Studies varied in the CV sequence used, pressure

peak inclusion criteria, number of syllables collected per trial, the specific peaks examined to obtain PTP values, syllable rate, and whether or not the nostrils were occluded during PTP data collection. The majority of studies used the CV sequence /pi/ and collected five or seven syllables per trial. The range of syllables collected during a single trial ranged from five to seven. 75% of the studies used a syllable rate of 1.5 syllables/sec. A variety of instruction sets were used to elicit the quietest phonation possible. The majority of respondents to the survey were women and the mean age was 42.25 years. 40.7% of respondents answered that they collected PTP data, 50% for clinical use, and 75% for research use. When asked about the number of consecutive syllables required when collected PTP data, 38% responded with five syllables, 4.8% responded with six syllables, and 28.6% responded 10 syllables. Respondents reported a variety of syllables used, including /pi/ (57%), /pae/ (19%), and /pa/ (9.5%). When asked about syllable rate, 19% did not control for rate, 61.9% reported a rate of 1.5 syllables/sec, 4.8% reported a rate of 3 syllables/sec, and 4.8% reported a rate necessary to acquire reliable results. 61.9% reported that they did account for frequency and 28.6% reported that they did not account for frequency. 61.5% reported that they collected PTP at a high F_0. When asked about environmental variables, 19% controlled for room humidity. 57.2% of respondents indicated that they considered hydration. Of these respondents, they considered hydration in the following ways: 83.3% by client report, 25% by visual inspection, 8.3% through controlling room humidity, and 8.3% by having the client drink water. 57.14% indicated that they take vocal training into consideration. 33.3 % of respondents indicated that they take hormonal changes into consideration. 71.4% considered the client's age; 61.9% considered vocal fold pathology; 52.4%

considered voice use prior to data collection and current respiratory infection; 42.9% considered history of smoking and LPR, as well as time of day; 23.8% considered native language; 38.1% considered alcohol consumption, history of respiratory infections, and profession; 33.3% considered history of asthma; and 47.6% considered client sex, medication use, and vocal fold function.

Conclusions: This article showed consistent scientific rationale for the use of phonation threshold pressure data across published studies.

Relevance to the current work: PTP is an important measure in the current study. This article provides a history of PTP, as well as rationale for its use and a standardized protocol for clinically estimating PTP.

Regner, M. F., Robitaille, M. J., & Jiang, J. J. (2010). Interspecies comparison of mucosal wave properties using high-speed digital imaging. *The Laryngoscope*, *120*(6), 1188-1194. https://doi.org/10.1002/lary.20884

Purpose of the study: The purpose of this study was to find which larynges were the best model for simulating the human larynx by comparing phonatory measurements from in vivo human larynges to those of ex vivo pig, dog, cow, and sheep larynges.

Method: This study consisted of excised larynges from seven canines, seven pigs, five sheep, and six cows. Larynges were frozen in saline and then slowly thawed in a cold-water bath before trials. Larynges underwent dissection to remove the epiglottis, corniculate cartilage, cuneiform cartilages, posterosuperior aspects of the thyroid cartilage, and associated tissues. Further dissection was completed to reveal a superior view of the vocal folds. The larynx was mounted on an excised larynx phonation system and secured using a metal hose clamp. Arytenoid cartilages were adducted to the point of

approximation of the vocal processes using three pronged micromanipulators. The laryngeal prominence of the thyroid cartilage was sutured to an anterior micromanipulator and adjusted to elongate the vocal folds to 105% of their length at rest. Air provided to the system passed through a humidifier and pseudolung before entering the larynx. During phonation trials, pressure measurements were collected using a digital pressure meter, acoustic data was measured using a Sony microphone, and high-speed video was recorded.

Results: The data reflected a statistically significant difference between the mean amplitude, phase difference, and median frequency between pig, dog, cow, and sheep larynges. The cow and sheep larynges exhibited oscillation frequencies lower than humans and pig larynges had a higher oscillation frequency. Dog larynges had a frequency range that was the most similar to humans. Cows and sheep had the largest oscillation amplitudes. The oscillation amplitudes of dogs and pigs were similar to each other, but pigs had an oscillation amplitude most similar to humans. The phase difference of pigs was significantly different from cows, dogs, and sheep.

Conclusions: Dog and pig larynges are the most similar to human larynges regarding physical vibration. They both provide a reliable model when studying laryngeal phonation.

Relevance to the current work: The current work will use pig larynges to study phonatory measures of the larynx, specifically focusing on PTP. Based on the findings of this study, the findings of the current work can eventually be applied to human subject phonation.

Sivasankar, M., & Fisher, K. V. (2002). Oral breathing increases Pth and vocal effort by superficial drying of vocal fold mucosa. *Journal of Voice*, *16*(2), 172-181. https://doi.org/10.1016/s0892-1997(02)00087-5

Purpose of the study: The purpose of this study was to investigate how P_{th} and vocal effort change due to oral and nasal breathing.

Method: This study consisted of 20 females between the ages of 21 and 36. They were randomly assigned to one of two groups: oral breathing or nasal breathing. Data collection was completed in a quiet room with consistent humidity. They wore a vented pneumotachograph mask with pressure transducers, and had a plastic tube inserted into their mouth to collect oral pressure. Tasks were completed to determine maximum and minimum pitch. Participants smoothly produced the syllable /pi/ five times in a row on a single breath. They were instructed to speak as softly as possible without whispering and to produce the syllables at a rate of 1.5 syllables/sec. Using a comfortable pitch they completed a suprathreshold syllable series and a softer syllable series just above a whisper. They then viewed their practice trials on a computer and were instructed to make the glottal volume velocity waveform as small in amplitude as possible, without being flat. They practiced these tasks until the oral pressure peaks were consistently near a minimum threshold, below which oscillation in the glottal volume velocity waveform was absent. They completed the pretreatment tasks by producing /pi/ five times at a comfortable pitch, a low pitch, and a high pitch. An estimate of patient-perceived vocal effort was collected by singing "Happy Birthday" in their quietest voice starting at the 50^{th} percentile of their pitch range, and then rating vocal effort on a magnitude estimation

scale. Then, depending on what group they were assigned to, they performed either oral or nasal breathing for 15 minutes. Then the P_{th} and vocal effort protocols were replicated.

Results: The effects that oral and nasal breathing had on P_{th} differed depending on the pitch. For the low pitch condition, nasal breathing decreased mean P_{th} by 0.7+/-0.4 cmH_2O and oral breathing increased the mean P_{th} by 0.8+/-0.4 cmH_2O. For the comfortable pitch condition, nasal breathing decreased mean P_{th} by 0.5+/-0.3 cmH_2O and oral breathing increased the mean P_{th}, by 0.8+/-0.4 cmH_2O. For the high pitch condition, nasal breathing significantly decreased the mean P_{th} by 0.9+/-0.5 cmH_2O and oral breathing caused a slight increase in mean P_{th} which was not significant. It was reported that subjects in the oral-breathing group experienced a mean increase in vocal effort (24+/-13.5) and subjects in the nasal-breathing group experienced a mean decrease in vocal effort (20+/-17).

Conclusions: The findings of this study exhibit that P_{th} is potentially regulated by the sol layer. Oral and nasal breathing have an effect on P_{th} and patient-perceived vocal effort. Oral breathing contributes to superficial laryngeal dryness. There is a need to develop strategies that can prevent the adverse effects of oral breathing.

Relevance to the current work: This article outlines the negative impact that dehydration has on PTP. The current work is interested in collecting consistent measures of PTP. The current study will aim to prevent dehydration in order to obtain more reliable measures of PTP.

Smitheran, J. R., & Hixon, T. J. (1981). A clinical method for estimating laryngeal airway resistance during vowel production. *Journal of Speech and Hearing Disorders*, *46*(2), 138-146. https://doi.org/10.1044/jshd.4602.138

Purpose of the study: The purpose of this study was to develop and test a new and noninvasive clinical method for estimating laryngeal airway resistance.

Method: This study utilized fifteen adult males. The participant sat upright and was instructed to repeat the syllable /pi/ seven times. This was repeated three times. They were also instructed to take a breath twice as deep as normal, to produce all seven syllables on a single, continuous expiration, and to use a normal loudness, quality, and pitch. They were instructed to put equal stress on each syllable and a metronome was used to pace the productions at 1.5 syllables/sec. An anesthesia mask was used to collect airway-opening flow and a pressure sensing catheter was positioned in the midline of the participant's oral cavity to record oral air pressure.

Results: The mean resistance values for the group is 35.7 cm H_2O/LPS, with a range of 30.0 cm H_2O/LPS to 43.1 cm H_2O/LPS. The standard deviation is 3.3 cm H_2O/LPS. The data suggests that there is no relationship between chronological age and the magnitude of laryngeal airway resistance during vowel production.

Conclusions: The data from this study supports the validity and reliability of the new and noninvasive clinical method for estimating laryngeal airway resistance during vowel production.

Relevance to the current work: The current study is preparatory research to determine a more consistent way to measure PTP within human subjects. This article discusses a new technique of estimating PTP within humans.

Stevens, K. A., Thomson, S. L., Jetté, M. E., & Thibeault, S. L. (2016). Quantification of porcine vocal fold geometry. *Journal of Voice*, *30*(4), 416-426. https://doi.org/10.1016/j.jvoice.2015.06.009

Purpose of the study: The purpose of this study was to determine the impact that freezing has on the geometry of the vocal fold tissue and the airway. It also aimed to compare the geometry of pig vocal folds to human vocal folds.

Method: This study utilized five pig larynges. The larynges were also imaged using a Siemens Inveon Acquisition workplace software. During imaging, the folds were in resting position. The posterior surface of the larynx was placed flat on the imaging bed of the scanner. Each larynx was frozen with isopentane, which was cooled over liquid nitrogen. After freezing, larynges were then scanned again. The scans were analyzed two ways. The geometry of the pig vocal folds was compared to that of other models by extracting the medial surface profiles of each larynx. Analysis of tissue and airway volumes was also completed to determine the effect that freezing had on the geometry of the tissues.

Results: The geometry of the pig larynges was similar to the human larynges. Some differences include the inlet and exit radii and the slopes of the inferior region of the folds. The superior folds oscillate in the pig larynges, but do not oscillate in human larynges. The percent change in volume between room temperature and freezing was 4.6% for larynx one, 5.1% for larynx two, 4.4% for larynx three, 5.4% for larynx four, and 3.0% for larynx five. The average change in volume was 5%. However, the change was not uniform throughout the tissues. After thawing, the larynges returned to 1-2% of their dimensions before freezing.

Conclusions: The geometry of the pig larynx is similar enough to the human larynx to be used as a model when studying phonation. There is a 1-2% distortion caused by freezing and thawing pig larynges.

Relevance to the current work: This article found that pig larynges can be used as models for studying phonation. The current work will utilize pig larynges in phonation trials. The current work will also keep in mind the 1-2% distortion that can be caused by freezing and thawing the pig larynges.

Tanner, K., Roy, N., Merrill, R. M., & Elstad, M. (2007). The effects of three nebulized osmotic agents in the dry larynx. *Journal of Speech, Language, and Hearing Research*, *50*(3), 635-646. https://doi.org/10.1044/1092-4388(2007/045)

Purpose of the study: The purpose of this study was to explore the effects of three different nebulized treatments (HS, IS, and SW) with different osmotic properties on PTP and PPE after participating in a laryngeal desiccation activity.

Method: This study consisted of 60 women who were randomly assigned to one of four groups, including a nontreatment group. They each attended a two-hour data collection session. They first participated in a laryngeal desiccation procedure that consisted of oral breathing medical-grade dry air for 15 minutes. After the desiccation procedure, they were administered 3 mL of either nebulized HS, IS, or SW, using a Micro Mist Nebulizer. The participants were instructed to breathe normally through the mouth while the nebulizer was applied. PTP and PPE ratings were taken before and after the laryngeal desiccation procedure. PTP measures were also collected after nebulization at 5 minutes and then every 15 minutes until 50 minutes.

Results: The data showed a significant increase in PTP between baseline and post desiccation by approximately 0.5 cm H_2O. The mean PTP values were consistently lower within the control group. The differences in PTP and PPE between the four groups were not statistically significant. PPE ratings significantly decreased between baseline

and post-desiccation. There was no significant correlation between the changes in PTP and PPE from baseline to immediately post desiccation.

Conclusions: PPT measures and desiccation events are related, in that PPT increases after desiccation. None of the three nebulized treatments made a significant positive impact on PTP after desiccation.

Relevance to the current work: The current work will utilize excised larynges that are prone to tissue dehydration during phonatory trials to measure PTP. Based on this research, the current study will aim to prevent desiccation from occurring by regularly applying hydration through externally spraying the larynx with a saline solution.

Witt, R. E., Taylor, L. N., Regner, M. F., & Jiang, J. J. (2011). Effects of surface dehydration on mucosal wave amplitude and frequency in excised canine larynges. *Otolaryngology Head Neck Surgery*, *144*(1), 108-113. https://doi.org/10.1177/0194599810390893

Purpose of the study: The purpose of this study was to examine the effects of dehydration of voice deterioration and estimate mucosal wave measurements of dehydrated vocal folds using high-speed video.

Method: This study utilized 10 excised canine larynges. After the larynges were excised, they were examined to ensure that there were not any diseased tissues or lesions. Larynges were placed in 0.9% saline solution and frozen. On the day of data collected, larynges were slowly thawed. The superior cornua and superior portions of the thyroid cartilage were dissected away. To expose the vocal folds, the epiglottis, ventricular folds, corniculate cartilages, and cuneiform cartilages were removed. The dissected larynx was mounted on an excised larynx phonation system. A hose clamp was used to clamp the trachea onto a pipe. Airflow was provided by an air compressor and flowed through two

heater-humidifiers. Vocal fold adduction and abduction at the point of glottal closure were controlled by two three-pronged micrometers that were inserted into the lateral aspect of the arytenoid cartilages. A suture ran through the anterior commissure of the thyroid cartilage to an anterior micrometer to control the elongation of the vocal folds. Eight of the larynges were used in dehydration and two were used in control trials. In the dehydration trials, air supplied to the larynges was warm, but was not humidified. Air was supplied to the vocal folds at a constant pressure of 20 cm H_2O until the larynx no longer phonated. Throughout the dehydration trials, no saline solution was applied to the larynges. During the control trials, air supplied to the larynges was warm and humidified. Airflow was held constant at 20 cm H_2O for 30 minutes. Throughout the control trials, the larynges were hydrated every 30 seconds using 0.9% saline solution.

Results: The data indicated that increased dehydration was associated with a decrease in both amplitude and frequency and an increase in viscosity and stiffness of the vocal fold tissue and mucosa. Data also exhibited a negative correlation between dehydration and mucosal wave amplitude. In dehydration trials, there was a significant decrease in measures of amplitude over time when compared to the control group. As dehydration increased, PTP also increased. In the dehydration group, there was also a negative correlation between level of dehydration and mucosal wave frequency.

Conclusions: There is an inverse relationship between dehydration and mucosal wave amplitude and frequency. Based on the findings of this study, voice deterioration occurs after extreme surface dehydration.

Relevance to the current work: The current article indicates that PTP can be influenced by dehydration of the vocal folds. Based on these findings, the current work

will intentionally avoid desiccation by applying surface hydration and eliminating variables that cause dehydration.

Yiu, M. L., Liu, C. C. Y., Chan, C. Y. P., Barrett, E., & Lu, D. (2021). Vibrational therapies for vocal fatigue. *Journal of Voice*, *35*(1), 29-39. https://doi.org/10.1016/j.jvoice.2019.07.009

Purpose of the study: The purpose of this study was to investigate the effects of vibrational therapy on young adults with a healthy voice.

Method: This study included 44 young adults between the ages of 19 and 25 years. Each participant participated in karaoke for at least 95 minutes until they could not sing anymore to induce vocal fatigue. After subjects reported they could not sing anymore, they were instructed to sing one more song. While singing, participants were not allowed to drink water. After karaoke, each participant was randomly assigned to one of three groups. The first group was whole body vibration (WBV). The second group was localized perilaryngeal vibration (LPV). The third group was a control group. Before intervention, each group was informed that the treatment they underwent might reduce their vocal fatigue. In the WBV group, each subject stood on the platform of the vibrational machine for ten minutes. The vibrational frequency was selected based on which frequency resulted in the largest vibrations in the individual's neck. In the LPV group, a Novafon Pro electric vibrational massager was applied to the neck for ten minutes. Participants were instructed to apply the device to several neck areas. First, they slowly moved the vibrator from the lower edge of the mandible, along the perilaryngeal muscles, to the top of the right and then the left clavicle, ten times in a row. Then they held the vibrator on the right side of the thyroid lamina for one minute and then the left

side of the thyroid lamina for one minute. They repeated these exercises throughout the ten minutes. The participants in the control group were provided with a hand-held vibrational device. Subjects were instructed to place the device on the left side of the thyroid lamina for one minute and then the right side of the thyroid lamina for one minute, for a total of ten minutes. Subjects were informed that the device would emit ultrasonic pulses, relaxing the laryngeal muscles. However, the device was never actually turned on. Participants in all groups were instructed to not talk during the intervention. Throughout the study, two primary outcome measures were collected. Participants produced a glissando task three times to measure the highest pitch produced as a vocal function test. A self-rating of vocal fatigue at the time of the study was also collected using a Vocal Fatigue Score from selected items on the VFI.

Results: After participating in karaoke, all subjects exhibited a decrease in vocal function. The WBV and LPV groups both showed significantly improved measures of highest pitch production and perception of vocal fatigue when compared to the control group.

Conclusions: Both localized and whole-body vibrational therapy is effective in improving measures of vocal fatigue.

Relevance to the current work: This article discusses the impact that an external vibration can have on muscles. Since the current work will apply an external vibration to the larynx, it is important to understand what additional influences the external vibration may have on the muscles.

APPENDIX B

Materials

Materials for Dissection

1. Stainless Steel Disposable Scalpels (No. 10)
2. Stainless Steel Disposable Scalpels (No. 11)
3. Stainless Steel Disposable Scalpels (No. 15)
4. Hemostatic Forceps (4)
5. Disposable nitrile gloves
6. Dissection table
7. Plastic table mats
8. Disposable plastic aprons
9. Protective eye goggles
10. Face masks
11. Lab sink
12. Room temperature water
13. Phosphate Buffered Saline (PBS) Solution
14. Dish brush
15. Antibacterial Dishwashing Liquid Dish Soap
16. Manicure scissors
17. Medical tweezers
18. Adjustable desktop light
19. Medical Suture (silk black braided 45 cm suture, 24 mm needle)
20. Gallon-sized Ziplock ™ bags
21. ThermoScientific Nalgene ™ bottles (1000 mL, closure diameter 63 mm, low density poly-ethylene)
22. Permanent Marker
23. Styrofoam™ cooler
24. Cryogenic gloves
25. Thermoscientific ™ Freezer
26. Food grade refrigerator
27. Liquid nitrogen (provided by BYU Chemistry Stockroom)
28. Red hazardous waste box (for scalpel and suture disposal)
29. Digital Caliper Micrometer Measuring Tool (Shenzhen Tengyes Technology Co. Model IP54)
30. Sani-Cloth™ germicidal disposal sanitizing wipes
31. Digital Scale (Ozeri Model ZK14-S™)

Materials for Data Acquisition

1. Dell ™ computer
2. Dell ™ computer monitor
3. Audio Output Extension
4. Bose ™ Amplifier
5. Cable ties
6. LabChart™ data acquisition software (AD Instruments)

7. PowerLab ™ data acquisition hardware (AD Instruments)
8. Microphone (Model SM-48, Shure, Niles, IL)
9. Medical-grade air tank (2) containing compressed, low humidity air (30 psi, <1% relative humidity)
10. Physiological pressure transducer (Model MLT844, AD Instruments)
11. Sphygmomanometer (AD Instruments)
12. Pressure calibration block
13. Syringe (25 cc/ml)
14. Velcro ™ for securing transducers during calibration and data collection
15. Gauze (used to decrease reverberation under the pressure transducer)
16. Pneumotach Calibration Unit (MCU-4, Glottal Enterprises)
17. AcuRite ™ Hygrometer (Model 01083M)
18. Seagate Portable 1 TB External Hard Drive HDD
19. SanDisk 128GB Cruzer USB 2.0 Flash Drive

Materials for Benchtop and Phonatory Trials

1. Screwdriver
2. Metal hose clamp
3. Disposable nitrile gloves
4. Bolts
5. Dampening rubber sheet
6. External oscillatory device
    a. High Power DC Motor (HC385MG-301)
    b. H-bridge Motor Driver (L293D)
    c. Hall Effect Sensory (A3144)
7. Control Panel
    a. Gitfun MTS102 2 Position 3 Pin Mini Toggle Switch
    b. Green LED
    c. White LED
8. 10K Ohm Resistor
9. 2K Ohm Resistor
10. 10K Ohm Linear Taper 3 Pin Rotary Potentiometer Resistor
11. Power Source (Atten Instruments PPS3005S Program DC Power Supply)
12. Leads
13. 4-Pin Molex connector
14. Velcro™ strap
15. Laptop
    a. Arduino Nano
    b. NanoCode
    c. Serial Monitor
16. Mini-USB cable
17. Distilled water
18. TheraHeat Humidifier™ (Model RC700000, Smiths Medical, Dublin, OH)
19. Phosphate Buffered Saline (PBS) Solution
20. Micropositioners located anteriorly (one) and laterally (two) (Model 1460, Kopf Industries)

21. Micropositioner triple prong attachments (Kopf Industries)
22. Tubing
    a. Vinyl: 1 ½” ID outer diameter (OD), 1” inner diameter (ID)
    b. Clear Vinyl: 1 1/8” OD, 7/8” ID; 1” OD, ¾” ID; ¾” OD, ½” ID; 7/8” OD, 5/8” ID; 5/8” OD, ½” ID; ½” OD, 3/8” ID; 3/8” OD, ¼” ID; 5/16” OD, 3/16” ID; 3/16” OD, 1/8” ID
23. Respiratory flow head transducer (Model MLT300L, AD Instruments, Sydney, Australia)
24. Flow head meters (Model MLT300L, AD Instruments)
25. Super Poligrip Original Formula Zinc Free Denture and Partials Adhesive Cream
26. Teflon tape™
27. 20 cm foam-insulated aluminum custom pseudolung

APPENDIX C

LabChart™ Protocol, Computer Set Up

1. Power on the computer (Dell ™) and monitor (Dell ™)
2. Then power on the PowerLab unit
3. Open the LabChart™ 8 Application (AD Instruments, 2015)
    a. See "Scanning for Devices" pop up to ensure that both "Powerlab 8/35" and "Playback File" are selected. If they are not, verify that the power to the PowerLab unit has been turned on and select "device scan" again
    b. Click "OK"
    c. On the Welcome Center screen, select the "Pig Data Charts Template"
    d. In the upper right corner of the screen, press the "start" button
        i. Allow LabChart™ to run for 15 minutes to give the program sufficient time to warm up
4. Input channel settings
    a. In the upper left corner, click on the "Setup" tab and then "Channel Settings"
        i. Ensure the following settings are applied:
            1. Microphone: 40 k/s: 10 mV: mV
            2. Pressure: 1 ks/: 50 mV: mmHg
            3. Flow: 1 k/s: 200 mV: mV (eventually units will change to L/sec, but do not worry about it at this point)
            4. High Speed Trigger: 1 k/s: 2 V: V
        ii. When the above-mentioned settings are accurate, press "OK" in the bottom right corner
    b. Insert a comment that the channel settings were double-checked
        i. In the upper right part of the screen there is a text box. In that text box, type in "settings"
            1. To the left of the text box, there is a drop-down box. Ensure that it is set to "All".
            2. Press the "Add" button to the right side of the text box
                a. Drag the comment closer to the actual moment of change by hovering the mouse over the small black box right below the comment (at the bottom of the screen). Once a white left/right arrow pops up, you can drag the comment
5. To get back to the live recording of data, press the button in the bottom right corner entitled "Show latest data" (hover your mouse over the button to see the label name)

APPENDIX D

Pressure Calibration Protocol

1. Zero the pressure transducer before collecting data
    a. Take the pressure transducer and attach it to the clear piece, with the white cap, by pinching the clear prongs together and fitting the circle around the pressure transducer's rim
    b. Attach the pressure transducer to the wooden block using Velcro™ for stability
    c. Attach the manometer (sphygmomanometer dial piece) via the blue stop cock (the tubing of the manometer should run parallel, not perpendicular, to the pressure transducer cable)
        i. The airtight screw end should attach to the outlet on the stop cock that is 180 degrees from the tube that attaches the manometer
        ii. Remove the white stop cock on the pressure transducer, opening it to atmospheric pressure
        iii. Ensure that the hand within the manometer dial is within the small rectangle at the bottom while zeroing
    d. Ensure that the pressure transducer is stable
    e. Press the start button on LabChart™ to collect data
        i. Collect data for 3 seconds before pressing "stop"
        ii. Highlight the most recent section of the blue (pressure) data
            1. Select "Pressure" from the drop down box on the right side of the screen
            2. Select "Bridge Amp"
            3. Set range to 500 mV
            4. Do not set a low pass value
            5. Do not check "Mains filter" box
            6. Press "zero" button
            7. Click "OK"
    f. Leave a comment on LabChart™ noting that zeroing has occurred by pressing Alt+p
    g. Return the white cap back to the clear piece
2. Take the syringe (25 cc/ml) and pull the plunger out to the end of the syringe
    a. Add the syringe to the open outlet on the blue stopcock
    b. Press "start" on the computer
    c. Depress plunger on the syringe until the dial reads 40 mmHg- hold this consistently for 5 seconds
        i. Add a comment by pressing Alt+4
    d. Press "stop" on the computer
    e. At the bottom of the screen, adjust the horizontal scaling to approximately 50:1 or until the "zero pressure" plateau and the 40 mmHg plateau are visible
    f. Highlight the two plateaus by starting at the "zero pressure" and finishing at the 40 mmHg plateau
    g. Click the pressure drop down box (on the right side of the screen)

i. On the bottom left side of the mini screen should be a + and – button. Press the + button until you can see the bump on the small graph.
    1. If data does not show up on the chart, make sure the zero pressure and 40 mmHg are both on the right side of the blue line on the data collection chart
ii. Click the Units Conversion “on” button in the upper right-hand corner
iii. Click your cursor on the first plateau
    1. Click the arrow button next to “Point 1”- a value should automatically appear
    2. Type a “0” into the next text box
    3. In the “Units” drop down box, click “mmHg”
iv. Click on the second plateau
    1. Click the arrow button next to “Point 2”- a higher value should automatically appear
    2. Type “40” into the next text box
v. Click “OK”
vi. Insert comment by pushing Alt+C

APPENDIX E

Flow Calibration Protocol

1. Zero the spirometer before collecting data
    a. Take the tubes off of both sides of the flow head meter
        i. Hold the flow head steady while you run 3 seconds of data collection
        ii. Click “stop”
        iii. Highlight the most recent flow signal (green line)
        iv. In the “Flow” dropdown box, click “Spirometer”
            1. Set the Range to 200 mV
            2. Set the Low Pass to 100 Hz
            3. Do not check the “Invert” box
            4. Click “Zero” button
            5. Click “OK”
        v. Leave a comment that zeroing occurred by pressing Alt+F
    b. Attach the flow head meter (via the blue piece) to the top of the pneumotach calibration unit. Ensure that the arrow on the flow head meter is point up
        i. Switch on the power of the back of the pneumotach calibration unit
        ii. Set the Flow rate to ½
        iii. Set the Liter to 1
        iv. Default mode should be on “Flow”
        v. Turn on “Start” on LabChart™
        vi. Flip up the “start” switch. The machine will produce three inhalations and three exhalations
        vii. Select the middle “up” whole single signal on LabChart™
        viii. Click the “Flow” dropdown box
        ix. Select “Spirometry Flow”
        x. Next to “Flow Head”, click MLT 300 L
        xi. Click “Calibrate”
        xii. Insert 1L in injected volume
        xiii. Click “OK”
    c. Leave a comment noting that calibration occurred by pressing Alt+1
2. Ensure that Channel 3 (Flow) is now in L/s
3. Reattach the flow head meter to the tubing under the benchtop setup. The arrow on the flow head meter should point in the direction of flow (left).
    a. Do not remove the clear tube attachments between the LabChart™ box and the flow head meter.

APPENDIX F

Pig Tissue Dissection and Preparation Protocol

Gross Dissection

1. Pick excised pig larynges up from Circle V Meats (609 Arrowhead Trail, Spanish Fork, UT 84660) within two hours postmortem.
2. Transport the excised larynges in a Styrofoam™ cooler to 106 Taylor Building Annex (106 TLRA).
3. Put on safety goggles, disposable plastic apron, and disposable gloves.
4. Cover the dissection table with dissection mats.
5. Using a No. 10 stainless steel disposable scalpel, carefully remove all extra-laryngeal structures and tissue, including the esophagus, lungs, vascularization, extrinsic laryngeal muscles, innervation, fat, and glands.
6. Cut the trachea to 50 mm if possible.
7. Examine all larynges for any damage to the superior 50 mm of the trachea, or arytenoid, cricoid, and thyroid cartilages.
    a. Discard any larynges that have superior damage to the trachea or perforations in the thyroid, arytenoid, or cricoid cartilages.
8. Briefly run each larynx under lukewarm running water to remove excess tissue or blood clots.
9. Place each larynx in its own gallon-sized plastic bag, or Nalgene™ bottle.
    a. If a plastic bag was used, place that plastic bag within another gallon-sized plastic bag so that the larynx is double bagged.
10. Pour PBS solution into the inner plastic bag or Nalgene™ bottle until the larynx is completely immersed.
11. In a Styrofoam™ cooler, transport all larynges that have undergone dissection to the BYU Chemistry in the Nicholes Building on BYU campus.

Flash Freezing Protocol

1. Immediately after completion of gross dissection, transport the larynges in a Styrofoam™ cooler to the BYU Chemistry Store in the Nicholes Building on BYU campus.
2. At the BYU Chemistry Store, ask an employee to fill a Styrofoam™ cooler with liquid nitrogen.
3. Put on cryogenic gloves.
4. While wearing cryogenic gloves, carefully lower the plastic bags into the liquid nitrogen. Hold the bag suspended in the liquid nitrogen without letting the bag touch the bottom or sides of the Styrofoam™ cooler to ensure larynges are evenly frozen.
    a. If the plastic bag breaks, place the ripped bag in a new bag and continue to freeze.
5. Begin a 7-minute timer.
6. When the timer ends, check the plastic bag for remaining PBS solution that has not completely frozen. If there is still liquid remaining, submerge the bag for another minute. Continue to check the bag each minute until the larynx and PBS solution are completely frozen.
7. If the larynx was placed in a Nalgene™ bottle, follow the same steps as listed for the plastic bag.

   a. Do not leave the bottles in the liquid nitrogen for longer than necessary as there is risk of the plastic exploding.
8. Place all of the frozen larynges in the Styrofoam™ cooler and transport back to the Taylor Building Annex.
9. Ask a BYU Chemistry Store employee to dispose of the excess liquid nitrogen safely.
10. Pay for the liquid nitrogen using the McKay School of Education Grant.
11. Place all of the frozen larynges in the ThermoScientific™ Freezer at -80˚C until they are to be used for data collection.
   a. Larynges should be frozen for at least 24 hours before they are used for data collection.

Fine Dissection

1. Two hours before fine dissection, remove the frozen larynges from the ThermoScientific™ Freezer. With the larynges still in the plastic bags, submerge them in a room temperature water bath in the lab sink to thaw.
2. Leave the larynges full submerged until they are fully thawed (approximately 1-2 hours).
3. Cover the dissection table with dissection mats.
4. Prepare appropriate instrumentation for dissection: hemostatic forceps, manicure scissors, medical tweezers, and stainless steel disposable scalpels (No. 10, 11, 15).
5. Put on plastic gloves, safety goggles, and a disposable plastic apron.
6. Before any fine dissection, use the digital scale and digital calipers to measure the following for each larynx: weight (in ounces) before dissection, length of thyroid cartilage from the thyroid prominence to bottom, and length of thyroid cartilage from top to bottom.
7. Using a No. 10 stainless steel disposable scalpel, remove the superior portion of the epiglottis, taking care not to cut into the arytenoid cartilages.
8. Using the same scalpel, carefully cut away the muscles and fascia that connect the thyroid cartilage to the ventricular folds.
9. Make an incision in the thyroid cartilage approximately .5 cm above the thyroid prominence. Continue to cut the thyroid cartilage bilaterally at a slight upward angle posteriorly. The cut portion thyroid cartilage should detach from the larynx.
10. Using a hemostat and No. 11 stainless steel scalpel, dissect away the ventricular folds. Take care to leave the arytenoid cartilage and true vocal folds intact.
11. Carefully separate the fascia on the superior surface of the thyroarytenoid muscle from the true vocal folds and dissect away the fascia.
12. Remove any leftover tissue superior and posterior to the vocal folds. This is removed to prevent vibration of tissue outside of the vocal folds.
13. Trim the inferior end of the trachea to 50 mm for ease in mounting the larynx on the benchtop.
14. Tie a large knot in the end of the suture thread. Using a hemostat to hold the suture needle, insert the suture needle just above the anterior commissure of the thyroid cartilage. Securely tie a knot in the thread to ensure that it will not pull through. Trim the suture needle off of the end of the thread and dispose of it properly in the red hazardous waste box.
15. Using the scale and digital calipers, measure the following for each larynx: weight (in ounces) after fine dissection, length of trachea, width of trachea, width of thyroid

cartilage, length of the thyroid cartilage from the top to the prominence, length of the vocal folds, 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).

16. Any unique physiology characteristics should also be noted.
17. Place the larynx in a plastic bag and fill with fresh PBS solution until the larynx is completely immersed. With a permanent marker, label the plastic bag with the corresponding larynx number. Place in the refrigerator until the larynx is mounted on the benchtop.

APPENDIX G

Data Acquisition Protocol

These procedures should occur immediately after fine dissection of the pig larynges and flow and pressure calibration. At least two research assistants are required to collect data on pressure and flow phonation, with one running (1) LabChart™ and the computer and the other performing (2) Mounting and Air responsibilities at the benchtop:

1. LabChart™ and Computer:
    a. Press "start" before the trial begins.
    b. Manually type "P# DEV Trial 1" if the device is being used for the trial. Type "P# NO Trial 1" if the device is not being used for the trial.
        i. Replace "#" with the corresponding pig number for each larynx.
        ii. Press enter to insert in channel 1 (microphone channel).
    c. At the onset of phonation, press Alt+O (pre-set comment: "Phonation Onset").
    d. At the steady-state of phonation, press Alt+S (pre-set comment: "Phonation Steady State").
    e. At the cessation of phonation, press Alt+T (pre-set comment: "Phonation Offset").
    f. Between each trial press "stop" and then "start" before the next trial.
        i. After every three trials, press "Save" to save the collected data.
    g. For each trial, adjust the text box comment to correspond with the appropriate trial number. Click enter to leave the comment before each trial.
        i. If a trial needs to be redone, add "redo" to the textbox.
            1. Ex: P1 NO Trial 4 redo
    h. Repeat until 15 trials are complete.
    i. Ensure signals look normal during phonation.
    j. Leave additional comments regarding difficulty in phonation, re-recording trials for irregular signals, etc.
    k. During trials, take notes on the data Excel™ sheet.
        i. Ex: initial and final humidity and temperature, airflow requirements, pressed phonation, notes on why a trial was redone, etc.
2. Mounting and Air:
    a. Mount the pig larynx on a custom benchtop setup. Use a metal hose clamp at the base of the trachea to secure trachea to airflow tube and prevent any air leakage.
        i. Insert micropositioners into the arytenoid cartilages at the same level. Tighten the micropositioners medially until the vocal folds are adducted and level.
        ii. Tie the suture thread to the anterior micropositioner and tighten anteriorly until the thread is taut, but not too tight.
        iii. Ensure that the larynx is sitting up straight and is secure.
    b. Gradually turn on air flow using hand-dial on the air tank until steady phonation is perceived. Allow the larynx to phonate for approximately 3 seconds and then gradually turn the air tank off.
    c. Between every three trials, lightly mist the larynx with PBS solution.

APPENDIX H

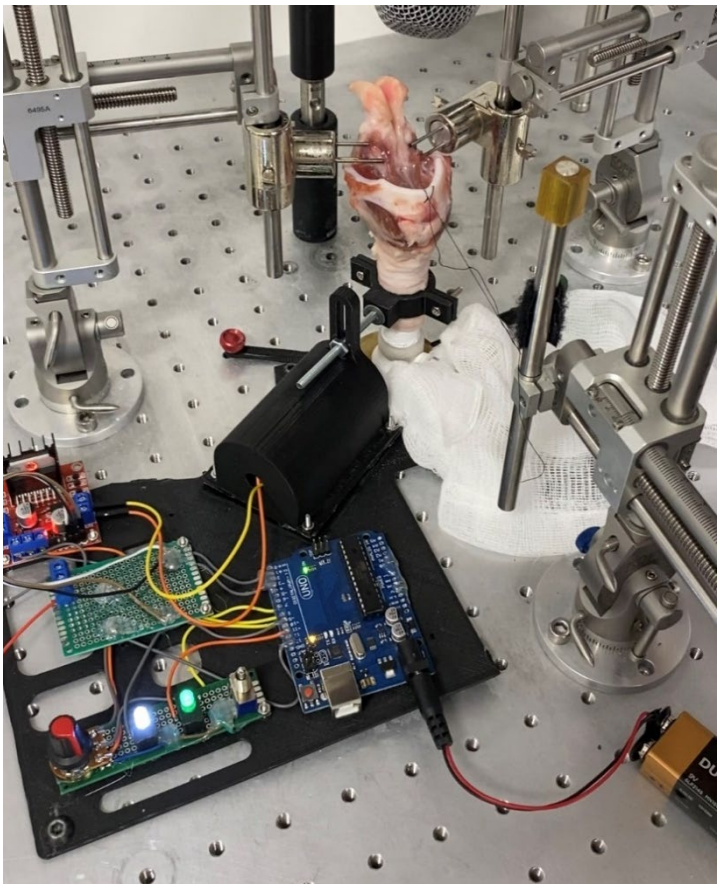
Pilot Trials and Data Collection

Prior to collection of the data used in the current study, pilot trials were conducted using the same methods described in this study. These trials were conducted primarily to test the prototype of the external oscillatory device and make revisions. Initially, the device consisted of a plastic clamp that was placed around the trachea. Bolts were used to tighten and hold the clamp in place. During pilot trials, it was concluded that the device should be applied to the larynx at the vocal folds, instead of the trachea, in order to produce the most significant impact. These pilot trials, also indicated that the plastic clamp was unable to attach securely around the larynx and would slip down during phonation trials. In conjunction with the Mechanical Engineering department at BYU, a revised model of the external oscillatory device was created. This resulted in the updated model of the device utilized in the current study.

In total, seven pig larynges were used to conduct pilot trials. Each larynx underwent 30 phonation trials, 15 with the external oscillatory device applied and 15 without the external oscillatory device applied. Figure 9 shows the prototype device attached to a pig larynx mounted on the benchtop.

Figure H1

Prototype of External Oscillatory Device With Mounted Larynx



APPENDIX I

Pig Phonation Trial and Error

Various methods were utilized to achieve phonation of the pig larynges. Phonation was best achieved when the vocal folds were on a level plane and were adducted when at rest. Micropositioners were used to approximate the arytenoid cartilages and adduct the vocal folds. In some larynges, maximum adduction of the vocal folds was achieved, but there was still air leakage through the posterior glottis, between the arytenoid cartilages. In these cases, denture cream (poly-grip) was inserted between the arytenoid cartilages to seal the posterior glottis and prevent the leakage of air posteriorly. In turn, this directed airflow through the vocal folds exclusively. The larynx was stabilized using both micropositioners and a suture attached from the anterior thyroid cartilage to an anteriorly located micropositioner, which moved anteriorly and posteriorly.

During some trials, air leakage occurred at other points within the benchtop setup. One point of such air leakage was out of the bottom of the trachea. A hose clamp was used to secure the tubing to prevent leakage of air. A loose tube in the airflow pathway could also be responsible for air leakage. If a larynx was not phonating properly, the airflow pathway was thoroughly checked to ensure that no tubes were leaking air. If an air leak was discovered, the tubing was either replaced or Teflon^{TM} tape was used to create an airtight connection.

APPENDIX J

Data Segmentation and Analysis Protocol

Segmentation

1. Selecting Signals for Segmenting
    a. Open LabChart™ version 8.
    b. Open the desired file from the Desktop folder "LabChart Data."
    c. Select the pre-collected pig larynx signals that you want to segment by clicking your mouse at the beginning of the data and dragging it until the end of the data.
        i. Save a segmentation selection file for each pig larynx for the trials with the device and other file for each pig larynx for the trials without the device.
    d. Select "File" and then "Save Selection."
        i. Rename file and save in designated folder.
            1. Rename file to "P# [DEV or NO] [Research Assistant's Initials] Segmentation."
        ii. Do not save changes to the main LabChart™ data file.
    e. Open new file to segment.
2. Marking Trial Errors
    a. Identify any trials where errors occurred, and the trial had to be repeated.
        i. For the trial with errors, delete all of the preplaced markers (trial label, phonation onset, phonation steady state, and phonation offset).
        ii. This is done so that the trial error will not be included when MatLab™ analysis is performed.
    b. Keep detailed notes on which trials were in error and where they are located in the data.
3. Placing Onset, Steady State, and Offset
    a. Zoom in to 2:1
    b. Analyze the waveform and place onset on the second peak after the waveform begins to appear semi-periodic.
        i. To move the previously inserted comment, right click on the black box and select "Move comment."
    c. Place steady state after onset where the signal appears periodic.
    d. Examine both periodicity and amplitude of waveform to determine where offset is and place the marker on the last semi-periodic peak before signal dies out.
    e. Note: You can use both the audio and visual signal from the microphone to help identify the approximate location of onset and offset.
4. Export Segments
    a. Click "File" > "Save" and save segmented file as a new file.
    b. Select "File" > "Export" to convert file to txt file.
    c. Save the txt files in the correct folder and upload to custom MatLab™ Program for further analysis.

Analysis

1. Open MatLab™ Application

   a. Click “Open File” > select desired segmented txt file.
   b. Drag the yellow boxes on the screen out of the way. These are unnecessary for analysis.
   c. Count trials to ensure that all 15 trials have been included in the txt file.
2. Selecting Results
   a. Move red markers on microphone signal data to surround one trial of phonation.
      i. Note the placement of the vertical lines between pressure signal peaks. The red markers should be placed as close to these lines as possible but must be within the vertical markers.
   b. Press “play” in order for application to register line placement
3. Select “save”
   a. Save as “rabbit#[DEV or NO]_trial#”. It will save as a CSV file (both sound and Excel file).
4. Open Excel file to view flow, pressure, and resistance values for phonation onset, phonation steady state, and phonation offset.

APPENDIX K

Thesis Timeline

- 9/20
  - Ordered materials for the lab
  - Created inventory of the lab
  - Initial discussion of thesis focus and the creation of the external oscillatory device
- 10/20
  - Discussion of appropriate COVID-19 protocols for the lab and data collection
- 11/20
  - Obtained excised pig larynges from local slaughterhouse
  - Received training on gross dissection of excised pig larynges and flash freezing
- 1/21
  - Collected excised pig larynges from local slaughterhouse
  - Gross dissection and flash freezing of excised pig larynges
- 2/21
  - Collected excised pig larynges from local slaughterhouse
  - Gross dissection and flash freezing of excised pig larynges
  - Consultation with research assistant from engineering lab regarding creation of the external oscillatory device
  - Trained in fine dissection of pig larynges
  - Trained in pressure and flow calibration
  - Trained in mounting and phonation of pig larynges
  - Trained in collection of acoustic, aerodynamic, and visual data for larynges with and without the external oscillatory device
  - Conducted data collection using the first model of the external oscillatory device
  - Segmentation and analysis of data
  - Modification of external oscillatory device
  - Further discussion of external oscillatory device creation and thesis method
- 3/21
  - Fine dissection, collection of acoustic, aerodynamic, and visual data for larynges with and without the external oscillatory device
  - Trained in data segmentation and analysis using LabChart™ and MatLab™
  - Ran trials using the second version of the external oscillatory device
  - Data segmentation and analysis using LabChart™ and MatLab™
- 4/21
  - Fine dissection of pig larynges
  - Collection of acoustic, aerodynamic, and visual data for larynges with and without the external oscillatory device
  - Ran trials using the final/third version of the external oscillatory device
  - Replaced the air tank used for phonatory trials within the lab
  - Replaced tubing in the benchtop setup due to air leakage
  - Data segmentation and analysis using LabChart™ and MatLab™
  - Collaboration with engineering department regarding modification of external oscillatory device and benchtop setup

- 5/21
  - Fine dissection, data collection
  - Data segmentation and analysis using LabChart™ and MatLab™
- 6/21
  - Discussion of how to analyze data
  - Data segmentation and analysis using LabChart™ and MatLab™
- 8/21
  - Write prospectus
- 9/21
  - Edit prospectus document
  - Complete Prospectus meeting with thesis committee, discussing specific thesis questions and the importance of current study
- 10/21
  - Edit prospectus documents according to feedback received from thesis committee
- 3/22
  - Prepare for thesis defense by completing first written draft of thesis document
  - Schedule oral thesis defense
- 5/22
  - Complete oral thesis defense