8-2016

Hydration and vocal loading on voice measures

Anusha Sundarrajan
Purdue University

Graduate School Form
30 Updated 12/26/2015

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By Anusha Sundarrajan

Entitled
HYDRATION AND VOCAL LOADING ON VOICE MEASURES

For the degree of Doctor of Philosophy

Is approved by the final examining committee:

Dr. Preeti M. Sivasankar
Chair

Dr. Anne Smith

Dr. Stacey Halum

Dr. Susan deCrane

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Approved by: Dr. Keith R. Kluender 7/7/2016
Head of the Departmental Graduate Program Date

HYDRATION AND VOCAL LOADING ON VOICE MEASURES

A Dissertation

Submitted to the Faculty

of

Purdue University

by

Anusha Sundarrajan

In Partial Fulfillment of the

Requirements of the Degree

of

Doctor of Philosophy

August 2016

Purdue University

West Lafayette, Indiana

To my Grandparents

ACKNOWLEDGEMENTS

“Karmanye vadhi ka rasthe, maa phaleshu kadachana,

ma karma phala hetur bhur, matey sangostva karmani”

Since my childhood, I have believed in this saying from the Bhagvad Geeta. This saying translates to “we as humans have the right to perform our actions, but we are not entitled to the fruits of the actions”. The meaning has definitely inspired me during my PhD. My motto is to keep working harder and harder, and not be deterred by any obstacles. In my opinion, perseverance is the key to success and ***SOME IMPORTANT PEOPLE*** help you achieve great heights. I have a long list of these important people in my life who have helped and supported me throughout these years. Above all, Lord Krishna has always been there for me and with me, and I thank him for his graciousness.

First and foremost, I would like to express my deepest sense of gratitude and sincere thank you to my mentor, Dr. Preeti Sivasankar. It would have been impossible without her constant support and belief in me. Thank you for the valuable guidance and everlasting encouragement throughout the PhD. You have been a source of inspiration to me always. You are a “star” and I wish I could be at least 5% of you some day.☺

I would like to thank my committee members Dr. Stacey Halum, Dr. Anne Smith, and Dr. Susan deCrane for providing feedback and input to better my dissertation in every possible way. All three are successful and accomplished researchers and specialists in their area and I am grateful to have each of you in my committee. Special thank you to Dr. Smith who is truly a very good listener.

My interest in aging stemmed from a course taught by Dr. Ken Ferraro. He encouraged me to pursue a minor in Gerontology (and I received it without doing extra coursework!!!). This was a great opportunity to meet and collaborate with the faculty in the Center of Aging and Life Course. I will definitely miss being part of the CALC family. Big thank you to Traci for helping me in older subject recruitment.

I would like to thank all my lab mates Rob, Anumitha, Sara, Abby, Tanaya, Xinxin, Abby D, and Nathan who have definitely helped me combine “FUN” and “LAB” together.☺ I have learnt small things from each one of you that have made me a better person every single day. I deeply cherish the times in lab specially our lab meetings which have been a great avenue for learning and improving my limited knowledge. Special thanks to Sara, who has contributed immensely to this project. I think she is the person who has seen me in every phase of this dissertation. I thank her for keeping me sane.☺ I have found a great friend in Rob and I hope we can go to conferences once every 6 months and continue to have interesting conversations. Anumitha-I absolutely have no words for you. I love how comfortable you are with me, be it in appreciating or criticizing my work. I would like to thank Jena and Logan in helping me with data collection. I would like to thank Chris (School of Nursing) for helping in participant recruitment. Special thank you to all my participants who made this project possible.

Music has always been a part of my life. I am thankful to ICMAP in reminding me of my strong Indian cultural and spiritual roots. Through ICMAP, I have made some wonderful friends-Shankar, Prasad, Adharsh, Upasna, and Swetha. Thank you all for the wonderful memories and experiences. I hope we all keep in touch and plan grand concerts together.☺

Purdue has given me an opportunity to meet some amazing people who are very special to me. Big thank you to my “PhD clan”-Janet, Cagla, Erica, Rana, Varsha, Anne, Saryu, Satya, Vijay, and Yue. You all have made my journey special and worth sharing. A special thank you to my friends, now my family- Ranjini, Pradeep, and Rohith. I can’t imagine a life without you guys. Love you all!!!

A very special thank you goes to the dearest persons in my life. Thank you Madhu and Prashant for playing a key role by being great mentors. Madhu-I cannot thank god enough for giving me the best sister. You have supported, helped, and encouraged me throughout. I love how you have motivated me to face challenges in life. I respect you for your practical and rightful thinking. You have for sure made me a better critical thinker. Prashant Bhaiya-Kudos to you for your patience!!!! Thank you to my friends-Subha, Nisha, Shumitha, Zainab, and Sivapriya for filling my life with laughter and happiness.

Very special thanks to my parents for providing me with the strength, encouragement and support. You both have stood by me in everything, both in personal and professional decisions. Appa and Amma, you both are the greatest treasure in my life. Thank you. Thank you, for everything…No one can replace you both!!! I would like to express my sincere gratitude and respect to my in-laws. Mama and Mami-you both have accepted me as your daughter whole-heartedly. I hope I have made you proud!!!

I owe my PhD success to my dearest husband Surya. I don’t think I would have been able to come this far without your love, trust, support, and understanding. We both have come a long way together and I hope our 50-50 deal never fades off.☺ Love you forever!!!!

Above all, as an inadequate expression of love and respect, I dedicate this dissertation to my Rangu Thatha who has instilled the strength and will in every phase of my life.

Life at Purdue would not have been easier without the people who have been a part of my life, who have shaped it, and who in turn created little pieces of the person I have become. I am indescribably grateful to everyone. As Oscar Wilde said "Experience is a teacher of all things". I think this process of PhD has been a wonderful learning experience in my life.

TABLE OF CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Anusha Sundarrajan, Ph. D., Purdue University, August 2016. Hydration and Vocal Loading on Voice Measures. Major Professor. Preeti M. Sivasankar.

Vocal loading adversely affects the healthy larynx. The negative effects of vocal loading are thought to be exacerbated in dry environments, noisy environments, using non-habitual speaking patterns, and voice quality. Advancing age is also thought to be a risk factor for the negative effects of loading. To systematically tease out the effects of these factors on the healthy larynx, three different experiments were conducted. In each experiment, healthy participants produced 45-minutes of child-directed speech. In experiment 1, older, healthy adults produced loud child-directed speech, in the presence of background noise, in both low and moderate humidities, and voice was assessed. In experiment 2, young, healthy adults produced loud child-directed speech, in the presence of background noise, in both low and moderate humidities, and voice was assessed. In experiment 3, young, healthy adults produced child-directed speech using low-effort whisper quality and voice was assessed. In each experiment, voice measures included Phonation Threshold Pressure, Cepstral Peak Prominence, self-perceived phonatory effort, and self-perceived vocal tiredness. These voice measures were collected at set points of the frequency range. Our data suggest that the aging larynx is negatively affected by 45-minutes of loud child-directed speech and that humidification is beneficial in reducing these negative effects. Younger adults are also negatively affected by 45-minutes of loud child-directed speech, but not whispered speech. Increasing ambient humidity does not minimize these effects. The adverse effects of loud speech are much greater than whispered speech. Overall, these data increase our understanding of factors that load the larynx and lay the foundation for developing clinical tests to identify speakers who are susceptible to voice problems.

CHAPTER 1. INTRODUCTION

Larynx is an important organ which serves as a sound generator during speech production (Kahane, 1981). Normal voice production requires the respiratory, laryngeal, and supralaryngeal systems to work in a coordinated fashion. Disruptions or breakdowns to any one or more speech subsystems can lead to voice problems. A voice disorder is defined “to be any time the voice does not work, perform, or sound as it normally should, so that it interferes with communication” (Roy, Merrill, Thibeault, Parsa, et al., 2004). Voice disorders are associated with symptoms like vocal fatigue, throat discomfort, hoarseness, reduced pitch, limited loudness range, increased vocal effort, weak voice, and/or changes in vocal quality, and loss of voice. Symptoms like social isolation, depression, impaired general and disease-specific quality of life, and work absenteeism commonly occur in individuals with voice disorders (S. Cohen, Dupont, & Courey, 2006; Roy, Merrill, Gray, & Smith, 2005; Roy, Merrill, Thibeault, Gray, & Smith, 2004). Specific vocal behaviors (i.e., loud talking, prolonged talking) can cause voice disorders (Roy et al., 2005). Exposure to low ambient humidity in speaking environments can also increase susceptibility to voice problems (M Sivasankar, Erickson, Schneider, & Hawes, 2008a).Vocal aging results in changes to the structure and function of the laryngeal system and can therefore make the larynx vulnerable to voice problems. There is an on going controversy on whether whispering is detrimental than normal speaking (A. Rubin, Praneetvatakul, Gherson, Moyer, & Sataloff, 2006), and authors claim that whispering induces abusive forces to the vocal folds (Griesman, 1943; Pressman, 1942) . Thus, voice production can be affected by loud talking, whispered speech, exposure to a low humidity environment, or with aging. The overarching goal of the present study was to determine the effects of vocal loading induced by a combination of loud speech, whispered speech, aging, and low ambient humidity on the larynx.

The following chapters provide pertinent background information on vocal loading, vocal fold hydration, and vocal aging. In particular, Chapter 2 consists of literature review on vocal loading and vocal aging specifically focusing on studies in non-dysphonic speakers using videostroboscopic assessments, acoustic and aerodynamic measures, and ratings of perceived phonatory effort. A review of vocal fold hydration is included in Chapter 2. The purpose of the study is then outlined. Chapter 3 provides data from older adults and Chapter 4 covers data from young adults.

CHAPTER 2. REVIEW OF LITERATURE

2.1 Vocal loading

Vocal loading is a concept defined by a "combination of prolonged voice use and additional loading factors (e.g. background noise, acoustics, air quality) affecting the fundamental frequency, type and loudness of phonation, or the vibratory characteristics of the vocal folds as well as the external frame of the larynx" (Vilkman, 2004). The four distinct phases involved in vocal loading are: warm up (adapting to the voicing task), performance (continuation of the voicing task), vocal fatigue (perceived increase of physical effort associated with voicing; physical changes to the larynx), and rest or recovery (Jilek, Marienhagen, & Hacki, 2004; Vintturi, Alku, Sala, Sihvo, & Vilkman, 2003).

Vocal loading is commonly induced in the laboratory with a prolonged reading task. Prolonged loud reading is commonly used to mimic excessive voice use and induce symptoms of vocal fatigue (Gelfer, Andrews, & Schmidt, 1996; Kelchner, Lee, & Stemple, 2003; Stemple, Stanley, & Lee, 1995; Vintturi et al., 2003). Table 2.1 provides an extensive review of the studies examining the effects of vocal loading using a prolonged reading task.

2.1. a. Duration of vocal loading

Vocal loading induced by prolonged speaking is a means to experimentally induce vocal fatigue. The duration of speaking tasks vary from 15-30 minutes (Akerlund, 1993; Linville, 1995; Niebudek-Bogusz, Kotylo, & Sliwinska-Kowalska, 2007; Stone & Sharf, 1973) to 45-90 minutes (Gelfer, Andrews, & Schmidt, 1991; Gelfer et al., 1996; Laukkanen et al., 2004; Lohscheller, Doellinger, McWhorter, & Kunduk, 2008; Neils & Yairi, 1987; Scherer et al., 1991; Sihvo, Laippala, & Sala, 2000; Vintturi et al., 2003) to 2 or more hours (Boucher & Ayad, 2010; Chang & Karnell, 2004; Kelchner, Toner, & Lee, 2006; Lauri, Alku, Vilkman, Sala, & Sihvo, 1997; Scherer et al., 1991; Solomon & DiMattia, 2000; Solomon, Glaze, Arnold, & Van Mersbergen, 2003; Stemple et al., 1995; Vilkman, Lauri, Alku, Sala, & Sihvo, 1999). Among the many above mentioned studies, of particular interest are the ones by Gelfer et al (1991, 1996) as these studies induced loading for 1-hour.

In a study by Gelfer et al. (1991), 26 vocally trained singers and 24 untrained women participated in a 1 hour prolonged loud reading task. Prior to and after the reading task, acoustic measures of voice were collected. These measures included fundamental frequency (F0), intensity, jitter ratio, shimmer, and signal-to-noise ratio. The results indicated that the jitter ratio increased and signal-to-noise ratio decreased in trained singers when pre vs. post loading data were compared. In addition, trained singers showed a general consistent pattern when vocal function was compared. Authors like Laukkanen et al. (2006) and Stemple et al. (1995) report that the acoustic changes of F0, signal-to-noise ratio, and intensity changes to be either warm-up or fatigue effects (Orlikoff & Baken, 1990; Orlikoff & Kahane, 1991).

Gelfer et al. (1996) investigated the effects of 1-hour prolonged loud reading on 8 young women singers and 8 young women with limited musical experience. Videostroboscopic examinations were conducted before and after the prolonged reading task. Three experienced judges evaluated the randomized pre-test and post-test videotaped samples. A total of 9 parameters were rated by the three judges. The parameters were: presence or absence of glottal closure at the most closed point in the vibratory cycle, glottal configuration at its most closed point, amplitude of excursion of the vocal folds at their most open point, asymmetry of amplitude and asymmetry of phase, presence or absence of various vocal fold features like injection (visible and presumably distended blood vessels) in the true and false vocal folds or the epiglottis, mucus on the true vocal folds, irregular vibratory cycles (visualized as a "fluttering" of the vocal folds during the stroboscopic recording). Greater amplitude of glottal opening was revealed for the untrained group when the pre-test condition was compared to the post-test condition. No significant pre-test to post-test changes were found for the singer group. The authors concluded that a 1-hour prolonged loud reading task was not sufficient to induce notable laryngeal alterations. However, others have reported vocal changes after 1-hour of loud reading (Chang & Karnell, 2004).

2.1. b. Whispered speech

A basic understanding of the perceptual characteristic of a whisper includes fast airflow through vocal tract constrictions resulting in aperiodic noise (Solomon, McCall, Trosset, & Gray, 1989). A whisper can be produced in two ways; a quiet whisper with low-effort, and loud whisper with high-effort (Brodnitz, 1958; Landes, 1977; Luchsinger

& Arnold, 1965; Pressman & Kelemen, 1955; H. Rubin & Lehrhoff, 1962). Two most common vocal fold configurations reported are the inverted “Y” which is oriented with the anterior larynx at the top and the posterior larynx at the bottom (Luchsinger & Arnold, 1965; Monoson & Zemlin, 1984; H. Rubin & Lehrhoff, 1962). The other commonly reported configuration is an inverted “V” which is bow shaped (Monoson & Zemlin, 1984; Sweat, 1983). Inconclusive findings are reported on the influence of effort level on the laryngeal configuration during a whisper production (Landes, 1977; Monoson & Zemlin, 1984; H. Rubin & Lehrhoff, 1962). To date, limited number of studies are available on whisper production. Studies have focused mainly on laryngeal configuration (Solomon et al., 1989), airflow (Weismer & Longstreth, 1980), resonance (Ito, Takeda, & Itakura, 2005), and speaker identification (Higashikawa, 1994).

From an aerodynamic perspective, mostly single subject studies have explored the relationships between subglottal pressure, glottal flow, and glottal area. Oral air pressure and oral flow data has revealed mixed results when whispering and speaking are compared. Greater peak flow and no difference in air pressure was reported for whisper as compared to normal speaking (Weismer & Longstreth, 1980). Stathopoulos and colleagues (1991) examined respiratory and laryngeal function in healthy adults during normal speaking and whispering. Chest wall kinematics, pressure, and flow data was obtained using magnetometers. Subjects read a paragraph using a comfortable whisper, at normal loudness and rate for the whispering task. For the speaking task, subjects read a paragraph in a comfortable voice. Measures of vital capacity, abdominal capacity, isovolume and relaxation maneuvers were obtained. Lower tracheal pressures, higher translaryngeal flows, and lower laryngeal airway resistance were observed in whispering than speaking. The authors concluded that chest wall function during whispering is different in normal subjects and in individuals with speech and voice disorders (Stathopoulos, Hoit, Hixon, Watson, & Solomon, 1991).

2.1. c. Measuring the effects of vocal loading in the laboratory

2. 1. c. i. Videostroboscopic assessment of vocal loading

The videostroboscopic assessments have not shown consistent changes in the glottic configurations after vocal loading (Gelfer et al., 1991, 1996; Kelchner et al., 2006; Stemple et al., 1995; Vilkman et al., 1999). Two recent studies have utilized high speed digital imaging with phonovibrograms (Doellinger, Lohscheller, McWhorter, & Kunduk, 2009; Lohscheller et al., 2008). Doellinger and colleagues (2009) investigated the effects of continuous voice use during a working day using high-speed digital imaging technique (HSI) and the phonovibrogram (PVG) on one healthy subject. The HSI and the acoustic recordings were obtained while the subject produced the vowel /i/ at a comfortable, normal pitch, and loudness level. The vowel was recorded 13 times within 2 consecutive days in the morning before and in the afternoon after vocal loading. PVGs were used to extract and visualize the vocal fold dynamics. The parameters analyzed were based on the vocal load, left-right PVG asymmetries, anterior-posterior PVG asymmetries, and opening-closing differences. Significant changes in the vibration behavior were obtained when recordings performed in the morning and recordings after load were compared. The PVG revealed left-right asymmetry of the vibration pattern being statistically significantly approved for the posterior closing process which confirms that the right and left vocal folds are not identical in their histological makeup. Different dynamics between opening and closing procedure as well as for anterior and posterior parts were obtained. The authors concluded that constant voice use stresses the vocal folds even in healthy subjects and PVG technique can be useful in detecting those changes. Additionally, the left-right PVG asymmetries may occur in a healthy voice to some extent.

2. 1. c. ii. Acoustic assessment of vocal loading

Increase in fundamental frequency (F0) and vocal intensity, and a decrease in perturbation measures like jitter and shimmer have been reported acoustically (Jónsdottir, Laukkanen, & Vilkman, 2002; Kelchner et al., 2006; Laukkanen, Ilomaki, Leppanen, & Vilkman, 2008; Lehto, Laaksonen, Vilkman, & Alku, 2006, 2008; Rantala, Vilkman, & Bloigu, 2002; Sodersten, Granqvist, Hammarberg, & Szabo, 2002; Stemple et al., 1995; Vilkman et al., 1999).

In a study by Stemple et al (1995), voice production was assessed in 10 female graduate students before and after a 2 hour reading task. The voice measures examined in this study included acoustic and aerodynamic measures. Fundamental frequency, jitter, maximum phonation time, phonation volume, and flow rate were obtained from sustained phonation of vowels /a, i, u/. Six dimensions of phonatory function were examined using videostroboscopy, these include; the configuration of glottic closure, the condition of the vocal fold edge, the amplitude of vocal fold movement, mucosal wave, phase closure, and phase symmetry. The study experiment was designed to obtain subjective ratings of level of effort after the first and second hours of reading. The results of this study revealed only one significant finding. Decreased jitter was obtained during the production of high pitched vowels when pre-test and post-test data were compared. Post-loading, the reading fundamental frequency significantly increased. In 6 out of 10 subjects anterior glottic chinks were observed. These subjects did not present a glottal gap during the pre-test recording. The study results contribute to existing body of literature on chronic laryngeal fatigue with better understanding of the possibility of TA muscle involvement.

Kelchner et al. (2006) studied the effects of vocal loading in 25 healthy pubescent males. The participants were randomly divided into an experimental (2-hour of continuous loud reading) and a control (silent reading with brief periods of conversation) group. Pre-post acoustic (average fundamental frequency and intensity assessed during reading the 'Rainbow passage'), video-endoscopic, and perceptual (self-ratings of vocal quality and physical effort) were measured. Significant changes in fundamental frequency, self-ratings of vocal quality, and physical effort were observed in the experimental group. The control group did not report of any significant changes. Authors concluded that prolonged loud reading can induce temporary but measurable changes in fundamental frequency and self-perception of vocal function.

A large body of literature has focused on assessing vocal function with vocal loading in non-dysphonic speakers using mainly acoustic measures like fundamental frequency, intensity, and perturbation measures of jitter and shimmer (Kelchner et al., 2006; Laukkanen et al., 2008; Lehto et al., 2006, 2008; Stemple et al., 1995; Vilkman et al., 1999). To our knowledge, there have been no studies using the cepstral measures of cepstral peak prominence (CPP) and low/high spectral ratio (LHR) examining vocal function with vocal loading. Cepstral analysis is a measure of voice quality for vowels and continuous speech that focuses on the shortcomings of traditional methods of acoustic analysis. Cepstrum, as defined by (Noll, 1967) is a Fourier transform of the logarithmic power spectrum of an acoustic signal. It is a process of extracting the fundamental frequency from the spectrum of a sound wave. A typical cepstrum shows a well-defined fundamental frequency and harmonic structure that corresponds to a more prominent cepstral peak, whereas signals like dysphonic voices have disturbed periodicity and are associated with a decrease in amplitude of the cepstral peak. The ratio of low-frequency versus high-frequency spectral energy has also shown to contribute to predict dysphonia severity. Typically in normal voice signals, most of the energy is centered close to the fundamental frequency which determines the pitch in the voice. The vocal spectrum can be arbitrarily divided to a low-frequency and a high-frequency region and a simple ratio called the low/high spectral ratio can be obtained. Normal voice signals are typically characterized by a high LHR which means that the low-frequency energy is substantially greater than the high-frequency energy. CPP and LHR have been widely used to analyze vowels and continuous speech samples in dysphonic voices (S. Awan & Roy, 2009; Heman-Ackah, Micheal, & Goding, 2002; Lowell, Colton, Kelley, & Hahn, 2011; C. Watts & Awan, 2011; R. Watts, Ronshaugen, & Saenz, 2015; Wolfe, Martin, & Palmer, 2000).

Watts and Awan (2011) evaluated the diagnostic value of spectral/cepstral measures to differentiate dysphonic speakers versus non-dysphonic voices using sustained vowels and continuous speech samples in 32 age and gender matched individuals. Subjects were recorded reading a standard passage (“The Rainbow Passage”) and sustaining the vowel /a/. Four spectral/cepstral measures (CPP, CPP SD, LHR, and LHR SD) were calculated for each subject’s continuous speech and vowel productions.

CPP and LHR was found to be significantly different between the groups in both speaking conditions, while CPP SD was significantly different in continuous speech only.

In a study by Watts et al (2015), the effects of aging were investigated by using acoustic measures. Participants included 60 males between the ages of 20 and 79 years, and were divided equally into two groups (young and old). All subjects were required to sustain the vowel /i/ and perform a connected speech task (sentence “We were away a year ago”) from the Consensus Auditory Perceptual Evaluation of Voice (CAPE-V) protocol. Cepstral/Spectral Index of Dysphonia (CSID) is a multi-parameter variable that was obtained from the sustained phonation of vowels and connected speech recordings. Additionally, the experiment included the vocal amplitude calculation for these tasks. Overall, study results suggested higher CSID values and slightly lower vocal amplitude for the older subjects. Study findings indicated a non-significant effect for sustained vowels when the two groups were compared. However, the authors concluded that CSID in connected speech (standard sentences) may be most sensitive to the physiological effects of vocal aging in non-dysphonic male speakers.

2.1. c. iii. Aerodynamic assessment of vocal loading

PTP has been used in studies involving prolonged speaking (Chang & Karnell, 2004; Milbrath & Solomon, 2003; MP Sivasankar & Erickson-Levendoski, 2012; Solomon & DiMattia, 2000; Solomon et al., 2003). In a study by Chang and Karnell (2004), the relationship between perceived phonatory effort (PPE), and phonation threshold pressure (PTP) was assessed. PPE has been defined as a subjective index of vocal fatigue and PTP is a quantifiable measure defined as the minimal lung pressure required to initiate and sustain vocal fold oscillation. A total of 10 healthy adults participated in this study with equal males and females. A prolonged reading task was used that involved reading aloud for two hours at 75-85 dB SPL. The microphone was kept at a distance of 18 inches from the speaker's mouth. The experiment was designed to obtain PTP at low, mid, and high pitches, and PPE measures prior to, during, and following the vocal loading task at 15 different time points. The study findings indicated that both PPE and PTP increased steadily during the vocal loading task. Specifically, PTP at high pitches returned to the baseline condition within 15 minutes of task completion. However, low and mid pitched PTP were more resistant returning to the baseline condition at the one hour follow up time point. In contrast, for PPE, participants recovered from vocal loading only after 2 hours. Correlation coefficients using the Fisher z transformation were computed to measure reliability. Good correlation was obtained between mean PTP and mean ratings of PPE at the low and mid pitches, but the correlation was poor at high pitches.

Solomon and colleagues (2000, 2003) investigated the effects of vocal loading and systemic hydration in 4 women (Solomon & DiMattia, 2000) and 4 men (Solomon et al., 2003) using phonation threshold pressure (PTP), ratings of speaking effort (PPE), and laryngeal imaging. Both the studies used similar protocol and dependent measures. PTP and PPE increased as a result of 2-hour loud reading. PTP increase was delayed or attenuated to some degree in 3 of the 4 women when they consumed ample amounts of water in the baseline condition. In the study with men, 2 of the 4 men benefitted from increased systemic hydration. On laryngeal examination, 2 men had an anterior glottal gap after loading. When both studies were compared, data collected from men was found to be more variable than women. Benefits of water intake were more prominent in women than men. The study findings confirmed that prolonged loud talking leads to detrimental voice effects.

Sivasankar and Levendoski (2012) studied the effects of obligatory mouth breathing on voice during daily activities in low and high humidity environments. 63 vocally healthy adults participated in three mouth breathing challenges in either low humidity (≤30%) or high humidity (≥53%). Mouth breathing during everyday activities was simulated using three challenges: 15 minutes of obligatory mouth breathing alone (15M), in loud reading (15R), and in exercise (15E). Subjects were instructed to read aloud at 70 dB in the 15R challenge. PTP (PTP at the 10^{th} and 20^{th} pitch of the frequency range) and PPE (using a 10-inch visual analog scale) were measured at baseline and after each challenge. The findings of the study indicated PTP increased in mouth breathing with loud reading and exercise challenge in comparison to mouth breathing alone. PPE did not significantly change for the loud reading and exercise challenge. The authors concluded that obligatory mouth breathing in loud reading and exercise have detrimental effects on PTP as compared to mouth breathing alone.

Vilkman et al (1999) investigated the effects of prolonged (5x45 minutes) of oral reading on fundamental frequency, sound pressure level, subglottal pressure, and two glottal flow waveform parameters (AC amplitude of glottal flow and negative peak amplitude of differentiated flow. 80 subjects participated in two rest and three loading sessions. The material recorded was three strings of five /pa:p:a/ words that were produced normally, as softly, and as loudly as possible in this order. The results indicated a vocal “warming-up effect” in normal phonation as all the parameters changed significantly post-loading. Intraoral pressure, negative peak of differentiated flow, and glottal flow were extremely sensitive in soft phonation. Sound pressure level increased post-loading in both the morning and afternoon sessions.

Hodge and colleagues (2001) studied the relationship between lung pressure, fundamental frequency, peak air flow, open quotient, and maximum flow declination rate and vocal intensity in young and old males. 11 young males were included in the control group and 11 elderly males formed the experimental group. Subjects produced a syllable train /baep/ at three intensity levels: 25% (soft), 50% (moderate), and 75% (loud) and threshold level (softest possible). The peak values of intraoral pressure during the /b/ and /p/ phonemes were averaged to provide an approximated measure of phonation threshold pressure. Sound pressure level and fundamental frequency were obtained from the vowel portion of the syllable. Mean values for lung pressure, excess lung pressure, phonation threshold pressure, sound pressure level, fundamental frequency, peak airflow, open quotient, and maximal flow declination rate were obtained at the three intensity levels and compared for the young and old groups. Significant increase was reported for lung pressure, excess lung pressure, fundamental frequency, peak airflow, open quotient, and maximal flow declination rate with intensity increase in both young and old adults. On group comparisons, open quotient was found to be significantly lower in the control group than the elderly group. Thus, there is definitely a difference in vocal intensity between young and elderly voices due to differences in lung pressure, peak airflow, and open quotient.

2.1. c. iv. Effects of loading on vocal effort

Significant increase in self-related voice symptoms have been observed during a working day (Laukkanen et al., 2008; Lehto et al., 2006, 2008) or after a loading task performed in a laboratory (Chang & Karnell, 2004; Kelchner et al., 2006; Laukkanen et al., 2004; Vintturi et al., 2003). Lehto et al (2006) investigated the relationship between subjective and objective measures in 24 female customer advisors during a working day. All subjects completed a questionnaire addressing vocal symptoms at four time points: in the morning, before lunch, after lunch, and at the end of working day. Objective acoustic measures were studied: fundamental frequency, sound pressure level, and the alpha ratio which is the ratio between spectral energy below and above 1000 Hz. The most interesting finding in this study was the significant change in self-perceived voice symptom during the working day. The fundamental frequency increase paralleled previous studies and the sound pressure level was lower for the customer advisors as compared to other occupational voice users (i.e. teachers).

Laukkanen and colleagues (2008) studied the relation between symptoms of vocal fatigue and acoustic variables to examine the effects of vocal loading in 69 female primary school teachers. The different tasks included: a 1-minutes text reading sample recorded at habitual loudness and loudly as in a large classroom, prolonged phonation of the vowel /a/ recorded at habitual speaking pitch and loudness, a questionnaire addressing voice quality, ease or difficulty of phonation and tiredness of throat. The vowel sample was analyzed for average fundamental frequency, sound pressure level, alpha ratio, jitter and shimmer. Of interest, the mean fundamental frequency and jitter rise correlated with throat tiring in loud reading. The authors concluded that in functionally healthy experienced female teachers, increase in jitter and throat tiring could possibly reflect muscle fatigue or inflammation of the vocal fold tissue.

2.2 Speaking intensity on vocal loading

The amplitude of vocal fold oscillations influences the intensity of voice produced. The medial compression of the vocal folds and shear stress in the ligament are affected by vocal intensity (Gunter, 2004; Titze, 1994). Background noise, room acoustics, number of listeners, and distance between the speaker and listener are some factors that influence vocal intensity (Sodersten, Ternstrom, & Bohman, 2005; Vilkman, 2004). Individuals naturally increase their vocal loudness in the presence of background noise which creates the “Lombard effect” (Sodersten et al., 2005; Vilkman, 2004). Occupational voice users are frequently exposed to speaking in background noise in classrooms that constitutes as a risk factor for voice disorders (Chen, Chiang, Chung, Hsiao, & Hsiao, 2010; Ternstrom, Bohman, & Sodersten, 2006; Vilkman, 2000, 2004). Increased fundamental frequency is found to correlate with higher vocal intensity (Gramming, Sundberg, Ternstrom, Leanderson, & Perkins, 1988). Even though certain studies have found vocal intensity and fundamental frequency correlations, Lindstrom et al found no general correlation between noise exposure, vocal intensity level, and average fundamental frequency in preschool teachers (Lindstrom, Waye, Sodersten, McAllister, & Ternstr€om, 2011). Therefore, it appears that vocal behavior consequent to noise exposure is individual specific.

2.3 Interaction of loading and vocal fold hydration

In everyday life, voice users (e.g. singers) regularly spend time breathing in fluctuating environmental humidities (Tanner et al., 2010). Voice production can be affected by alterations to environmental humidity (M Sivasankar, Erickson, Schneider, & Hawes, 2008b). The negative effects of decreasing humidity include a stiffer and more viscous vocal fold cover as compared to humid air (Hemler, Wienke, Lebacq, & Dejonckere, 2001). Increase in vocal fold tissue viscous properties requires larger lung pressures to initiate and sustain vocal fold vibration. This lung pressure is called phonation threshold pressure (PTP), which is theoretically proportional to vocal fold tissue viscous properties (Titze, 1988). PTP has been found to increase through dehydration challenges including reduced water intake (Verdolini-Marston, Sandage, & Titze, 1994), diuretics (Verdolini et al., 2002), mouth breathing, low humidity (M Sivasankar et al., 2008b), and accelerated breathing (M Sivasankar & Erickson, 2009) in healthy individuals. Increased environmental humidity (> 60%) effectively reverses the adverse voice effects (decrease in PTP) induced by oral breathing in low humidity (Levendoski, Sundarrajan, & Sivasankar, 2014). Additionally, hydration treatments such as increased water intake may help in decreasing vocal fold tissue viscous properties (Verdolini, Sandage, & Titze, 1994). Practically, in everyday life it becomes difficult to avoid dehydrating conditions like mouth breathing or low humidity environment. Humidification is a common, practical, and cost-effective technique adopted by voice clinicians to reduce and prevent vocal fold drying. However, till date, there is limited literature available in human subjects on the beneficial effects of increased humidity

(Tanner, Roy, & Merrill, 2013; Tanner, Roy, Merrill, & Elstad, 2007; Tanner et al., 2010).

There have been few studies exploring the effects of vocal loading and dehydration (Chang & Karnell, 2004; Erickson-Levendoski & Sivasankar, 2011; Solomon & DiMattia, 2000; Solomon et al., 2003).

Solomon and DiMattia (2000) assessed PTP, effort for speaking, and vibratory closure pattern in 4 women with normal untrained voices after 2 hours of loud reading. The speaking and non-speaking tasks were comfortable reading for 10 minutes, then loud reading for 2 hours, followed by vocal silence for 15 minutes. PTP was obtained at the conversational pitch, 10%, 50%, and the 80% of the pitch range. Overall, PTP increased at all pitches after 1-hour of loud reading for 80% of the trials, and increased for every trial at the 80^{th} pitch. PTP values at the 80^{th} pitch were higher during the low-hydration as compared to the high-hydration condition. Subject's perception of effort increased after 1-hour of loud reading, increased further after an additional hour of loud reading, and decreased after 15 minutes of vocal silence. Additionally, all subjects reported being exhausted by the end of each experimental session.

Solomon et al (2003) assessed vocal function in 4 vocally normal men after a 2-hour loud reading task. Vocal function was examined using PTP, effort ratings, and laryngeal imaging after the reading challenge. Subjects read aloud (Harry Potter: The Sorcerer's Stone at 75-80 dB at 45 cm) for one hour, then paused for PTP and effort data collection, further resumed the loud reading task for an additional hour. PTP was obtained at the 10^{th}, 50^{th}, and the 80^{th} pitches. Effort ratings followed the PTP task at each pitch where the subjects rated "how effortful it was to perform the PTP task" on a 20 cm visual analog scale. PTP increased at several pitches after loud talking for the four young subjects. On an average, PTP changed 1 to 2.5 H_2O after 1 hour and 1.8 to 3.7 H_2O after 2 hours of the loud reading task. 2 out of the 4 men benefitted from increased systemic hydration. The vocal effort ratings had good correlations with the PTP data, with variations across subjects and pitches. The laryngeal imaging results suggest reduced amplitude of vocal fold vibration in three men when under-hydrated as compared to well-hydrated after loud reading. The authors concluded that prolonged loud reading results in detrimental voice effects.

Erickson-Levendoski and Sivasankar (2011) investigated the effects of caffeine on voice production. They also evaluated whether caffeine worsens the ill effects of vocal loading. 16 vocally healthy adults participated in two sessions; one where they consumed caffeine (caffeine concentration=480 mg) or sham (caffeine concentration=24 mg). PTP and PPE data were collected for both sessions in a humidity controlled environment (70%±6%). Voice measures were collected at three time points (preloading, after 35 minutes of reading aloud in background noise, after an additional 35 minutes of vocal loading). PTP data was collected at the 10^{th} and 80^{th} percent pitch of each subject's maximum pitch range. To rate PPE, subjects sang "Happy Birthday" in a soft voice starting at the 50^{th} percent pitch of the maximum phonation range. Subjects rated the effort for singing on a 10 inch visual analog scale with anchors "no vocal effort" and "maximum vocal effort". The results indicated that caffeine consumption did not have any ill-effects on voice production. No significant differences between the caffeine and sham conditions for PTP_{10}, PTP_{80}, and PPE were obtained. Caffeine or sham consumption did not worsen the effects of vocal loading, however vocal loading alone significantly increased PTP_{10} and PTP_{80} but not PPE. The authors suggested that a high dose of caffeine does not adversely affect PTP and PPE in the timeline examined in this study.

In sum, voice measures like PTP and PPE increased after a 1-hour prolonged reading task in non-dysphonic speakers reported in the studies discussed above. However, data on the beneficial effects of humidity are mixed. Table 2.2 summarizes the studies on vocal fold hydration.

2.4 Vocal aging

Human aging causes laryngeal and respiratory system changes. These alterations manifest as vocal aging by changes to laryngeal function and voice quality (R. Watts et al., 2015). Changes in the laryngeal mechanism like calcification and ossification of laryngeal cartilages, degeneration of laryngeal muscles which includes thickening of connective tissue of lamina propria, thinning of intermediate layer of lamina propria, densening of deep layer of lamina propria have been reported (Colton & Casper, 1996; Kahane, 1987; Mueller, 1997; Sinard, 1998). Studies have reported laryngeal changes such as loss of vocal folds bulk due to atrophy of muscle and loss of fat pad around the vocal folds. This results in inability to get complete glottal closure and presence of a gap in the middle third of the vocal folds. This condition is called bowing of the vocal folds and it is the most common benign pathology of the aging voice. These changes further lead to vocal impairment and handicap with frequent or extended vocal demands (R. Watts et al., 2015).

Vocal fold paralysis and paresis, laryngopharyngeal reflux, cancer, inflammation, and neurological disorders are some other common laryngeal disorders reported in the elderly (Coyle, Weinrich, & Stemple, 2001). In addition to the age-related changes in pitch, loudness, and quality, the elderly are affected by comorbid conditions like pulmonary disease and hypertensive cardiac disease for which they consume multiple medications (Woo, Casper, Colton, & Brewer, 1992). These medications may also have adverse effects on the voice. Thus, it is challenging to determine the actual impact of age alone on voice (Gregory, Chandran, Lurie, & Sataloff, 2012). There is a substantial increase in the proportion of older adults due to decreased birth rates and increased longevity in developed countries. According to the U.S. Census Bureau current population survey 2012, there is a predicted two-fold increase by the year 2030 and the elderly will constitute over 20% of the US population. In developed countries, it is expected that there will be older adults than children by 2050 (J. Cohen, 2003).

Generally, a limited body of literature is available on vocal function in old non-dysphonic speakers, and acoustic measures have been their prime focus (S. Awan, 2006; Goy, Fernandes, Pichora-Fuller, & van Lieshout, 2013; Maslan, Leng, Rees, Blalock, & Butler, 2011; Stathopoulos, Huber, Sussman, & Lafayette, 2011) that have been summarized in Table 2.3. Several studies have unanimously reported differences in fundamental frequency, perturbation measures like jitter and shimmer, harmonic to noise ratio between young and old individuals (Brown, Morris, & Michel, 1989; da Silva, Master, Andreoni, Pontes, & Ramos, 2011; Decoster & Debruyne, 1997; Ferrand, 2002; Linville, 2002; Stathopoulos et al., 2011; Xue & Deliyski, 2001; Zraick, Smith-Olinde, & Shotts, 2011). Recently, Goy and colleagues (2013) reported no age related changes in harmonic to noise ratio for both males and females. Only few studies have focused on cepstral and spectral based analyses (S. Awan, Helou, Stojadinovic, & Solomon, 2011; Hillenbrand, Cleveland, & Erickson, 1994; Peterson et al., 2013; C. Watts & Awan, 2015). Cepstral/spectral acoustic analysis was applied to derive a multiparametric measure of Cepstral/Spectral Index of Dysphonia (CSID) in young and old male adults (R. Watts et al., 2015). The individual parameters CPP and LHR to determine age-related effects have been rarely used. Studies using aerodynamic measures like PTP and MFDR have included healthy adults with mean age of 24, 28, 36.1 years (Hemler, Wienke, & Dejonckere, 1997; Levendoski et al., 2014; Tanner et al., 2007), females between 22-29 years (Solomon & DiMattia, 2000), males between 19-29 years (Solomon et al., 2003), singers; 30.2 years (Tanner et al., 2010). Hodge and colleagues (Hodge, Colton, & Kelley, 2001) studied the relation between MFDR and vocal intensity in young and old men, but did not find any significant group effects. Perceptual measures of perceived phonatory effort (PPE) have been used in the elderly population (Gorman, Weinrich, Lee, & Stemple, 2008; Ziegler, Verdolini-Abbott, Johns, Klein, & Hapner, 2014).

Therefore, it is important to study vocal function using a variety of measures (i.e. acoustic, aerodynamic, and perceptual ratings) in old non-dysphonic speakers to better distinguish physiological aging and disease processes, and compare the young to old speakers to examine group differences if any. Additionally, voice production is affected by aging, low humidity, and prolonged speaking. Even though, studies have focused on these parameters individually, interactions between these factors have not been systematically investigated.

2.5 Purpose

The proposed research focused on understanding how the healthy, young and old larynx responds to the effects of unhealthy, and excessive use of the laryngeal mechanism. Vocal loading tasks are a means to experimentally replicate excessive and unhealthy voice production. Factors such as loud talking, speaking in a whisper, exposure to a low humidity environment, and aging may induce vocal loading. In order to fully understand the laryngeal adaptations to such factors individually or in combination, the project was divided into three experiments.

The first goal of experiment 1 was to examine the effects of vocal loading on the aging larynx. The second goal of experiment 1 was to determine if speaking in moderate-high humidity could attenuate the detrimental effects of vocal loading. Vocal function was assessed before and after a vocal loading task (loud child-directed speech pattern) using an aerodynamic measure (PTP), acoustic measure (CPP), self-perceived phonatory effort, and vocal tiredness ratings. We hypothesized that vocal function will be adversely impacted after vocal loading in low humidity as compared to vocal loading in moderate-high humidity.

Since, the first experiment targeted old non-dysphonic speakers, it was important to obtain information about how vocal loading affects young healthy individuals. In addition, we wanted to explore the effects, if any, of altered vocal quality in this demographic. Therefore loud child-directed speech and whispered child-directed speech were used as vocal loading tasks. The goals of the second and third experiments were to investigate whether speaking in child-directed speech and whispered speech patterns would induce vocal loading. We hypothesized that speaking in child directed speech would induce vocal loading and that this detrimental effect would be greater at low humidity. We also hypothesized that a whispered vocal quality would negatively affect the voice.

CHAPTER 3. HUMIDITY AND VOCAL LOADING ON THE AGING VOICE

3.1 Introduction

The United States has approximately 41.5 million older adults (> 65 years old) (Census Bureau, 2012). The elderly population is projected almost to double by the year 2030, at which time it will comprise over 20% of the US population (Census Bureau, 2012). Advancing age is a significant risk factor for voice impairment with prevalence estimates of voice disorders ranging from 19% to 29% (Golub, Chen, Otto, Hapner, & Johns, 2006; Marino & Johns III, 2014; Plank, Schneider, Eysholdt, Schutzenberger, & Rosanowski, 2011; Roy, Stemple, Merrill, & Thomas, 2007; Schneider, Plank, Eysholdt, Scutzenberger, & Rosanowski, 2011; Turley & Cohen, 2009). With the increased prevalence of voice problems in a growing aging population, it is important to understand factors that increase vulnerability to voice problems and the functional ramifications of structural changes in the aging larynx.

Human aging alters the laryngeal and respiratory sub-systems (R. Watts et al., 2015). These changes include calcification and ossification of laryngeal cartilages (Yerman, Werkhaven, & Schild, 1988), thinning of the lamina propria of the vocal folds (Hammond, Gray, Butler, Zhou, & Hammond, 1998a, 1998b; Ximenes Filho, Tsuji, do Nascimento, & Sennes, 2003), and vocal fold atrophy

(Hirano, Kurita, & Nakashima, 1983; Honjo & Isshiki, 1980; Kahane, 1987; Mueller, 1997; Sinard, 1998; Ximenes Filho et al., 2003). The respiratory changes include decreased chest wall compliance, reduced lung elasticity, and lower elastic recoil forces (Frank, Mead, & Ferris Jr, 1957; Mittman, Edelman, Norris, & Shock, 1965). Other causes of voice disorders in the elderly population include intake of medications (Woo et al., 1992), laryngopharyngeal reflux, and neurological disorders (Coyle et al., 2001). Extrinsic factors that predispose the larynx to voice problems include loud speaking, noisy environments, and non-habitual speech patterns. These factors may interact with intrinsic factors like normal age-related processes and increase susceptibility to voice problems in the aging population. The overall goal of this paper was to investigate the influence of these extrinsic factors on the aging larynx with a vocal loading task in the laboratory.

Vocal loading combines prolonged voice use and environmental conditions which make the larynx vulnerable to voice problems (Vilkman, 2004). Intrinsic factors such as vibration modes (Doellinger et al., 2009; Lohscheller et al., 2008), phonatory duration (Boucher & Ayad, 2010; Chang & Karnell, 2004; Gelfer et al., 1991, 1996; Remacle, Finck, Roche, & Morsomme, 2012; Remacle, Schoentgen, Finck, Bodson, & Morsomme, 2014; Vilkman, 2004), and normal age-related processes (S. Awan, 2006; Goy et al., 2013; Hodge et al., 2001; Maslan et al., 2011; Stathopoulos et al., 2011) affect vocal loading. In addition, extrinsic factors like room acoustics (Sodersten et al., 2005; Vilkman, 2004; Vintturi et al., 2003) background noise (Chen et al., 2010; Ternstrom et al., 2006; Vilkman, 2000), and hydration levels (Solomon & DiMattia, 2000; Solomon et al., 2003) may induce vocal loading. There is a vast literature on the effects of vocal loading in young non-dysphonic speakers (Boucher, Ahmarani, & Ayad, 2006; Kelchner et al., 2006; Lauri et al., 1997; Linville, 1995; Lohscheller et al., 2008; Neils & Yairi, 1987; Remacle, Morsomme, & Finck, 2014). The effects of vocal loading on the aging voice have been minimally studied. Prolonged reading is commonly used to load the larynx (Gelfer et al., 1996; Kelchner et al., 2006; Stemple et al., 1995; Vintturi et al., 2003). Our study used a novel task, child-directed speech to load the larynx. Child-directed speech has a different prosodic organization when compared to adult speech, including the use of higher pitch, slower tempo, and exaggerated intonation (Blount & Padgug, 1976; Ferguson, 1964; Fernald & Simon, 1984). Although child-directed speech is a non-habitual speech pattern, it may be used in everyday situations by older speakers in conversations with their grandchildren.

Alterations to environmental humidity can also adversely affect voice production (M Sivasankar et al., 2008a). Decreases in environmental humidity can cause increased vocal fold cover stiffening and increased viscosity (Hemler et al., 1997; Hemler et al., 2001). Vocal folds with higher viscous properties need larger lung pressures to initiate and sustain vocal fold vibration. This lung pressure is called phonation threshold pressure (PTP), and is theoretically proportional to vocal fold tissue viscous properties (Titze, 1988). PTP has been shown to be sensitive to dehydration induced by mouth breathing (Levendoski et al., 2014; M Sivasankar et al., 2008a; M Sivasankar & Fisher, 2002), desiccated air (Tanner et al., 2013; Tanner et al., 2007; Tanner et al., 2010), reduced water intake (Verdolini, Titze, & Druker, 1990; Verdolini, Titze, & Fennel, 1994) and accelerated breathing (M Sivasankar & Erickson, 2009). Although, emerging evidence suggests that systemic hydration can partially alleviate the ill-effects of vocal loading

(Solomon & DiMattia, 2000; Solomon et al., 2003) few studies have explored the effects of vocal loading and systemic or surface dehydration. Also, it is noteworthy that these studies have focused solely on young adults (Levendoski et al., 2014). Limited literature is available on how vocal loading impacts non-dysphonic older speakers and whether surface hydration treatments can prevent any potential adverse effects. This study focused on the interaction of surface hydration and vocal loading in older individuals.

PTP has been reported to increase in low ambient humidity conditions (M Sivasankar & Erickson, 2009) and decrease in high ambient humidity conditions (Levendoski et al., 2014). Increase in PTP post vocal loading (1-hour prolonged reading task) has been observed in non-dysphonic speakers (Solomon & DiMattia, 2000; Solomon et al., 2003). Measures of self-perceived phonatory effort and perceived tiredness have been reported to increase with vocal loading (Chang & Karnell, 2004; Kelchner et al., 2006; Laukkanen et al., 2004; Vintturi et al., 2003). Traditional acoustic measures of jitter and shimmer yield mixed results with vocal loading (Stemple et al., 1995; Verstraete, Forrez, Mertens, & Debruyne, 1993). It is difficult to separate results from the sensitivity of the acoustic measures obtained and the effect of the vocal loading task itself. Cepstral peak prominence (CPP) has been widely used to analyze vowels and continuous speech samples in dysphonic voices (S. Awan & Roy, 2009; Heman-Ackah et al., 2002; Lowell et al., 2011; C. Watts & Awan, 2011; R. Watts et al., 2015; Wolfe et al., 2000) but it is unknown whether this measure is sensitive to effects induced due to vocal loading.

The primary objective of the present study was to investigate the effects of vocal loading on the aging larynx. The second goal was to determine if speaking in moderate-high humidity could reduce the negative effects of vocal loading. We hypothesized that vocal function will be adversely impacted after vocal loading in low humidity as compared to vocal loading in moderate-high humidity.

3.2 Methods

3.2.a Participants

Thirteen healthy adults (5 males and 8 females) participated in this investigation following procedures approved by the Purdue University Institutional Review Board. Participants were between 65-78 years of age (mean age: 72 years) and denied a history of laryngeal and respiratory disease. All participants reported general good health, perceptually normal speech and voice, no history of voice problems, and smoking.

3.2.b Screening

The screening protocol included the Vocal Fatigue Index-Part I (Nanjundeswaran, Jacobson, Gartner-Schmidt, & Verdolini-Abbott, 2015), the Reflux Symptom Index (RSI) (Belafsky, Postma, & Koufman, 2002), audiometry (Diagnostic Audiometer AD229e, Interacoustics A/S, Assens, Denmark), videostroboscopy (9100 KayPENTAX Videostrobe, Lincoln Park, NJ), and refractometry (ATAGO refractometer, Bellevue, WA). All participants passed hearing screening with a hearing threshold of 40 dB HL at 500 Hz, 1 KHz, and 2 KHz. All participants presented with normal appearing larynges during screening. Only one participant had an RSI score of 14 (a score of 13 was the cut-off). Before each experimental session, urine hydration was measured using refractometry. Threshold hydration criterion was set at $\leq$ 1.02g/ml. If participants did not meet the criterion, they were offered water and testing were repeated within 20 minutes.

All 13 participants met criterion before participating in the rest of the study. Diet and voice use was monitored through verbal questions prior to day 1 and using a log before day 2. Overall, participants had similar general food and water intake prior to both sessions.

3.2.c Experimental Design

Participation in the study involved two sessions scheduled on consecutive days at similar times of the day (± 1hr). Participants were asked questions about their average voice use and, food, and water intake. A log was used to monitor similar patterns of voice use and diet before both sessions. Participants were screened before starting each session (described below). After passing the screening, they participated in the experimental sessions. The experimental protocol was similar on both days, except for the ambient humidity for the vocal loading task. The ambient humidity during each session was set to low (<30% relative humidity) or moderate-high (52%-70% relative humidity) with commercially available dehumidifiers and humidifiers (Siemens®) inbuilt in the experimental rooms. The order of the ambient humidity was randomized for all participants. Participants were not aware of manipulation in ambient humidity levels. At the start of the session, the 10^{th}, 20^{th}, and 50^{th} percent pitches were established from the maximum frequency range. A laryngeal microphone was placed around the subject's neck on either side of the thyroid notch. Participants were asked to glide on a soft /i/ sound to their highest and lowest frequencies. The highest and lowest frequencies were recorded in Hertz and converted into semitones using a keyboard. Next, the 10^{th}, 20^{th}, and the 50^{th} percent pitches were calculated from the semitone range. These pitches were further utilized for voice measures. Prior to the start of the session, participants sat in the ambient humidity room (low or moderate-high) for 20-minutes allowing for thermal equilibration to the pre-established environmental condition (Sandage, Connor, & Pascoe, 2014). We expected that the 20-minutes equilibration time would also enable humidity acclimation. Next, baseline voice measures were collected and participants completed a 45-minutes vocal loading challenge. Finally, voice measures were re-obtained. The dehumidifiers and humidifiers used to control ambient humidity were turned off during data collection to minimize background noise.

3.2.d Vocal loading Challenge

Participants read aloud for 45-minutes in the presence of multi-talker babble background noise (AUDiTEC of St. Louis) using child-directed speech. The noise was played on a computer and the sound pressure level (SPL) of the noise was set to 65-decibels (dB). The distance between the microphone that the participant wore, and the sound level meter (RadioShack 22-806, Fort Worth, TX) was approximately 2 feet. Participants were required to read in child-directed speech with the following instructions: “For this task you will be asked to read few children books (Froggy series, Jonathan London Puffin Books, 1992). I want you to read in an appropriate character voice that you think would keep the child interested. If you would like, I can play a sample. You should be loud enough to be heard outside this room. You will be reading in the character’s voice for 45-minutes with background noise.” The books used were directed to 3-to 8-year old children. All participants were able to read the books successfully in child-directed speech. Three female subjects reported prior experience in speaking in such a voice with their grandchildren during initial training. Participants were trained to produce child directed speech consistently while the investigators provided feedback and cues to elicit the desired voice quality. These cues included non-verbal signs which were shown if the participants couldn't meet the desired voice (perceptually determined by the investigators). The intensity of their child-directed speech was monitored throughout the challenge and recorded every 15-minutes using a sound level meter. Participants were shown non-verbal signs of increasing their loudness as necessary. The same task was repeated on day 2 and participants were monitored for consistent production of the child-directed speech. We defined consistent production by intensity and usage of the child-directed speech. Two undergraduate researchers, with prior experience in listening to infant-directed speech rated whether participants produced child-directed speech or not. A 5-minutes speech sample was obtained from the middle of the 45-minutes vocal loading task for the rating. The two researchers listened to all samples and provided a consensus rating of 0/1/2 (where 0 indicated no child-directed speech, 1 indicated some child-directed speech, and 2 indicated more child-directed speech). All participants had more child-directed speech, except one (S10) who had some child-directed speech on day 1.

3.2.e Voice measures

The following voice measures were collected prior to and following the vocal loading challenge.

PTP. A circumferentially vented pneumotachograph mask fitted with low band-width and wide band-width differential pressure transducers (Glottal Enterprises PTL-1 and PTW-2) was used. The PTL-1 was used for measuring oral pressure and PTW-2 for airflow. The flow and pressure signals were calibrated before starting data collection (MCU-4 Calibration system, Glottal Enterprises, Syracuse, NY, USA). The differential pressure transducers are connected to a measurement system (MSIF-2 system, Glottal Enterprises, Syracuse, NY, USA). A digital multichannel hardware system (PowerLab 16/30 ADInstruments, Sydney, Australia) was used for acquiring data. The sampling rate used was 4k/s. A 2" length plastic tubing was connected to the mask that collected oral pressure from just inside the lips. Before starting data collection, participants were trained on the PTP task per validated procedures (Fisher & Swank, 1997; Verdolini et al., 1990). The face mask was held firmly by the investigator around the participant's mouth and nose. The participants wore nose plugs and were instructed to produce the syllable string /pi pi pi pi pi / at 1.5 syllables/second "as smoothly as possible on a single breath and as quietly as possible without whispering". The task was modeled multiple times and verbal feedback regarding the rate, loudness, and consistency was provided by the investigator. The rate was monitored using a metronome Seiko Digital Metronome (Model# DM50; Seiko Sports Life Co., Ltd, China). Additionally, visual feedback regarding the within-trial pressure peaks and oral flows was provided from the data collection monitor. Participants practiced until they were considered trained. The training criteria was defined as (a) within-trial pressure peaks of the same height and (b) oral flows reaching 0 ml/s during the /p/ production. After PTP training, a minimum of 5 trials were collected at the 10^{th} (PTP_{10}) and 20^{th} (PTP_{20}) pitches. Each trial included seven /pi/ productions. Trials comprised of intermittent phonation, syllables produced at supra-threshold levels, or syllables produced with high oral flows were not included for data analysis. Data for one subject could not be included for analysis due to oral flows higher than 0 ml/s. There is a direct dependence of the fundamental frequency on PTP; therefore the investigator monitored the pitches perceptually throughout data collection (Fisher & Swank, 1997;

Verdolini et al., 1990; Verdolini, Titze, et al., 1994). Data was analyzed using Lab Chart 7 (Power Lab 16/30 ADInstruments). During analysis, the investigator manually selected the three middle /p/ peaks from each trial (Fisher & Swank, 1997; Holmberg, Hillman, & Perkell, 1988). The average lung pressure across five trials determined PTP_{10} and PTP_{20}.

CPP. The instrumentation included a head-mounted microphone (AKG C555 L, AKG, Vienna, Austria) placed at a distance of 4 cms from the participant's mouth and was kept constant throughout data collection. The microphone was connected to a mixer (XENYX 1202/1002/802/502, Behringer, Road Town, Tortola, British Virgin Islands) and the audio signal was routed to the computer through an A/D converter (PowerLab 16/30 ADInstruments, Sarasota, FL). The speech recordings were made at a sampling rate of 44.1 kHz. Participants read the first paragraph of the Rainbow passage (Fairbanks, 1960) at their habitual pitch and loudness level. CPP measure (CPP_{RAIN}) was computed on the second and third sentences of the Rainbow passage (S. Awan, 2011) by selecting the appropriate protocol on the Analysis of Dysphonia in Speech and Voice program (ADSV Model 5109, KayPENTAX, Montvale, NJ).

Self-perceived phonatory effort. Participants rated their self-perceived phonatory effort on a visual analog scale (VAS) while singing "Happy Birthday" as softly as possible starting at the 50^{th} percent pitch. The participants were provided a 9-inch VAS with two anchors ("no effort" and "maximum effort") and were asked to draw a vertical line on the scale corresponding to their perceived effort during the preceding singing task. Data for self-perceived phonatory effort were analyzed by measuring the distance in inches and averaged to obtain the mean across all participants.

Vocal tiredness. Participants rated their vocal tiredness on a 9-inch VAS before and after the vocal loading challenge. Participants rated vocal tiredness after the completion of other voice measures. The VAS anchors were labeled ("not tired" and "tired") and participants drew a vertical line to indicate their extent of vocal tiredness. Data for vocal tiredness were analyzed by measuring the distance in inches and averaged to obtain the mean across all participants.

3.2.f Statistical analysis

SPSS-22 software (IBM SPSS Statistics Armonk, New York, USA) was used for the analysis. PTP_{10}, PTP_{20}, CPP_{RAIN}, self-perceived phonatory effort, and vocal tiredness data were subjected to Shapiro-Wilk normality tests. Data for PTP_{10}, PTP_{20}, CPP_{RAIN}, self-perceived phonatory effort, and vocal tiredness were not normally distributed and were therefore transformed to meet the assumptions of normality. PTP_{10} data were log transformed, PTP_{20} data were inverse transformed, CPP_{RAIN} data were reflected and log transformed, self-perceived phonatory effort and vocal tiredness data were square root transformed. Following transformations, a two-factor repeated measure analysis of variance (ANOVA) was utilized with loading challenge (pre/post) and humidity (low/moderate-high) as the within subject factors. Separate ANOVAs were performed for all the dependent variables. The alpha level was 0.05.

3.2.g Reliability

10% of samples were analyzed for interarater reliability. Interrater reliability was determined using interclass correlation coefficients (ICCs). A 2-way mixed, absolute single measures ICC was conducted for all variables. The values of ICCs were in excellent range (>0.9) for all the variables suggesting adequate reliability.

3.3 Results

Effects of Loading and Humidity

There was a significant interaction effect for loading and humidity for PTP_{10} (F = 4.978, df = 1, 11, p = 0.047, Figure 1). PTP_{10} increased to a greater extent in low humidity (average change of 1.32 ± 1.40) as compared to moderate-high humidity (0.47 ± 0.45) after vocal loading. There was a significant interaction effect for loading and humidity for PTP_{20} (F = 5.091, df = 1, 11, p = 0.045, Figure 2). PTP_{20} increased to a greater extent in low humidity (average change of 1.31± 0.94) compared to moderate-high humidity (0.77±0.74) after vocal loading. No significant interaction effects of loading and humidity were observed for $CPP_{RAIN,}$ self-perceived phonatory effort, and vocal tiredness.

Effects of Loading

A significant main effect for loading was observed for PTP_{10}, $PTP_{20,}$ $CPP_{RAIN,}$ self-perceived phonatory effort, and vocal tiredness. PTP_{10} significantly increased from baseline (Mean ± SD: 6.070 ± 1.969) to post loading (6.965 ± 2.637; F = 65.568, df = 1, 11, p = 0.00, Figure 1). Similarly, PTP_{20} significantly increased from baseline (5.58 ± 1.58) to post vocal loading (6.62 ± 1.96; F = 42.466, df = 1, 11, p = 0.00, Figure 2). CPP_{RAIN} significantly increased from baseline (5.69 ± 0.94) to post loading (5.84 ± 0.85; F = 42.466, df = 1, 12, p = 0.00, Figure 3). Significant main effects for loading for self-perceived phonatory effort and vocal tiredness were also obtained. Self-perceived phonatory effort significantly increased from baseline (2.29 ± 1.95) to post loading (3.95 ± 2.38, F = 42.466, df = 1, 12, p = 0.00, Figure 4). Vocal tiredness significantly increased from baseline (1.89 ± 1.59) to post loading (4.22 ± 2.50, F = 42.466, df = 1, 12, p = 0.00, Figure 5).

Effects of Humidity

There was a significant main effect for humidity for self-perceived phonatory effort and vocal tiredness. Self-perceived phonatory effort increased from baseline by (2.91 ± 0.4) at low humidity and (1.69 ± 0.08) at moderate-high humidity ($F = 7.281$, $df = 1, 12$, $p = 0.019$). Likewise, vocal tiredness increased from baseline by (2.54 ± 2.02) at low humidity and at moderate-high humidity (2.10 ± 1.81), ($F = 8.01$, $df = 1, 12$, $p = 0.015$). No significant effects of humidity were obtained for PTP_{10}, PTP_{20}, and $CPP_{RAIN.}$

3.4 Discussion

The present study investigated the effects of vocal loading on the aging larynx and whether humidity would affect the extent of vocal loading. Vocal loading was induced by 45-minutes of reading aloud using child-directed speech in 65 dB background noise. Overall, our data suggest that loading was successfully induced in participants, as measured using phonation threshold pressure, cepstral peak prominence, self-perceived phonatory effort, and vocal tiredness. We obtained a statistically significant loading and humidity interaction effect for PTP suggesting a greater magnitude of vocal decrement in low humidity as compared to moderate-high ambient humidity. Thus, increased humidification was helpful in alleviating the ill-effects of loading. Vocal loading induced higher self-perceived phonatory effort and vocal tiredness ratings in low humidity as compared to moderate-high humidity. These findings further our understanding of how the non-dysphonic aging larynx responds to vocal loading. Further, humidification may be an easy and practical treatment to minimize the detrimental effects of vocal loading.

In our study, PTP at the 10th and 20th percent pitches increased after the loading challenge, with a greater magnitude of increase in low humidity as compared to moderate-high humidity. We hypothesized that vocal function to be adversely impacted after vocal loading in low humidity as compared to vocal loading in moderate-high humidity. Decreasing humidity results in an increase in viscoelastic properties (Hemler et al., 2001) and therefore increased pressure is required to initiate vocal fold vibration. Increasing ambient humidity effectively reverses the adverse voice effects (lower PTP) induced by oral breathing in low humidity (Levendoski et al., 2014). Our finding supports that increasing surface hydration (moderate-high ambient humidity) may hydrate the airway to alleviate the ill-effects of vocal loading in old non-dysphonic speakers. Practically, in everyday life it becomes difficult to avoid dehydrating conditions like mouth breathing or a low humidity environment. Humidification is a common, practical, and cost-effective technique adopted by voice clinicians to reduce and prevent vocal fold drying. Results also suggest that the aging larynx is susceptible to vocal loading and increasing hydration may be a beneficial treatment option in this population. It might be useful to advise older individuals to use humidifiers at home that aid in surface hydration. It can also be inferred that as age advances, the healthy larynx starts deteriorating and becomes less robust and susceptible to changes in environmental humidity.

It is noteworthy that some voice measures were affected by ambient humidity and some were not. Specifically, self-perceived phonatory effort and vocal tiredness showed statistically significant differences in low humidity as compared to moderate-high humidity. Subjects perceptually felt tired and had increased perception of their speaking effort to a greater extent in low as compared to moderate-high humidity. Our results parallel work by Solomon and colleagues who assessed effort ratings in four men and women with normal voices (Solomon & DiMattia, 2000; Solomon et al., 2003). Subjects had a higher perception of effort after a 2-hour vocal loading task. We observed similar voice related symptoms in our old participants after a 45-minutes vocal loading task. Symptoms like frequent throat clearing, throat pain, vocal tiredness, scratchy voice quality, throat soreness, throat and mouth dryness were commonly reported. Self-perceived phonatory effort was rated on a visual analog scale while singing “Happy Birthday” as softly as possible starting at the 50th percent pitch. Four subjects (S1, S5, S7, and S12) reported this task to be “extremely hard” and it was difficult to reach the “high notes”. The study results support previous data on the use of soft phonation tasks (such as Happy Birthday) to detect changes due to vocal loading. Soft phonation tasks in the clinic are reliable in detecting vocal injury (Bastian, Keidar, & Verdolini-Marston, 1990).

It is noteworthy that CPP measure collected on the rainbow passage (CPP_{RAIN}) showed a significant loading effect. The CPP_{RAIN} increased from baseline (5.69 ± 0.94) to post loading (5.84 ± 0.85) which is a minimal change. We hypothesized that CPP_{RAIN} would decrease following vocal loading, and the effects would be greater in low humidity compared to moderate-high humidity. The reason for an increase in CPP_{RAIN} could be that the subjects compensated the effects of loading by increasing their pitch post loading. This may be indicative of a carry-over of increased pitch during spontaneous speech as the vocal loading task involved speaking in child-directed speech. Previous literature supports subjects increasing fundamental frequency as a compensatory mechanism during connected speech (Stemple et al., 1995). CPP has been commonly used to differentiate normal and dysphonic voices (S. Awan & Roy, 2009; Heman-Ackah et al., 2002; Lowell et al., 2011; C. Watts & Awan, 2011; R. Watts et al., 2015; Wolfe et al., 2000), but there is limited literature on standardizing CPP as a reliable acoustic measure for non-dysphonic speakers. Additionally, CPP has been reported to be more sensitive to breathiness (S. Awan, 2011). A variable Cepstral/Spectral Index of Dysphonia (CSID) obtained on connected speech has been reported to be sensitive to the physiological effects of vocal aging in non-dysphonic male speakers (R. Watts et al., 2015). Future studies in our laboratory will include CSID as a measure to detect vocal loading in aging non-dysphonic speakers.

It is also important to mention that we used an innovative vocal loading task, and there is no data to compare our findings. Participants were instructed to read children books using child-directed speech. This task was relevant because older individuals may use child-directed speech in their everyday communication. Our novel task involved normal pitch and loudness variations by employing child-directed speech to load the larynx.

3.5 Conclusions

In conclusion, the aging larynx is vulnerable to changes induced by a 45-minutes vocal loading task. By altering voice quality and phonatory duration, the laryngeal mechanism can be successfully loaded. Measures of PTP, CPP, self-perceived phonatory effort, and vocal tiredness demonstrated statistically significant loading effects. PTP was sensitive to a loading and humidity interaction effect. Increasing environmental humidity is a potential, cost-effective option to alleviate the negative effects of vocal loading.

Table 3.1. Participant Demographics

Subject Number	Gender	Age (years)	Refractometry	Hearing	VFI part 1*	RSI
S1	Female	72	+	P	18	2
S2	Male	72	+	P	3	3
S3	Female	78	+	P	4	7
S4	Female	73	+	P	0	9
S5	Female	65	+	P	4	14
S6	Female	66	+	P	3	7
S7	Female	77	+	P	0	0
S8	Male	75	+	P	10	13
S9	Male	65	+	P	6	1
S10	Male	70	+	P	17	0
S11	Male	77	+	P	3	2
S12	Female	70	+	P	5	8
S13	Female	77	+	P	1	8

+ Indicates urine hydration threshold ≤1.02 g/ mL

P indicates hearing threshold at 40 dB HL at frequencies 500 Hz, 1 KHz, and 2 KHz

*Scores <24 are considered within normal range

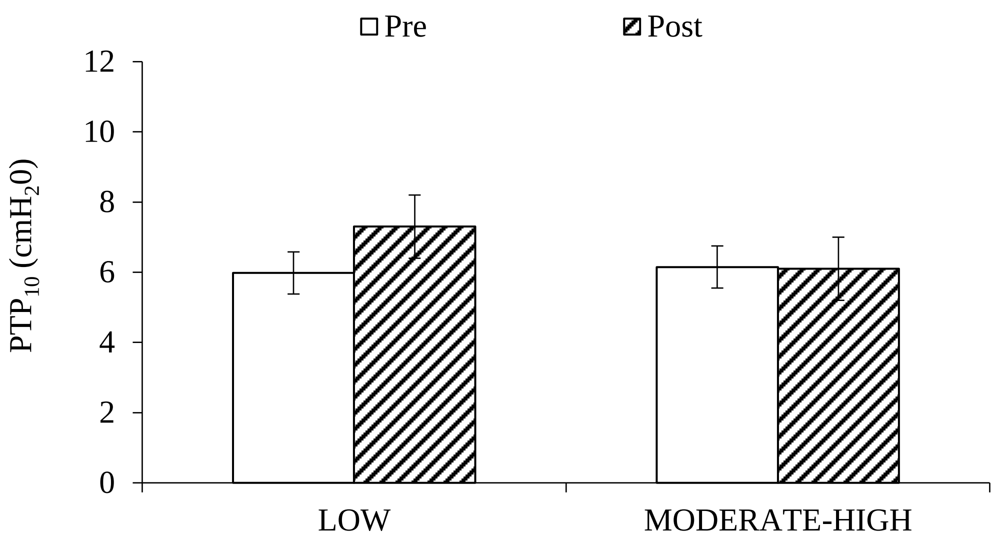

Figure 3.1

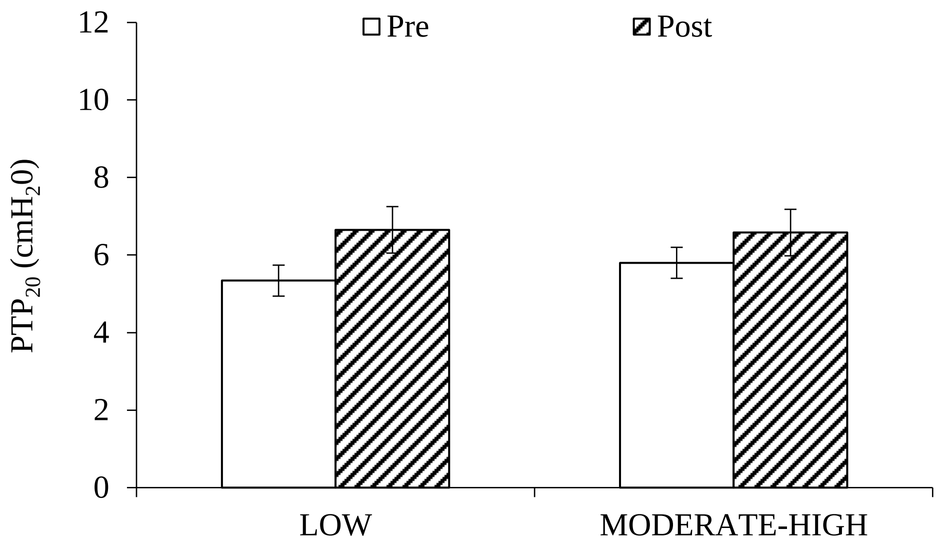

Figure 3.2

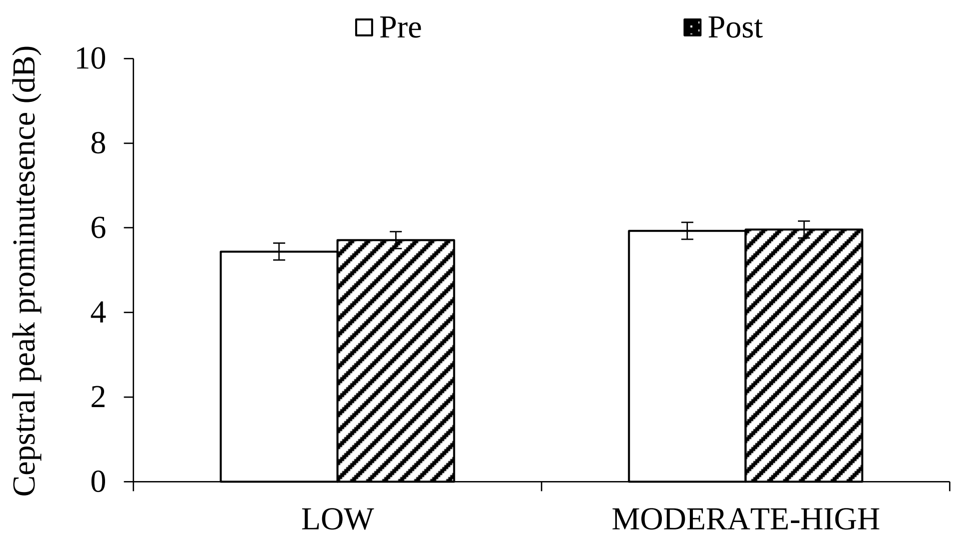
Pre
Post
Cepstral peak prominutesence (dB)
10
8
6
4
2
0
LOW
MODERATE-HIGH

Figure 3.3

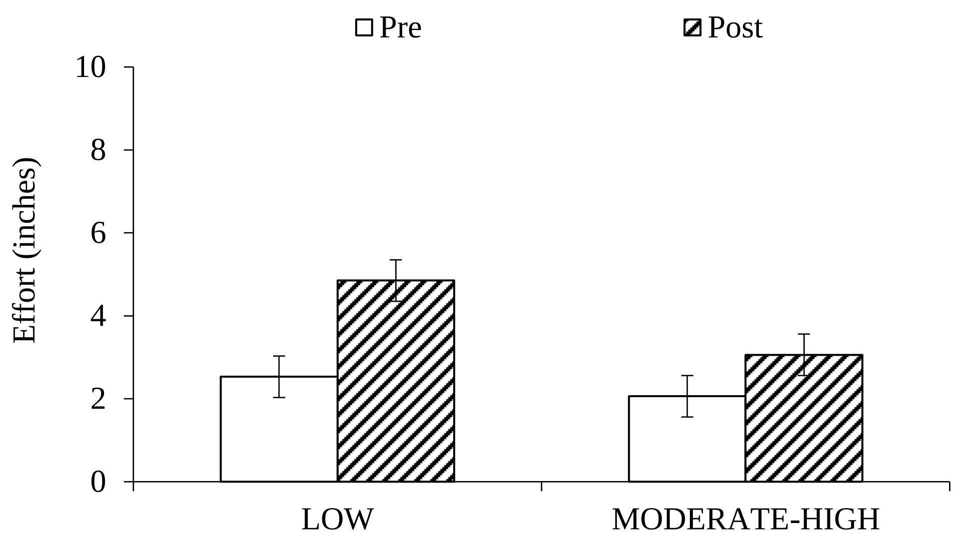

Figure 3.4

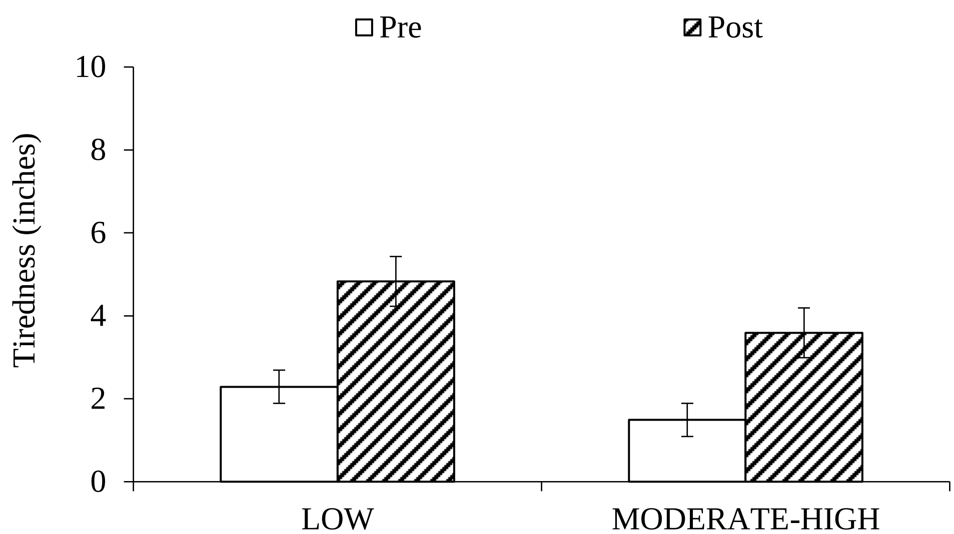

Figure 3.5

CHAPTER 4. AN INNOVATIVE METHOD OF VOCAL LOADING WITH CONSIDERATION TO FACTORS OF HYDRATION AND VOICE QUALITY

4.1 Introduction

The concept of vocal loading refers to the demands posed by voice use on the vocal organ (Vilkman, 2004). Loading factors can be intrinsic or extrinsic in nature. Intrinsic factors such as using high intensity speech (Chen et al., 2010; Gramming et al., 1988; Ternstrom et al., 2006; Vilkman, 2000, 2004), altering laryngeal configuration while speaking (Gelfer et al., 1991, 1996; Kelchner et al., 2006; Stemple et al., 1995; Vilkman et al., 1999), increasing phonatory duration (Boucher & Ayad, 2010; Chang & Karnell, 2004; Gelfer et al., 1991, 1996; Remacle et al., 2012; Remacle, Schoentgen, et al., 2014; Vilkman, 2004), and altering fundamental frequency influence the extent to which the larynx is loaded (Jónsdottir et al., 2002; Kelchner et al., 2006; Laukkanen et al., 2008; Lehto et al., 2006, 2008; Sodersten et al., 2002; Stemple et al., 1995; Vilkman et al., 1999). Vocal loading increases fundamental frequency (Stemple et al., 1995) and vocal intensity (Vilkman et al., 1999), and causes abnormal decrease in perturbation measures like jitter (Laukkanen et al., 2008). Literature is not consistent with changes in laryngeal configurations after vocal loading tasks (Gelfer et al., 1991, 1996).

In addition to the intrinsic factors, there is also research on extrinsic factors that may load the larynx. These include background noise (Chen et al., 2010; Ternstrom et al., 2006; Vilkman, 2000), hydration levels

(MP Sivasankar & Erickson-Levendoski, 2012; Solomon & DiMattia, 2000; Solomon et al., 2003), caffeine intake (Erickson-Levendoski & Sivasankar, 2011), and room acoustics (Sodersten et al., 2005; Vilkman, 2004; Vintturi et al., 2003). Speaking in background noise increases the risk of voice disorders, particularly for teachers in the classroom (Chen et al., 2010). Work by Solomon and colleagues reported that a minimum consumption of 5, sixteen ounce bottles of water reduced the negative effects of loading on voice measures like phonation threshold pressure (PTP) in three out of four female participants (Solomon & DiMattia, 2000).

In this study, we investigated the influence of some of these intrinsic and extrinsic factors on vocal loading. Specifically, we examined the effects of ambient humidity (extrinsic) and voice quality (intrinsic). We briefly summarize the literature on hydration and vocal loading, and the effects of whispering on voice below.

In everyday life, voice users regularly spend time breathing in fluctuating environmental humidities (Tanner et al., 2010). Voice production can be affected by changes in environmental humidity (M Sivasankar et al., 2008b). The negative effects of decreasing humidity include a stiffer and more viscous vocal fold cover as compared to that observed in humid air (Hemler et al., 2001). Increase in viscoelastic properties requires larger lung pressures to initiate and sustain vocal fold vibration. This lung pressure is called phonation threshold pressure (PTP), which is theoretically proportional to vocal fold tissue viscous properties (Titze, 1988). PTP has been found to increase following dehydration challenges including reduced water intake (Verdolini-Marston et al., 1994), diuretics (Verdolini et al., 2002), low humidity (M Sivasankar et al., 2008b), and accelerated breathing (M Sivasankar & Erickson, 2009) in healthy individuals.

Increased environmental humidity (> 60%) effectively reverses the adverse voice effects (decrease in PTP) induced by oral breathing in low humidity (Levendoski et al., 2014). Limited evidence is available on the effects of vocal loading and dehydration (Chang & Karnell, 2004; Erickson-Levendoski & Sivasankar, 2011; Solomon & DiMattia, 2000; Solomon et al., 2003). However, to date, there is limited literature available in human subjects on the beneficial effects of increased humidity (Tanner et al., 2013; Tanner et al., 2007; Tanner et al., 2010). Solomon and DiMattia (2000) assessed PTP, effort for speaking, and vibratory closure pattern in 4 women with normal untrained voices after 2-hours of loud reading. Overall, for 80% of the trials, PTP increased at all pitches after 1-hour of loud reading. Subject’s perception of effort increased after 1-hour of loud reading, increased further after an additional hour of loud reading, and decreased after 15-minutes of vocal silence. In a similar study, Solomon et al (2003) assessed vocal function in 4 vocally healthy men. The authors concluded that prolonged loud reading results in detrimental voice effects. In summary, voice measures like PTP and self-perceived phonatory effort increased after a 1-hour prolonged reading task in non-dysphonic speakers reported in the studies discussed above. However, data on the beneficial effects of increased humidity are limited. Voice patients are warned that whispering causes more trauma to the larynx than normal speech (A. Rubin et al., 2006). There is an existing controversy on including whisper in a program of voice rest (Solomon et al., 1989). Various claims exists on whether whispering induces (Griesman, 1943; Pressman, 1942), or actually reduces (Landes, 1977) abusive forces to the vocal folds. Whisper, is perceptually characterized by rapidly flowing air through constrictions in the vocal tract, which results in an aperiodic noise (Solomon et al., 1989). A whisper can be produced in two ways: (a) a quiet whisper, produced in a relaxed, comfortable, low-effort manner (Brodnitz, 1958; Landes, 1977; Luchsinger & Arnold, 1965; Pressman & Kelemen, 1955) and (b) a relatively loud whisper, produced in a forced, strained, high-effort manner (Brodnitz, 1958; Landes, 1977; Luchsinger & Arnold, 1965; H. Rubin & Lehrhoff, 1962). Due to limitations like small sample size and less sophisticated visualization techniques, it has been difficult to characterize changes in aerodynamic and laryngeal configuration during whispered voice (A. Rubin et al., 2006). Additionally, many studies have been performed in healthy adults with no voice complaints. Therefore, it is difficult to derive conclusions regarding the detrimental effects of whispering. From an aerodynamic perspective, measures like translaryngeal flow are higher during whispering than speaking (Monoson & Zemlin, 1984; Schwartz, 1971; Stathopoulos et al., 1991). Factors like high tracheal pressures, low laryngeal airway resistance, or combination of these factors results in high translaryngeal flow in whispered speech. It is unclear how these variables differ in whispering and speaking (Stathopoulos et al., 1991). Evidence on how pressure and flow data change during whispered production is minimal (Sundberg, Scherer, Hess, & Mueller, 2010). Among those studies mixed results are present on the oral air pressure data when whispering and speaking are compared; some studies report higher (Klich, 1982; Murry & Brown, 1976), lower (Murry & Brown, 1976; Stathopoulos et al., 1991), or no different in pressures (Weismer & Longstreth, 1980) and the data depends on individual subjects. To the best of our knowledge, there is no data on the effects of low-effort whispered voice on vocal loading measured using different voice measures. It is important to examine how the normal healthy laryngeal mechanism responds to vocal loading using whispered voice. In addition, it is critical to develop evidence-based clinical recommendations on the beneficial or harmful effects of whispering.

Vocal loading is typically measured using aerodynamics (Chang & Karnell, 2004; Milbrath & Solomon, 2003; MP Sivasankar & Erickson-Levendoski, 2012; Solomon & DiMattia, 2000; Solomon et al., 2003), acoustics (Kelchner et al., 2006; Laukkanen et al., 2008; Lehto et al., 2008; Stemple et al., 1995; Vilkman et al., 1999), and self-perceived effort (Kelchner et al., 2006; Laukkanen et al., 2008; Laukkanen et al., 2004; Lehto et al., 2006, 2008; Vintturi et al., 2003). We used several measures (phonation threshold pressure-PTP, cepstral peak prominence-CPP, self-perceived phonatory effort, and vocal tiredness rating) to study whether loading was accomplished using child-directed speech and whispered speech. PTP has been used in studies with vocal loading and hydration (Erickson-Levendoski & Sivasankar, 2011; Solomon & DiMattia, 2000; Solomon et al., 2003). To date, few studies have measured oral pressure in whispered voice (Klich, 1982; Murry & Brown, 1976; Stathopoulos et al., 1991; Weismer & Longstreth, 1980). CPP has been used to differentiate normal and dysphonic voices in sustained phonation and connected speech tasks (Awan, Roy, & Dromey, 2009; Heman-Ackah et al., 2003; Peterson et al., 2013; C. Watts & Awan, 2015). It is unknown whether CPP as an acoustic measure is sensitive to the effects of vocal loading using child-directed speech and whispered speech.

We addressed these questions in two experiments. The first experiment examined the interaction of hydration with vocal loading. Subjects were vocally loaded by reading loudly using child-directed speech in low humidity and moderate-high ambient humidity. Child-directed speech uses pitch and loudness variations-specifically higher pitches and exaggerated intonation patterns (Ferguson, 1964; Fernald & Simon, 1984). We thought child-directed speech would load the larynx as it taps into normal pitch and loudness variations. The second experiment investigated whether altering vocal quality in young individuals would induce vocal loading. Subjects read for 45-minutes using low-effort whisper quality. Hydration levels and voice quality were specifically investigated because these factors are thought to load the larynx. We hypothesized that speaking in child directed speech would load the larynx and that this detrimental effect would be greater at low humidity. Additionally, whispering in low humidity would also induce vocal loading.

4.2 Method

4.2.a Participants

The Purdue University Institutional Review Board approved all study procedures. A total of 32 non-smoking adults, between the ages of 18 and 30 years (mean age: 22 years) participated in experiments 1 and 2. Equal number of males and females participated in the two experiments. The inclusionary criteria were identical for both experiments. All participants reported general good health, perceptually normal speech and voice, and no history of voice problems (information obtained using a screening form). The exclusionary criteria included smoking and vocal training. All female participants took part in the study during the follicular phase (days 1-15) of the menstrual cycle to control for hormonal effects on voice. Participants were not taking any medicines except birth control at the time of the study. All participants were screened (as described below) before they could participate in the study.

4.2.b Screening

The screening protocol for experiment 1 and 2 was identical and included the Vocal Fatigue Index-Part I (Nanjundeswaran et al., 2015), the Reflux Symptom Index (Belafsky et al., 2002), spirometry (Discovery Spirometer, Futuremed America, Inc., Granada Hills, CA), videostroboscopy (9100 KayPENTAX Videostrobe, Lincoln Park, NJ), and refractometry (ATAGO refractometer, Bellevue, WA). Only one participant in experiment 2 was not able to tolerate the endoscope. Before each experimental session, urine hydration was measured using a refractometer. Threshold hydration criteria was set at $\leq$ 1.02g/ml. If participants did not meet the criterion, they were offered water and testing was repeated within 20 minutes. 31 participants met the criterion before participating in the rest of the study. We were not able to test urine hydration for one subject due to equipment error in experiment 1. Consistency in diet and voice use was monitored through verbal questions and a log to ensure similar voice and diet profile. Overall, participants had similar general food and water intake prior to the sessions. Once subjects passed the screening they participated in experiment 1 or 2 as described below.

4.2.c Procedures

General protocol: Before the start of each experiment, questions were asked verbally regarding average voice use (in hours), food, and water intake. A log was given to subjects in experiment 1 to monitor voice use and diet prior to the second session. In both experiments, subjects were exposed to the selected ambient humidity for 20 minutes to enable humidity acclimation (Sandage et al., 2014). Participants performed a vocal loading task in ambient humidity conditions, and they were blinded to the humidity condition. Further, voice measures were collected before and after the vocal loading challenge.

Experiment 1 protocol: For experiment 1, subjects completed the study in low (15%-27% relative humidity) and moderate-high (51%-70% relative humidity) with the order of humidity conditions randomized across subjects. The sessions were scheduled on consecutive days at similar times of the day (± 1 hour).

Experiment 1 vocal loading task: For experiment 1, to elicit child-directed speech, participants were instructed to read few children's books (Froggy series, Jonathan London Puffin Books, 1992) for 45-minutes while wearing a microphone (AKG C555 L, AKG, Vienna, Austria). Participants were asked to read in an appropriate character voice that would keep a child interested if they were reading these books to him/her. An audio sample was provided if participants needed a model to practice. Additionally, they were instructed to read "loudly enough to be heard outside this room". During the training phase, investigators provided feedback and cues to correctly and consistently produce the child-directed speech pattern. Non-verbal signs were shown to those participants who couldn't read in the target voice during the task. Participants observed the signs and continued producing child-directed speech following that. The intensity of their child-directed speech was monitored for the entire challenge and recorded every 15-minutes using a sound level meter (RadioShack 22-806, Fort Worth, TX). Participants repeated the same vocal loading task on the second day and were monitored for consistent production. Consistency was defined by intensity (monitored using the sound-level meter) and presence of the child-directed speech (perceptual assessment by the investigator). Two undergraduate researchers in the laboratory, rated whether all participants produced child-directed speech or not. Both the researchers had prior experience in listening to infant-directed speech. A 5-minutes sample was clipped from the 45-minutes vocal loading task (between 20-25 minutes). The researchers listened to these speech samples and provided a consensus rating of 0/1/2. 0 indicated no child-directed speech, 1 indicated some child-directed speech, and 2 indicated more child-directed speech. All participants had a rating of 2 except S7 and S13 (rating of 1).

Experiment 2 protocol: Subjects completed the study in low humidity in one session.

Experiment 2 vocal loading task: For experiment 2, participants were instructed to read the same books as experiment 1, by combining child-directed speech and whispered speech patterns. The ambient humidity during the loading challenge was set to low level (<28% relative humidity). The subjects were instructed to read using a low-effort whispered voice. During the training phase, to elicit whispered voice quality, subjects were required to produce three different effort levels (low/moderate/high). At first, subjects read with high effort, and slowly transitioned to moderate, and low. All participants were successful in producing a low-effort whispered voice that was comfortable for them. The investigators provided feedback consistently and monitored the use of whispered speech throughout the task.

4.2.d Voice measures

All voice measures were collected before and following the vocal loading task and were identical in experiments 1 and 2.

PTP. PTP varies with vocal fundamental frequency (Solomon, Ramanathan, & Makashay, 2007; Verdolini, Titze, et al., 1994). Consequently, at the start of the session, the 10^{th}, 20^{th}, 50^{th} and 80^{th} percent pitches were established from the maximum frequency range. A laryngeal microphone was placed around the subject's neck on either side of the thyroid notch. Instruments were calibrated before starting each session (MCU-4 Calibration system, Glottal Enterprises). The primary instrumentation included a circumferentially vented pneumotachograph mask fitted with differential pressure transducers (PTL-1 and PTW-2, Glottal Enterprises) for the collection of oral pressure and oral flow respectively. The differential pressure transducers are connected to a measurement system (MSIF-2 system, Glottal Enterprises, Syracuse, NY). A digital multichannel hardware system (PowerLab 16/30 ADInstruments, Sydney, Australia) was used for acquiring data. A 2" length plastic tubing was connected to the mask that collected oral pressure from just inside the lips. Before starting data collection, participants were trained on the PTP task per validated procedures (Fisher & Swank, 1997; Verdolini et al., 1990). During training, the investigator firmly held the mask around the subject's mouth and nose. The participants wore nose plugs and were cued to the target pitch. They were asked to repeat the syllable /pi/ at 1.5 syllables/second about 7 times "as smoothly as possible on a single breath and as quietly as possible without whispering". The production of 7 /pi/ syllables at minimal vocal loudness constituted 1 syllable string. The task was modeled multiple times and verbal feedback regarding the rate, loudness, and consistency was provided by the investigator. The rate was monitored using a metronome Seiko Digital Metronome (Model# DM50; Seiko Sports Life Co., Ltd, China). Additionally, visual feedback regarding the within-trial pressure peaks and oral flows was provided from the data collection monitor. Participants practiced until they were deemed trained. The training criteria was defined as (a) within-trial pressure peaks of the same height and (b) oral flows reaching 0 ml/s during the /p/ production. Upon the completion of training, a minimum of 7 syllable strings were collected at the 10th (PTP_{10}), 20th (PTP_{20}) and the 80th (PTP_{80}) pitches. Trials comprising of intermittent phonation, syllables produced at supra-threshold levels, or syllables produced with high oral flows were not included for data analysis. Data was analyzed using Lab Chart 7 (Power Lab 16/30 ADInstruments). During analysis, the investigator manually selected the three middle /p/ peaks from each trial (Fisher & Swank, 1997; Holmberg et al., 1988). The average lung pressure across five trials determined PTP_{10}, $PTP_{20,}$ and PTP_{80}.

CPP. Participants wore a head-mounted microphone (AKG C555 L, AKG; Vienna, Austria). The microphone signal was routed through a mixer (XENYX 1202/1002/802/502, Behringer, Road Town, Tortola; British Virgin Islands) to an A/D converter (PowerLab 16/30 ADInstruments). The speech recordings were made at a sampling rate of 44.1 kHz. The microphone was placed 4 cm, from the participant's mouth and this distance was kept constant throughout data collection. Participants read the first paragraph of the Rainbow passage (Fairbanks, 1960) at their habitual pitch and loudness level. CPP measure was computed on the (i) second and third sentences of the Rainbow passage (S. Awan, 2011) (CPP_{RAIN}), and (ii) sustained /i/ phonation at the 80th percent pitch ($CPP_{/i/80}$) using the Analysis of Dysphonia in Speech and Voice program (ADSV Model 5109, KayPENTAX, Montvale, NJ).

Self-perceived phonatory effort. Participants sang "Happy Birthday" as softly as possible, starting at the 50th percent pitch and provided ratings of perceived phonatory effort on a 9-inch visual analog scale. Participants were asked to draw a vertical line on the scale corresponding to their perceived effort. Data were analyzed by measuring the distance in inches and averaged to obtain the mean across all participants.

Self-perceived vocal tiredness. Participants rated their vocal tiredness on a 9-inch VAS after the completion of other voice measures. Participants drew a vertical line to indicate their extent of vocal tiredness. Data were analyzed by measuring the distance in inches and averaged to obtain the mean across all participants.

Speaking effort. Ratings of speaking effort were obtained at baseline, 15^{th} minute, 30^{th} minute, and post loading only for experiment 2. Participants rated their speaking effort using the Adapted Borg CR-10 (Borg & Borg, 2002). They were instructed to point to the number corresponding to their speaking effort (range: 0 to 10).

4.2.e Statistical analysis

Data were organized as Means ± SD. The dependent variables included PTP_{10}, PTP_{20}, PTP_{80}, CPP_{RAIN}, $CPP_{/i/80}$, self-perceived phonatory effort and vocal tiredness. Parametric statistical analyses were run using the SPSS-22 software (IBM SPSS Statistics Armonk, New York, USA) after assessing normality distribution (Shapiro-Wilk normality tests). A two-factor repeated measure analysis of variance (ANOVA) was applied to the dependent variables in experiment 1 with loading challenge (pre/post) and humidity (low/moderate-high) as within subject factors. Separate ANOVAs were performed for all the dependent variables. The significance level (α level) was 0.05. Paired t-tests were run on all the dependent variables in experiment 2.

4.2.f Reliability

10% of samples for each measure were selected. Interrater reliability (the two raters were authors in this study) was determined using interclass correlation coefficients

(ICCs). A 2-way mixed, absolute single measures ICC was conducted for all variables. The values of ICCs were in excellent range (>0.9) for all the variables suggesting adequate reliability.

4.3 Experiment 1: Results

Effects of Vocal Loading and Humidity

There was a significant interaction effect for vocal loading and humidity for $CPP_{/i/80}$ (Figure 2). $CPP_{/i/80}$ decreased in low humidity (average change of -0.69 ± 0.72) and increased in moderate-high humidity (+0.63 ± 0.78) after vocal loading. This interaction effect was significant (F = 6.706, df = 1, 15, p = 0.021). No significant interaction effects of vocal loading and humidity were observed for any of the other variables.

Effects of Vocal Loading

A significant main effect for vocal loading was observed for PTP_{10}, PTP_{20}, PTP_{80}, self-perceived phonatory effort, and vocal tiredness. PTP_{10} significantly increased from baseline (Mean ± SD: 5.181 ± 1.053) to post vocal loading (6.067 ± 1.075; F = 24.535, df = 1, 15, p = 0.00, Figure 1). Similarly, PTP_{20} significantly increased from baseline (5.12 ± 1.2) to post vocal loading (5.88 ± 1.23; F = 70.772, df = 1, 15, p = 0.00, Figure 1). PTP_{80} significantly increased from baseline (8.327 ± 3.5) to post vocal loading (9.39 ± 3.58; F = 18.215, df = 1, 15, p = 0.001, Figure 1). Significant main effects for vocal loading for self-perceived phonatory effort and vocal tiredness were obtained. Self-perceived phonatory effort significantly increased from baseline (2.45 ± 1.72) to post vocal loading (3.77 ± 2.05, F = 17.219, df = 1, 15, p = 0.001, Figure 4). Self-perceived vocal tiredness significantly increased from baseline (2.43 ± 1.65) to post vocal loading (5.24 ± 2.19, F = 89.716, df = 1, 15, p = 0.00, Figure 5). CPP_{RAIN} decreased from baseline (6.56 ± 0.79) to post vocal loading (6.42 ± 0.87; F = 1.211, df = 1, 15, p = 0.289, Figure 3) but the effect was non-significant.

Effects of Humidity

There were no significant main effects of humidity for any of the dependent variables.

4.4 Experiment 2: Results

Effects of Vocal Loading

Increased PTP_{10}, $PTP_{20,}$ $CPP_{RAIN,}$ self-perceived phonatory effort, and vocal tiredness were observed post vocal loading. PTP_{10} significantly increased from baseline (Mean ± SD: 5.21 ± 0.74) to post vocal loading (5.83 ± 0.89; p = 0.002, Figure 5). Similarly, PTP_{20} significantly increased from baseline (5.15 ± 0.76) to post vocal loading (5.49 ± 0.99; p = 0.016, Figure 5). However, no significant effects were observed for PTP_{80} (p = 0.101). CPP_{RAIN} significantly decreased from baseline (5.97 ± 1.03) to post vocal loading (5.62 ± 1.13; p = 0.032, Figure 6). Self-perceived phonatory effort significantly increased from baseline (3.23 ± 1.7) to post vocal loading (4.19 ± 1.72, p = 0.00, Figure 7). Self-perceived vocal tiredness significantly increased from baseline (1.5 ± 1.33) to post vocal loading (2.69 ± 1.7, p = 0.00, Figure 8).

4.5 Discussion

The present study investigated how some intrinsic and extrinsic factors affect vocal loading. We conducted two experiments. In the first experiment, we loaded the larynx by manipulating intensity, frequency, and vocal fold vibratory characteristics using child-directed speech. Vocal loading was induced by 45-minutes of reading aloud in 65 dB background noise in low and moderate-high ambient humidity. Study findings suggest that loading induced detrimental vocal changes, as measured by phonation threshold pressure at the 10th, 20th, and 80th pitches, and self-perceived ratings of phonatory effort and vocal tiredness. We obtained a statistically significant loading and humidity interaction effect for $CPP_{/i/80}$ suggesting a smaller magnitude of change with loading in moderate-high humidity as compared to low ambient humidity. However, this magnitude of change was minimal and averaged at 0.6 dB. The magnitude of vocal decrement was similar for all other variables at both low and moderate-high ambient humidity. Additionally, no significant main effects of humidity were observed for other variables. Therefore, it appears that increasing ambient humidity was not helpful in alleviating the negative effects of vocal loading in young non-dysphonic speakers.

In a second experiment, we loaded the larynx using a low-effort whispered voice quality by manipulating ambient humidity and vocal fold vibratory characteristics. Vocal loading was induced by 45-minutes of reading in low ambient humidity. The results from this experiment suggest that loading induced negative changes in voice, measured by PTP_{10}, PTP_{20}, CPP_{RAIN}, self-perceived phonatory effort, and vocal tiredness. Additionally, all participants reported increased speaking effort from baseline (0.5 ± 0.5) to post loading (3.3 ± 1.8) measured using the Adapted Borg CR-10. Hence, overall it appears that a low-effort whispered voice quality produced in low humidity was successful in vocally loading the larynx.

Both tasks i.e. child-directed speech and whispered speech were successful in inducing vocal loading. In addition to increased perception of phonatory effort and vocal tiredness, participants reported various voice-related symptoms including “tight muscles”, “gravelly voice quality”, “throat soreness”, “face and jaw muscle tension”, “hoarseness”, and “dry mouth and voice”. Increased perception of phonatory effort has been reported after a 2-hour prolonged vocal loading task (Solomon & DiMattia, 2000; Solomon et al., 2003). Participants rated self-perceived phonatory effort while singing “Happy Birthday” as softly as possible starting at the 50^{th} percent pitch. Using soft phonation tasks are clinically applicable in identifying vocal injury perceptually (Bastian et al., 1990). At least half of our participants reported their voice being more tired after the vocal loading task on both days. Three participants (S2, S12, and S22) in experiment 1 reported tiredness being carried-over from previous day.

The current study demonstrates that PTP_{10} and PTP_{20} significantly increase following vocal loading tasks. PTP has been used in studies involving prolonged speaking (Chang & Karnell, 2004; MP Sivasankar & Erickson-Levendoski, 2012; Solomon & DiMattia, 2000; Solomon et al., 2003). Prolonged speaking was a means to quickly induce vocal loading in the current study. However, non-significant humidity and loading interaction effects indicate that increasing ambient humidity did not significantly reduce the detrimental loading-induced voice changes. Our subjects included young healthy adults which may indicate that the healthy larynx is adequately robust in tolerating changes to ambient humidity. This observation is supportive of recent findings in our laboratory that as age advances, the laryngeal mechanism starts deteriorating and becomes vulnerable to challenges like environmental humidity and prolonged speaking (Sundarrajan, Loerch, Fujiki, & Sivasankar, 2016). The loading task using child-directed speech was conducted in low and moderate-high ambient humidity. On some experimental days, the ambient humidity in the room was at 51% relative humidity. It is also possible that the proximity between low (<27%) and moderate (>51%) relative humidity levels could have obscured the effects.

It is noteworthy that CPP measure collected on the rainbow passage (CPP_{RAIN}) showed a statistically significant loading effect during whispered speech production. The CPP_{RAIN} decreased from baseline to post loading by 0.3 dB which is a minimal functional change. This finding is interesting because CPP strongly correlates with breathiness (Hillenbrand et al., 1994). It is possible that a breathy voice quality may have been induced following vocal loading using whispered voice.

We used two innovative tasks to induce vocal loading in this study. Participants were instructed to read children's books using child-directed speech and whispered speech patterns. To the best of our knowledge, these tasks have not been studied in the context of vocal loading thus far. The results contribute to the understanding of the influence of different loading tasks on vocal health. Further, it is important to inform voice clinicians on the negative effects of whispering on vocal health.

4.6 Conclusions

In conclusion, the normal laryngeal mechanism is vulnerable to changes induced by a 45-minutes vocal loading task. Both tasks (child-directed speech and whispered speech) significantly loaded the larynx. Measures of PTP, CPP, self-perceived phonatory effort, and vocal tiredness demonstrated statistically significant loading effects. CPP on sustained phonation was sensitive to a loading and humidity interaction effect when child-directed speech was used. These vocal loading tasks lay groundwork for optimally testing the vocal mechanism. Further, the negative effects of vocal loading were not attenuated by increased ambient humidity.

Table 4.1. Participant Demographics

Subject Identifier	Gender	Age (years)	Refractometry	VFI part 1*	RSI	Spirometry
S1	Female	22	+	5	0	P
S2	Female	27	+	15	1	P
S3	Male	22	+	7	7	P
S4	Female	21	+	24	8	P
S5	Male	26	+	2	0	P
S6	Female	21	+	2	1	P
S7	Female	21	+	18	3	P
S8	Male	18	+	4	3	P
S9	Male	18	+	4	6	P
S10	Male	22	CNT	3	1	P
S11	Male	22	+	10	0	P
S12	Female	21	+	21	11	P
S13	Male	20	+	17	12	P
S14	Female	22	+	5	4	P
S15	Female	27	+	2	0	P
S16	Male	28	+	12	19	P
W1	Female	22	+	11	14	P
W2	Male	21	+	23	7	P

W3	Male	30	+	37	14	P
W4	Male	21	+	12	8	P
W5	Female	19	+	14	2	P
W6	Female	21	+	14	12	P
W7	Female	20	+	11	3	P
W8	Female	27	+	11	0	P
W9	Female	22	+	7	1	P
W10	Male	21	+	0	5	P
W11	Female	18	+	26	10	P
W12	Female	27	+	6	3	P
W13	Male	22	+	2	4	P
W14	Male	21	+	13	4	P
W15	Male	20	+	27	13	P
W16	Male	20	+	14	14	P

+ Indicates urine hydration threshold ≤1.02 g/ mL

*Scores <24 are considered within normal range

P indicates SVC and FEV scores above 80%

CNT indicates could not be tested

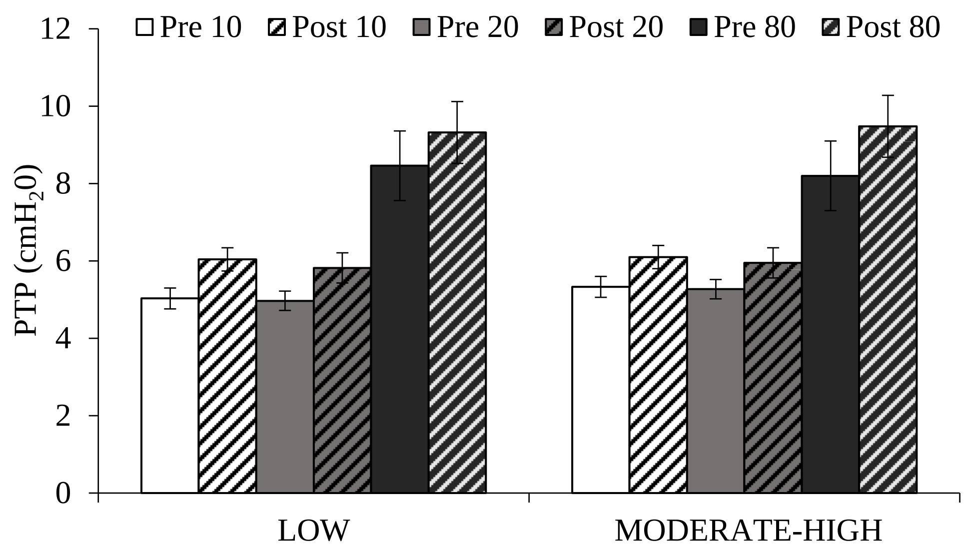
Pre 10
Post 10
Pre 20
Post 20
Pre 80
Post 80
12
10
8
6
4
2
0
PTP (cmH20)
LOW
MODERATE-HIGH

Figure 4.1

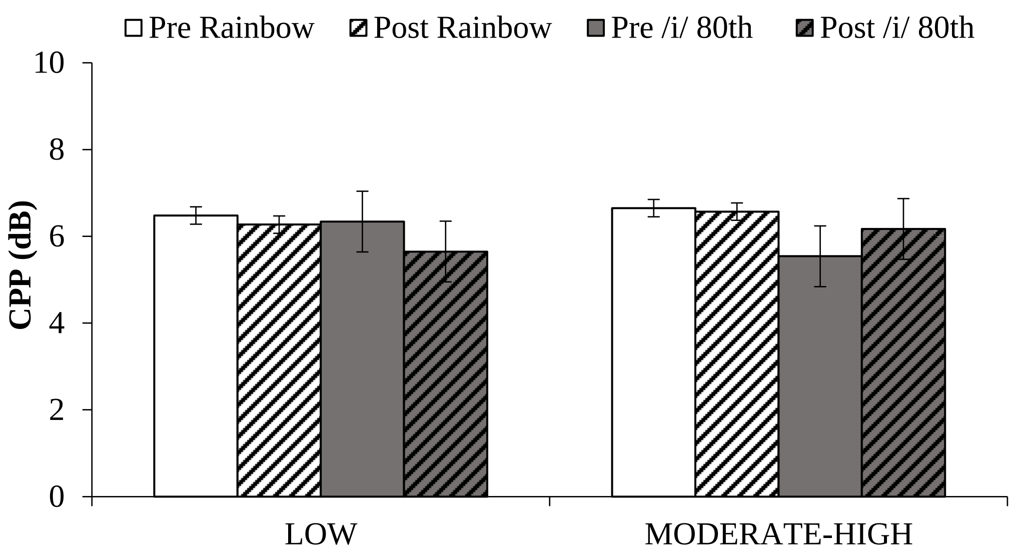
Pre Rainbow
Post Rainbow
Pre /i/ 80th
Post /i/ 80th
CPP (dB)
10
8
6
4
2
0
LOW
MODERATE-HIGH

Figure 4.2

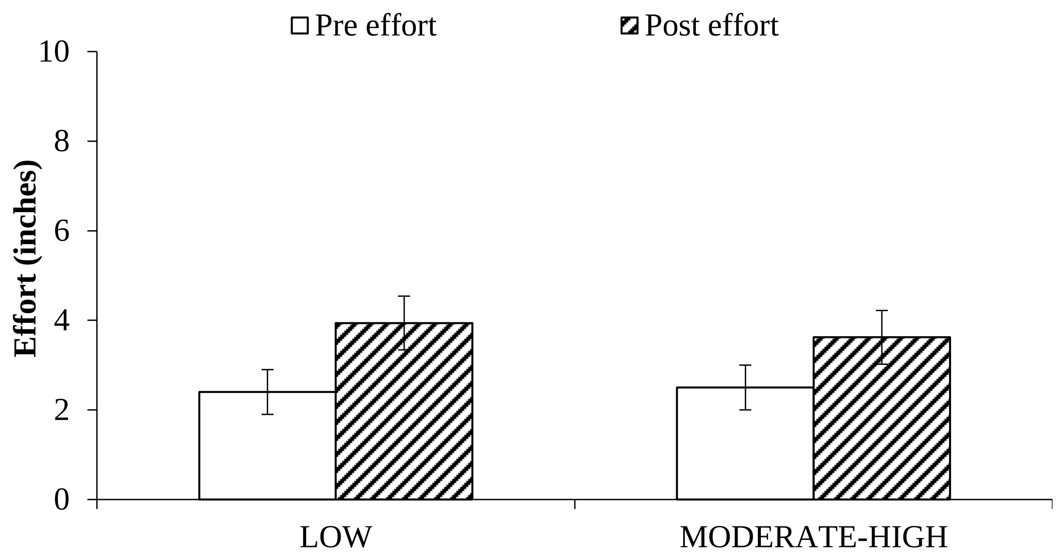
Pre effort
Post effort
Effort (inches)
10
8
6
4
2
0
LOW
MODERATE-HIGH

Figure 4.3

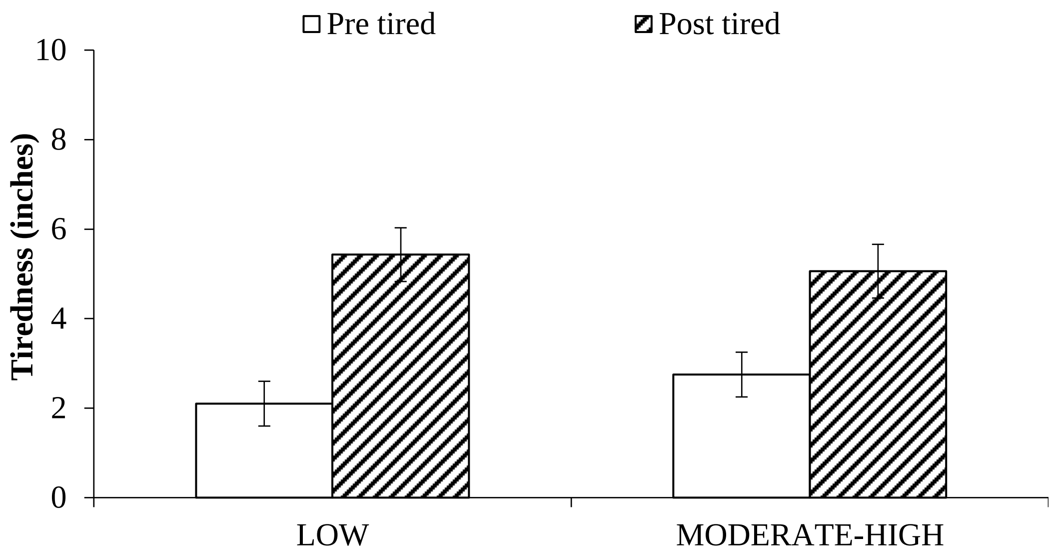
Pre tired
Post tired
Tiredness (inches)
10
8
6
4
2
0
LOW
MODERATE-HIGH

Figure 4.4

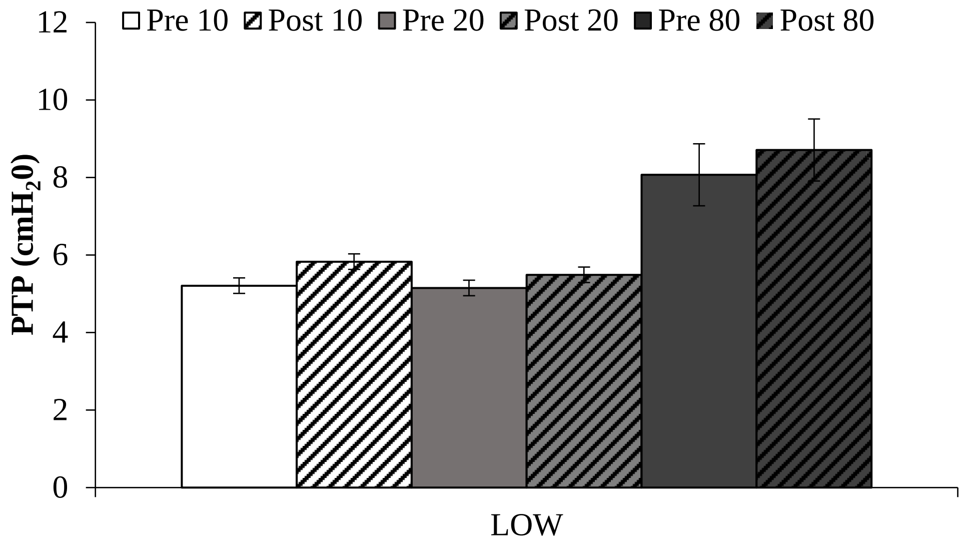
Pre 10
Post 10
Pre 20
Post 20
Pre 80
Post 80
PTP (cmH$_2$0)
0
2
4
6
8
10
12
LOW

Figure 4.5

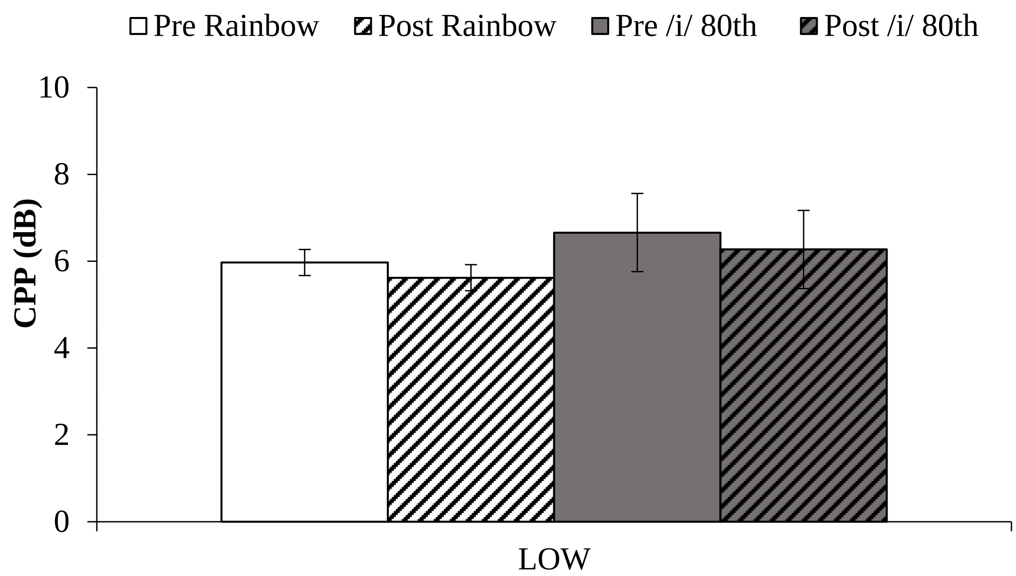
Pre Rainbow
Post Rainbow
Pre /i/ 80th
Post /i/ 80th
10
8
6
4
2
0
CPP (dB)
LOW

Figure 4.6

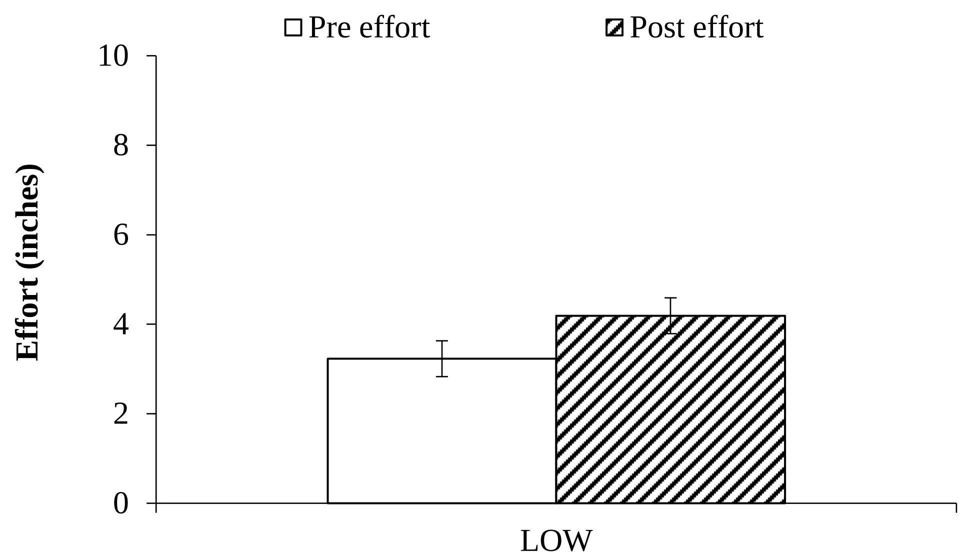

Figure 4.7

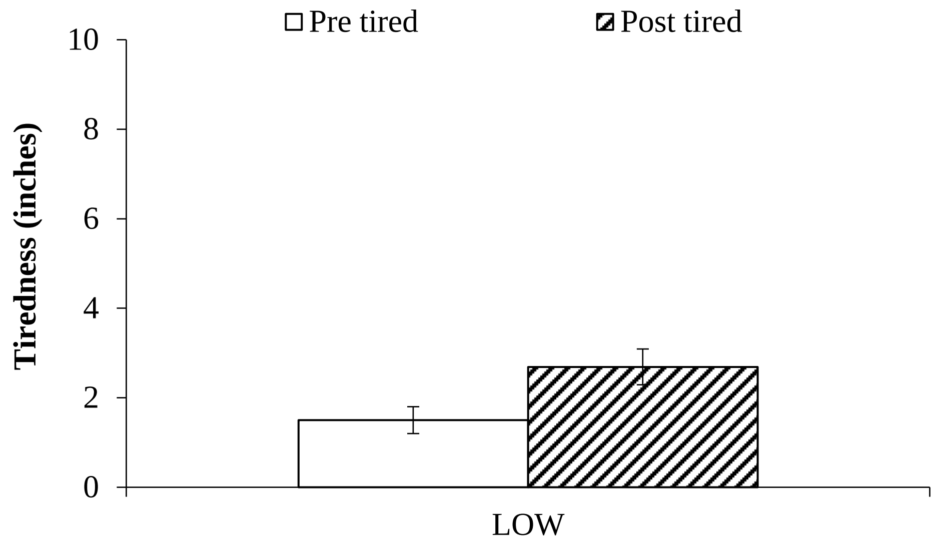

Figure 4.8

OVERALL SUMMARY

This study examined the effects of hydration and vocal loading on voice measures in young and older, healthy individuals. Factors such as speaking in dry environments, noisy environments, using altered voice quality, and non-habitual voice patterns negatively affect vocal loading. Three experiments were conducted to understand the effects of these factors. The vocal loading task involved child-directed speech or low-effort, whispered quality in child-directed speech. Voice was assessed using measures such as Phonation Threshold Pressure, Cepstral Peak Prominence, self-perceived phonatory effort, and vocal tiredness. A 45-minutes vocal loading task negatively affected the voice in both young and older speakers. Humidification attenuated the negative effects of vocal loading in older speakers, but not in younger speakers.

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APPENDICES

Appendix A Vocal Loading Using Prolonged Reading

Appendix Table A. Studies examining the effects of vocal loading using a prolonged reading task

Authors & Year	Subjects	Protocol	Measures	Findings
(Neils & Yairi, 1987)	6 vocally healthy females	45 minutes of reading in speech babble noise (50, 70, or 90 dB A). 1) recorded a 32-word portion of the "Grandfather Passage" and three CVC syllables; /pip/ was the target 2) airflow during /a/ phonation 3) speech sample for listener ratings	1) Mean fundamental frequency 2) Mean air flow rate in each noise level 3) Voice quality	Inconsistent trends were observed in perceived vocal quality over the seven samples. Changes in fundamental frequencies varied significantly between subjects and noise levels. The mean airflow rates increased after the reading period and decreased after the resting period for the 50- and 70 dB (A) condition.

(Gelfer et al., 1991)	50 women, 26 were trained singers and 24 were individuals with limited singing experience	Reading aloud for 1 hour at 80% of subject's speaking intensity range	Pre and post test data were collected on: 1) Sustain vowels /i/, /a/, and /u/ at a comfortable speaking pitch and relatively soft intensity level 2) Sing "The Star-Spangled Banner" on comfortable starting pitch using the syllable /lu/ Fundamental frequency (F0), intensity, jitter ratio, shimmer, and signal-to-noise ratio (SNR)	For the trained singers, 1) F0 and intensity remained stable. 2) Jitter ratio and shimmer values for most vowel productions decreased slightly. The only significant pre to post difference was jitter ratio on /i/. For the untrained subjects posttest measures showed higher jitter ratios and lower SNRs.
(Linville, 1995)	12 vocally healthy women	15 minutes of loud reading and subjects were seated ~ 15 feet from the examiner	1) Pre and post loud reading stroboscopic examination 2) Subjects sustained the vowel /i/ for a minimum of 3 seconds at three pitch levels (high, middle, and low) and at three loudness levels (medium loud, loud, and soft)	Post loud reading, altered glottic configurations were found across phonatory conditions particularly during high-pitched phonation Specifically, glottal closure increased in five subjects, glottal opening increased in one subject, identical pre vs. post configuration was maintained in two subjects, and four subjects demonstrated no consistent pattern.

(Stemple et al., 1995)	10 vocally healthy females	The vocal loading task involved reading at 75-80 dB for 2 hours with a 5 minute break after 1 hour	Measures were obtained before and after the loading task- 1) Acoustic measures included - Fundamental frequency, jitter, and frequency range 2) Aerodynamic measures included- Maximum phonation time, phonation volume and flow rate for the vowels /a, i, u/ at 2 different pitch levels 3) Video stroboscopic evaluation 4) Self reports of effort	The results obtained post-test were as follows: 1) Increased mean fundamental frequency; lower mean jitter were obtained 2) There was no difference for other acoustic and aerodynamic measures except the ones mentioned above 3) Video stroboscopic evaluation revealed appearance of glottal chinks and abnormal glottal closure in 9/10 subjects 4) 8/10 subjects reported 100% maximum effort at the end of 2 hours of reading i.e. at post loading time point

(Gelfer et al., 1996)	8 young women singers and 8 young women with limited musical experience	1 hour prolonged loud reading	Pretest and posttest videostroboscopic examinations were compared. Three experienced judges evaluated various aspects of laryngeal appearance and vibratory characteristics	In the untrained group, a small but significant increase in amplitude of vocal fold excursion was found. No significant differences were noted in the trained singer group. The study findings indicated that a 1 hour reading task was not sufficient to induce notable laryngeal alterations in the subjects.
(Lauri et al., 1997)	40 male and 40 female young university students with little musical knowledge	5x45 minutes of prolonged reading in <40 dB background noise. Two rest (morning and noon) and three loading (two in the morning and one in the afternoon) sessions were conducted.	Three strings of five /pa:pea/ words (Finnish for 'grandpa') produced normally (females on average 71 and males 74 dB SPL), as softly (females 60, males 63 dB SPL) and as loudly (females 93, males 97 dB SPL) Time based parameters analyzed from the estimated glottal waveform: (1) length of fundamental period (T); (2) open quotient (OQ); (3) speed	Loading affected only the T and OQ values of normal phonation during morning data collection. All the quotient values changed statistically in loud and normal phonation for the afternoon data collection. Increased SQ and decreased CIQ may have led to hyperfunction due to vocal loading.

			quotient (SQ); (4) closing quotient (CIQ)	
(Buekers, 1998)	20 females with vocal fatigue and 12 vocally healthy females	The loading task was accomplished using a voice endurance test. The test comprised of the following tasks and was called the Voice Interval test: tasks included reading aloud in a whisper, reading aloud in a low creaky voice, subjects were asked to make noises, then sing at different pitches, and cough three times. The tasks were completed for 5 minutes each and the total duration of the test was 30 minutes.	Voice measures included 1) Relative average perturbation (RAP), Pitch period perturbation quotient (PPQ), Shimmer, smoothed amplitude perturbation quotient (sAPQ) and NHR. 2) Pain, fatigue, discomfort, and globus sensation were evaluated on a visual analog scale	1) Study results indicated no changes in any of the voice measures before and after a working day as well as on the voice interval test in the vocally fatigued group. There were no differences obtained between the healthy and vocal fatigue group. 2) No change was observed in ratings for pain, fatigue, discomfort, and globus sensation before and after working days in the vocal fatigue group, but reports of increase in symptoms after the voice interval test. Fatigue increased significantly in vocal fatigue group after a working day, and after the voice interval test.
(Solomon & DiMattia, 2000)	4 young vocally healthy females	Speaking and non-speaking tasks: comfortable	1) Phonation threshold pressure (PTP) at conversational	1) PTP increased after the vocally fatiguing task at conversational

		reading (10 minutes), loud reading (2 hours), and vocal silence (15 minutes)	pitch, 10%, 50%, and 80% of the pitch range 2) Effort for speaking on 20 cm line with extremes “no effort” and “extreme effort” 3) Laryngeal imaging reviewed by 3 speech language pathologists	pitch, and 10%, 50%, and especially at the 80% of the pitch range 2) Effort for speaking increased consistently throughout the loud reading task and subsequently decreased after 15 minutes of vocal silence 3) 3 subjects demonstrated spindle-shaped vibratory closure patterns after loud reading on videostroboscopic examination
(Solomon et al., 2003)	4 young vocally healthy males	Initial session-training and practicing PTP and effort ratings 4 remaining sessions: 10 minutes of reading aloud at a comfortable level (to “warm-up” the voice), collection of 3-5 sets of baseline PTP and effort data, and laryngeal imaging, data collection pre and post a 2 hour loud reading task, 15 minutes of vocal silence,	1) Phonation threshold pressure (PTP) at conversational pitch, 10%, 50%, and 80% of the pitch range 2) Effort for speaking on 20 cm line with extremes “no effort” and “extreme effort” 3) Laryngeal imaging reviewed by 3 speech language pathologists 4) Visual-perceptual ratings	1) PTP increased after the loud reading task and correlated with PPE. 2) PPE varied across subject and pitch 3) Laryngeal endoscopy revealed an anterior glottal gap in two mean after loud reading. Amplitude of vocal fold vibration reduced in three subjects when under hydrated and one

		and then final data collection		subject when well hydrated
(Vintturi et al., 2003)	80 vocally healthy men and women (40 each)	The novel vocal loading task comprised of 5x45minutes of reading. The subjects were given a lunch break for 45 minutes and another 45 minutes of rest after the 5th loading session. Subjects were exposed to low and high humidity, soft and loud output level and two postures; sitting or standing. There were 5 in each cell combination (gender vs. every exposure group)	The variables included 17 questions related to symptoms factored into 5 factors a) central fatigue, b) shoulder, neck, and back, c) drying in the mouth and throat, d) symptoms of the throat, and e) symptoms of voice	Study results indicated women to have more loading related symptoms than men. In addition, women were greatly impacted by the different ambient humidity conditions as compared to men. Also, subject had more difficulty while reading in a standing posture as compared to reading while sitting.
(Chang & Karnell, 2004)	10 vocally healthy men and women (5 each)	The vocal loading task involved reading for two hours at a speaking intensity of 75-85 dB. Measures were collected every 30 minutes during the vocally fatiguing task and at time points of 15 minutes, 1 hour, 2 hour, 24 hour and 72 hour post loading	1) PTP was measured during CVC repetitions at 3 different pitch levels. 2) PPE was used to correlate with PTP	1) Findings indicated an increase in PTP during the fatiguing task compared to baseline at all three pitch levels. PTP was significantly higher for the high pitch condition. PPE had good correlations with PTP at low and comfortable

				conditions only. No gender effects were obtained.
(Laukkanen et al., 2004)	24 females with varied vocal training and were in teaching or service profession that required great deal of speaking daily	Read aloud from a book for 45 minutes (70 dB at 40 cm distance from the mouth) 1) Questionnaire on sensations of throat and voice 2) Acoustic measurements were made for the 1st, 5th, 15th, 30th and the 45th minutes of loading and for text reading before and after	1) Voice characteristics rated on visual analog scale: difficulty/ease of phonation, tiredness of throat, tickling of throat, pain in throat, lump in throat, need to cough, hoarseness, roughness, and breathiness of the voice 2) Average F0, SPL, and alpha ratio	1) F0, SPL, and alpha ratio increased during the vocal loading task Subjects with more vocal training had a lower F_0 in the loading test. SPL seemed to reflect variation of symptoms during loading. 2) Changes in acoustic parameters did not distinguish between subjects with most and fewest symptoms of fatigue in this test
(Boucher et al., 2006)	7 vocally healthy, 5 men and 2 women	12-14 hour protocol with 2 breaks to eat. Every 12-15 minutes, 2 tasks: a) vocal effort task- reading aloud at 74 dB for 3 minutes b) vocalization task- six tokens of /a/ at modal and high pitch	1) EMG spectral compression	1) EMG spectral compression was variable, but was observed at the end of the test period for all participants, indicating fatigue with effortful voicing task.

(Kelchner et al., 2006)	25 pubescent males, 15 in experimental group and 10 as controls	2-hour reading at an intensity of 75 dB SPL to 80 dB SPL. Every 15 minutes, Ratings of vocal quality and physical effort using 7-point equal interval scale	1) Average reading fundamental frequency (F0) and intensity on Rainbow passage 2) Frequency range on /i/ glides 3) MPT on /a/ 4) Voice quality and phonatory effort ratings 4) Post video endoscopic examinations	Post reading, the experimental group showed changes in F0, self- ratings of voice quality, and physical effort. No significant differences in either the perceptual quality of the experimental group's voices or their videoendocopic images.
(Niebudek-Bogusz et al., 2007)	51 full-time female teachers with functional voice disorders participated in this study	The task involved a vocal loading test; subjects read aloud text for a duration of 30 minutes in the presence of 80 dB SPL white background noise. The tasks included: 1) Voice handicap questionnaire 2) Video stroboscopy 3) Sustained phonation of /a/ at comfortable pitch and loudness	Measurements comprised of acoustic analysis such as the LTAS and FFT on phonation of vowel /a/. post analyses 17 parameters were Extracted, these include average fundamental frequency (F0), standard deviation of the fundamental frequency(sdev F0), pitch perturbation quotient (jitter), relative average perturbation (RAP) and pitch period perturbation quotient (PPQ), amplitude perturbation	Study findings indicated glottal insufficiency with bowed vocal folds in 35.2% of the participants, soft vocal nodules in 31.4% of the participants, and hyperfunctional dysphonia with a tendency towards vestibular phonation in 19.6% of the patients on videostroboscopic examination. Voice Handicap Index scores revealed that about 66% of the female teachers estimated their own voice problems to be of moderate disability. Post

			quotient (shimmer) and amplitude perturbation quotient (APQ) harmonic perturbation measurements: harmonic perturbation quotient (HPQ); harmonic perturbation quotient for high frequency (HPQh); residual harmonic perturbation quotient (RHPQ); residual harmonic perturbation quotient for high frequency (RHPQh); residual to harmonic (R2H), non-harmonic to harmonic (U2H); U2Hl (for low frequency); U2Hh (for high frequency), subharmonic to harmonic (S2H); and noise to harmonic ratio (NHR).	loading there was an increased rate of abnormal frequency perturbation parameters (pitch perturbation quotient (Jitter), relative average perturbation (RAP), pitch period perturbation quotient (PPQ).

(Lohscheller et al., 2008)	3 female subjects (2 investigated in the morning, and 1 in the afternoon)	Read loudly magazine articles for 2 hours at sound level between 75-80 dB at a distance of 30 cm from the mouth High speech digital imaging (HSDI) acquired while producing the vowel /i/ before reading loudly, at least 1.5 hours after completion of the reading task, and at least 30 minutes after completion of the reading task	Phonovibrograms (PVG) obtained from HSDI analysis represents a comprehensive image of patterns of vocal fold opening and closing and the dynamic behavior of the both the right and left vocal folds Linear discrimination analysis performed to reveal differences between subjects	The effects of vocal loading were reflected by alterations to the PVG parameters representing the posterior opening and closing dynamics Within subject evaluations revealed slight asymmetric vibratory behavior between the left and right vocal folds
(Hunter & Titze, 2009)	87 elementary and secondary school teachers	Reading aloud from a book for 2 hours Every 15 minutes, pause and perform 3 short vocal tasks and perceptual ratings	Perceptual ratings 1) Current speaking effort level (EFFT, 1-10 scale; 1 for no effort, 10 for an extreme effort to speak 2) Inability to produce soft voice (IPSV, 1-10 scale; 1 used for unproblematic soft voice, 10 for extreme problems with producing a specific soft voice task 3) Laryngeal discomfort (DISC, 1-10 scale; 1 for no discomfort, 10 for	Two types of post-loading response: 1) warm-up effect which was characterized by a general improvement of voice after fatigue and lasting for 3 days 2) voice being rated continually worse in the hours after the vocal loading exercise Vocal recovery trajectory was highlighted which is similar to a dermal wound healing trajectory

			extreme discomfort)	
(Echternach, Nusseck, Dippold, Spahn, & Richter, 2014)	101 senior student teachers	Read a standardized text (in German) for 10-minutes VLT (80 dB at a distance of 30 cm) and a real 45-minutes teaching lesson using a portable VoxLog system Multifactorial voice profile: videostroboscopy, roughness-breathiness-hoarseness scale, Voice Handicap Index, voice range profile, maximum phonation time, multidimensional acoustic analysis, Dysphonia Severity Index	1) Phonation time 2) Cycle dose 3) Energy dissipation dose 4) Radiated energy dose 5) F0 and SPL	A VLT of 10 minutes with 80 dB at 30 cm distance showed only small differences of vocal doses in comparison to a real teaching situation of 45 minutes. Male subjects showed a higher time dose in the teaching condition. Higher F0, SPL, and relative phonation time was associated with the VLT as compared to the real teaching lesson.
(Remacle, Schoentgen, et al., 2014)	50 vocally healthy women	Reading a novel aloud for 2 hours in two sessions Vocal intensity between 60-65 dB (A) in the first session and 70-75 dB (A) in the second	Seconds sentence from reading of a phonetically balanced text (French sentence) 1) Perceptual judgment task on the GRBAS scale to evaluate breathiness by 10 experienced judges with theoretical knowledge and regular practical experience in	Breathiness was significantly lower post loading suggesting vocal improvement and an adaptation of voice to loading

			perceptual voice analysis using the paired stimuli approach	

Appendix B Vocal Fold Hydration

Appendix Table B. Summary of the studies on vocal fold hydration

Authors & Year	Subjects	Protocol	Measures	Findings
(Verdolini et al., 1990)	6 vocally healthy non-singers and 6 singers	Pre and post measurement 1. Dry exposure (30-35% with no fluid 3 tsp decongestant-Dimetapp) 2. Wet exposure (85-100% humidity with water and 2x2 tsp mucolytic (Robitussin) 3. Normal exposure (40-55% humidity with no fluid control	PTP at low, mid, and high pitches	1. There were minimal differences between the dry, wet, and control conditions with increases in PTP with pitch rise 2. PTP was lowest in the wet condition at high pitch 3. Minimal increase in PTP in dry condition compared to the wet condition

(Verdolini-Marston et al., 1994)	6 females with vocal fold nodules or polyps	1. Hydration exposure (8x16 oz water and 90-100% humidity with 3x1 tsp mucolytic) 2. Control (3x1 tsp cherry syrup) medications intake – 6 hourly and each treatment was for 5 consecutive days	1. PTP obtained at low, mid, and high pitches 2. PPE 3. Laryngeal imaging on a 5 point scale 4. Perceptual rating on a 5 point rating scale 5. Acoustic measures including jitter, shimmer, Signal/Noise ratios	Improvement in both the hydration and control conditions with hydration reported to be superior. No significant differences in PTP, some trend observed at high pitch. No significant differences in perceptual rating Less severe rating on laryngeal imaging No clear hydration effects present for acoustic measures, but treatment effects present Inconsistent results across subjects
(Verdolini, Titze, et al., 1994)	9 vocally healthy females, and 3 males	1. Hydration condition (90% humidity with water and 2x2 tsp mucolytic) 2. Dehydration (10-20% humidity with no fluid and 2x2 tsp decongestant 3. Control (50% humidity and no fluid control 2x2 tsp cherry syrup	1. PTP at 10th, conversation pitch, and 80th pitch 2. PPE	Inverse relationship between PTP and hydration level With pitch increase, sensitivity of PTP increased Significant increase in PPE in dry condition as compared to control and wet conditions Conclusion: changes in PTP with hydration

		Humidity exposure for 4 hours, and mucolytic intake at start and 30 minutes prior, decongestant intake at 60 minutes prior and control 120 minutes prior		level are pitch dependent with greatest impact seen at higher pitches, PPE changes are less sensitive with changes in hydration
(Roy, Tanner, Gray, Blomgren, & Fisher, 2003)	18 healthy vocally normal female university students	Evaluated once per week for 3 consecutive weeks, before and after administration of one of three nebulized agents (sterile water, 12.5% Mannitol, and Entertainer's Seconds Throat Relief)	PTP at the 80th pitch obtained 6 times (baseline measurements: 1 and 2 performed at 15 minutes prior and immediately prior to nebulization. 3rd observation was performed 5 minutes after nebulization, 4th, 5th, and 6th observations were performed at 20 minutes, 35 minutes, and 50 min after nebulization	Mannitol, an agent that facilitates osmotic water flux to the luminal airway surface decreased PTP immediately after its administration for high pitch productions Sterile water and Entertainer's Second Throat Relief did not show any significant post administration PTP effects
(Tanner et al., 2007)	60 vocally healthy women 15 subjects per group	3 out of 4 groups received nebulized isotonic saline, hypertonic saline or sterile water, and 4th	PTP and PPE collected for high pitch productions at baseline, immediately post-desiccation,	Significant increase in PTP for all the groups after the desiccation challenge On an average, PTP was 0.5 cm

		group served as a non-treatment control	and at 5, 20, 35, and 50 minutes post nebulization	H_2O higher post-desiccation than baseline A temporary trend toward a PTP reduction was seen for the isotonic saline group PPE ratings decreased significantly after the desiccation challenge Poor correlation between PTP and PPE
(M Sivasankar et al., 2008b)	16 females divided into two groups: 8 reporting a history of vocal fatigue, and 8 matched controls	Participation in three experimental sessions on different days Exposed to three humidity levels (low: 20±5%), moderate: 50±5%), and high: 70±5%) in a counterbalanced order	PTP and PPE collected at pre-challenge baseline, after 15 minutes of oral breathing, and after 15 minutes of nasal breathing PTP was obtained for 10^{th}, 20^{th}, and the 80^{th} pitch PPE was obtained after participants sand "Happy Birthday" in a soft voice starting at the 50^{th} percent of their pitch range Respiratory frequency was measured across the oral	15 minutes of oral breathing in low humidity significantly increased PTP, more increase in the vocal fatigue group as compared to controls PTP did not increase in either participant group after oral breathing in a humid environment Poor correlation between PTP and PPE

			and nasal breathing challenges	
(Yiu & Chan, 2003)	10 male and 10 females healthy amateur singers	1. Hydration and voice rest with 100 ml water 2. No hydration and voice rest and no fluids Fluid intake after each song performance	Pre and post Acoustic measures including F0, jitter, shimmer, noise-to-harmonic ratio, perceptual ratings of roughness and breathiness on VAS, phonetogram	Significant differences in the hydration with voice rest group, there was increase time till fatigue occurred, increase in jitter in speech of the no hydration and voice rest group after 10 songs Decrease in highest frequency for females in the no hydration and voice rest group Authors recommended hydration and voice rest for singers as preventative measures to reduce vocal fatigue
(Franca & Simpson, 2009)	19 vocally healthy females	1. Abstain from food and water intake before testing 2. Intake 1L of water 14 h fast before testing 1 L water in 20 minutes Test 90 minutes after ingestion	Acoustics Jitter and shimmer times Pre ✗ Post U Initial Ax in dehydrated state after fast	Improved jitter and shimmer in rehydrated condition, but not for all participants
(Selby & Wilson, 1997)	8 healthy males	All participants abstained from	Participants water intake	Lx imaging 1/8 participants

	18–31 years	alcohol and caffeinated drinks Testing at 20 degree C no air conditioning 1. Dehydration Abstain from all fluids and food before testing 2. Rehydration Ingest 2 L electrolytic fluid timing 1 d prior abstinence from alcohol and caffeine Dehydration-abstain fluid from 4 pm and food from 6 pm a day before testing (~18 h) Rehydration-ingest 20-minutes period, testing 1 h after	0.25–2 L/d 50% often Thirsty Laryngograph Lx imaging Acoustics Jitter, F0 mode, range, irregularity timing Pre ✗ Post U Initial Ax in dehydrated state post abstinence	excluded due to sulcus vocalis No diff. between smokers and nonsmokers Acoustics Sig. [modal F0 in conversation after rehydration Hydration status does not have a marked influence on F0 mode, range and regularity
(Fujita, Ferreira, & Sarkovas, 2004)	Subjects included 6 males aged ranged between 28–36 years. Healthy professional voice users	Subjects were asked to remain in workplace environment (except transit) and were required to	Laryngeal imaging was accomplished by a technique called Video-kymography. The measures included:	No statistical analysis was completed on the measures. The open phase time/closed phase time decreased after hydration

	who had low humidity working environments.	abstain from systemic medications, coffee, alcoholic drinks, and irregular diet changes. The protocol included: 1. Dehydration Induced by abstaining all liquids 2. Rehydration was induced by ingesting 200 mL room temperature aqueous solution with electrolytes and in addition inhalation of 0.9% saline. The design comprised of an unknown general abstinence period, followed by no fluid intake 4 hours before testing. Fluid ingestion immediately followed the initial Ax. The saline inhalation lasted for 10 minutes.	open phase time/ closed phase time, and dB and Hz timing The time points included: Pre ✗ Post U Initial Ax in dehydrated state post abstinence	in 80% of participants, but not in the remaining 20% of the participants.

(Roh, Kim, & Kim, 2006)	20 healthy males between the ages of 21–24 years	The subjects were asked to avoid consumption of caffeine, alcohol, high sodium food, drugs, dehydrating substances, excessive eating or drinking of water, and strenuous voice use. The subjects were either given the xerostomia-intramuscular injection 0.3 mg (1.5 mL) Glycopyrrolate or the control-intramuscular Injection which was 1.5 mL saline for general avoidance were not specifically stated. The time points for the injections were at baseline, and at 3 hours post	Saliva volume by modified swab was measured. Participant Perception on how dry their mouth was – measured using a visual analog scale. The ratings were taken before and every 30 minutes for 3 hours after injections. The voice measures included: acoustic variables such as fundamental frequency, jitter, shimmer noise-to-harmonic ratio, voice range profiles. The Aerophone II was used to measure maximum phonation time, average airflow, subglottal pressure, PTP, and	Study findings indicated higher saliva flow rates 50% after glycopyrrolate injection which was measured post 30–60 minutes, Lowest saliva flow was observed 90–120 minutes after injection. Significantly dry mouth increased 30 minutes post, and the highest level was at 120 minutes. PPE and PTP was higher for both groups post 3 hours, significant results obtained for the treatment group. Voice range profile indicated significant pitch and loudness effects in the treatment group No significant changes were observed for variables such as Maximum phonation time, average

			PPE was measured using VAS 20 minutes post reading. Laryngeal imaging was used to describe variables like vibratory closure pattern, supraglottic activity, presence of mucus, color, mucosal wave, amplitude and symmetry.	airflow, and videostroboscopy ratings.
(Akhtar, Wood, Rubin, O'Flynn, & Ratcliffe, 1999)	8 healthy Males 4 Females 27–55 years	250 mg pure caffeine (53 Proplus tablets) Baseline and post-baseline Measures were obtained	Laryngograph irregularity of F0 Free speech Reading passage ''Happy Birthday''	Sig. effects Between participants, not within participants (pre and post) in all three conditions Caffeine mg/L varied between participants Reading- substantial F0 variation in each task across participants before caffeine ingestion.

Appendix C Vocal Aging

Appendix Table C. Studies on vocal aging in non-dysphonic speakers

Authors & Year	Subjects	Protocol	Measures	Findings
(S. Awan, 2006)	10 vocally healthy female subjects in 5 groups; the subjects had ages between 18-30 years, 40-49 years, 50-59 years, 60-69 years, and 70-79 years.	The subjects were asked to produce a continuous speech sample which was (The Rainbow passage) and three repetitions of sustained vowel phonation of /a/ at comfortable pitch and loudness levels. In addition to the voice parameters, respiratory variables included vital capacity (VC), maximum phonation time (MPT), and phonation quotient (PQ) which was collected using a spirometer.	Measures of mean speaking fundamental frequency (SFF) and SFF standard deviation were obtained from the second sentence of the Rainbow passage. The other voice measures of jitter, shimmer, pitch sigma, and signal-to-noise ratio obtained from the middle 1 second portion of the vowel /a/.	The study findings indicated that SFF, MPT, VC, and pitch sigma significantly changed between the aging groups. The authors suggested that decrease in SFF may not be completely due to changes during menopause, but may occur in individuals who are at the pre-menopausal stage and in decades well beyond menopausal completion. The other reasons suggested for increased pitch sigma and long-term instability may be due to neuromuscular

				control variations. In addition, these changes could be due to changes in the mechanical properties of the vocal folds post-menopausal stage. A reduced vital capacity may be attributed to a number of age-related morphological and histological changes in the aging larynx.
(Goy et al., 2013)	159 healthy young undergraduate students and 133 community-dwelling older adults	Habitual pitch task: sustained /a/ phonation for 8 seconds at 75 dB (C) Maximum pitch: phonate /a/ for 8 seconds at high pitch Minimum intensity: phonate /a/ for 8 seconds in normal, most comfortable pitch as softly as possible Maximum phonation time: phonate /a/ at normal, most	Mean intensity (SPL), mean fundamental frequency (F0), Jitter, jitter percent, relative average perturbation, pitch perturbation quotient, shimmer percent, amplitude perturbation quotient (APQ) calculated over 3, 5, and 11 cycles (APQ3, APQ5, and APQ11), HNR, and	Older females had a lower F0 and SF0 and smaller SF0SD than younger females, but younger and older males did not differ. Shimmer increased with age for males, but neither jitter nor shimmer increased with age for females, whereas noise measures were similar for both ages.

		comfortable pitch for as long as possible	NHR calculated from habitual pitch trials Dysphonia severity index (DSI) calculated using max F0 From the reading passage, speaking fundamental frequency (SF0), the standard deviation of speaking fundamental frequency (SF0SD), and mean speech intensity were calculated	Younger and older males had a similar DSI, whereas older females had a higher DSI than younger females Age related differences were found for males and females
(Maslan et al., 2011)	69 healthy adult volunteers, with 34 mean and 35 women (15, 26, and 28 in the seventh, eighth, and ninth decades of life respectively)	MPT task while sitting upright, three times /a/ MPT with a 1 minute rest period between each trial	Means and standard deviations calculated for MPT by age, group, gender, and trial	No statistical significance for age, group, gender, trial and their interactions MPTs were longer for subjects older than 65 years Stable measurements across three trials of MPT s in a single short sampling session
(Stathopoulos et al., 2011)	192 participants between 4-93 years of age	Sustain /a/ for a comfortable period of time	Middle interval of each vowel production	In males, F0 declined steadily from 4

		using their comfortable everyday pitch and loudness levels	(500 ms for the 4-25 year olds, 2000 ms for the 30-93 year olds) Measures included: Average F0, SPL, and SNR	to 50 years of age and then increased slowly, in females F0 declined steadily from 4 to 60 years with a shallower slope than males SPL increased as the subjects aged thus suggesting average SPL does not reflect the declining laryngeal system SNR was lower for young and older female subjects indicating more additive noise in the voice signal, males demonstrated a linear increase in SNR with age Authors concluded that F0, SPL, and SNR followed nonlinear trends, higher at younger and older ages

VITA

VITA

Anusha Sundarrajan

Department of Speech, Language, and Hearing Sciences, Purdue University
715 Clinic Drive, West Lafayette, IN 47907

RESEARCH INTERESTS:

Understanding the physiology of the aging voice using aerodynamic and acoustic measures

EDUCATION:

2011- Present — **Purdue University, Indiana**
Ph.D. in Speech Language Pathology
Minor in Gerontology (Centre of Aging and the Life Course)

- Dissertation: “Hydration and vocal loading on voice measures”- The primary aim of the study was to understand how the healthy, young and old larynx responds to the effects of unhealthy, and excessive use of the laryngeal mechanism.

2010 — **Manipal University, India**
Masters of Audiology and Speech Language Pathology (M.A.S.L.P)

2008 — **Ramachandra University, India**
Bachelor of Audiology and Speech Language Pathology (B.A.S.L.P)

RESEARCH EXPERIENCE:

Jan 2015- Present — **Graduate research assistant, Laryngeal Research Laboratory, Purdue University**

- Assessed voice function for young and old adults; designed experiments, collected, and analyzed aerodynamic, acoustic, and perceptual data

Jan 2015- Jan 2013 — **Graduate research assistant: Speech Physiology Laboratory, Purdue University**

- Collected and analyzed laryngeal and respiratory kinematic data from typically aging older adults

Sept 2011-
Dec 2012

Graduate research assistant, Laryngeal Research Laboratory, Purdue University

- Collected and analyzed aerodynamic and perceptual data from young adults

Aug 2009-
June 2010

Graduate student, Manipal University

Thesis: "Prevalence of Voice Problems in University Teachers"

- Conducted a voice survey using questionnaires in 100 college level teachers

Jan 2007-
June 2008

Undergraduate researcher, Sri Ramachandra University

Conducted a vocal hygiene awareness program for school teachers

PEER-REVIEWED PUBLICATIONS:

Sundarrajan, A., Levendoski, E., & Sivasankar, M. (2015). A preliminary investigation of potential biases in Phonation Threshold Pressure analysis. *Journal of Voice, 29*(1), 22-25.

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WORK IN PROGRESS:

Sundarrajan, A., Loerch, S., Fujiki, R., & Sivasankar, M. An innovative method of vocal loading with consideration to factors of hydration and voice quality

Sundarrajan, A., Loerch, S., Fujiki, R., & Sivasankar, M. Humidity and vocal loading on the aging voice.

Fujiki, R., Chapleau, A., Sundarrajan, A., & Sivasankar, M. (manuscript submitted to Journal of Voice). The interaction of surface hydration and vocal loading on voice measures.

Sundarrajan, A., Huber, J., & Sivasankar, M. Effects of aging on the laryngeal and respiratory system.

CONFERENCE PRESENTATIONS:

Sundarrajan, A., Fujiki, R., & Sivasankar, M. (2016). Humidity effects on an innovative vocal loading challenge. Poster presented at the Combined Otolaryngology Spring Meetings (COSM), Chicago, IL.

Sundarrajan, A., Fujiki, R., & Sivasankar, M. (2016). Humidity and Vocal Loading on the Aging Voice. Talk presented at the Voice Foundation Annual Symposium, Philadelphia, PA.

Fujiki, R., Sundarrajan, A., & Sivasankar, M. (2016). The Role of Surface Hydration on a 30-Minutes Vocal Loading Challenge. Talk presented at the Voice Foundation Annual Symposium, Philadelphia, PA.

Herndon, N., Sundarrajan, A., Huber, J., & Sivasankar, M. (2015). Respiratory and Laryngeal Interactions in Determining Phenotypes for Voice Disorders. Poster presented at the American Speech Language-Hearing Association Conference, Denver, CO.

Sundarrajan, A., Erickson, E., Sivasankar, M. (2014). Does Knowledge of the Experimental Hypothesis Bias PTP Analysis? Poster presented at the Voice Foundation Annual Symposium, Philadelphia, PA.

Sundarrajan, A., Huber, J, & Sivasankar, M. (2014). Effects of aging on the laryngeal and respiratory system. Talk presented at the American Speech-Language-Hearing Association Conference, Orlando, FL.

Sundarrajan, A., Erickson, E., Sivasankar, M. (2012). Experimental considerations in using Phonation Threshold Pressure (PTP): Investigating the effects of humidity. Poster presented at the American Speech-Language-Hearing Association Conference, Atlanta, GA.

TEACHING EXPERIENCE:

2012-2013	Graduate Teaching Assistant, Purdue University SLHS 302: Acoustics of Speech and Hearing SLHS 115: Introduction to Communication Disorders SLHS 420: Developmental Speech and Language Disorders SLHS 430: Speech-Language Disorders in Health Care Settings
2015-2016	Graduate Teaching Assistant, Purdue University SLHS 303: Anatomy and Physiology of the Speech Mechanism SLHS 449: Introduction to Clinical Practice in Communication Disorders
2012-2016	Guest lectures SLHS 532: Voice and its Disorders (1 lecture) SLHS 536: Speech Sciences (2 lectures) SLHS 115: Introduction to Communication Disorders (2 lectures) SLHS 430: Introduction to Clinical Practice in Communication Disorders (3 lectures)

SCHOLARSHIPS & AWARDS:

2014 & 2015	Wilson Scholarship in the Department of Speech, Language, and Hearing Sciences, Purdue University
2015	Indiana Lion's Club McKinney Research Award
2013	Ringel Research Scholarship
2011	First Prize for Oral Presentation titled "Prevalence of Voice Problems in University Teachers" at PHONOCON 2011, Army R&R Hospital (New Delhi)
2009	Received the Best Journal Club Award titled "Voice Production during a Weightlifting and Support Task"

PROFESSIONAL AFFILIATIONS:

Life member of the Indian Speech and Hearing Association (ISHA)
Member of National Student Speech Language Hearing Association (NSSLHA)
Member of the Asian Indian Caucus (AIC), a multicultural constituency group of ASHA
Member of the Pan American Vocology Association (PAVA)