A Comparison of the Effects of Phonation into a Positive Expiratory Pressure Device and Silicone Tube in Water on the Vocal Mechanism

*,†Pedro Amarante Andrade, †Marek Frič, ‡Brian Saccente-Kennedy, and †Viktor Hruška, *Australia, †Czechia, and ‡London, UK

Summary: Objectives. Positive expiratory pressure (PEP) devices have become an additional therapeutic approach for treating voice disorders. Similar to water resistance therapy (WRT), phonation in a PEP device introduces a secondary source of vibration within the vocal tract. This investigation aimed to compare the effects of phonation using a PEP device and silicone tube phonation (STP) commonly used in WRT on the vocal mechanism during phonation.

Methods. Three normophonic subjects participated in the study. High-speed videoendoscopy, pressure, airflow, electroglottography, and acoustic recordings were collected.

Results. The results demonstrated that phonation using both the PEP device and silicone tube induced alterations in glottal behavior. The PEP device produced more pronounced and consistent pressure oscillations, impacting the glottal cycle and influencing parameters including contact quotient (CQ), fundamental frequency, glottal area, pressure, and airflow. The regular vibratory mechanism of the PEP device systematically modified the glottal cycle. In STP, regular bubbling at lower depths of submersion produced higher CQ values, supporting the efficacy of deep bubbling exercises for inducing glottal adduction.

Conclusions. The findings suggest that phonation using PEP devices has a more pronounced impact on the vocal tract and glottis. It also provides a stronger massage effect that directly affects the glottal source. Phonation with a silicone tube produces similar results, although to a lesser extent and with lower regularity. These findings offer guidance in the selection of voice therapy devices.

Key Words: Semi-occluded vocal tract exercises–Positive expiratory pressure–Tube phonation–Water resistance therapy–Acapella–Shaker–Vocal tract impedance–Periodogram.

INTRODUCTION

Semi-occluded vocal tract exercises (SOVTEs) have proven to be a well-established therapeutic approach for treating voice disorders. The core principle of SOVTEs involves increasing the impedance of the vocal tract to match that of the glottis.[1–5] This, in turn, enhances vocal economy, allowing for maximum vocal output with minimal stress on the vocal folds.[6,7] Increasing the impedance of the vocal tract can be achieved by narrowing the vocal tract or elongating it artificially. This enhances vocal fold vibration by approximating the impedance of the vocal tract to that of the glottis, consequently increasing the positive (inertive) reactance, thereby aiding vocal fold vibration.[2,5] Furthermore, some SOVTEs incorporate a lower-order secondary source of vibration in the vocal tract, such as lip and tongue trills or water bubbling.[8] The addition of a secondary source of vibration in the vocal tract produces large oscillations in the mean intraoral pressure, not typically observed during regular phonation. These oscillations manifest as mechanical vibrations of the vocal tract and laryngeal structures. This additional effect, often referred to as the 'massage effect' in previous literature[8–12] can be regarded as an ancillary therapeutic outcome since it is not directly associated with the constriction or elongation of the vocal tract, as defined by the term SOVTEs.

A more recent alternative method of introducing a secondary source of vibration in the vocal tract is the use of a vibratory positive expiratory pressure (PEP) device.[13–19] PEP devices were originally designed for bronchial hygiene and aim to mobilize secretions from the lungs and trachea in conditions such as cystic fibrosis and neurogenic diseases, hence they are commonly used without vocalization. PEP devices are composed of a mouthpiece connected to a tube with an oscillatory valve at its distal end (Figure 1). As such, PEP devices work similarly to water resistance therapy (WRT) (ie, phonation into a long silicone tube with the distal end submerged under water), as both techniques artificially lengthen the vocal tract while also introducing a secondary source of vibration at its distal end (water bubbling for WRT and a flapping/bouncing mechanism for PEP devices). Different mechanisms of pressure and airflow modulation generated by PEP devices have been developed. For example, the Shaker device (POWERbreathe International Ltd., Warwickshire, UK), uses a plastic cone containing a metal sphere that is vertically displaced by the

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From the *Curtin School of Allied Health, Faculty of Health Sciences, Curtin University, Australia; †Musical Acoustics Research Centre, Academy of Performing Arts in Prague, Czechia; and the ‡University College London Hospitals NHS Foundation Trust, London, UK.
Address correspondence and reprint requests to Pedro A. Andrade, Curtin School of Allied Health, Faculty of Health Sciences, Curtin University, Kent St, Bently, WA 6102, Australia. E-mail: pedro.andrade@curtin.edu.au
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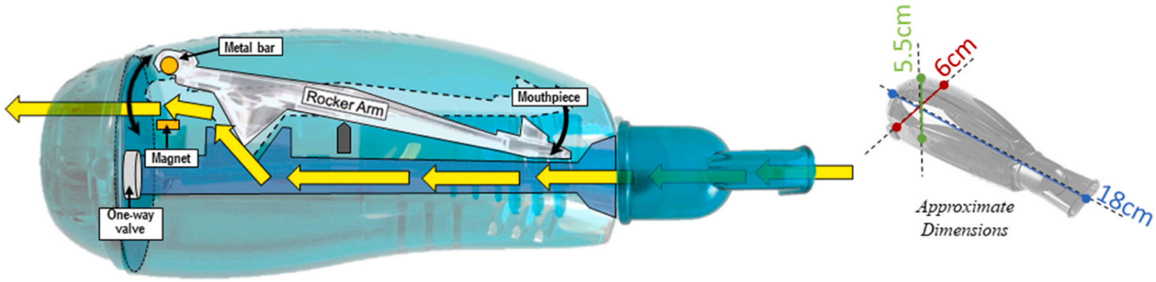

FIGURE 1. PEP device. Acapella Choice (from Saccente et al, 2022).

airflow,[20] while the Acapella Choice (henceforth Acapella) is composed of a tube with a distal oscillatory arm that closes and opens with airflow (Figure 1). The impact on the voice by these mechanical differences between PEP devices still needs to be investigated during phonation.

With regard to the Acapella device, Andrade 2022[14] found that the low-frequency modulation of pressure and airflow values in the vocal tract significantly affects the vibration pattern of the vocal folds. Specifically, the Acapella device causes regular changes in the impedance of the vocal tract, which alternates the vibration pattern of the vocal folds between aided and hindered. When the Acapella's arm is closed, pressure is at its maximum, causing the vocal fold vibration to be hindered. Conversely, when the Acapella's arm opens, intraoral pressure drops to minimum values, causing the vocal folds to vibrate with maximum amplitude, which in turn leads to larger modulations of the high-frequency component of pressure; related to the vocal cycle. This slow (relative to the frequency of phonation) pressurization and depressurization of the vocal tract is responsible for the previously mentioned massage effect. The authors also offer the possibility of an unanticipated therapeutic effect with the use of Acapella devices in which small adjustments of the intrinsic laryngeal muscles may take place to counteract the changes in impedance. Although undocumented, these adjustments may impact on the dexterity and strength of the intrinsic muscle activity of the larynx. These assumptions should be tested in future studies.

Similarly, to PEP devices, WRT also produces lengthening and a lower frequency modulation of pressure in the vocal tract and therefore changes in its impedance, which is likely to affect vocal fold vibration.[9,21] However, as the vocal tract pressure modulation by the WRT's water bubbling can present different regimes (regular, bimodal, and chaotic[22]), it is unclear whether it can produce similar changes in vocal fold vibration pattern as PEP devices.[14] The aims of this study are a) to compare the behavior of pressure and airflow profiles in the vocal tract between Acapella and WRT, b) specifically, to compare the massage effect driving pressure caused by the lower-order frequency modulation produced by each technique, and c) to assess how these changes affect vocal fold vibration. As we do not use a WRT protocol in this study, we prefer the term "silicone tube phonation" (STP) as it better describes the methodology employed in our analysis. Therefore, this study assesses vocal changes during exercise, rather than the therapeutic effects of WRT. We hypothesize that changes from the baseline in glottal behavior will be more pronounced during phonation into the Acapella device due to the previously observed larger pressure modulation.[13]

METHODS

Participants

Three cisgender normophonic subjects, aged between 40 and 50, participated in the study, including two males (M1 and M2) and one female (F1). All participants confirmed having no previous history of vocal pathologies and expressed their ability to produce a normal voice. Throughout the data collection process, the researchers, who were a speech pathologist and a vocologist, did not observe any auditory-perceptual deviations in the participants' voices. These individuals were recruited using a convenient sampling method and had prior experience with data collection at the Academy of Performing Arts in Prague. Initially, two additional volunteers were considered for inclusion in the study; however, they were subsequently excluded due to challenges in visualizing the larynx during videoendoscopy caused by the positioning of their epiglottis, which obstructed the view path. All participants were familiar with SOVTEs, however, only M1 (the first author of this study) had previously used the Acapella device. All participants provided consent for the data collection and were instructed by the first author on how to perform the required tasks appropriately. All participants provided informed consent before taking part in this study.

Device

The Acapella device (Acapella Choice, Smiths Medical ASD, Inc, Rockland, Massachusetts) (Figure 1) was chosen for this study due to its pressure-airflow values being less susceptible to changes caused by the device's position, including the angle between the device and the floor.[20] Additionally, it's noteworthy that the Shaker devices require an 8 cmH_2O threshold pressure before oscillation begins, limiting their comparability to STP only at deeper tube submersions. For STP a 30 cm silicone tube with 9 mm of inner diameter at water depths ranging between 2 and 12 cm was used.

Equipment

High-speed videoendoscopy (HSV)

The high-speed videoendoscopy (HSV) was recorded at 6000 fps using a VisionResearch Phantom V611 camera (VisionResearch Phantom, New Jersey, USA) with an Olympus CLV-S45 (300 W) light source (Olympus Corporation, Tokyo, Japan). The camera was mounted on a tripod and the rigid endoscope was set at a comfortable height and angle for the tested subjects. Half-second segments were recorded at different time intervals using a manual trigger, and the recorded moments were captured at stable parts of the phonation when unobstructed views of the glottis were possible. Glottal area waveform (GAW) signals were obtained using the Glottal Analysis Tools 2020 software (GAT) (University Hospital Erlangen, Erlangen, Germany). The GAW was resampled to 48 kHz using MATLAB. Digital videokymograms (VKG) were produced from the HSVs using the ImageJ software version 1.53c (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2018).

Pressure and airflow data

Pressure was measured using a digital manometer, Honeywell ASDXAVX001PD2A3 (Honeywell International Inc, North Carolina, USA). A 10 cm long measuring probe was placed at the T-shaped mouthpiece junction (see Figure 2). Airflow was measured using a Sensirion SFM3000 digital airflow meter (Sensirion AG, Staga, Switzerland), placed between the mouthpiece and the Acapella or silicone tube. Pressure and airflow measurements were recorded at 2 kHz and resampled to 48 kHz using MATLAB (The MathWorks, Inc. 2023,

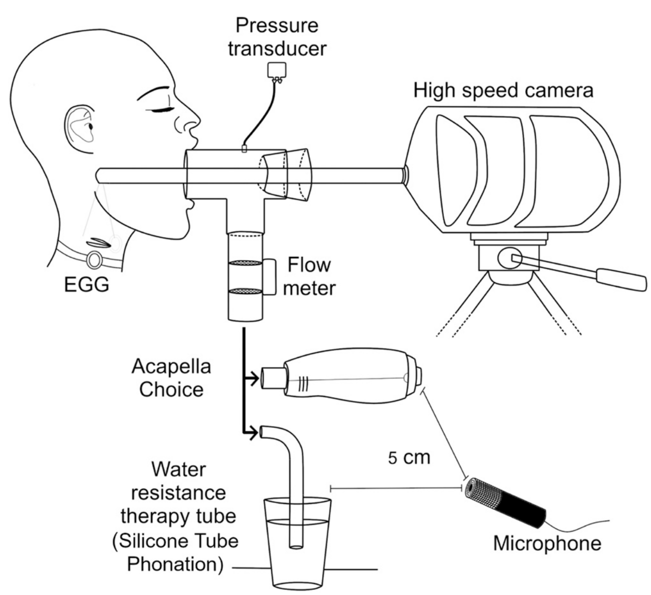

FIGURE 2. Data collection setup. For clarity, the T-shaped mouthpiece is shown vertically, however, during the experiment, the airflow meter and Acapella device were kept horizontally. Different depths of water were tested for STP.

Massachusetts, USA). The rigid endoscope was placed across the straight portion of the T-shaped mouthpiece and sealed with a rubber bung at the distal end to avoid air leakage (see Figure 2). The pressure probe was placed through a small hole in the outside part of the perpendicular joint in the T-shaped tube. Vaseline was applied to all joints to avoid air leakage.

Electroglottographic and acoustic data

Electroglottographic (EGG) signals were recorded using a Laryngograph D-200 device (Laryngograph, Wallington, UK), and the contact quotient (CQ) of the EGG signal was calculated using a hybrid algorithm as described by Howard et al (1995).[23] Acoustic data were obtained using a Sennheiser ME 62 microphone (Sennheiser, Wedemark, Germany), placed 5 cm from the distal end of the Acapella device or the upper edge of the container used for WRT (see Figure 2). EGG and audio signals were recorded synchronously (48 kHz, 24-bit). The audio recording was used to evidence the behavior of the flapping mechanism of the Acapella device as it was noted that airflow can sometimes be produced in its absence. An attempt was made to quantify the sound pressure level (SPL) differences among utterances; however, the clicking sound of the rocker arm interferes with the audio signal, making SPL data unreliable. The audio recording was also used for annotation purposes during the experiment. Data were collected in a sound-treated room.

Procedures

Videoendoscopic procedure

To obtain clear videos of the larynx, the tested subjects were standing directly in front of a reference monitor to visually manage the laryngeal viewing area. The participants were instructed to align the center of the endoscopic view with the larynx to obtain a full view of the glottis while phonating into the mouthpiece. They were advised to position their tongue inside the T-shaped mouthpiece to enhance visualization of the glottis. Although the /i/ vowel was the target vowel because it promotes a high position of the tongue with an improved laryngeal view, distortions in sound quality were allowed due to the presence of the endoscope. Topical anesthesia was not administered, and despite the complexity of the procedure, the subjects managed to tolerate it with minimal discomfort.

Data collection protocol

Data for the Acapella device was collected first to minimize the overlapping effects of repeated STP recordings at the various water depths used in this study. As mean intraoral pressure during STP is mainly determined by the depth of water submersion of the distal end of the silicone tube,[21,22,24] water depths ranging from 2 to 12 cm were investigated. This range would enable the selection of the mean intraoral pressure during STP that best corresponds to mean intraoral pressures generated during phonation into the Acapella device. The use of mean intraoral pressure as an independent variable in this analysis is because it can be easily manipulated by changes in water depth during STP, but more importantly, it has been shown to correlate with oscillatory pressure and airflow rate during STP and PEP phonation.[14,20,25,26] To account for possible effects of fundamental frequency on the data, male subjects were asked to sustain phonation at an E3 while the female subject was asked to use an A3. These pitches were reported to be comfortable for the participants. Data were extracted from the central 4 seconds of sustained vowels.

Data processing and analysis

To examine the isolated effects of the devices on the voice, the raw pressure and airflow data were filtered to obtain values related to the mean, device, and glottal components. The mean values, used to pressurize the PEP device and silicone tube prior to oscillation, were derived from the central 90% of each token's signal data. The low-frequency modulation by the PEP device or STP's water bubbling was obtained using a 0.002-second moving average filter. The higher frequency component related to the glottal cycle (note: although the term glottal is used, pressure and airflow data are obtained outside the mouth and not at the level of the vocal folds) was obtained using a polynomial filter to remove the drifting effects caused by phonation into the devices. The data were visually inspected in all analyses, and adjustments to the filtering settings were made as necessary to ensure that the correct filtering was achieved without introducing changes in the phase relations among variables. To quantify regularity in the modulation of pressure by the PEP device and STP, the peak of the power spectral density ($Pressure_{PSD\_Peak}$) was calculated using a periodogram.[27] Additionally, for the same purpose, the regularity in the envelope signals of the EGG ($EGGEnvelope_{Sample\_Entropy}$) and GAW ($GAWEnvelope_{Sample\_Entropy}$) were measured using a sample entropy method based on Richman and Moorman (2000)[28] and implemented as a MATLAB function by Víctor Martínez-Cagigal.[29] Furthermore, the phase delay between the pressure and airflow signals was analyzed.

The data analysis was conducted in two stages. First, we examined a subset of the data consisting of a single STP water depth that matched the mean pressure produced by the Acapella device. This analysis focused on observing changes over time and provided insights into the behavior of pressure and airflow profiles in the vocal tract and their influence on the glottal cycle. Second, a more extensive analysis was performed to assess the differences between STP and Acapella, considering multiple water depths for STP. This comprehensive analysis provides a broader understanding of how STP compares to Acapella in terms of its effects on the vocal tract and glottal cycle.

RESULTS AND DISCUSSION

Consideration of the acoustic characteristics of the data collection setup

Before interpreting the relationship between the obtained signals, it is crucial to consider the acoustic properties of the vocal tract when elongated by either the Acapella device or the 35 cm silicone tube. The calculation for determining the first resonance frequency (f_{R1}) of a uniformly shaped tube involves dividing the speed of sound (346 m/s) by four times the length of the tube (in meters). Assuming a reference length of the human vocal tract as 0.17 m, the f_{R1} of the vocal tract is approximately 500 Hz. By adding a 35 cm length tube to the vocal tract (0.17 m + 0.35 m), the resonance of the vocal tract plus the tube is estimated to be around 166 Hz. Likewise, considering the added length by the Acapella device (18 cm), the f_{R1} is calculated to be 247 Hz. However, it is important to note that these calculations do not account for the additional length of the T-joint and pressure flow meter (10 cm) used in this study, which lowers these values to 54 Hz and 192 Hz, respectively. Furthermore, although less quantifiable for STP, the Acapella device exhibits a noticeable variation in the shape of its distal end, alternating between open and closed positions. Consequently, it can be inferred that the overall configuration of the combined tube (comprising the vocal tract, Acapella device, and T-joint) transitions between closed-open and closed-closed ends conditions. This variation has implications for f_{R1}, which is expected to span a range of approximately 140 Hz (closed-open) to 280 Hz (closed-closed), with an average value of around 210 Hz. Consequently, it is likely that phonation for the female subject occurred above f_{R1}, while the male subjects may have been below this value (more probably for the Acapella). This disparity in phonation frequencies between males and females has significant implications for the interactions between the source and filter. Specifically, for the female, the vocal tract can be considered compliant, whereas, for males, it is more likely to be inertive at their respective fundamental frequencies (for more details on vocal tract resonance, refer to Wolfe 2009[30]). These distinctions in vocal tract behavior can affect the non-linear interactions between the vocal fold vibration and the vocal tract, ultimately influencing the vibratory characteristics of the vocal folds.

Presentation of time domain analysis

Firstly, the time domain illustrations of the effects of the oscillatory pressure and airflow profiles on the glottis are shown. Figures 3–8 show subsets of the data for the PEP phonation and STP for the three subjects (Figures 3 and 4 show the data for the female subject producing an A3 (approx. 216 Hz) during Acapella and STP respectively. Figures 5–8 show the males producing the same exercises at a E3 (162 Hz). Synchronous EGG, VKG, glottal area, pressure, and airflow data are displayed. A longer time interval window showing the normalized low-pass filtered data for pressure and airflow (this refers to the low-frequency modulation produced by the Acapella flapping mechanism and the water bubbling for STP) synchronous with the electroglottography (EGG) envelope ($EGG_{envelope}$), glottal area waveform envelope ($GAW_{envelope}$), contact quotient (CQ), and the fundamental frequency (F_0) are also shown. Finally, an extract of low-pass filtered data for pressure and airflow with the superimposed EGG tracing showing three glottal cycles is displayed at instances when the intraoral pressure modulation produced by the

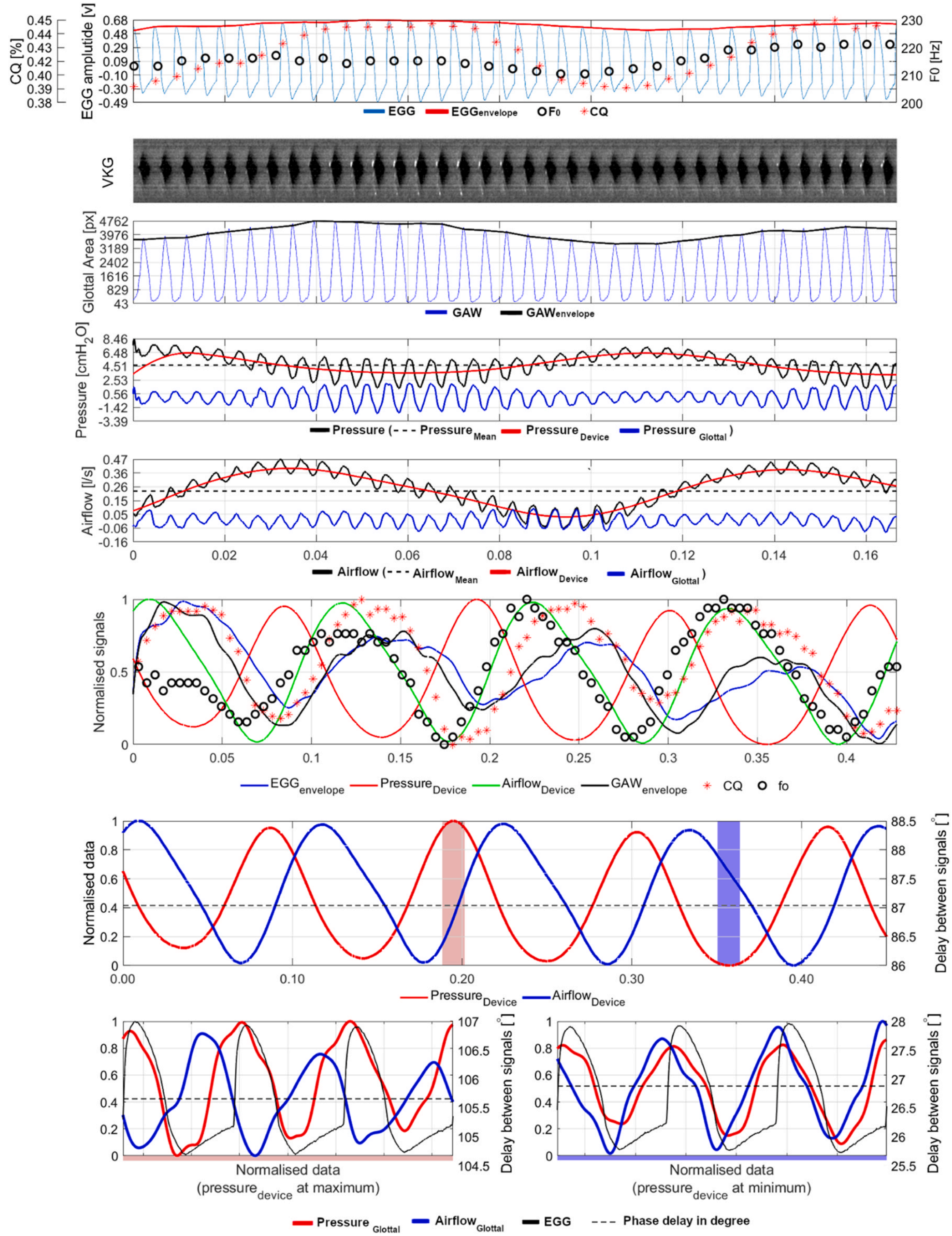

FIGURE 3. Data for a female participant (F1) producing an A3 at a habitual level during phonation into the Acapella device. From top to bottom, the figure displays EGG and CQ, VKG, glottal area, pressure, airflow, normalized signals (This visualization allows for the inspection of the phase relationship between pressure and airflow) and subsets of the data during maximum and minimum pressure device values. All presented data are shown for a duration of 0.15 seconds, except for the normalized signals, which are displayed for approximately 0.4 seconds and data at maximum and minimum pressure device values.

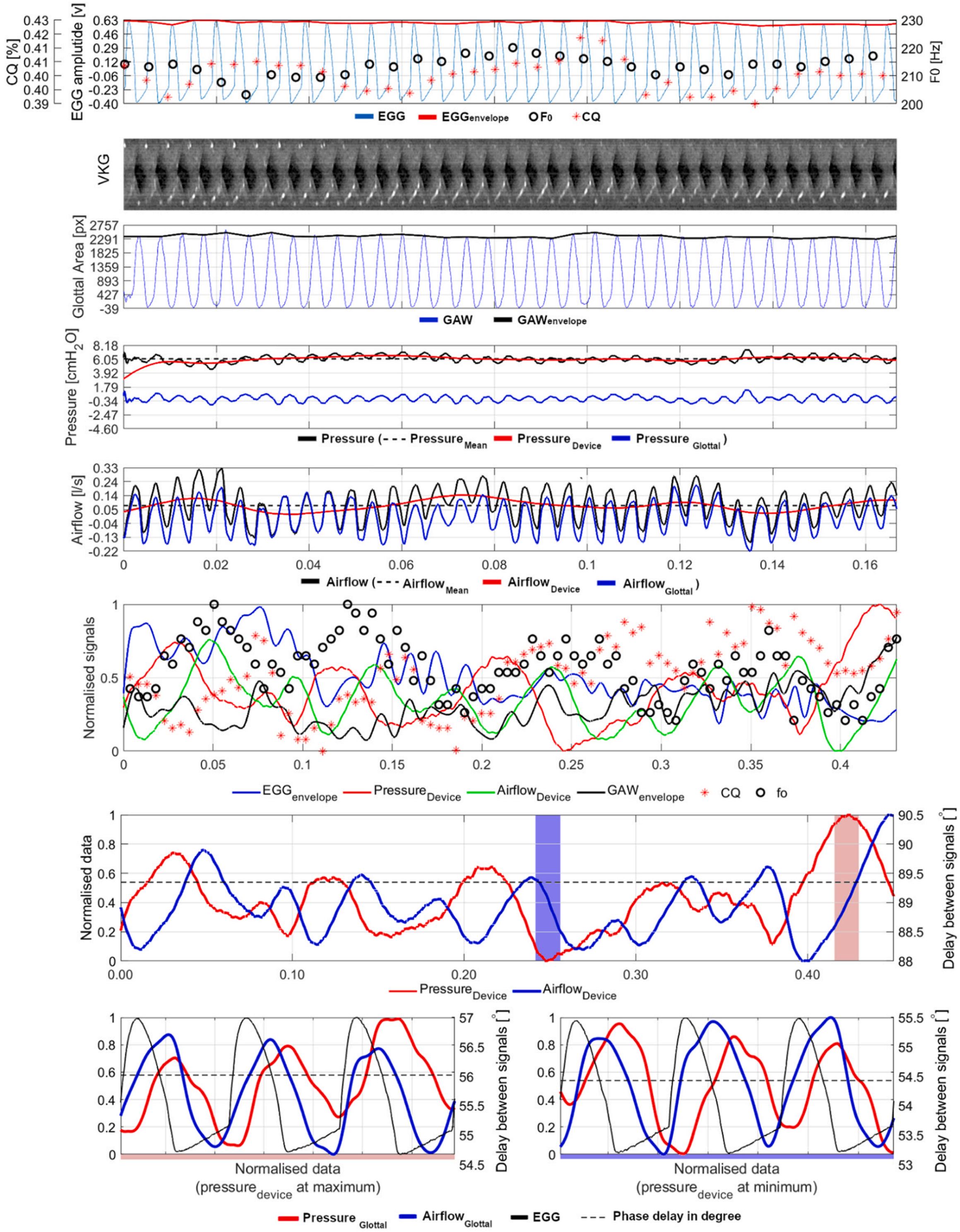

FIGURE 4. Data for a female participant (F1) producing an A3 at a habitual level during silicone tube phonation. From top to bottom, the figure displays EGG and CQ, VKG, glottal area, pressure, airflow, normalized signals (This visualization allows for the inspection of the phase relationship between pressure and airflow), and subsets of the data during maximum and minimum pressure device values. All presented data is shown for a duration of 0.15 seconds, except for the normalized signals, which are displayed for approximately 0.4 seconds, and data at maximum and minimum pressure device values.

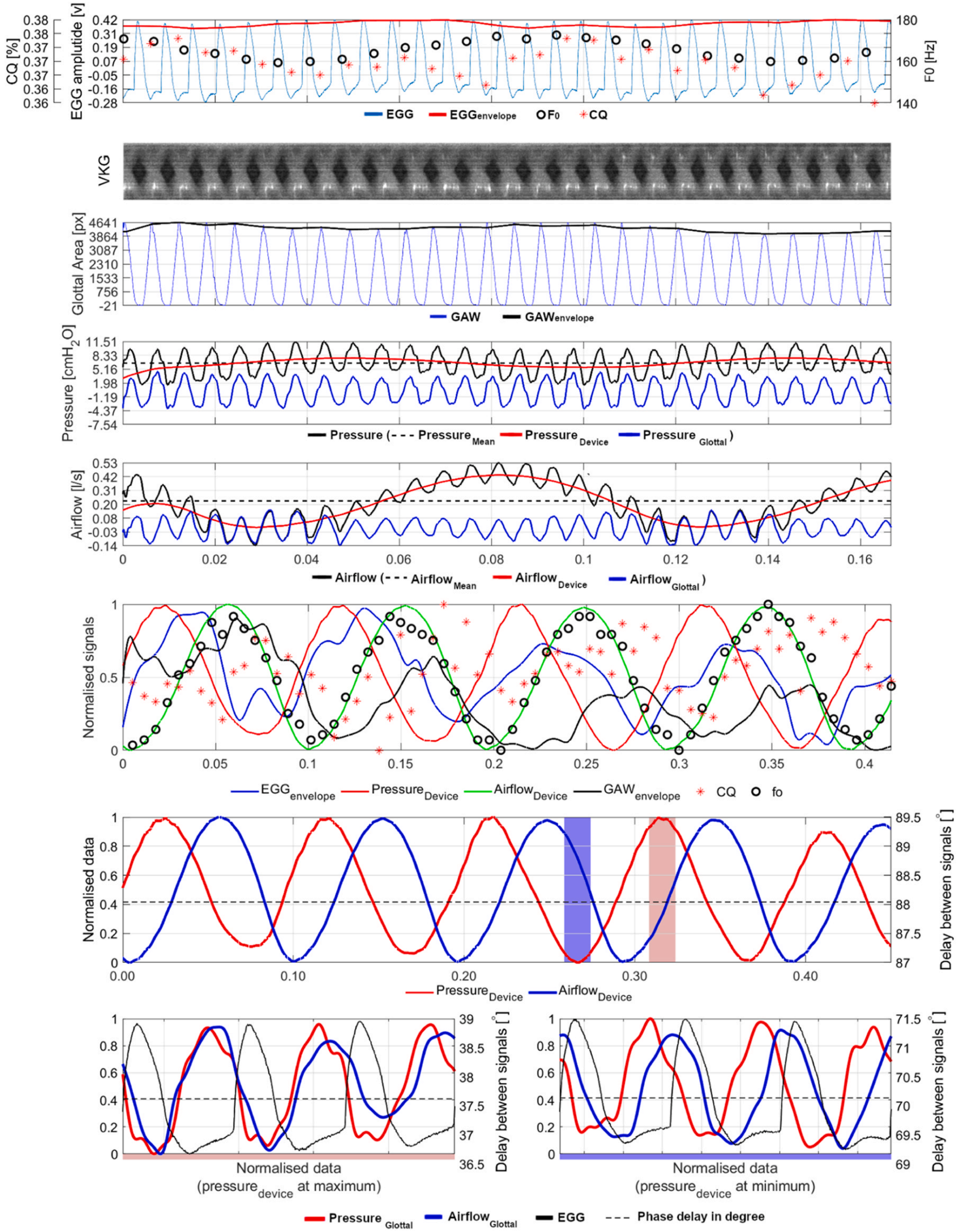

FIGURE 5. Data for a male participant (M1) producing an E3 at a habitual level during phonation into the Acapella device. From top to bottom, the figure displays EGG and CQ, VKG, glottal area, pressure, airflow, normalized signals (This visualization allows for the inspection of the phase relationship between pressure and airflow), and subsets of the data during maximum and minimum pressure device values. All presented data are shown for a duration of 0.15 seconds, except for the normalized signals, which are displayed for approximately 0.4 seconds, and data at maximum and minimum pressure device values.

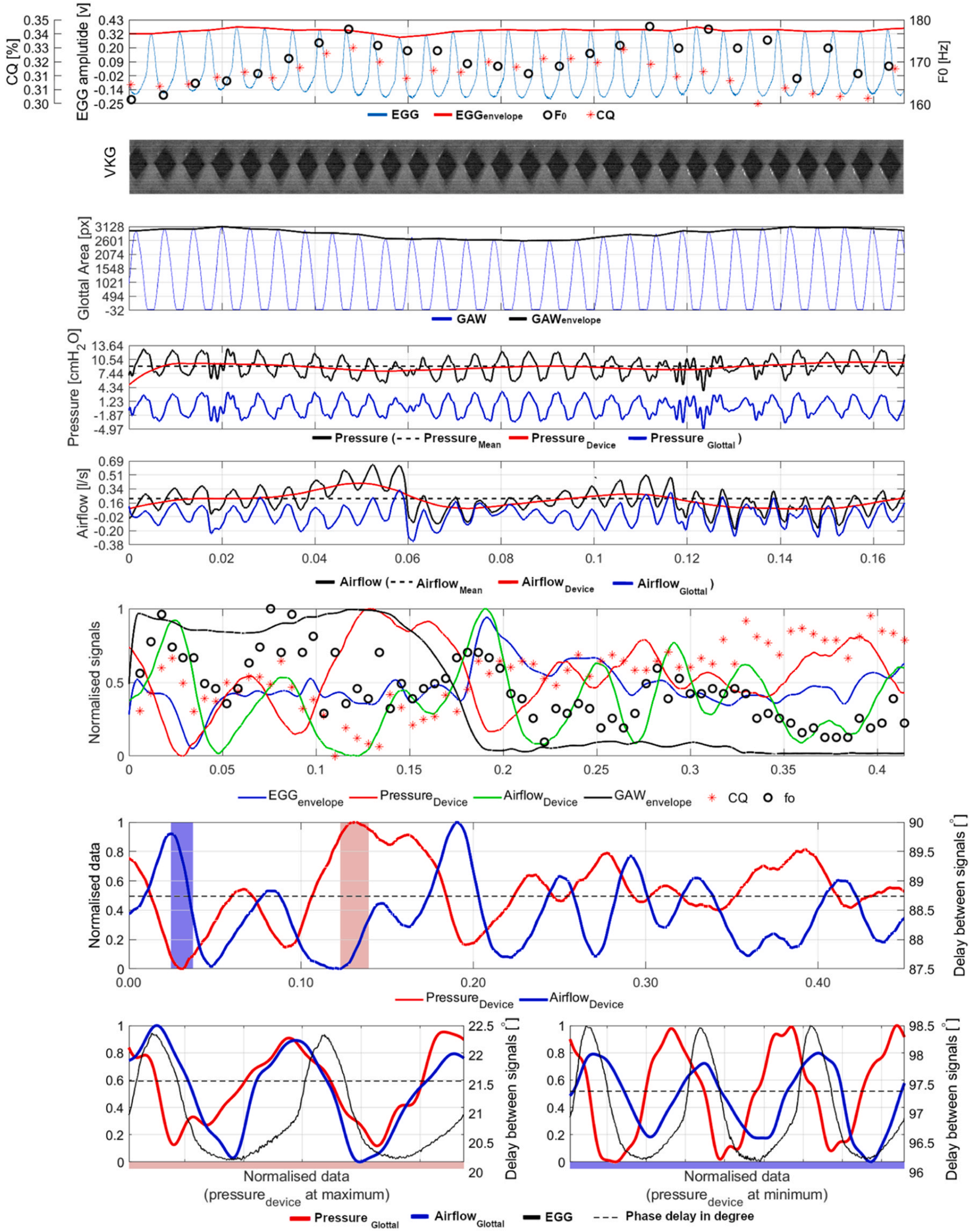

FIGURE 6. Data for a male participant (M1) producing an E3 at a habitual level during silicone tube phonation. From top to bottom, the figure displays EGG and CQ, VKG, glottal area, pressure, airflow, normalized signals (This visualization allows for the inspection of the phase relationship between pressure and airflow), and subsets of the data during maximum and minimum pressure device values. All presented data are shown for a duration of 0.15 seconds, except for the normalized signals, which are displayed for approximately 0.4 seconds, and data at maximum and minimum pressure device values.

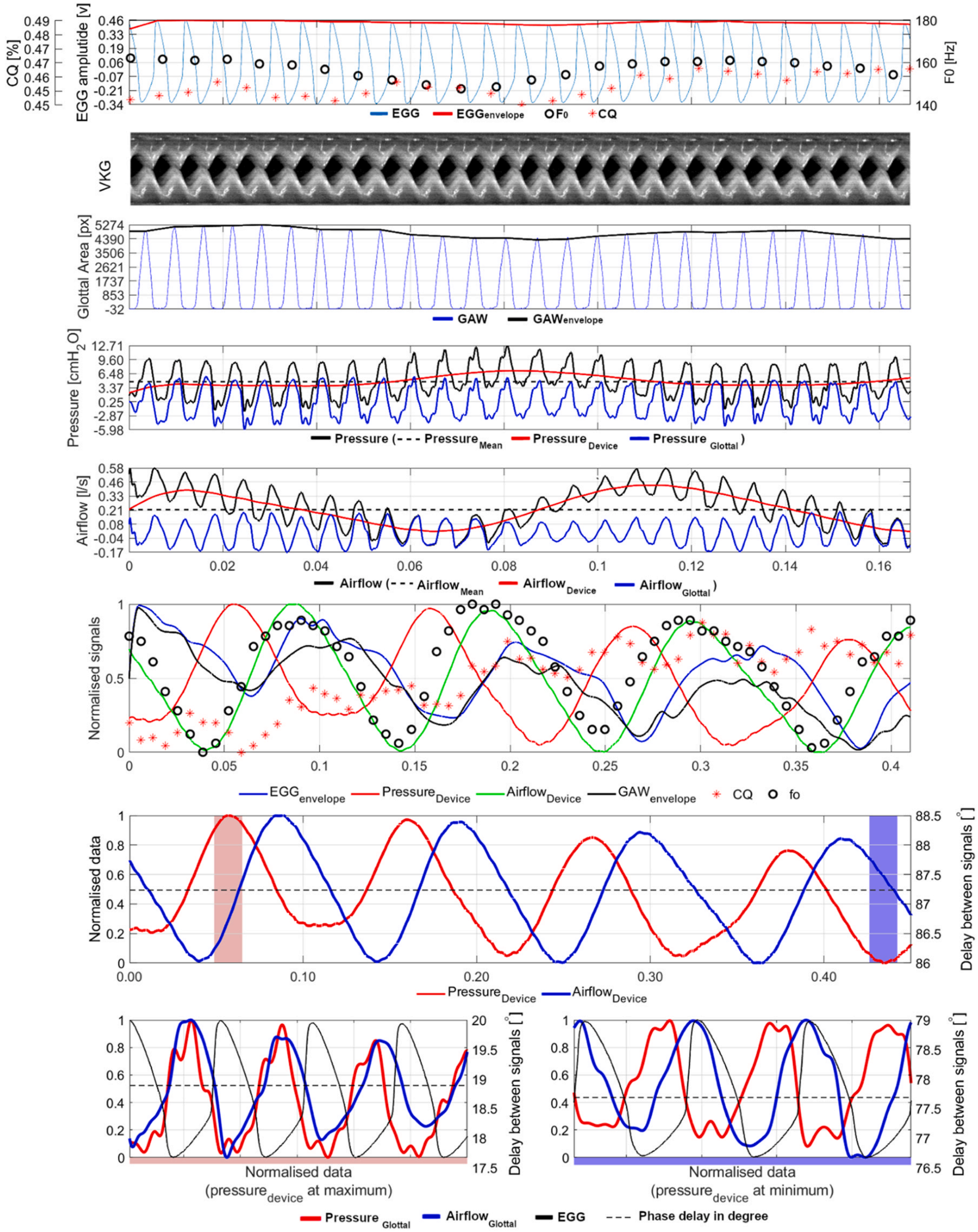

FIGURE 7. Data for a male participant (M2) producing an E3 at a habitual level during phonation into the Acapella device. From top to bottom, the figure displays EGG and CQ, VKG, glottal area, pressure, airflow, normalized signals (This visualization allows for the inspection of the phase relationship between pressure and airflow), and subsets of the data during maximum and minimum pressure device values. All presented data are shown for a duration of 0.15 seconds, except for the normalized signals, which are displayed for approximately 0.4 seconds, and data at maximum and minimum pressure device values.

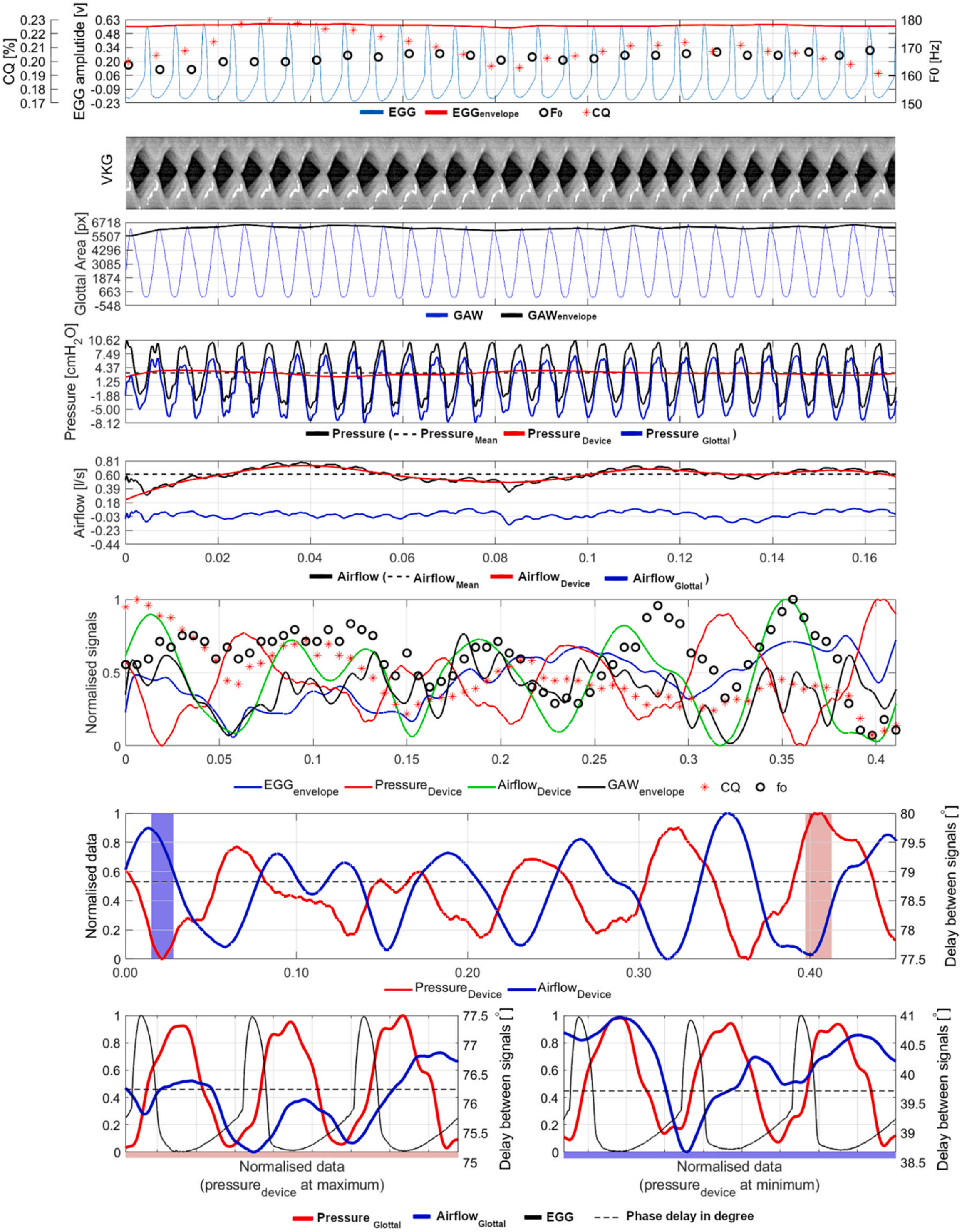

FIGURE 8. Data for a male participant (M2) producing an E3 at a habitual level during silicone tube phonation. From top to bottom, the figure displays EGG and CQ, VKG, glottal area, pressure, airflow, normalized signals (This visualization allows for the inspection of the phase relationship between pressure and airflow) and subsets of the data during maximum and minimum pressure device values. All presented data are shown for a duration of 0.15 seconds, except for the normalized signals, which are displayed for approximately 0.4 seconds and data at maximum and minimum pressure device values.

devices reaches maximum and minimum values (lower panel, left, and right, respectively). A summary of the data obtained from Figures 3–8 is presented in Table 1.

Time domain analysis: comparison of low-frequency pressure modulation effects on the voice by both devices

Observation of the data displayed in the time domain showed important patterns. It can be seen that changes in the $pressure_{Device}$ by both exercises caused changes in all glottal parameters. However, these changes are more pronounced during the regular and larger modulation of pressure caused by the flapping mechanism of the Acapella device compared to STP. This result demonstrates the mechanistic effect of the Acapella device, which influences the aerodynamic properties of the system and consequently affects the glottal cycle, corroborating previous findings.[13,14] In the case of the Acapella device, maximum pressure occurs when the rocker arm is lowered, obstructing the airflow outlet. This is primarily caused by the downward force exerted on the rocker arm by the magnetic field generated by the two magnets at its distal end. As pressure builds up, the airflow pushes the rocker arm upward, opening the airflow outlet resulting in an increase in airflow. After the airflow reaches its peak, the rocker arm reverses its motion, reducing the airflow outlet and subsequently increasing the pressure for the next pressure/airflow cycle modulated by the Acapella device. This distinct pattern can be observed in the normalized signals (sixth and seventh graphs from top to bottom in Figures 3, 5, and 7), where pressure leads flow by approximately 90 degrees.

Similarly, changes in pressure and airflow caused by the flapping mechanism of the Acapella device also influence the modulation of the glottal cycle, as evidenced by the glottal and EGG envelopes, as well as the CQ and F_0 data. Although a slight delay is introduced in the signals due to the distance between the pressure and airflow meters from the glottis, it is evident that CQ generally tracks the modulation of airflow during Acapella usage. However, for STP, the trends in the data, including the modulation of F_0, EGG and glottal area waveform envelopes, are not clearly discernible, as illustrated in Figures 4, 6, and 8. Nonetheless, it is worth noting that the $pressure_{Device}$ consistently leads the $airflow_{Device}$ by approximately 90 degrees for both devices, as indicated in Table 1.

When comparing the data from STP to the Acapella device, it is evident that changes in $pressure_{Device}$ are negligible and primarily associated with the mean pressure ($Pressure_{Mean}$).This is due to the nature of the exercise, where oral pressure must overcome the resistance exerted by the water column above the distal end of the tube before bubbling begins, resulting in a prescribed static pressure in the vocal tract (referred to as $pressure_{Device}$ in this study). Consequently, lower, and more irregular changes are observed in the EGG, VKG, glottal area waveform, and airflow data for STP. Table 1 indicates that STP yields lower values for CQ, $pressure_{Device}$, $pressure_{PSD\_Peak}$, and

TABLE 1.
Summary Data for Devices Matched by Mean Static Pressure

Subject	F1		M1		M2	
device	PEP	$STP_{5\,cm}$	PEP	$STP_{4\,cm}$	PEP	$STP_{4\,cm}$
CQ [%]	0.42 (0.02)	0.41 (0.01)	0.37 (0.00)	0.34 (0.02)	0.47 (0.01)	0.2 (0.01)
$EGGEnvelope_{Sample\_Entropy}$	0.0037	0.0119	0.0051	0.0062	0.004	0.0043
f_o [Hz]	216.52 (4.68)	213.11 (3.63)	166.82 (4.62)	163.87 (5.97)	156.91 (4.38)	165.19 (3.22)
$GAWEnvelope_{Sample\_Entropy}$	0.0039	0.0119	0.0050	0.0061	0.0040	0.0043
$Pressure_{Mean}$ [cmH_2O]	4.67 (1.51)	6.1 (0.61)	6.53 (2.64)	5.79 (2.54)	4.7 (3.31)	3.18 (4.79)
$Pressure_{Device}$ [cmH_2O]	3.28 (0.12)	0.55 (0.28)	2.28 (0.13)	2.26 (0.75)	3.56 (0.38)	0.92 (0.52)
$Pressure_{Glottal}$ [cmH_2O]	2.61 (0.86)	1.2 (0.42)	7.07 (1.04)	6.85 (1.32)	8.18 (2.61)	13.57 (0.86)
$Pressure_{PSD\_Peak}$ [dB/Hz]	-6.57	-18.41	-8.14	-18.19	-6.91	-14.13
$airflow_{Mean}$ [L/s]	0.22 (0.14)	0.08 (0.1)	0.22 (0.18)	0.19 (0.16)	0.21 (0.17)	0.61 (0.1)
$airflow_{Device}$ [L/s]	0.36 (0.01)	0.08 (0.02)	0.42 (0.00)	0.33 (0.03)	0.38 (0.02)	0.21 (0.08)
$airflow_{Glottal}$ [L/s]	0.11 (0.03)	0.24 (0.05)	0.2 (0.04)	0.26 (0.08)	0.26 (0.05	0.07 (0.04)
Pressure/airflow Phase $Delay_{Device}$ [degrees]	87.04 (1.87)	89.34 (0.35)	88.04 (1.44)	88.74 (0.57)	87.23 (1.86)	78.82 (2.55)
Pressure/airflow Phase $Delay_{Device\_Pmax}$ [degrees]	-105.56 (23.49)	-55.95 (19.93)	37.52 (12.38)	21.48 (4.78)	18.22 (12.58)	76.15 (13.34)
Pressure/airflow Phase $Delay_{Device\_Pmin}$ [degrees]	-26.79 (21.82)	-54.35 (23.18)	70.04 (29.81)	97.30 (42.93)	77.59 (22.17)	39.62 (40.03)

The subscripts after STP indicate the water depth submersion used for comparison. The subscripts: Mean (static pressure), Device (Oscillatory pressure due to the second source of vibration in the vocal tract) and Glottal (Oscillatory pressure related to the glottal cycle), refer to the specific elements of the pressure and airflow signals analyzed. Phase delay values are displayed as positive numbers when pressure is ahead of flow and negative values when airflow leads pressure.

airflow compared to the Acapella device across all subjects. The differences in the regularity of pressure modulation by the two devices can be quantified by the peak value in the power spectral density of their respective periodograms ($Pressure_{PSD\_Peak}$). In this regard, the STP generates values as low as half of those obtained with the Acapella, indicating the chaotic nature of the water bubbling and its irregular impact on the glottal cycle. This is evident when visually examining the data for STP. For example, minimal to no observable variation in the VKG patterns is seen throughout the duration of the STP exercise. This finding highlight that clinically, the Acapella device has the capacity to produce a stronger and more consistently regular massage effect in the vocal tract compared to STP.

Time domain analysis: Low-frequency pressure modulation effects on glottal behavior (differences between male and female subjects)

When examining the modulation of $pressure_{Glottal}$ and $airflow_{Glottal}$ by the $pressure_{Device}$, as well as their phase relationships (depicted in the left and right panels at the bottom of Figures 3, 5, and 7), the impact of the Acapella device on vocalization becomes more evident. In the case of the female subject (Figure 3), at maximum Acapella pressure, $airflow_{Glottal}$ leads $pressure_{Glottal}$ by approximately 105 degrees (as shown in Table 1). This leads to a shorter glottal contact duration, resulting in slightly lower values of CQ compared to the average (refer to CQ values in the top graph of Figure 3). Furthermore, during maximum pressure, the glottal area waveform is reduced, indicating a narrower lateral opening of the glottis (also visible in the VKG data). The reduced amplitude of glottal area waveform and lower CQ values indicate hindered vocal fold vibration. This is further confirmed by the lower F_0, reduced peak-to-peak values of $pressure_{Glottal}$, and lower overall airflow values. Still for the female subject, when the Acapella pressure drops to its minimum (note that the pressure is still positive in this case, as indicated by the $pressure_{Device}$ data in Figure 3), CQ, F_0, EGG, and GAW envelopes increase. At this point, the glottal cycle becomes more efficient, resulting in larger peak-to-peak pressure values ($pressure_{Glottal}$) within the vocal tract. The increased amplitude of vibration during minimum $pressure_{Device}$ is also observable in the VKG image. Hence, vocal fold vibration increases when $pressure_{Device}$ is minimum.

Overall, the same pattern is seen in the data for the male subjects with $pressure_{Device}$ leading $airflow_{Device}$ by approximately 90 degrees. This causes similar modulations of glottal cycle for the male subjects. However, the phase relationship between $pressure_{Glottal}$ and $airflow_{Glottal}$ at maximum and minimum $pressure_{Device}$ does not follow the same pattern as for the female subject. For males, when $pressure_{Device}$ is maximum, the phase delay between pressure and airflow is minimum, resembling a resistive system in which energy is dissipated. When $pressure_{Device}$ is minimum, $pressure_{Glottal}$ leads $airflow_{Glottal}$ by approximately 75 degrees changing the impedance of the vocal tract into inertive. Here, the glottal cycle is aided as it can be seen by the increased CQ, F_0, and the GAW and EGG envelopes. However, the peak-to-peak $pressure_{Glottal}$ does not display the same gain as for the female subject.

For both male and female subjects, changes in glottal cycle are primarily influenced by the $pressure_{Device}$ when maximum pressure makes it harder for the vocal folds to vibrate while minimum $pressure_{Device}$ values, in comparison, facilitate the glottal cycle. However, it is interesting to note the differences between female and male subjects regarding the likely changes in the acoustic impedance of the vocal tract during Acapella phonation. When considering the impact of the maximum and minimum $pressure_{Device}$ instances on the glottal cycle, the female subject exhibits a transition from a compliant vocal tract (where $airflow_{Glottal}$ leads $pressure_{Glottal}$), with hindered vocal fold vibration, to a resistive condition (where $airflow_{Glottal}$ and $pressure_{Glottal}$ are in phase) when larger modulations of $pressure_{Glottal}$ and $airflow_{Glottal}$ by the glottal cycle occur. This transition can be clearly observed by examining the contextualized $pressure_{Glottal}$ signal in the fourth graph from top to bottom in Figure 3. On the other hand, for male subjects, the vocal tract impedance appears to transition from resistive (when $pressure_{Device}$ is at its maximum and $pressure_{Glottal}$ and $airflow_{Glottal}$ are in phase) to inertive (when $pressure_{Device}$ is at its minimum and $pressure_{Glottal}$ leads $airflow_{Glottal}$). This indicates that the compliant condition found only in the female subject led to a greater hindrance of vocal fold vibration. This is evident when looking at changes in $pressure_{Glottal}$ by the $pressure_{Device}$ shown in the pressure graph (fourth from top to bottom) in Figure 3. The variations in $pressure_{Glottal}$ peak-to-peak values are more significant for females, while the changes are not as extreme for males. As mentioned before, this difference between males and females is likely due to the F_0 being above the first formant for female subjects, whereas for male subjects, it is likely below the first formant. This observation is consistent with the findings in the STP as well, although the values obtained are less extreme. For a detailed explanation acoustic impedance refer to Titze, 2001[31] or Wolfe 2009.[30] When visually inspecting the data, please be aware that the $airflow_{Glottal}$ values obtained for M2 during STP may be unreliable due to the buildup of humidity in the airflow meter device, which was noticed only after data collection. For completeness, it should be noted that our assessments of phase differences are affected by uncertainty stemming from the fact that pressure and airflow measurements are not taken at precisely the same place. The average estimation of this uncertainty is around 10 degrees, confirming that conclusions about general trends and robust phenomena are well supported.

Comparison among water depths for STP and Acapella

To obtain a more comprehensive understanding of the differences between Acapella and STP, the entire dataset was analyzed to identify overarching patterns across the exercises, encompassing both the Acapella device and STP

TABLE 2.
Descriptive Data for CQ, Pressure and Airflow Data

Device	$Pressure_{Mean}$ Mean (SD) [cmH_2O]	$Pressure_{Device}$ Mean (SD) [cmH_2O]	$Airflow_{Mean}$ Mean (SD) [L/s]	$Airflow_{Device}$ Mean (SD) [L/s]	CQ Mean (SD) [%]
Acapella	6.82 (0.98)	3.85 (1.03)	0.26 (0.06)	0.43 (0.007)	0.40 (0.01)
$STP_{2\,cm}$	3.37 (1.13)	2.49 (0.46)	0.2 (0.04)	0.35 (0.05)	0.34 (0.05)
$STP_{3\,cm}$	3.44 (0.85)	1.58 (0.86)	0.47 (0.03)	0.2 (0.03)	0.24 (0.01)
$STP_{4\,cm}$	5.19 (1.48)	2.68 (1.66)	0.36 (0.05)	0.36 (0.06)	0.3 (0.03)
$STP_{5\,cm}$	6.30 (1.63)	1.74 (0.68)	0.22 (0.05)	0.23 (0.06)	0.39 (0.01)
$STP_{6\,cm}$	7.19 (0.23)	1.7 (0.73)	0.15 (0.024)	0.14 (0.02)	0.47 (0.02)
$STP_{7\,cm}$	8.18 (0.49)	1.93 (1.57)	0.13 (0.03)	0.16 (0.03)	0.42 (0.03)
$STP_{8\,cm}$	9.1 (1.19)	1.73 (0.85)	0.18 (0.06)	0.18 (0.08)	0.43 (0.001)
$STP_{9\,cm}$	10.3 (1.12)	1.72 (0.92)	0.16 (0.06)	0.02 (0.05)	0.37 (0.02)
$STP_{10\,cm}$	11.3 (1.09)	1.99 (0.71)	0.2 (0.06)	0.18 (0.07)	0.43 (0.01)
$STP_{11\,cm}$	12.6 (0.6)	1.76 (1.26)	0.14 (0.03)	0.15 (0.02)	0.43 (0.05)
$STP_{12\,cm}$	13.6 (0.12)	1.12 (0.34)	0.12 (0.12)	0.08 (0.01)	0.45 (0.01)

Notes: The subscripted values related to the depth of water submersion during STP.
Abbreviation: STP, silicone tube phonation.

across water depths ranging from 2 to 12 cm. However, due to time constraints, certain water depths were not recorded for M2, with only odd depths (plus 5 cm) being captured. The analysis presented below specifically focuses on the relationships between pressure and airflow and the valving mechanism of the vocal folds, as exemplified by measures of CQ. A summary of the mean values for each variable is presented in Table 2.

Mean pressure and airflow relationships among exercises

Figure 9 illustrates the correlations between the mean pressures and mean airflows for the Acapella device and STP at different water depths. A highly significant correlation ($r = 0.95$, $P < 0.00$) was observed between these variables for the Acapella device (see Table 3). Significant linear correlations were also found for STP at 2 cm, 5 cm

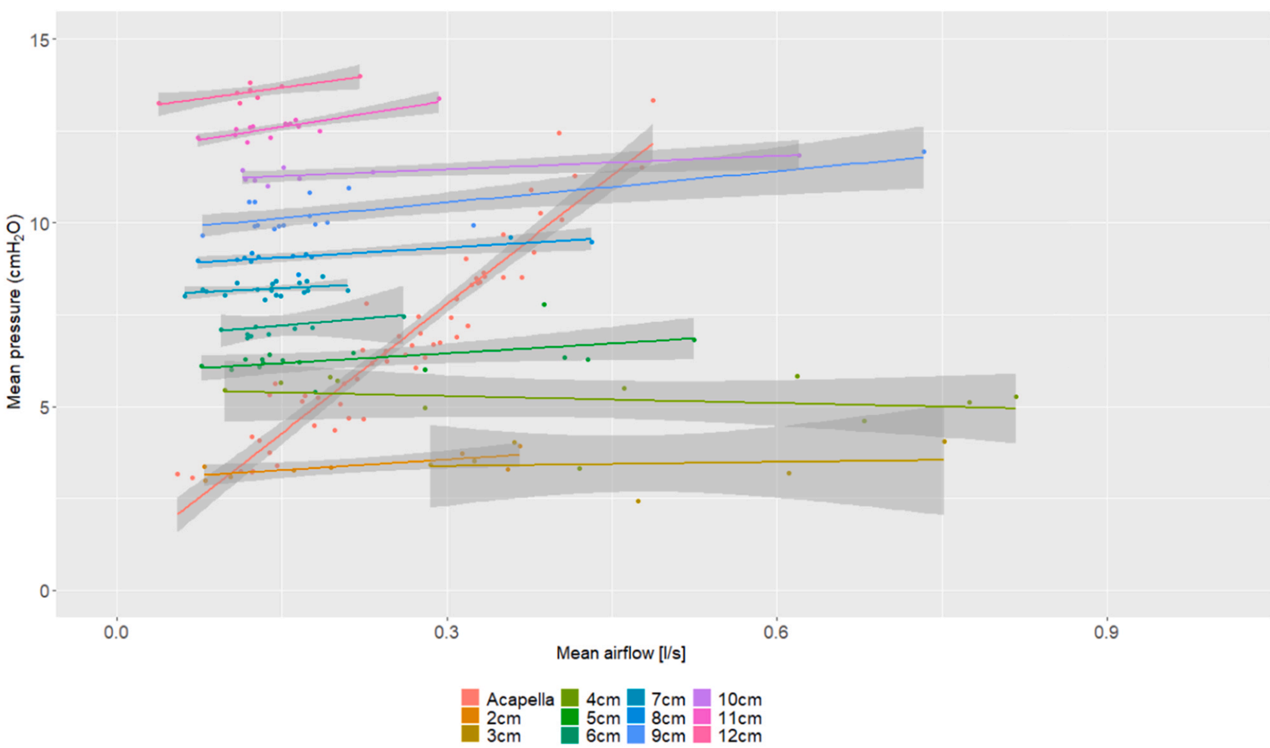

FIGURE 9. Correlation between mean airflow and mean pressure for Acapella and $STP_{2–12\,cm}$. Trend lines and 0.95 confidence intervals are also displayed for visual support.

TABLE 3.
Correlation Values Between Pressure, Airflow, and CQ Datasets

Device	Correlation between $airflow_{Mean}$ and $pressure_{Mean}$ R (*P*-value) [β]	Correlation between $pressure_{Mean}$ and $pressure_{Device}$ R (*P*-value) [β]	Correlation between $airflow_{Mean}$ and $pressure_{Device}$ R (*P*-value) [β]	Correlation between $airflow_{Mean}$ and $airflow_{Device}$ R (*P*-value) [β]	Correlation between $pressure_{Mean}$ and CQ values R (*P*-value)
Acapella	0.95 (0.000)*** [23.3]	0.54 (0.000)*** [1.23]	0.52 (0.000)*** [0.05]	0.86 (0.000)*** [0.63]	-0.35 (0.005)** [−5.6]
$STP_{2\,cm}$	0.75 (0.03)** [1.93]	0.13 (0.75) [0.08]	-0.37 (0.2) [−0.09]	0.23 (0.58) [0.28]	0.02 (0.695) [0.21]
$STP_{3\,cm}$	0.11 (0.83) [0.36]	0.24 (0.64) [0.17]	-0.75 (0.22) [−0.16]	-0.48 (0.33) [−1.31]	0.49 (0.32) [7.3]
$STP_{4\,cm}$	-023 (0.49) [−0.64]	0.21 (0.46) [0.08]	-0.62 (0.1) [−0.1]	-0.39 (0.16) [−0.96]	-0.04 (0.88) [−0.19]
$STP_{5\,cm}$	0.51 (0.04)** [1.8]	0.35 (0.17) [0.26]	0.7 (0.007)* [0.14]	0.88 (0.000)*** [1.05]	-0.17 (0.52) [−0.79]
$STP_{6\,cm}$	0.3 (0.39) [2.56]	0.3 (0.4) [0.16]	0.77 (0.014)* [0.05]	0.97 (0.000)*** [0.77]	-0.46 (−0.18) [−2.38]
$STP_{7\,cm}$	0.34 (0.17) [1.46]	0.15 (0.53) [0.02]	0.15 (0.34) [0.00]	0.4 (0.07) [0.29]	-0.1 (0.65) [−0.12]
$STP_{8\,cm}$	0.74 (0.006)** [1.78]	0.76 (0.003)** [0.23]	0.94 (0.000)*** [0.12]	0.97 (0.000)*** [0.73]	-0.56 (0.06) [−1.47]
$STP_{9\,cm}$	0.75 (0.001)** [2.83]	0.73 (0.000)*** [0.4]	0.66 (0.13) [0.1]	0.88 (0.000)*** [0.81]	-0.3 (0.18) [−0.85]
$STP_{10\,cm}$	0.8 (0.02)** [1.22]	0.77 (0.02)* [0.28]	0.72 (0.35) [0.17]	0.93 (0.000)*** [1.24]	-0.13 (0.77) [−0.31]
$STP_{11\,cm}$	0.85 (0.000)*** [4.72]	0.4 (0.13) [0.09]	0.25 (0.66) [0.01]	0.37 (0.18) [0.28]	0.06 (0.83) [0.09]
$STP_{12\,cm}$	0.79 (0.02)** [4.1]	0.53 (0.17) [0.41]	0.56 (0.19) [0.08]	0.79 (0.02)* [1.52]	-0.68 (0.06) [−2.04]

Notes: The subscripted values related to the depth of water submersion during STP. Significant levels: * < 0.05, ** < 0.01, and *** < 0.001.
Abbreviation: STP, silicone tube phonation.

and for depths beyond 8 cm (specific r, p, and β values are provided in Table 3). While both devices ($STP_{2,5,8–12\,cm}$ of water) demonstrated a significant correlation between mean pressure and mean airflow, it is noteworthy that the rate of change for the Acapella device (represented by the slope of the regression line and expressed as the beta coefficient (β) = 23.3) was approximately 10 times larger than that of STP ($\beta_{mean(2,5,8–12\,cm)}$ = 2.62 ± 1.32). This indicates that even small changes in mean airflow can lead to substantial changes in mean pressure for the Acapella device, whereas the same level of change in mean airflow has a lesser impact on mean pressure for STP. The clinical relevance of this finding is that it highlights the Acapella device's capacity to pressurize the vocal tract more effectively, benefiting from larger intraoral pressure, with a reduced requirement for increased airflow compared to STP. As previously reported,[22,24] the mean pressure for STP is primarily influenced by the water depth at which the distal end of the tube is submerged. This additional information aligns with our findings from the time domain analysis, where we observed minimal changes in airflow with variations in $pressure_{Device}$ during STP exercises.

Relationships between mean pressure and airflow values and device-specific pressure and airflow

Furthermore, $pressure_{Mean}$ in our data showed a correlation with changes in $pressure_{Device}$ for both Acapella and STP. However, significant correlations were observed for STP only at 8–10 cm of water depth (Table 3). Figure 10 illustrates the relationship between these variables, with marker size indicating normalized airflow values across the dataset. The results confirm that for the Acapella device, $airflow_{Mean}$ is correlated with $pressure_{Mean}$, which, in turn, is correlated with $pressure_{Device}$. This information holds clinical significance, as it suggests that the more a patient blows into the Acapella device, the greater the overall pressure produced, including static and peak-to-peak pressure, resulting in a more pronounced massage effect. Additionally, a stronger relationship between $airflow_{Mean}$ and $pressure_{Mean}$ can be observed for greater water submersion depths with STP. Similar to the relationship between mean pressure/airflow, the rate of change in $pressure_{Device}$ relative to $pressure_{Mean}$ for the Acapella device was approximately four times greater (β = 1.23) than the average change observed for STP ($\beta_{mean(8–10\,cm)}$ = 0.3 ± 0.08). Notably, this calculation only includes significant results for the relationships between mean and device pressures at water depths between 8 and 10 cm (see Table 3). Given the significant relationships observed among $airflow_{Mean}$, $pressure_{Mean}$, and $pressure_{Device}$ for both Acapella and $STP_{8–10\,cm}$, a direct comparison becomes necessary. As previously suggested, a direct causal link appears likely for the Acapella, where $pressure_{Mean}$ is determined by $airflow_{Mean}$, impacting the flapping mechanism of the Acapella and consequently, the $pressure_{Device}$. Conversely, in $STP_{8–10\,cm}$, as $airflow_{Mean}$ increases, $pressure_{Mean}$ tends to rise until a plateau determined by water submersion. $Airflow_{Mean}$ also affects $pressure_{Device}$ through larger bubbling frequencies and sizes,[22] exhibiting correlation with

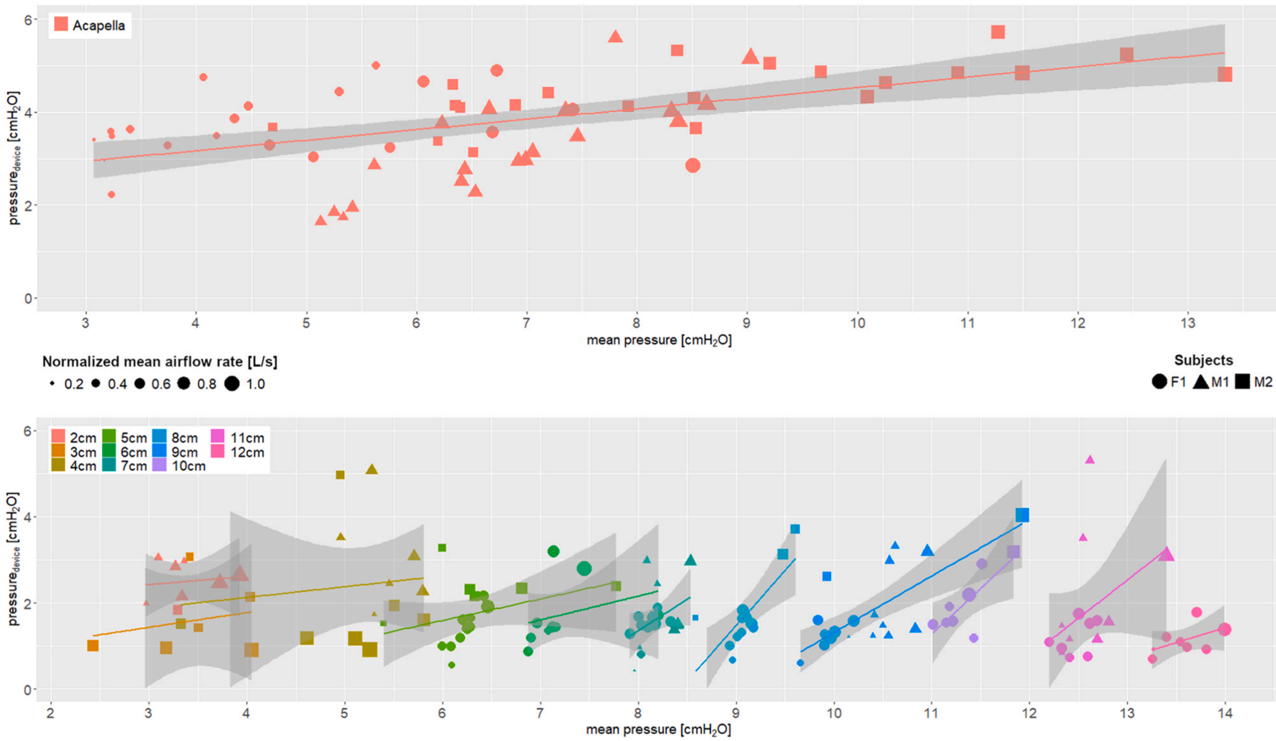

FIGURE 10. Scatter plot illustrating the relationship between $pressure_{Mean}$ and $pressure_{Device}$ values for the Acapella and STP exercises. Trend lines and 0.95 confidence intervals are included to provide visual support and aid in understanding the data.

this parameter as well. Significant strong correlations were also found between $airflow_{Mean}$ and $airflow_{Device}$ for both Acapella and STP at depths of 5, 6, 8–10, and 12 cm, confirming the relationship between input airflow and variations in bubbling frequency and bubble size, allowing for proportional air volume release. This mechanism may counterbalance unyielding $pressure_{Device}$ values determined by the depth of the silicone tube submersion. These findings align with Wistbacka,[22] who reported a similar pattern when studying bubbling formation during STP.

Relationships between mean pressure and airflow values and CQ values

With regards to laryngeal function inferred from the CQ data, a noticeable trend of CQ increasing with water depth is observed (Figure 11). CQ values exhibit an increase from 3 cm to 5 cm of water depth, reaching a plateau around 0.43% (Table 2). Comparatively higher CQ values are observed for deeper bubbling phonation (10 cm and deeper) when compared to lower depths and the Acapella device. This can be attributed to the increased loading above the

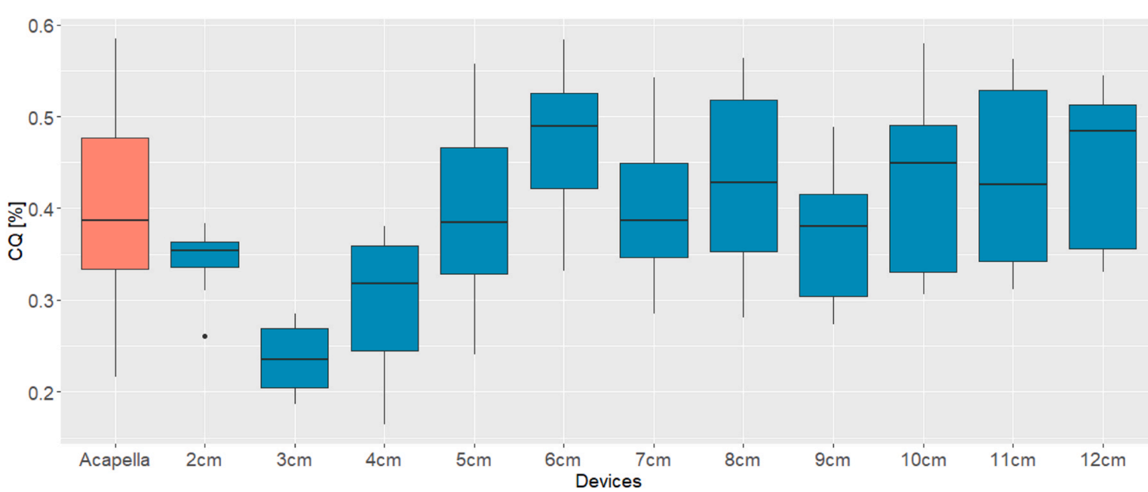

FIGURE 11. Boxplot of CQ data for Acapella and STP at different water depths.

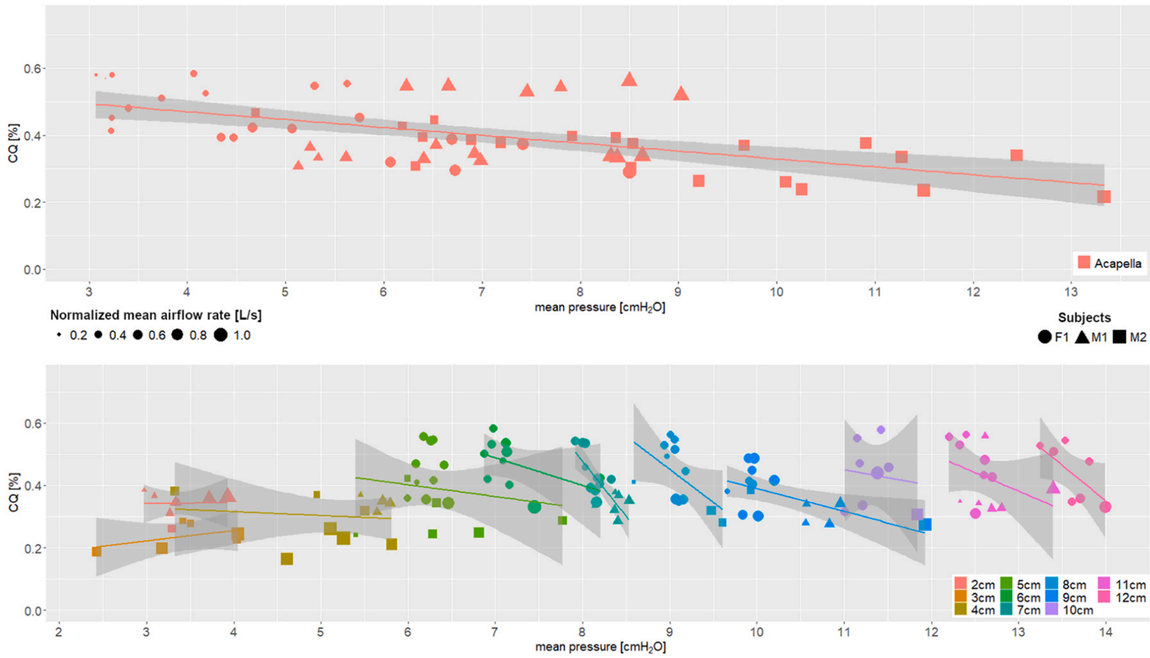

FIGURE 12. Scatter plot of mean pressure and CQ values for the PEP and STP. Trend lines and 0.95 confidence intervals are also displayed for visual support.

vocal folds, resulting in more effortful phonation. Consequently, greater tension is generated in the glottis to counteract the larger back pressure generated by the water column. This finding supports clinical practice, which uses deep bubbling as a means to promote glottal adduction.

Figure 12 illustrates the overall trends in CQ as a function of $\text{pressure}_{\text{Mean}}$ for both devices, with markers indicating normalized airflow values. Similar patterns are observed for the correlation between CQ and $\text{pressure}_{\text{Mean}}$, where the relationship is predominantly negative for Acapella and for any given STP depth. Significant results (Table 3) were found for the Acapella but not for STP, reinforcing our interpretation of the stronger effect of Acapella phonation on the vocal apparatus due to the device's mechanistic behavior. This interpretation is further supported by the highly significant results across all correlation analyses for Acapella data in Table 3. Although significant differences were not reached for STP, noticeable trends between CQ and $\text{pressure}_{\text{Mean}}$ can be observed for most tube submersions (Figure 12). For Acapella, two possible explanations can be given for the relationship between $\text{pressure}_{\text{Mean}}$ and CQ. Drops in CQ may be a byproduct of increments in $\text{airflow}_{\text{Mean}}$ that lead to higher pressure in the vocal tract (ie, higher supraglottal pressure). This, in turn, would raise intraglottal pressures, leading to the observed drops in CQ. As such, a back pressure component of the $\text{pressure}_{\text{Mean}}$ would be the cause of this negative correlation. Alternatively, specific glottal adjustments related to the valving mechanism of the larynx are controlling the transglottal airflow to produce the resulting $\text{pressure}_{\text{Mean}}$, and therefore this relationship is a direct consequence of controlling the valving mechanism via the CQ. Both assumptions are possible; however, some insights can be gleaned from the time domain analysis of the Acapella exercises (Figures 3, 5, and 7 normalized signals), where the $\text{pressure}_{\text{Device}}$ tracing (relative to the flapping cycle of the Acapella device) is preceded by changes in CQ, which are in phase with the $\text{airflow}_{\text{Device}}$. As such, this suggests that the valving mechanism of the glottis closely regulates airflow leading to changes in pressure, rendering the second explanation more plausible for the relationship between CQ and $\text{pressure}_{\text{Device}}$. This finding is consistent with what is expected during normal phonation, where the glottis plays a crucial role in controlling airflow. However, with SOVTE, this relationship may be influenced by increased vocal loading due to higher impedance in the system, and potential compensatory mechanisms like increased subglottal pressure. For STP, the relationship between $\text{pressure}_{\text{Mean}}$ and CQ is more complex, as CQ increases with tube submersion (related to $\text{pressure}_{\text{Device}}$, which is determined by the water depth), but decreases overall within each water depth (see Table 3 for the relationships between $\text{airflow}_{\text{Mean}}$ and $\text{pressure}_{\text{Mean}}$ for STP, which are mainly positive apart from 4 cm). This implies that regardless of the water depth during STP, increased blowing through the silicone tube leads to reduced CQ levels. This finding carries clinical implications, as prescribing regular bubbling at deeper submersions could enhance glottal adduction, making it suitable for hypofunctional disorders. In contrast, shallow tube submersions with lively bubbling may help hyperfunctional disorders, as it promotes lower CQ values. This can also be achieved using the Acapella with increased airflow, generating larger intraoral pressure and decreasing CQ while also producing a stronger massage effect. It is important to bear in mind that our data demonstrates general trends with considerable variability among subjects and water depths for STP, which can be attributed to the limited number of participants in this study.

Clinical application

Overall, our data revealed that the Acapella device produces more pronounced effects on the voice, characterized by larger pressure profiles in the vocal tract and a greater impact on the glottal cycle. In contrast, STP exhibits higher variability among subjects and water depths, which is expected due to the chaotic nature of water bubbling. Additionally, it appears that airflow plays a significant role in determining the pressure profiles in the vocal tract for Acapella, whereas for STP, it primarily relates to low-frequency modulation of pressure and flow associated with water bubbling, especially at lower submersion depths. These results are valuable in clinical practice, guiding exercise selection. The larger modulation of pressure by the Acapella device can induce more substantial changes in the vocal tract and glottal parameters, associated with a stronger massage effect. Consequently, we recommend employing this exercise approach for the treatment of hyperfunctional voice disorders. Conversely, STP can be utilized to prescribe a specific level of effort based on the water depth submerged, thereby facilitating better control over the patient's effort level. Furthermore, in the case of STP, deep bubbling can be employed to generate higher intraoral pressures characterized by larger peak-to-peak amplitude of oscillations and increased CQ values. Therefore, individuals with hypofunctional voice disorders may find therapeutic value in practicing STP exercises at deeper water depths. Our data also indicates that a massage effect may be achieved at all levels of water submersion, making it potentially beneficial for hyperfunctional voice disorders as well. Additionally, the STP data showed a stronger correlation between $\text{airflow}_{\text{Mean}}$ and $\text{airflow}_{\text{Device}}$, regardless of water depth. This suggests that a greater massage effect may be experienced when blowing harder into the tube; however, additional data is necessary to fully validate this point.

LIMITATIONS OF THE STUDY

Despite the valuable insights obtained from this study, certain limitations must be acknowledged. One significant limitation is the small sample size, with only three subjects (one female and two males) participating. This limited number of participants restricts the scope of the study, particularly concerning the comparison between male and female $\text{airflow}_{\text{Glottal}}$ and $\text{pressure}_{\text{Glottal}}$ relationships. Therefore, future studies should investigate this relationship in more detail with a larger and more diverse participant pool to draw more robust and generalizable conclusions. The complexity of the data collection, particularly the need for endoscopy during the exercises, contributed to the difficulty in recruiting suitable participants. Despite this limitation, we believe that the observed patterns across individuals provide a meaningful representation of the phenomena under investigation, offering insightful knowledge about the different impacts produced by phonation into the Acapella device versus STP. These findings offer valuable information for understanding the effects of these devices on glottal function and may guide further research and clinical applications in the field of voice therapy.

CONCLUSION

This study aimed to investigate the impact of phonation in the Acapella device and STP on glottal function and phonation. The findings revealed that the Acapella device generated more regular and pronounced oscillatory pressure, resulting in clear effects on the glottal cycle, as evidenced by changes in CQ, F_0, glottal area, pressure, and airflow modulation. Furthermore, the Acapella device demonstrated a strong correlation among variables, indicating that increased airflow resulted in a more pressurized vocal tract and enhanced pressure modulation. In other words, the stronger a subject blow into the device, the more pressure is generated in the vocal tract, leading to a stronger massage effect. In contrast, STP showed similar patterns but with higher levels of irregularity and lower intensity, attributed to the nature of the exercises, where mean pressure is primarily determined by water depth.

Additionally, the study indicated that for STP, CQ values increased with deeper water submersion, supporting the utilization of deep bubbling exercises for glottal adduction. Higher CQ values were observed with lower airflow levels compared to higher airflow levels within the same greater depths of submersion. These results have potential clinical applications, guiding device selection in voice therapy. Beyond the scope of this study was a detailed investigation of changes in vocal tract impedance with slowly changing boundary conditions due to the presence of the devices. We consider this an interesting direction for future research, especially for numerical simulations of the vocal tract. Our study has demonstrated the non-negligible importance of this phenomenon.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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