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**Article** *in* Journal of Voice · July 1996

DOI: 10.1121/1.414146 · Source: PubMed



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*Journal of Voice*
Vol. 10, No. 2, pp. 129–138


# Bifurcations in Excised Larynx Experiments

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**Summary:** Bifurcation analysis was applied to vocal fold vibration in excised larynx experiments. Phonation onset and vocal instabilities were studied in a parameter plane spanned by subglottal pressure and asymmetry of either vocal fold adduction or elongation. Various phonatory regimes were observed, including single vocal fold oscillations. Selected spectra demonstrated correspondence between these regimes and vocal registers noted in the literature. To illustrate the regions spanned by the various phonatory regimes, two-dimensional bifurcation diagrams were generated. Many instabilities or bifurcations were noted in the regions of coexistence, i.e., regions in which the phonatory regimes overlap. Bifurcations were illustrated with spectrograms and fundamental frequency contours. Where possible, results from these studies were related to clinical observations. **Key Words:** Excised larynx—Asymmetry—Bifurcations—Phonation onset—Vocal folds—Registers—Instabilities.

Given the nonlinearities associated with aerodynamically coupled oscillations, a nonlinear dynamics approach is essential in any study of vocal fold vibrations. While nonlinearities are routinely implemented in the *simulation* of vocal fold movement, it is less common to use a nonlinear approach in the *analysis* of vocal fold vibrations. The bifurcation diagram (explained below) is a crucial element in nonlinear analysis, especially when the system is expected to be governed by low-dimensional dynamics.

In relation to vocal fold dynamics, a bifurcation is a sudden qualitative change in the vibratory pattern of the folds, usually induced by a small change of some parameter such as lung pressure, vocal fold tension, or asymmetry. One common bifurcation (induced by gradually increasing the subglottal pressure) is phonation onset, an initialization of self-sustained vocal fold oscillations from a state of rest (1–3). Other common bifurcations include frequency jumps to new periodic oscillations or to subharmonic oscillations ($F_0/2$, $F_0/3$, etc.), onset of low-frequency modulations into the vibration pattern, and jumps to irregular oscillations associated with chaos (4–9).

The purpose of the bifurcation diagram, especially the two-dimensional bifurcation diagram explored in this article, is to document the regions in which distinct vibration patterns occur. Regions that might have physiological relevance include the parameter space spanned by subglottal pressure and vocal fold tension and the region spanned by subglottal pressure and an asymmetry parameter, such as the ratio of tensions between the left and right folds.

For nonlinear systems such as vocal folds, regions of different vibratory regimes may overlap. This overlap between regions poses serious consequences for vocal control: wherever an overlap occurs, more than one vibration pattern may result from the same vocal fold configuration. In such regions, involuntary, spontaneous jumps from one vibration pattern to another may occur, as well as all

Accepted September 5, 1995.
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of the bifurcations just enumerated. Consequently, bifurcation diagrams have immediate clinical relevance for pin-pointing the regions wherein instabilities, voice breaks, and other transitions may occur. They are also useful for documenting the overall range and capabilities of a particular voice.

A two-dimensional bifurcation diagram often used in connection with voice training and voice analysis is the voice range profile (VRP). In the plane spanned by fundamental frequency and intensity, the VRP maps out the region in which phonation is possible. Consequently, a central focus of the VRP is the Hopf bifurcation—the bifurcation associated with phonation onset. In generating the VRP, usually no attempt is made to maintain a particular voice type or vocal fold configuration. Rather, just one cumulative region is noted to encompass the entire vocal range of the subject.

When a co-existence of phonatory regimes appears, it is sometimes of interest to delineate the phonatory regimes with basin boundary diagrams. For a fixed set of parameters (an isolated point within the bifurcation diagram), a basin boundary denotes the set of initial conditions that eventually leads to a specific phonatory regime. In a recent study by Steinecke and Herzel (3), extensive bifurcation diagrams and one basin boundary diagram were generated for an asymmetric two-mass model of the folds. It was shown how basins of co-existing regimes (attractors) may be intertwined in a complicated manner. While the results of that study have implications for vocal fold paralysis, they need to be substantiated with models more directly related to vocal fold morphology. In our investigation, we focused on bifurcation diagrams generated from excised larynges.

First, we describe the methods for generating phonation from the excised larynges, and the protocol for data collection for the bifurcation diagrams. Next, an illustration of Hopf bifurcations (bifurcations associated with phonation onset) is presented. A qualitative description is given of the vibration patterns observed in the investigations, as well as a comparison of these patterns to registers discussed in the literature. In another section, bifurcations of relatively symmetric folds are presented. Finally, two bifurcation diagrams are shown for bifurcations induced by both adduction and elongation asymmetries. All observations are discussed and compared with clinical observations and with results from biomechanical simulations.

## METHODS

Larynges of five large mongrel dogs (each on the order of ~25 kg) were obtained from the cardiac unit of the University of Iowa Hospitals and Clinics. Animals were killed for other purposes, but the tissue was made available to us post mortem. Each larynx was dissected and trimmed and the thyroid cartilage bracketed and firmly mounted on a tube supplying heated and humidified air. One suture was attached to the anterior arch of the cricoid cartilage, allowing the vocal folds to be symmetrically elongated before asymmetries were applied, i.e., an upward pulling on the suture would elongate both folds, while a downward pulling would shorten both folds.

To permit asymmetrical adductory adjustments, sutures $A_1$ and $A_2$ were attached to the muscular processes of the arytenoid cartilages (Fig. 1). As shown, the sutures coursed anteriorly, medially, and inferiorly to approximate the action of the lateral cricoarytenoid muscle during adduction. Sutures L and S (Fig. 1) were attached to the left arytenoid cartilage, enabling asymmetrical elongation of the folds. All sutures were attached to micrometers for precise control in manipulating the degree of asymmetry.

All experiments in this study were recorded using a Super VHS color video system and an audio recorder with a Sennheiser-MD441U3 microphone, displaced from the glottal opening approximately 6 in vertically and 4 in horizontally. Selected portions of audio signals were later digitized with 16-bit resolution and a sampling rate of 22.1 kHz (antialiasing filters were set at 10 kHz). In general, the

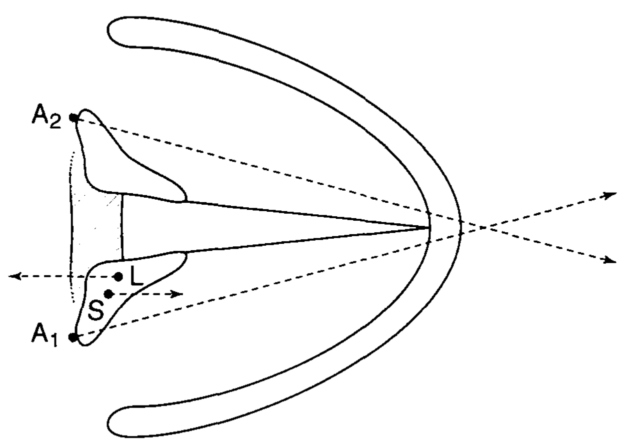


FIG. 1. A schematic of the sutures used to induce the asymmetries on the excised larynx.

following protocol was used: (a) Micrometer settings were adjusted (typically in 1 mm increments) and documented. (b) Subglottal pressure was gradually increased from 0 to 20–30 cm $H_2O$. A special note was made of pressures associated with phonation onset, jumps from one vibration pattern to another, instabilities, etc. Where possible, an attempt was made to strobe the various phonatory regimes. (c) Subglottal pressure was gradually decreased back to 0. Again, it was noted where phonation stopped and where instabilities and transitions occurred.

The process was repeated until all desired asymmetries had been investigated on five different larynges. No instabilities or bifurcations were noted for the asymmetries applied to larynx no. 1 or 2 other than those associated with phonation onset. "Symmetric" bifurcations related to vocal fry-like regimes were noted for larynx no. 3, especially at high pressures. For larynx no. 4, extensive bifurcations were induced with adduction asymmetries. Finally, bifurcations due to elongation asymmetries were observed in the investigations of larynx no. 5. All findings will be presented in the following sections on phonation onset, symmetric bifurcations, bifurcations due to asymmetric adduction, and bifurcations due to asymmetric elongation of the folds.

### Phonation onset

How much pressure is required to initiate and sustain phonation? In a more formal sense, where does the Hopf bifurcation (phonation onset) occur within a given parameter space? This question has immediate clinical relevance for investigating the "ease of phonation" under various conditions, including hydration level (10), pre-phonatory glottal shaping (11), vocal fold tension or fundamental frequency, and degree of disorder (e.g., to initiate phonation with unilateral paralysis, a relatively large subglottal pressure would be required). For computer models of phonation, related questions have already been carefully documented (2, 3, 12, 13).

It was also of interest to know whether the Hopf bifurcation is supercritical (a soft onset without hysteresis) or subcritical (an abrupt onset with hysteresis). For the latter case, two thresholds exist: one associated with phonation onset as subglottal pressure is gradually increased and one corresponding to the cessation of phonation as subglottal pressure is decreased (14). Examples of subcritical Hopf bifurcations from larynx no. 2 are shown in Fig. 2.

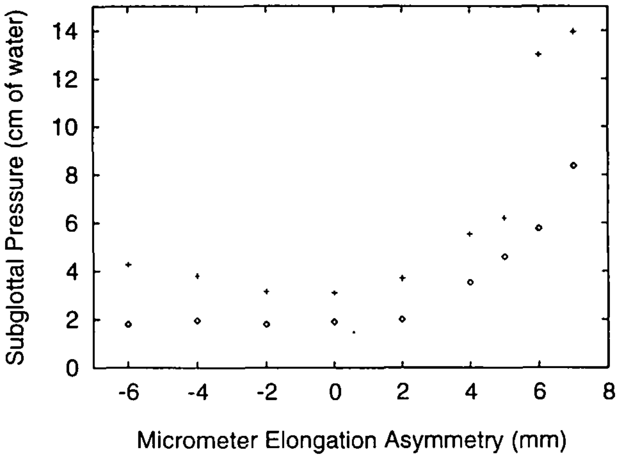


FIG. 2. Phonation threshold pressures for larynx no. 2. The horizontal axis displays the micrometer readings associated with suture S for negative elongation values, and suture L for positive values.

The upper line, denoted by "+" symbols, indicates the sudden onset of phonation as pressure is increased; the lower line, denoted by "◇" symbols, indicates where phonation stopped as the pressure was lowered.

Both the upper and lower thresholds shown in Fig. 2 represent the average value of at least two repeated measurements for larynx no. 2. The mean difference of repeated measures was ~0.35 cm $H_2O$. Over most of the range of induced asymmetries, there was a 1–2 cm $H_2O$ hysteresis observed in the curves separating aphonia from phonation. However, for large asymmetries, this number increased substantially. Also, as might be suspected, the phonation threshold increased as the elongation asymmetry was increased.

The area between the upper "+" and the lower "◇" symbols represents the region in which aphonia and phonation coexist for larynx no. 2. In this region, small perturbations (i.e., suture adjustments or variations in subglottal pressure) were observed to induce or terminate phonation. Such findings are reminiscent of the involuntary and intermittent aphonia observed in patients with vocal paralysis. Although the details of Fig. 2 are not necessarily general observations, all five larynges showed some hysteresis in connection with phonation onset.

### Vibration patterns and phonatory regimes

Through systematic and extensive variations of subglottal pressure, and asymmetric adduction and elongation, a wide variety of vibratory regimes have been explored in canine larynges, reminiscent of the wide range of vocalizations possible in the human voice. Although not always strictly defined, the concept of registers is often used to describe different vibratory regimes (15–19). Some of the registers that are commonly distinguished include vocal fry or pulse register, chest, falsetto or head, and whistle, flageolet, or flute register. Below, we present regimes that might fit within these general classifications, although no rigorous justification will be given for assigning a particular vibration pattern to a particular register. Rather, assignments will be based upon perceptual evaluations and qualitative differences in spectra.

Figure 3 shows spectra for chest-like phonation, falsetto-like phonation, and flute-like phonation. Spontaneous jumps were observed between chest-like and falsetto-like phonation, and just a small variation in elongation asymmetry (i.e., a 1 mm increase) induced the jump to flute-like phonation. The vibration pattern for chest-like phonation revealed large amplitudes of vibration, complete closure, a pronounced mucosal wave, and relatively intense higher harmonics (Fig. 3A). The falsetto-like phonation was characterized by small amplitude vibrations, incomplete closure, weak phase delay between upper and lower edges of folds, and less intense higher harmonics (Fig. 3B). The flute-like phonation had nearly double the frequency of the other two vibrations patterns (~200 Hz instead of 100 Hz), despite similar conditions. It had small vibrations, weak glottal opening, no visible mucosal wave, and very weak harmonics (Fig. 3C). Water on the folds appeared to be rotating synchronously with vocal fold oscillations, giving an indication of vorticity at the same frequency as vocal fold oscillations. Virtually the same characterization has been given by Keilmann and Michek (17) for stroboscopic observations of whistle register in the soprano voice. A preliminary explanation consistent with the observations would be an interpretation of "vortex-induced vibrations" (20), which might be related to the still controversially discussed flute or whistle register.

Finally, we presented a regime comparable to vocal fry phonation, taken from larynx no. 3 at 19 cm $H_2O$. The spectrum in Fig. 4 displays strong subharmonics at multiples of 50 Hz leading to a rough sounding phonation. Using a synchronization of ~100 Hz, a videostroboscopic recording of this regime was also obtained, revealing an alternation of large and small glottal openings, a phenomenon often associated with vocal fry phonation.

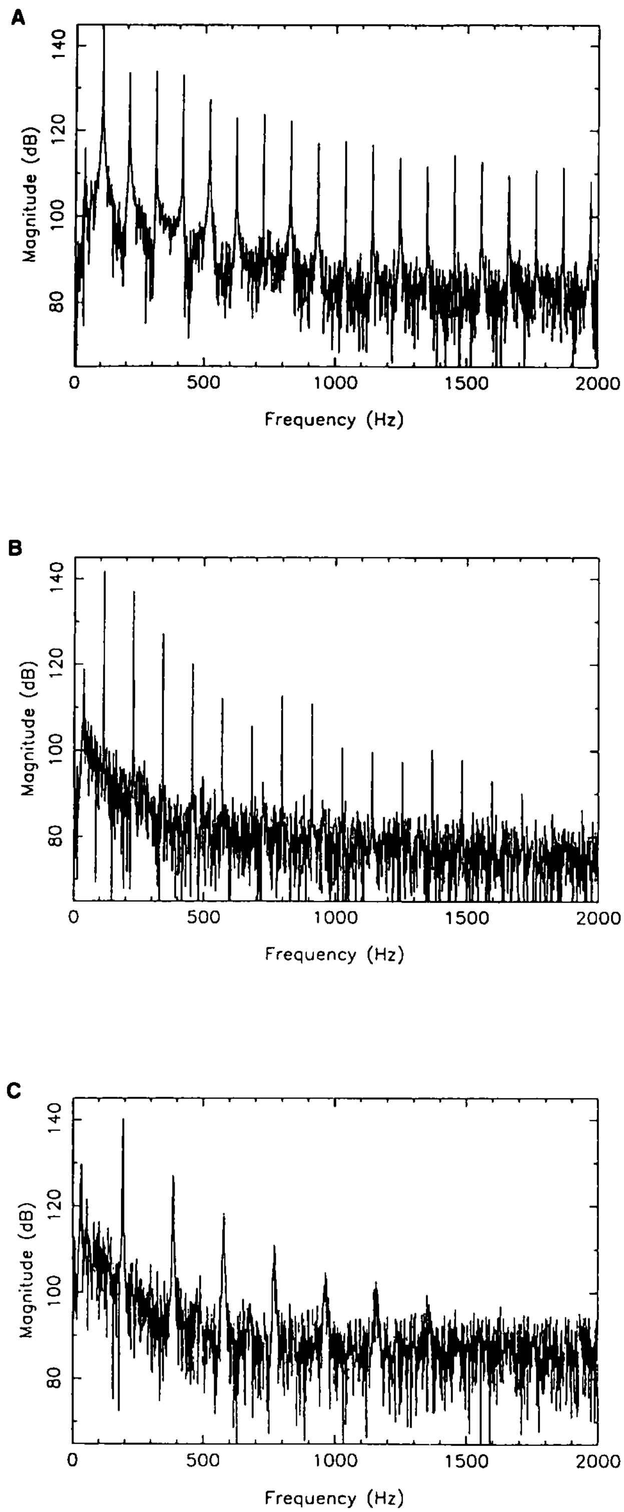


FIG. 3. Spectra from larynx no. 5 for (A) chest-like vibrations, (B) falsetto-like vibrations, (C) vortex-induced vibrations (related to flageolet, flute, or whistle register).

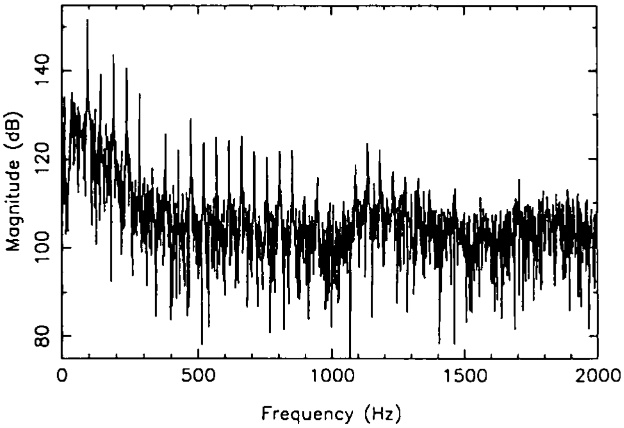


FIG. 4. Spectrum from larynx no. 3 for vocal fry-like vibrations.

**Bifurcations of symmetric folds**

Although instabilities generated by artificially induced asymmetries were the focus of this study, instabilities were occasionally observed for symmetric folds, especially at large subglottal pressures. Such observations have been noted in previous studies (Scherer RC, Austin SF, unpublished observations). In this section, characteristic bifurcations of larynx no. 3 for nearly symmetric conditions are presented. By symmetric folds, we mean that no artificial asymmetry was intentionally induced on the folds. It is readily acknowledged that, in practice, true symmetry cannot be achieved.

The spectrogram in Fig. 5 shows a transition to a subharmonic regime with peaks at multiples of $F_0/2$. This bifurcation was observed while slowly increasing subglottal pressure to ~16 cm $H_2O$. Qualitatively, this transition might be described as a jump between chest-like phonation and vocal fry-like phonation. A detailed spectrum of the vocal-fry-like phonation is shown in Fig. 4. For even larger subglottal pressures (~26 cm $H_2O$), intermittent jumps between normal phonation and highly irregular phonation occurred, as depicted in Fig. 6. The five dark vertical bars (occurring at 0.3, 1.1, 4.5, 5.8, and 7.2 s) represent irregular phonation.

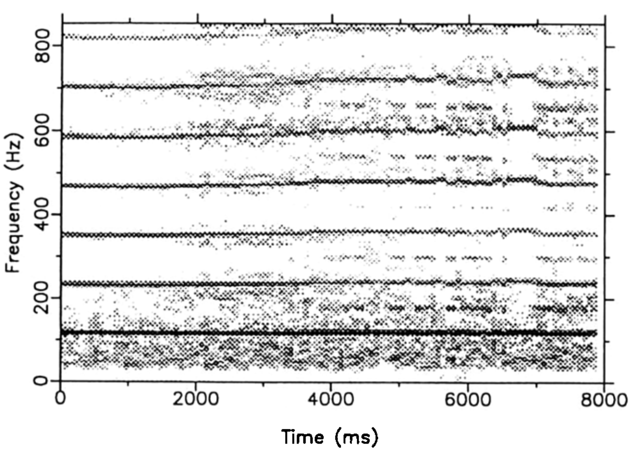


FIG. 5. Spectrogram from larynx no. 3 showing a transition from chest-like regime (1–2 s) to vocal fry-like regime (2–8 s), accompanied by a period-2 subharmonic, as indicated by the additional spectral lines at $3F_0/2$ (~180 Hz), $5F_0/2$ (~300 Hz), $7F_0/2$ (~420 Hz), etc.

**Bifurcations for asymmetric adduction**

To systematically vary the asymmetry of adduction, adjustments were made in the micrometer settings of sutures $A_1$ and $A_2$ (Fig. 1). Suture $A_1$ was varied from 2 to 10 mm, while suture $A_2$ remained constant at 2 mm. Micrometer asymmetry, i.e., the difference between the two micrometer readings, is shown as the horizontal axis in Fig. 7. Given the experimental constraints associated with this study, i.e., there was only a limited time to make measurements because of tissue dehydration and most threshold measurements had to be made at least twice to examine repeatability issues, only a 1 mm resolution was possible along the horizontal axis.

In this initial study of bifurcation analysis, no attempt was made to relate micrometer settings to specific glottal geometries. Presumably, one could investigate such a correspondence by analyzing the video recordings of these experiments. However, for the purposes of this study, bifurcation diagrams are generated directly from the micrometer readings.

Figure 7 shows the locations of various vibratory regimes of larynx no. 4 in the parameter plane spanned by adduction asymmetry and subglottal

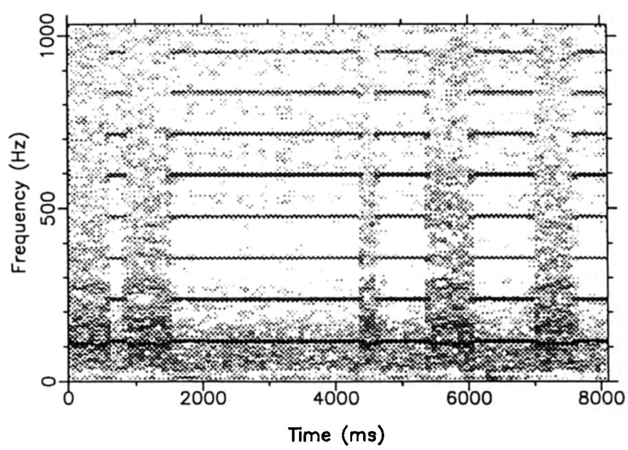


FIG. 6. Spectrogram from larynx no. 3 illustrating intermittent jumps from chest-like vibrations to irregular vibrations.

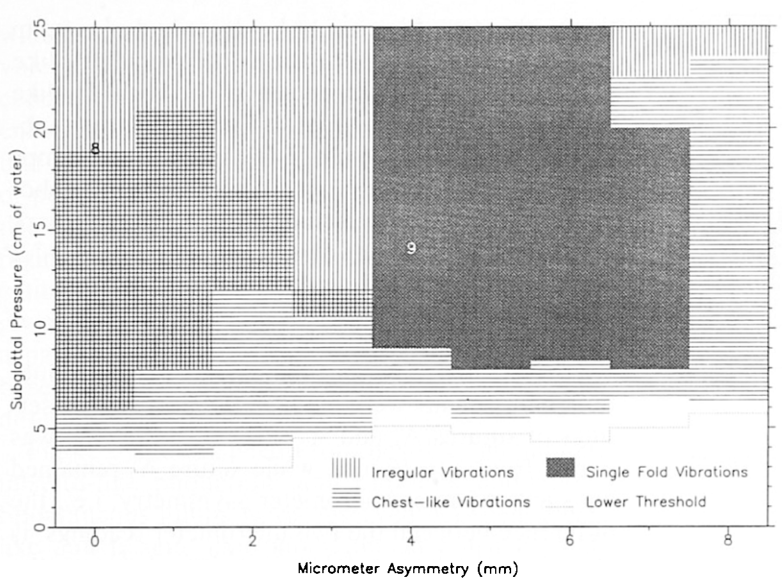


FIG. 7. Bifurcation diagram for adduction asymmetries applied to larynx no. 4. Square-like patterns indicate the overlap of the regions associated with chest-like vibrations and irregular vibrations. The dotted line indicates the lower phonation threshold for chest-like vibrations. The numerals "8" and "9" are positioned to indicate the laryngeal parameters that correspond to the signals used to generate Figs 8 and 9.

pressure. The observed regimes included chest-like vibrations, irregular vibrations, and periodic single vocal fold oscillations (the other fold appeared to be stationary). The area of overlap of horizontal and vertical lines, occupying a relatively large portion of the bifurcation diagram for low asymmetry, represents the region of coexistence of chest-like vibrations and irregular vibrations. The number "8" appears along the upper edge of this region of coexistence, indicating the parameter values of the folds associated with the audio signal analyzed in Fig. 8.

Typical of this region of coexistence, Fig. 8A shows the spectrogram of a transition from chest-like vibrations to irregular vibrations. Note that at ~1 s, the transition to irregular vibrations was initiated by a period-doubling, as indicated by the spacing of the harmonics. To examine this region more closely, a high precision fundamental frequency contour was generated (Fig. 8B) using GLIMPSES, a speech analysis package (21). The zig-zag pattern found in the contour from cycle ~32–52 is characteristic of a period-doubling. Because period-doublings are known to be common precursors of chaos, this gave an indication that the irregular oscillations may be associated with chaos. However, a closer examination of the spectrogram (Fig. 8A) showed that the "irregular" vibrations were not totally random. For example, immediately following the transition, hints of a fundamental frequency ~130 Hz ($F_0$), as well as a period-3 subharmonic (as manifested by the spectral lines at $F_0/3$, and $2F_0/3$, and $4F_0/3$) was seen.

As indicated by the symbol "9" in the bifurcation

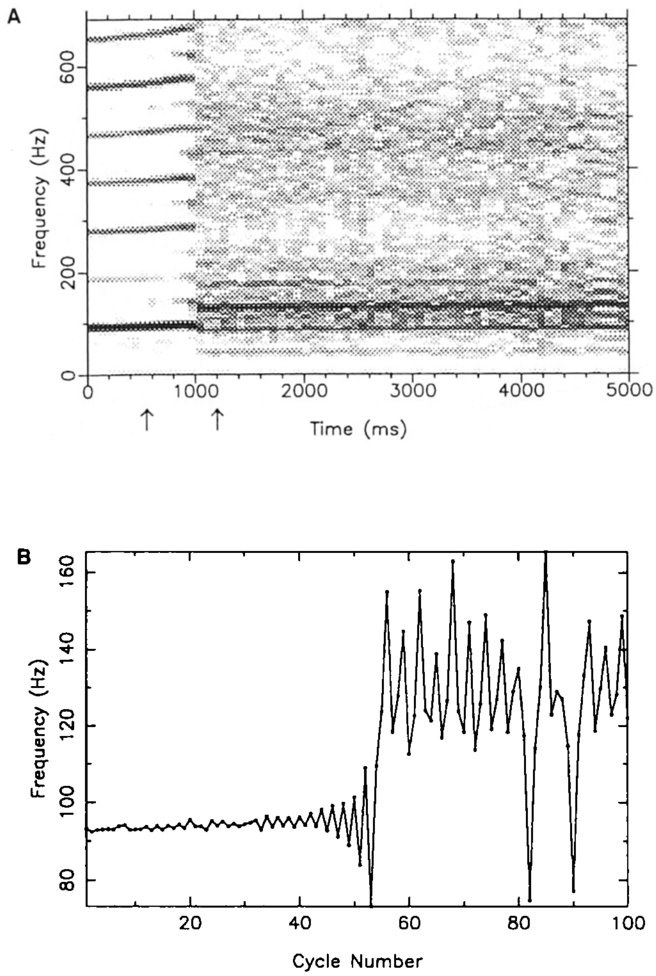


FIG. 8. A: Spectrogram from larynx no. 4 illustrating a transition from chest-like vibrations to irregular vibrations. B: Magnified view of the fundamental frequency contour near the transition in (A), as indicated by arrows.

diagram (Fig. 7), Fig. 9 was generated with parameter values associated with single vocal fold vibrations. Although too complicated to be displayed in the bifurcation diagram, the region of periodic single vocal fold vibrations showed considerable overlap with chest-like and irregular vibrations. A transition from single vocal fold vibrations to chest-like is shown in Fig. 9. The transition occurred at ~2.5 s, as manifested by the sudden drop in fundamental frequency. Single vocal fold vibrations were accompanied by a slight modulation, as depicted by the sidebands surrounding the harmonics during the first 2 s.

**Bifurcations due to elongation asymmetry**

To systematically vary the asymmetry of elongation, adjustments were made in the micrometer settings of sutures L and S. The employment of suture L was referred to as a positive micrometer asymmetry, and that of suture S as a negative micrometer asymmetry. For three separate larynges (nos. 1, 2, and 5), systematic lengthening and shortening of the left vocal folds were performed, with micrometer asymmetries ranging from 13 to −5 mm.

In this preliminary study of bifurcation analysis, no attempt was made to precisely correlate micrometer readings with more kinetic measures, such as force or tissue strain. Nevertheless, two microsutures were placed along the midlength of both folds, allowing strain to be calculated. Several spot computations revealed a general correspondence between micrometer asymmetry and tissue strain. However, for the purposes of this study, only micrometer asymmetries are reported.

With the applied elongation asymmetries, the only instabilities observed for larynges nos. 1 and 2 were those associated with the transition from aphonia to phonation and vice versa. In the case of larynx no. 1, perhaps the applied asymmetries were simply not great enough to induce further instabilities. For the relatively large asymmetries applied to larynx no. 2 (Fig. 2), a significant coexistence region existed between aphonia and phonation. As noted before, small perturbations were observed to both induce and terminate phonation, reminiscent of the intermittent involuntary aphonia often associated with vocal fold paralysis.

Larynx no. 5 demonstrated many instabilities, which are summarized in Fig. 10. Near the number "3", a small co-existence region of chest-like and falsetto-like vibrations is denoted by the overlapping horizontal and vertical lines. In this region, the previously mentioned spontaneous jump from falsetto-like vibrations (Fig. 3A) to chest-like vibrations occurred (Fig. 3B). Figures 11 (Figs. 3C and 11 correspond to the same audio recording) and 12 are taken from the gray region where a whistle or flageolet-like register was observed (possibly related to vortex-induced vibrations). Although an audible high-pitched whistling was present for elongation asymmetries of >7 mm, it is not indicated in Fig. 10 since no vocal fold vibrations were observed. Although too complex to be shown, the indicated whistling-region had considerable overlap with the falsetto and chest-like regions. The complicated bifurcations shown in Fig. 11 were associated with the co-existence of whistle-like phonation (fundamental frequency ~200 Hz) and falsetto-like phonation (fundamental frequency ~100 Hz). Another observation from the same region is depicted in Fig. 12. Here, the co-existence induced two independent frequencies, as demonstrated by the two sets of spectral lines, one set remaining constant and the other set gradually increasing.

Although the black region (speckled with white dots) of Fig. 10 is just another co-existence region of chest and falsetto-like vibrations, it is specifically denoted as an instability region. This particular region was the most remarkable of all the instability regions studied on the five larynges. A few typical examples are shown in Figs. 13 and 14.

The modulations shown in Fig. 13 appeared as precursors of the jump from falsetto to chest-like phonation. Although vibrato in a true singer's voice is probably induced by changes in muscle activity (22), our studies demonstrated that comparable

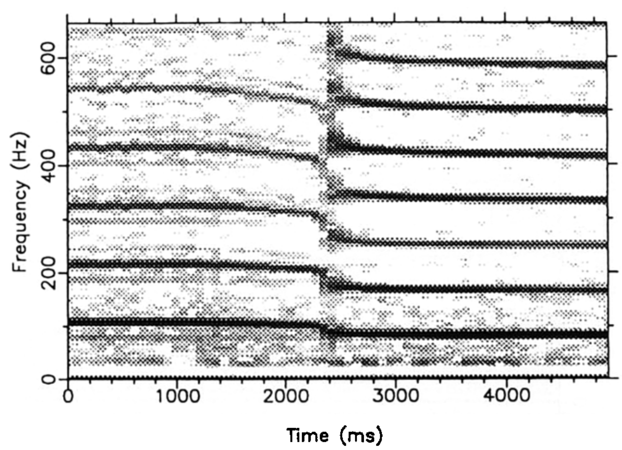


FIG. 9. Spectrogram from larynx no. 4 illustrating a transition from single vocal fold vibrations (first half) to chest-like vibrations (second half).

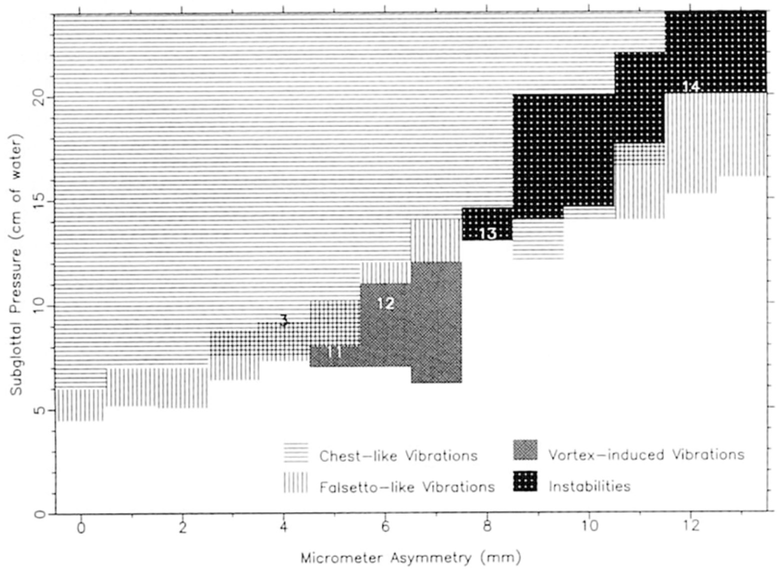


FIG. 10. Bifurcation diagram for elongation asymmetries to larynx no. 5. The numerals "11" and "14" are positioned to indicate the laryngeal parameters that correspond to the signals used to generate Figs. 11–14.

modulations may also be induced by biomechanical-aerodynamic instabilities. However, the observed modulation frequencies were often well above an acceptable vibrato range. For example, the sidebands in Figs. 9 and 14 suggest modulation frequencies of ~30 and 15 Hz, respectively.

At parameter values marked "14" in Fig. 10, >20 spontaneous jumps occurred between chest and falsetto-like phonations. A few examples of such spontaneous jumps are shown in Fig. 14. The falsetto-like phonation (upper frequency) was accompanied by a low-frequency modulation, as indicated by sidebands in the spectrogram of Fig. 14**A** and by the $F_0$ modulation in the fundamental frequency contour of Fig. 14**B**.

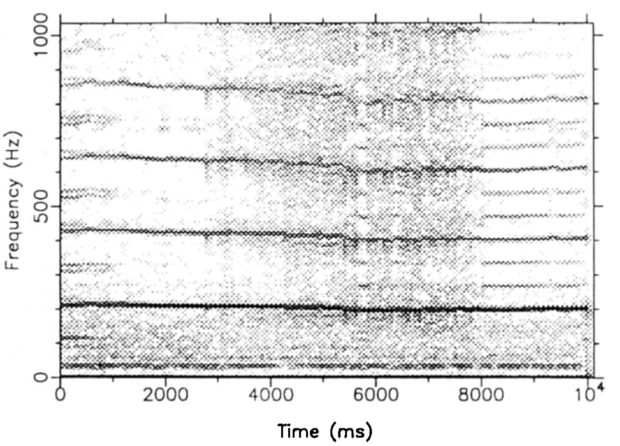


FIG. 11. Spectrogram from larynx no. 5 illustrating jumps from $F_0/2$ to $F_0$ to irregular vibrations to $F_0/3$.

## SUMMARY AND CONCLUSIONS

This investigation complements bifurcation analysis in computer models (5,9,23–25) and observations of bifurcations in voice disorders (5–8). In comparison to computer models, excised larynges are more representative of true vocal fold morphology. Furthermore, because the parameters of interest can be carefully monitored and controlled in excised larynx experiments, the researcher can pinpoint the sources of irregularity with greater confidence than would be possible in human subjects. However, we note that because of histological differences between canine and human vocal folds, the specific parameter values at which bifurcations occur cannot be applied to human folds.

Typical nonlinear phenomena observed in earlier studies (subharmonics, modulations, and transitions to irregular phonation associated with chaos) were also observed in the excised larynx experiments. For the most part, instabilities were observed in parameter regions in which phonatory regimes co-existed. In these regions, small parameter changes were seen to induce bifurcations. Sometimes jumps to new regimes occurred in these regions without any external intervention. Additionally, it was shown how many of these phonatory regimes might be related to the common vocal registers noted in the literature.

Sufficient asymmetric adduction was seen to induce single vocal fold oscillations. Clinically, such observations are found in unilateral vocal fold paralysis. Further asymmetric studies of this type can

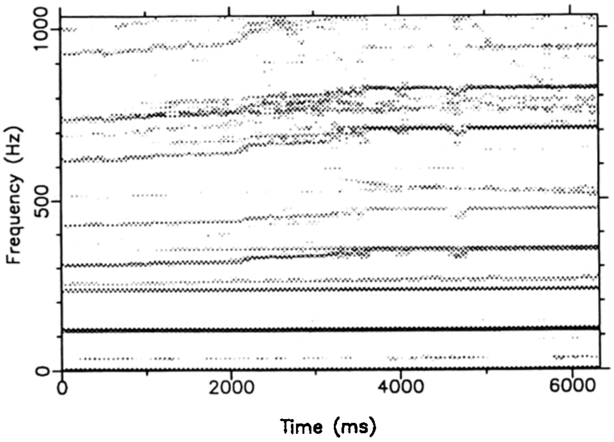


FIG. 12. Spectrogram from larynx no. 5 illustrating a whistle-like register with two independent frequencies, i.e., some spectral lines remain constant, while others increase in frequency.

be used to better quantify the nature of this disorder and to estimate the amount of surgical correction that might be necessary to restore normal, chest-like phonation under similar dysphonic conditions.

At various elongation asymmetries, several vocal registers were observed, including chest, falsetto, and flageolet. In this regard, Fig. 10 represents a comprehensive illustration of our most interesting results. In general, falsetto was observed at lower subglottal pressures than were chest-like vibrations. For relatively low asymmetries, the co-existence region of chest and falsetto was either very small or nonexistent. At larger elongation asymmetries, the co-existence region became very significant, and many spontaneous jumps were observed between chest and falsetto-like vibrations.

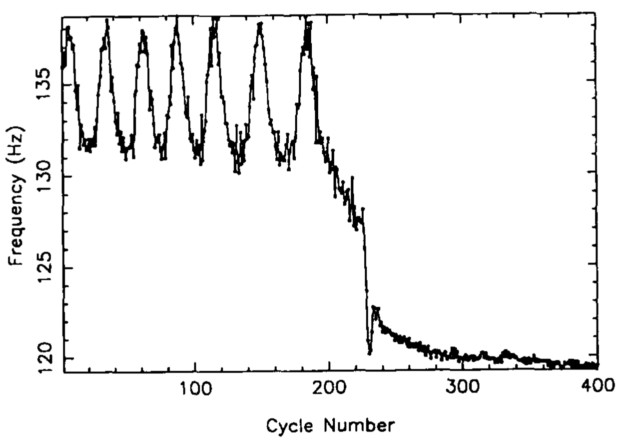


FIG. 13. Fundamental frequency contour from larynx no. 5 illustrating a spontaneously induced "vibrato" (i.e., a low frequency modulation) for fixed laryngeal parameters followed by a sudden decrease in fundamental frequency.

Only a small region corresponded to flageolet or whistle register. Although a high frequency whistling sound was present for higher asymmetries as well, vibrations did not appear to be induced at the higher frequencies. This high-pitched whistling might be comparable to the "whining" elicited from anesthetized dogs (26). We expect that pure whistling and whistle or flageolet register (i.e., vortex-induced vibrations) are closely related, both being induced by vorticity above the glottis. Indeed, if plotted in a bifurcation diagram (such as Fig. 10), these two phenomena would occur at neighboring parameter regions.

Although elongation asymmetries were often not visible (they increased the tension of the folds, but usually did not significantly alter the vocal fold geometry), experimental results indicated their profound influence on the overall vocal fold vibration

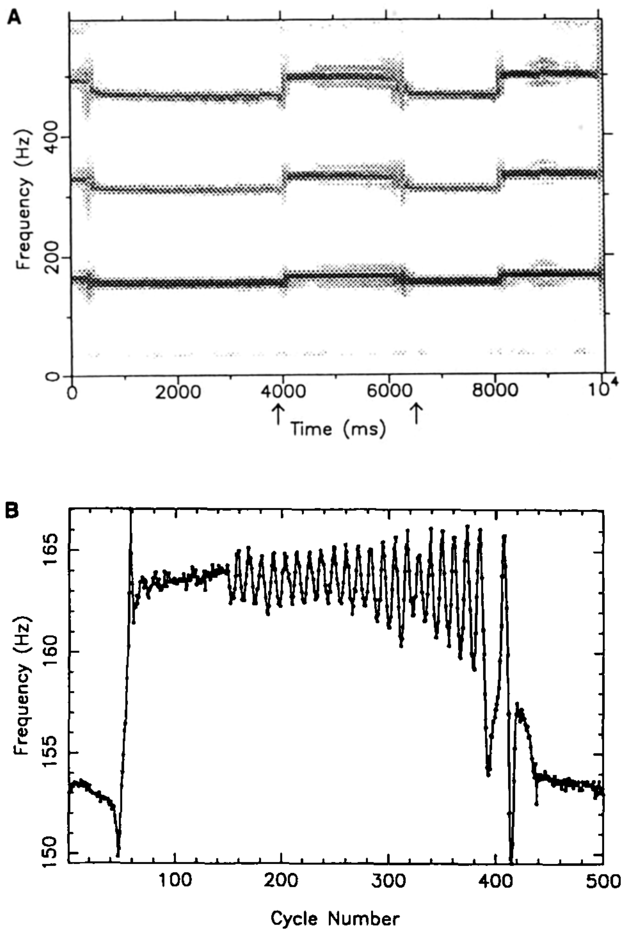


FIG. 14. A: Spectrogram from larynx no. 5 illustrating spontaneous jumps from chest-like (lower frequency) to falsetto-like (upper frequency with modulations) phonations. B: Fundamental frequency contour near the mid-portion of the spectrogram is indicated by arrows.

patterns. Thus, in the treatment of unilateral paralysis, the influence of elastic asymmetries needs to be considered in addition to the more obvious geometric asymmetries.

In summary, bifurcation diagrams have proven useful for studying vocal fold dynamics, just as they have for many other nonlinear systems. We envision that the systematic framework demanded in the generation of bifurcation diagrams will guide many future studies of vocal fold oscillations.

**Acknowledgment:** This work was supported by grant no. P60 00976 from the National Institute on Deafness and Other Communication Disorders and by the Deutsche Forschungsgemeinschaft. The authors thank Lucy Karnell for assistance with experimental design, dissection, and suturing.

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