Anatomy and Functional Morphology of the Mysticete Rorqual Whale Larynx: Phonation Positions of the U-fold.

Juliette Damien[a], Olivier Adam[b,c], Dorian Cazau[d], Paul White[a], Jeffrey T. Laitman[e], Joy S. Reidenberg[e]

[a] Institute of Sound and Vibration Research (ISVR), University of Southampton, Southampton, UK

[b] Sorbonne Universités, UPMC Université Paris 06, CNRS, UMR 7190, Institut Jean Le Rond d'Alembert, F-75005 Paris, France

[c] Institute of Neurosciences Paris Saclay, CNRS UMR 9197, Université Paris Sud, Orsay, France

[d] ENSTA Bretagne Lab-STICC, CNRS UMR 6285, Université Européenne de Bretagne, Brest, France

[e] Center for Anatomy and Functional Morphology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

Corresponding author: Juliette Damien, 0032499122806, jouliette-damien@hotmail.com.

Abstract

Many Mysticetes (baleen whales) are acoustically active marine mammals. This is epitomized by rorquals, and specifically male humpback whales *(Megaptera novaeangliae*) whose complex songs comprise a wide range of vocalizations. The sound production mechanism of odontocetes (toothed whales, including dolphins and porpoises) is well described, in contrast to that of mysticetes whose vocalization mechanism remains a subject of active scientific investigation. Anatomical observations and acoustic signal processing have led to divergent hypotheses under the framework of a production-based approach. We attempt to unify these hypotheses by broadening existing data with our new anatomical investigation, interpreted in light of known acoustical properties of mysticete vocalizations. We examined 15 specimens of four rorqual species: sei whale *(Baleanoptera borealis)*, fin whale *(Baleanoptera physalus),* minke whale *(Baleanoptera acutorostrata),* and humpback whale *(Megaptera novaeangliae)*. Based on these data and on previous literature, we propose a description of three functional positions (rest, breathing and recirculation), unidirectional egressive airflow for sound production (from lungs to laryngeal sac), and new nomenclature for different parts of the U-fold (distal section, midsection, corniculate flaps). Each of these sections has specific morphological and acoustical properties that support the concept of "mode variation" in baleen whale vocalizations.

The vocalizations of mysticetes (baleen whales) vary considerably. The size of their vocal repertoire ranges greatly between species. For example, the blue whale (*Balaenoptera musculus*) has a limited repertoire (McDonald et al., 2006) compared to the famous songs of humpback whales (*Megaptera novaeangliae*). Male humpbacks produce highly complex songs in the breading season (Payne and McVay, 1971), and both genders employ a rich set of social calls (Dunlop et al., 2007). The rorqual whales (baleen whales with expandable throat pleats) represent a subclass of mysticetes that includes blue, fin, sei, Bryde's, Omura's, minke, and humpback whales. All rorquals are acoustically active, but the majority of species have a small acoustic repertoire of highly stereo-typed calls (Zimmer, 2011). The humpback whale is the singular exception to this. Nevertheless, while the sound production mechanism of odontocetes (toothed whales, including dolphins and porpoises) is quite well understood, largely due to captive experimental studies (e.g., Cranford et al., 2011), the mechanism of mysticete vocalization is difficult to study in live, wild animals and still needs further scientific investigation. This study aims to examine the sound production mechanism in mysticetes based on anatomical analysis of the larynges of several rorqual species.

Mysticetes, typical of most mammals, appear to use the larynx for sound production. It is well known that the geometry and physical properties of the vibrating elements of the larynx (namely the vocal folds) determine the types of sounds generated. A better understanding of these features is needed to understand the mysticete sound production system. Geometry of the apparatus and tissue properties should be correlated with other physical features of the whale (e.g., sex, age, length, robustness, fitness). This, in turn, would open the possibility of expanding the role of acoustic methods to study these animals in the wild. For example, one could remotely assess the size of singing individuals, and explore whether the ability to control of singing is a measure of fitness or sexual maturity.

Anatomical observations and acoustic signal processing have already led to hypotheses under the framework of a production-based approach (Mercado et al., 2010; Cazau, 2012; Adam et al., 2013; Cazau et al., 2013). This has resulted in a better understanding of the morphology needed for mysticete sound generation, and helped to identify the factors constraining sound output. The production-based approach involves assessing acoustic characteristics of the vocal organs in order to: (1) understand the way mysticetes generate and emit sound (i.e., physical interactions between gas and tissue that determine how vocal organs affect flow characteristics, vibrate, filter, and how energy is transmitted into the surrounding medium), and to (2) identify the external factors that are not directly controlled by the whale (e.g., depth related pressure, concentrations of respiratory gases, composition of the whale's body in terms of internal resonance and acoustic impedance, filtering of the sound produced by the vocal apparatus before its propagation in the sea water) (Mercado and Handel, 2012). The production-based approach examines these interactions in order to define the anatomy underlying specific vocal behaviors, specifically to determine intentionality (voluntary control) of vocalizations under the regulation of external constraints (Mercado et al., 2010; Cazau et al., 2013).

This work also uses a production-based approach, focusing on laryngeal anatomy of rorqual mysticetes. We explore the geometry and tissue properties of the U-fold, as it was previously described as being the source of vibration in mysticete sound production (Reidenberg and Laitman, 2007a). This article examines: (1) the movements of the arytenoids at the cricoarytenoid joint and how these cartilages may be positioned to effect various phases of respiration and vocalization, (2) the different regions of the U-fold to determine whether changes in shape can affect the types of sounds produced, (3) the role of the various lumenal surface projections on the control of airflow, and (4) the major acoustic properties that can be generated by these features. This information will elucidate how rorqual mysticetes use laryngeal cartilage movements to produce vocalizations, manage and direct airflow, or regulate volumes and pressures of air (technically, it is respiratory gas, but we will refer to it as "air" throughout the article). Abbreviations used through this article are listed in Table 1.

BACKGROUND

Vocal folds and sound production in terrestrial mammals

The larynx of terrestrial mammals is composed of cartilages, muscles, and connective tissues. There are nine main cartilages: three unpaired (cricoid, thyroid, epiglottic) and six paired (arytenoids, corniculates, and cuneiforms). (Note that throughout this paper the word "thyroid" refers to the laryngeal cartilage, and not to the thyroid gland.) Laryngeal muscles are extrinsic (connecting the larynx to neighboring structures such as the hyoid bone and sternum) and intrinsic (arising and inserting within the laryngeal apparatus). The intrinsic muscles are the main muscles employed in phonation, and include: posterior cricoarytenoid (PCA), lateral cricoarytenoid (LCA), interarytenoid (IA), oblique arytenoid (OA) and thyroarytenoid (TA). The vocal folds (VF) each consist of a fibrous ligament covered with soft tissues that attaches dorsally to the vocal process of the arytenoids and ventrally to the thyroid. The muscles attaching to the arytenoids can abduct (PCA) or adduct (LCA, IA, OA) the VF by moving the arytenoids at the cricoarytenoid joint. The cricothyroid muscle (CT) pulls the thyroid ventrally, moving at the cricothyroid joint, resulting in stretching and tension of the attached vocal ligament. The vocalis muscle (part of the TA) is situated around the ligament and can therefore stiffen the VF (Harrison, 1995).

The vibration of the VF is described by the myoelastic-aerodynamic theory of voice production (Van den Berg, 1958). This describes a passive vibration mechanism. The properties of the vibration are determined actively by adduction, tension-elongation and stiffness of the VF tissues (Dickson and Maue-Dickson, 1982; Titze, 1988; Riede et al., 2010; 2011). The vibration then propagates into the supralaryngeal vocal tract (pharyngeal, oral and nasal cavities) (Fitch, 2006). These cavities vibrate preferentially at different formant frequencies according to their characteristics (length and shape) (Taylor and Reby, 2010) and contain features that can influence the source (Finnegan and Alipour, 2009). Therefore, the supralaryngeal cavities act as a filter and shape the spectrum of the vocal output. The interaction between the VF and the vocal tract is called the source-filter interaction or coupling (Fant, 1960; Titze et al., 2010; Riede et al., 2010).

Mysticete sound generator anatomy

Baleen whales were historically thought to have no VF (see review in Reidenberg and Laitman, 2007a), leaving this sound production system with no vibrator. Several authors have described laryngeal anatomy of mysticetes (Hosokawa, 1950; Henry et al., 1983; Quayle, 1991; Reeb and Best, 1999). But it is only in 2007 that the source of mysticete vocalizations had been proposed: the newly identified U-fold, homologous to mammalian VF (Reidenberg and Laitman, 2007a). Their article was followed by Schoenfuss et al. (2014) that identified the same U-fold structure in bowhead whales (*Balaena mysticetus*). Figure 1 presents a schematic view of the cartilage and muscle framework of the mysticete larynx. The description of the surrounding cartilages, muscles, ligaments, and tissues is homologous to the well-studied terrestrial mammal larynx, where the laryngeal VF are the source of sounds.

However, mysticetes present some differences from the typical mammalian pattern: (1) a vibratory organ that is not a free fold of tissue, but rather is completely supported by the fused corniculate and arytenoid cartilages, (2) an accessory air reservoir (diverticulum) ventral to the VF called the laryngeal sac (LS), and (3) two main resonators (LS and nasal cavities (NC)). These are addressed below.

(1) Vibratory tissue supported by cartilage:

The mysticete U-fold, shown in Figure 2, is previously described (Reidenberg and Laitman, 2007a) as the homolog of the mammalian VF. It is a thick, folded, curved, lip-like, mucosal structure that provides a large area with variable textures and degrees of opposing surfaces (in comparison, human VF are smooth and linear, with opposition all along one sharp edge). The orientation of the U-fold is approximately parallel to the trachea, with the caudal edge of the U-fold abutting the rostral edge of the ventral trachea. The U-fold is supported by cartilage along its entire length (arytenoid cartilages caudally, corniculate cartilages rostrally). Some free tissue projects rostrally or ventrally from the cartilages. The arytenoid and corniculate cartilages are fused to each other at approximately the midpoint of each "arm" of the U-fold. The line of fusion was never visualized, but is assumed to be superior to both the muscular process of the arytenoid and the synovial joint that articulates the arytenoid with the cricoid cartilage. The left and right "arms" of the U-fold are united caudally to form the bottom of the "U", and are supported by a free-floating caudal termination of the arytenoid ligament (joining the arytenoid cartilages caudally) that fuses into the raphe and the LS lumen. This changes the boundary conditions for the vibration of the VF compared to other mammals, where the vocal ligament attaches firmly and ventrally to the thyroid cartilage. The role of the vocal ligament extending longitudinally through the VF is replaced by cartilage, changing the oscillating characteristics of the U-fold. The corniculate flaps are also described as a second vibratory organ situated along the rostral part of the larynx, supported by the corniculate cartilages (Reidenberg and Laitman, 2007b; Cazau et al. 2013). These flaps are the most rostral extensions of the U-fold.

(2) Laryngeal sac:

The LS is positioned ventrally to the U-fold. It can be seen as a soft balloon that inflates and deflates elastically with airflow (Reidenberg and Laitman, 2008; Cazau, 2012; Adam et al., 2013). The LS walls are composed of soft tissues that fuse in the mid-line into the raphe, and attach dorsally to the trachea's ventral surface. It is surrounded by an extensive layer of muscles most likely homologous to the TA muscle and possibly one of the CT muscle bellies (Reidenberg and Laitman, 2007a). The LS is thought to play an important role in the circulation/recycling of air (Fitch, 2006; Reidenberg and Laitman, 2007b; 2008), in extra-pulmonary air storage for additional oxygen extraction and compensation for pressure-induced volume loss of the respiratory system at depth (Reidenberg and Laitman, 2007b; 2008), in sound production in mysticetes (Aroyan et al., 2000; Reidenberg and Laitman, 2007a; Mercado et al., 2010; Adam et al., 2013; Cazau et al., 2013; Samaran et al., 2013; Gandilhon et al., 2015), and regulation of the internal pressure in the respiratory tract (changing the whale's buoyancy) (Gandilhon et al., 2015).

(3) Two resonators:

Only one direction of airflow (egressive) is used for phonation in most terrestrial mammals. For mysticetes, the necessity for air recycling (i.e., sending air back to be re-used, rather than expelling it and losing it) opens the possibility of a bi-directional pathway that could be used for phonation during both egressive and ingressive airflow. The different characteristics of the vocal tract components during hypothetical bidirectional airflow have been explored by Cazau et al. (2013), Adam et al. (2013), and Mercado et al. (2010), considering two air sources (lungs and LS) and two resonators (LS and NC).

MATERIALS AND METHODS

Specimens

Rorqual mysticete specimens 1 to 14 in the Table 2 derive from United States (U.S.) waters of the Northwest Atlantic Ocean, where they are protected from hunting by U.S. law. Dissection and tissue acquisition from carcasses is regulated by US law, and thus all specimens were donated through the Marine Mammal Health and Stranding Response Program (U.S.) and were collected under a letter of authorization (issued to Joy Reidenberg) by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration (U.S.). Specimen 15 was collected in Sambava, Madagascar, by the scientific team of the Cetamada organisation. This is the first scientific specimen of mysticete larynx collected in the Indian Ocean. No whales were killed for this study; all specimens were obtained post mortem from carcasses that were discovered floating, brought into port by large ships that accidentally struck them, or found naturally stranded on beaches.

Fresh carcasses are rare as the whales are often found several days to weeks or even months after their death. Bacterial fermentation of the soft tissues degrades their characteristics within the first 24 hours (Gray et al., 2000) and as a result most of the information required to study the elasticity of the U-fold tissues is lost in many specimens. Furthermore, while humpback whale adult females, juveniles and calves swim close to the coast when they migrate, adult males travel further from the coast (Miksis-Olds et al., 2008). When an animal dies, the body initially sinks but comes back to the surface several days later due to buoyancy of decomposition gases, and is then washed to the coast. When the body sinks in deep water, the pressure may inhibit bacterial fermentation and prevent the build-up of buoyant decomposition gas. Thus, the carcass remains lost at depth. This could explain the lack of adult male humpbacks in our sample, and the preponderance of female and juvenile specimens stranding along the U.S. coast.

In this paper, we use mysticete specimens of both genders and a range of ages in the *Baleanopteridae* family (rorqual whales) including: minke whale (*Balaenoptera acutorostrata*), sei whale (*B. borealis*) and fin whale (*B. physalus*), and humpback whale (*Megaptera novaeangliae).* No gender difference in the ability to produce sound is considered, as social calls are produced by both male and female individuals and, for humpbacks, contain a diversity comparable to the song sound unit (Dunlop et al., 2007).

Measurements

Measurements were taken on all the specimens, with particular attention focused on specimen 8 as it was the only adult humpback specimen available presented in a preservation state adequate for measurement of the soft tissues. Most of the measurements were completed using a measuring tape during specimen dissection. Additional ones were collected using the scale bar on pictures taken of the specimens. The soft tissues on the arytenoids were measured and described using 2 parameters: A) the length of the tissue surface that is not supported by cartilage, and B) the size of the cartilage extension supporting the tissue. This protocol and the 2 parameters (A and B) are presented in Figure 7 and Table 4.

The directionality and amplitude of movements of the arytenoid cartilages within the laryngeal lumen were examined on different specimens. The arytenoids were moved at their joint surface with the cricoid in order to approximate the range of adduction, abduction and rocking motions. Manual retraction was performed on muscles or at the points of insertion into cartilages to determine directionality of movements. Other muscle movements/actions were inferred from geometry of muscle fiber alignment.

The volume of the LS of specimen 3 (sei whale) was measured. The method utilized a plastic bag that was fitted into the LS of the specimen and filled with water using a hose. A similar method was also used on specimen 15, but the LS wall tore during expansion, due to its state of degradation.

RESULTS

Measurements for all specimens are presented in Table 3.

Laryngeal sac measurement

The maximum volume of water that could fit in the inflated sei whale LS was 11.06 kg (after the weight of the bag, container and tools were subtracted), corresponding to 11.06 L or 0.011 m^3. This measurement likely corresponds to an underestimation of an adult whale's maximum LS volume, as the specimen measured was a juvenile and the pressure used to inflated the LS (water hose pressure) is likely less than the pressure that could be exerted by muscular contractions of the thoracic wall forcing air from the lungs into the LS.

Movements of the arytenoids at the cricoarytenoid synovial joint

The muscles present in the larynx of rorqual mysticetes are the LCA, IA, PCA, TA and CT muscles. The OA has not yet been specifically identified. The inclination, fibre direction and positions of the laryngeal muscles are illustrated in Figure 3. The intrinsic laryngeal muscles (LCA, IA, PCA) attach on the arytenoid portion of a fused aryteno-corniculate cartilage. The insertions of these muscles are onto a large projection of this fused cartilage called the muscular process. It projects dorso-laterally from the arytenoid portion, and is located near the midpoint of the fused aryteno-cricoid cartilage along the lateral aspect. Immediately medial and ventral to this muscular process is the curved (concave) joint surface of the cricoarytenoid joint. The convex surface of the cricoarytenoid joint is situated on the dorso-rostral end of the cricoid. The joint has an oblique shape, bending caudally with progression laterally from the dorsal midline. Manual abduction of the arytenoids also moves them both laterally and caudally along this diagonal slope of the joint surface on the cricoid.

Manual retraction of intrinsic muscles, or their points of insertion into the cartilage, also affects the positions of the caudal tip (i.e., vocal process) of each arytenoid as it projects into the laryngeal lumen. The IA is situated between the joints, and pulling these muscles together adducts the arytenoids towards the midline. This action could also adduct the corniculate flaps. Movement of the LCA resulted in three main actions: (1) pulling the joints rostrally along the cricoid slope, (2) adduction of the arytenoids, and (3) tilting the muscular process rostroventrally, bringing the caudal tip of the arytenoid dorsally. Furthermore, because the LCA also attaches on the lateral aspect of the arytenoids, a coupled movement of the LCA and IA rotates the arytenoids along their longitudinal axes causing adduction of the caudal tip of the arytenoid towards the midline in the coronal plane. This adduction also narrows the gap between the arms of the U-fold, thereby closing the entrance into the LS. The PCA is attached dorsally and can therefore rock the muscular process dorsocaudally, bringing the caudal tip of the arytenoid ventrally, opposing the movement #3 (described above) of the LCA. Tilting the arytenoid will affect the dorsoventral diameter of the trachea and the orientation of LS aditus relative to the tracheal lumen. The PCA is also attached along the lateral aspect of the muscular process of the arytenoid. Pulling caudally on the lateral aspect of the arytenoid’s muscular process rotates it at the synovial cricoarytenoid joint. This abducts the arytenoids and opens the gap between the arms of the U-fold, thereby opening the entrance into the LS.

The TA and CT muscles do not attach to the muscular process of the arytenoid. TA attaches between the thyroid and the arytenoids. It may help the LCA when lifting the caudal tip of the arytenoids dorsally. For other terrestrial mammals, the TA is the muscle stiffening the VF. We did not observe a direct insertion into the homologous VF tissues (the U-fold). However, it is possible that the TA may have become the inner layer of the circular muscle that surrounds the LS. It is also possible that the TA muscle is the unnamed muscle found nested in the groove of the fused aryteno-corniculate cartilage, near the L-shaped bend where there is a transition between the arytenoid and corniculate cartilages (see Fig. 8). The CT is traditionally a muscle with two bellies pulling the thyroid cartilage ventrally relative to the cricoid cartilage, therefore tensing the VF and elevating the pitch. In rorquals, one of the CT bellies attaches between the thyroid and the cricoid (Fig. 3). However, the rorqual U-fold does not attach directly to the thyroid. Additionally, the second CT belly may have moved caudally and incorporated into the TA circumferential muscle to form the outer muscular layer of the LS. Thus, both CT and TA contraction likely collapses LS volume instead of tensing the VF.

Additional feature affecting the airflow through the U-fold

The laryngeal "cushion" is a thick, fat tissue pad situated on the ventral surface of the cricoid (see Figs. 4 and 9). This position places it directly over the gap between the arms of the U-fold in the caudal region. As it contacts the gap, it can separate the trachea and the lungs from the rest of the respiratory system by sealing the U-fold when the arytenoids are rocked dorsally. Furthermore, the degree of contact between the cushion and the U-fold affects the diameter of the beginning of the trachea. This, in turn, can direct, concentrate, or elevate the velocity of the airflow onto different parts of the U-fold depending on the dorso-ventral position of the arytenoids.

The dorso-rostral edge trachea is fused to the dorso-caudal edge of the cricoid. It looks like a large plate of cartilage lying in the coronal plane of the torso, but the lateral edges are tapered to several bar-like processes. Further caudally, the tracheal cartilage is segmented into several C-shaped cartilages. Interestingly, these cartilages are open ventrally, rather than either the typical mammalian pattern of dorsally incomplete rings or the odontocete pattern of complete O-shaped rings. The open ventral aspect is covered with a flexible ventral membrane. When the LS is inflated, it pushes this membrane into the tracheal lumen, closing the lumen along the midline as shown in Figure 5. We also observed raised, thin folds in the tracheal lumen (see Fig. 6). These folds extend perpendicularly across the tracheal ring cartilages and are more pronounced laterally. They extend rostro-medially to the midsection of the U-fold, and appear to be comprised of connective tissue and mucosa with no muscle or cartilage.

Sections of the U-fold

According to the observations made during the dissections, three sections of the U-fold with different characteristics have been identified. Measurements on specimen 8 are presented in Table 4. Figure 7 presents the measurement protocol and a schematic view of these three sections called: "distal section," "midsection," and "corniculate flaps." All three sections are supported by cartilage. The distal section and midsection comprise the "arms" of the U-fold and are supported by the arytenoid cartilage. The corniculate flaps are supported by the corniculate cartilage. Note that the arytenoid and corniculate cartilages are fused to each other in the region just rostral to the midsection. Figure 8 presents the sagittal cross-sections of the distal section and midsection.

The distal section is situated at the caudal end of the U-fold and is supported by a caudal extension of the arytenoid called the vocal process. There, the tips of vocal processes are connected to each other by a thick interarytenoid ligament. The region, the bottom of the "U" of the U-fold, is positioned between the trachea and the LS. The tissues in this region are thick, loose and folded connective tissue. No muscle has been observed in this region during the dissection apart from the muscular cover of the LS. The abundance of connective tissue and lack of muscle suggests this region is very difficult to control during vibration. Further, it was not possible to adduct completely the arytenoid cartilages in this region using only manual movements in our specimens. Presumably, this means air can leak through the distal section between the lungs and the laryngeal sac even while the three sections are fully adducted. In order to seal the distal section, the arytenoids must be lifted dorsally against the cushion by relaxing the PCA muscle and tensing the ventral extension of the LCA.

The midsection of the U-fold is characterized by a very smooth and thin tissue cover on the dorsal and medial surfaces of the arytenoids. Ventrally, a thickened ridge with a rounded edge can be observed running longitudinally (rostro-caudally) along the length of this midsection. It can be opposed against a similar ridge of the other side, and thus they appear as a pair of lips. This region is located ventral to the cricoarytenoid joint and muscular process, at the junction between the arytenoid and the corniculate cartilages. Most of the muscles controlling the arytenoid movement on the cricoid (IA, LCA and PCA) are attached to this muscular process. It is therefore likely that the midsection can be controlled with fine accuracy and increased strength so that it can be adducted hermetically. Additionally, it is where the fused aryteno-cricoid cartilage starts to curve in an L shape and transition to its rostral extension, the corniculate cartilage. In the concavity of this L curve is a deep groove on the lateral aspect, and a muscle was observed lying within it. No muscle was observed between the cartilage and the tissue forming the ventral lips.

The corniculate flaps are situated on the corniculate cartilages at the rostral end of the U-fold. They are oriented in the sagittal plane and when opposed towards each other in the midline, they can close the airway leading to/from the trachea. They are long and thin compared to the distal and midsections of the U-fold, and, for some specimens, covered with small papillae. It is not clear whether this texture is present in the living whale. Perhaps the outer layer of mucosa has sloughed off, or maybe the papillae supported a thick mucus coating. The corniculate cartilage supporting the flaps is thinner and less rigid than the cartilage supporting the distal and midsections of the U-fold. The corniculate flaps can be adducted (IA tension), and likely abducted, but may only be closed hermetically when the epiglottis is lifted dorsally to oppose them. Humpback whale flaps are proportionally very short compared to those of fin, sei, and minke whales.

DISCUSSION

Measurements

Differences between rorqual species can be seen in the measurements in Table 3. In particular, there are variations in corniculate flaps size (larger length for sei, fin and minke whales, smaller length for humpback whales) and amount of medial cartilage extension into the midsection of the folds. These variations can affect the degree of control over vibrating surfaces, and could be the explanation for the different vocal behaviors observed for the various rorqual species. To sharpen the use of Table 3, two approaches can be considered. If measures are used to predict frequency or amplitude of sound produced, the absolute size presented in Table 3 is relevant (Frey and Riede, 2013). However, if measures are used to compare between species, ages or genders, then relative size is more important and would have to factor in the length (and ideally the body-mass) of the specimen (Fletcher 2004). Then, measures can be compared between individuals, once the yare size standardized. Nevertheless, a crude comparison based on scaled data shows that measures do not seem to scale only on animal length (ie. larger whale do not simply have larger measurements), neither between nor within species. This supports the individual signature hypothesis (Adam et al., 2013) and suggests that further analysis should be performed on the data before comparison.

Functional positions

Three functional positions are described in Figure 9 as: (1) the rest position, (2) the breathing (inhale/exhale) position and (3) the recirculation position.

(1) The rest position is likely to be the position that provides the best protection of the trachea and lungs, sealing them from the remainder of the laryngeal cavity and avoiding any water penetrating the respiratory tract. Sealing is performed initially by closure of the blowholes, the interlock of the epiglottis and the soft palate, and

opposition of the arytenoids against the cricoid cushion. Concerning the arytenoids, the rest position is likely to be obtained by relaxing the muscles. Adducted arytenoids (relaxation of the LCA) have their caudal tip lifted dorsally (relaxed PCA). This brings the adducted U-fold arms close to the cushion. In this position, the cushion plugs the gap between the U-fold arms. These interlocked tissues block the entrance to the LS and may also block the tracheal lumen.

(2) Breathing in mysticetes is an active, fast, and powerful event both for exhalation and inhalation. The laryngeal cavity is one of the narrowest portions of the respiratory tract and therefore has to withstand the highest flow velocity (Schoenfuss et al., 2014). As this is the shortest and straightest pathway between the blowhole and the lungs, we assume that there is laminar airflow through the nasal cavities and between the corniculate flaps. The U-fold arms have to be held abducted. The corniculate flaps are then maximally opened (with the flap tissues opposed laterally against the nasopharyngeal walls). The rest of the U-fold is pulled ventrally (PCA contraction) in order not to be damaged by the strong airflow through a maximally dilated trachea. This movement also collapses the vertical height of the laryngeal sac, thus preventing air from entering it. The above tissue positions would be adopted for both expiration and inspiration. It is unlikely that a healthy whale would produce sound when breathing because this would be counter-productive for the efficiency of the vital air exchange. Audible sounds would indicate that some tissue is obstructing the flow of air, thus reducing efficiency. After emptying the lungs during exhalation, and filling the lungs during inhalation, the whale likely expands the laryngeal sac. For this, the U-fold arms would remain abducted, but there would be a change in U-fold position so the caudal tip is elevated dorsally to accommodate expansion of the LS. This expansion will maximize the "reserve air space" after the breathing cycle (Schoenfuss et al., 2014) and be used to mitigate volume collapse due to increased ambient pressure acting on the respiratory tract at depth (Gandilhon et al., 2015).

(3) During a dive, whales need to optimize their use of the oxygen content in a single volume of air. Therefore, air recirculation or “rebreathing” allows air to be mixed and sent from the LS to the lungs. This allows extraction of oxygen from the extra air volume previously stored in the LS. The U-fold is likely to be elevated (mild PCA contraction) to be positioned coronally (parallel to the long axis), U-fold arms abducted (LCA abduction), and corniculate flaps adducted (IA contraction). This

position allows a large opening between the LS and trachea, but prevents airflow superiorly to the nasal cavities.

Egressive sound production

Several authors have proposed bidirectional airflow during phonation both in mysticetes generally and in humpback whales specifically (Reidenberg and Laitman, 2007a; Mercado et al., 2010, Mercado and Handel, 2012; Cazau et al., 2013; Adam et al., 2013). In this case, the air would flow through the different regions of the U-fold from the lungs to the LS (egressive) or from the LS to the lungs (ingressive). Different muscles would generate opposing flows, and produced sounds are presumed to have distinguishable characteristics. Schoenfuss and colleagues (2014), in their work on bowhead whales (a non-rorqual mysticete), propose that the sound production mechanism is solely ingressive, with the LS as principal air source and pressure regulator. Our observations on rorqual mysticetes revealed a clear dorso-ventral asymmetry of the midsection and distal section of the U-fold. This asymmetry makes ingressive flow in sound production anatomically unlikely (see Figs. 7 and 8). This is because the ventral position of the lips in the midsection and the loose tissues on the ventral side of the distal section (compared to the smooth dorsal side) would be flattened or even inverted with ingressive flow. This would thus act like a plug when the U-fold arms are adducted for phonation. Therefore, our observations support the unidirectional hypothesis, specifically that sound production only occurs during egressive airflow. As air flows past the projecting lips, they could vibrate and produce sound (see below). Perhaps ingressive airflow is a sound production mechanism unique to bowhead whales and possibly other non-rorqual mysticete species. Unidirectional sound production would explain the silence between sound units in humpback whale songs (Payne and McVay, 1971) likely occurring as air is directed back to the lungs after the LS is totally inflated. Further acoustical study on silences, preceding and following the different sound units, could give considerable information on this particular process.

Tracheal ridges

The observation made on the trachea (ventrally open C-shaped cartilages and folds perpendicular to the tracheal rings) is supported by previous publications (Reidenberg and Laitman, 2007a, 2013, 2014; Schoenfuss et al., 2014). An inflation of the LS or a high ambient pressure can cause tracheal collapse along the ventral midline membrane. In either scenario, two air passages are then conserved on each side of the trachea, albeit probably with less volume. These two passages direct airflow laterally around the dorsally placed cushion and its interlock with the ventrally placed U-fold. The tracheal ridges have been observed in many other cetacean species (Reidenberg and Laitman, 2013, 2014). It is possible that they help expand and strengthen the tracheal walls, and direct airflow towards the U-fold (Reidenberg and Laitman, 2013, 2014). They may also reduce airflow turbulence (Reidenberg and Laitman, 2013, 2014; Schoenfuss et al., 2014) and therefore increase airflow efficiency. As they extend rostrally to the midsection of the U-fold, they could also direct airflow towards this region when the cushion is obstructing the distal section of the U-fold.

Sound production from different regions of the U-fold

The U-fold is homologous to the VF of terrestrial mammals (Reidenberg and Laitman, 2007a), although there appears to be a major difference from the typical system for mammal frequency modulation. We consider two resonators, LS and NC, following the principles described in Adam, et al. (2013) and Cazau, et al. (2013).

The distal section of the U-fold is difficult to control and adduct completely. It is sealed by lifting the folds against the cushion. The egressive sound is produced through this section of the folds by lowering the caudal tip of the arytenoids in the coronal plane (while the corniculate flaps and the midsection are strongly adducted and therefore closed). No muscle capable of tensing the tissue coverings of the U-fold has been found in this region. The ventral mucosa could nevertheless be tensed when the LS is inflated, affecting the vibrating properties of the distal section. Air expelled from the lungs inflates the LS, and if the epiglottis and corniculates are open it will also flow up to the NC.

Although the NC is largely enclosed in the bone of the skull, the contiguous inferior portion (nasopharynx) has flexible walls bounded by mucosa covering pharyngeal muscles. This portion can expand or contract with volume changes. In addition, the air volume in the lumen of the NC may be regulated by vascularized tissue (similar to that found in the southern right whale, Buono, et al., 2015) that can inflate with blood to expand and fill the void created by collapsing air under pressure during diving. If air is shunted from the LS or lungs to the NC, the increased pressure may force blood out of these lining tissues, thus expanding the capacity of the NC.

The air-filled LS and NC can act as resonators, the LS resonant characteristics also being influenced by its inflation state. A low-frequency noisy source signal with many formants is expected, due to the thick and folded characteristic of the mucosa in this region producing a complicated and variable glottal pulse shape (broadband spectrum) coupled with LS and NC resonance. Because this low frequency sound requires a minimum airflow pressure (called Phonation Threshold Pressure in literature on human speech (Titze, 1992)), a lower pressure passing through the distal section is expected to produce only a blowing noise.

The midsection of the folds can be sealed by strong adduction of the arytenoids. It is likely to produce an egressive sound when the distal section is lifted against the cushion, the corniculate flaps are strongly adducted, and the midsection is adducted but not sealed. The epiglottis has to be pulled ventrally leaving the rostral opening unobstructed. A raised epiglottic position opposing the corniculate flaps would bring the ventral membrane of the laryngeal lumen too close to the midsection of the U-fold and would thus dampen the vibration of the lips ventrally. The midsection is thought to be the most controllable part of the U-fold. Muscles were found on the muscular process (IA, PCA and LCA) and in the L-curve of the arytenoid cartilage. This last muscle could have an impact on lip adduction by pulling directly on the cartilage supporting the lip. It may be the TA muscle, arising ventro-laterally from the thyroid cartilage and inserting medio-superiorly onto the lip. Nevertheless, no muscle was observed between the cartilage and the tissue forming the ventral lips and therefore, we hypothesize that the tissues cannot be stiffened (as in other mammals, where VF can be tensed by the TA). As the lips are resting on cartilage and not on a flexible surface (such as a vocal ligament), it is reasonable to think that they cannot be tensed either. Therefore, they must vibrate passively as air flows between the lips and pushes them apart. The degree of vibration can thus be controlled by regulating the gap between the lips. The different positions that the arytenoid can take affects lip positions and shapes to produce a range of frequencies. The initial vibration could be controlled by changing the spacing between the lips, the shape of the air channel, the length available to vibrate, and dampening of tissue movements.

A musical analogy can be considered based on guitar strings (Rossing, 2010). One can change the frequency of the string's vibration by rotating the tuning machine pegs on the guitar's head, thereby changing the tension of the string. The frequency can also be changed by pressing a string against the frets on the guitar neck, effectively shortening the string. Harmonics can be produced by vibrating and gently touching the string at specific locations that divide the vibrating length in fractions.

It is presumed that mysticetes do not change the tension of the U-shaped fold, but instead use the equivalent of guitar frets to change the length of fold. Dampening variable portions of the U-fold effectively shortens the length available for vibration and thus alters frequency. The length of vibrating tissue that can change ranges from a few cm to about 15 cm (maximum length of the ventral lip region, with the total length of one side of the U-fold being around 40 cm for adults, see Table 3). This assumes that the rest of the length that is left open is dampened by the cushion without changing the tension of the folds. Additionally, the abducted space between the arms of the U-fold will also have an impact on the vibration. Slight touching at specific locations along the vibrating U-fold arm or the lip could also produce harmonics. Figure 10 presents the movements that can adduct the lips with a different adducted length and change the shape of the channel for the airflow. The sound produced is likely to have a high diversity of frequency modulation and a clear glottal pulse shape producing clear harmonics coupled with LS and NC resonance.

Sound may also be produced through vibration of the corniculate flaps when the epiglottis is at least half lowered away from the flaps, and air is flowing from the lungs to the NC through nearly adducted corniculate cartilages. In this case, the arytenoids are lifted against the cushion and the air is flowing in the lateral tracheal channels. Air flowing rostrally can be stored in the expandable nasopharynx (and not the NC because it is a rigid space), and sent back to the LS through the ventro-rostral laryngeal aditus when the nasopharyngeal muscles contract this space. The major resonator in this system is the NC. The sound produced by the corniculate flaps is likely to be a periodic low frequency pulse. Humpback whale flaps are very short compared to those of fin, sei, and minke whales, implying a different frequency (probably higher) or degree of control (probably better) of the sound they produce with the corniculate flaps.

Modes

We have been describing different types of sounds and the regions of the U-fold that are likely to produce them. Therefore, it is now possible to define modes in the function of this acoustic generator, as it has been done for other mammals. The so-called “modes”, or “registers” in human singing, are obtained by changing the stiffness and the tension of the folds, allowing different thicknesses of the folds to vibrate during phonation (the higher the pitch, the smaller the thickness). In humans, changing mode has been proven to create frequency jumps or irregularities when performed by untrained singers (Roubeau et al., 2009). In the case of mysticetes, it is likely that the vocal tract has evolved into a more stable configuration. Vocal structures are separated from the primary laryngeal passageway for air during breathing, and soft tissues are directly attached to cartilages instead of a vocal ligament. These features help withstand the high airflow rate, great changes in internal pressure, and large displacements of tissues (Schoenfuss et al., 2014). Therefore, we hypothesize that if rorqual mysticetes are not able to change the stiffness and the tension of their folds, they can instead change the section of the U-fold that they will activate (thicker for the distal mucosal folds, thinner for the middle lips). Changing which section vibrates implies a change in the laryngeal configuration. This is likely to require experience for the singers to accomplish successfully, and thus may explain the occurrence of frequency jumps in humpback whale sound units - presumably being made by younger (inexperienced) singers. Additionally, while arytenoid adduction controls the frequency of the sounds produced, the power of the egressive airflow has an impact on the sound intensity (amplitude). Different configurations for sound emission can be described as: (1) blowing sound, when the power of the airflow is not sufficient for the tissue to vibrate, (2) pulsed sounds, when the airflow is low and the arytenoids are not strongly adducted, (3) vocalization, when the airflow and arytenoids adduction are adapted to the tissue vibration conditions, and (4) high pitch vocalization (or whistling by acoustical analogy) in the case of strong adduction and high airflow.

CONCLUSIONS

This work combines anatomy and acoustics in order to give a multidisciplinary approach to solving the mystery of rorqual sound production mechanisms. The various cavities, soft tissues, and cartilages are controlled by specific intrinsic laryngeal musculature. These produce different functional positions (rest, breathing, recirculation) for the arytenoid cartilages supporting the U-fold. The U-fold contains three regions (distal section, midsection, and corniculate flaps) that are responsible for generating different sounds (noisy for the distal section, tonal for the midsection, and pulsed for the corniculate flaps). Sound output is coupled to resonant cavities. There does not appear to be any mechanism for adjusting tension or stiffness of the U-fold. The sound frequency produced by the distal section and the corniculate flaps therefore is controlled only by adduction, while the sound frequency produced through the midsection is controlled by adjusting adduction and length of the vibrating surface. The sound production system of the rorqual whales is therefore fundamentally different from that of terrestrial mammals.

ACKNOWLEDGEMENT

Partial support for this work was provided by the Office of Naval Research, and the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) Prescott Stranding Grant. Specimens 1-14 were collected under MMHSRP's MMPA/ESA Enhancement and Scientific Research Permit NMFS Permit No. 932-1905/MA-009526, and the NMFS (NOAA Fisheries) Letter of Authorization (to possess samples from marine mammals for scientific study) to Dr. Joy S. Reidenberg. The authors wish to thank the following institutions for precious help in collecting samples: Foundation for Marine Research and Preservation (formerly Okeanos Ocean Research Foundation) in New York; of Marine Mammal Stranding Center in New Jersey; Mystic Aquarium in Connecticut; New England Aquarium in Massachusetts; International Fund for Animal Welfare (formerly Cape Cod Stranding Network) in Massachusetts; Maryland Department of Natural Resources in Maryland; University of North Carolina Wilmington in North Carolina; Cetamada, east coast of Madagascar. We also thank Anjara Saloma, Schédir Marchesseau, Jessica Delarbre and Magalie Chaigneau, members of Cetamada, for their work on specimen 15.

REFERENCES

Adam O, Cazau D, Gandilhon N, Fabre B, Laitman JT, Reidenberg JS. 2013. New acoustic model for humpback whale sound production. Applied Acoustics, 74(10), 1182-1190.

Aroyan JL, McDonald MA, Webb SC, Hildebrand JA, Clark D, Laitman JT, Reidenberg JS. 2000. Acoustic models of sound production and propagation. In Hearing by whales and dolphins (pp. 409-469). Springer New York.

Buono, MR, Fernández, MS, Fordyce, RE and Reidenberg, JS. 2015. Anatomy of nasal complex in the southern right whale, *Eubalaena australis* (Cetacea, Mysticeti). J. Anat., 226: 81–92.

Cazau D. 2012. Acoustics of the mysticete cetacean (baleen whale) vocal production system. Master's thesis, Université Pierre and Marie Curie, Paris, France.

Cazau D, Adam O, Laitman JT, Reidenberg JS. 2013. Understanding the intentional acoustic behavior of humpback whales: A production-based approach. The Journal of the Acoustical Society of America, 134(3), 2268-2273.

Cranford TW, Elsberry WR, Van Bonn WG, Jeffress JA, Chaplin MS, Blackwood DJ, Carder DA, Kamolnick T, Todd MA, Ridgway SH. 2011. Observation and analysis of sonar signal generation in the bottlenose dolphin (*Tursiops truncatus*): Evidence for two sonar sources. J. Exp Marine Bio Ecology 407:81-96.

Dickson D R, Maue-Dickson W. 1982. Anatomical and physiological bases of speech. Little Brown & Company.

Dunlop RA, Noad MJ, Cato DH, Stokes D. 2007. The social vocalization repertoire of east Australian migrating humpback whales (Megaptera novaeangliae). The Journal of the Acoustical Society of America, 122(5), 2893-2905.

Fant G. 1960. Acoustic theory of speech production. The Hague: Mouton.

Finnegan EM, Alipour F. 2009. Phonatory effects of supraglottic structures in excised canine larynges. Journal of Voice, 23(1), 51-61.

Fitch WT. 2006. Production of vocalizations in mammals. Visual Communication, 3, 145.

Fletcher NH. 2004. A simple frequency-scaling rule for animal communication. The Journal of the Acoustical Society of America 115(5), 2334-8.

Frey R, Riede T. 2013. The anatomy of vocal divergence in North American elk and European red deer. Journal of morphology 274.3, 307-319.

Gandilhon N, Adam O, Cazau D, Laitman JT, Reidenberg JS. 2015. Two new theoretical roles of the laryngeal sac of humpback whales. Marine Mammal Science, 31(2), 774-781.

Gray SD, Alipour F, Titze IR, Hammond TH. 2000. Biomechanical and histologic observations of vocal fold fibrous proteins. Annals of Otology, Rhinology & Laryngology, 109(1), 77-85.

Harrison DFN. 1995. The anatomy and physiology of the mammalian larynx. Cambridge University Press.

Henry RW, Haldiman JT, Albert TF, Henk WG, Abdelbaki YZ, Duffield DW. 1983. Gross anatomy of the respiratory system of the bowhead whale, Balaena mysticetus. The Anatomical Record, 207(3), 435-449.

Hosokawa H. 1950. On the Cetacean Larynx, with Special Remarks on the Laryngeal Sack of the Sei Whale and the Aryteno-Epiglottideal Tube of the Sperm Whale.

McDonald MA, Mesnick SL, Hildebrand JA. 2006. Biogeographic characterization of blue whale song worldwide: using song to identify populations. Journal of cetacean research and management 8.1.

Mercado III E, Schneider JN, Pack AA, Herman LM. 2010. Sound production by singing humpback whales. The Journal of the Acoustical Society of America, 127(4), 2678-2691.

Mercado III E, Handel S. 2012. Understanding the structure of humpback whale songs (L). The Journal of the Acoustical Society of America, 132(5), 2947-2950.

Miksis-Olds JL, Buck JR, Noad MJ, Cato DH, Stokes MD. 2008. Information theory analysis of Australian humpback whale song. The Journal of the Acoustical Society of America, 124(4), 2385-2393.

Payne RS, McVay S. 1971. Songs of humpback whales. Science, 173(3997), 585-597.

Quayle CJ. 1991. A dissection of the larynx of a humpback whale calf with a review of its functional morphology. Memoirs of the Queensland Museum 30.2: 351-354.

Reeb D, Best PB. 1999. Anatomy of the laryngeal apparatus of the pygmy right whale, Caperea marginata (Gray 1846). Journal of morphology, 242(1), 67-81.

Reidenberg JS, Laitman JT. 2007a. Discovery of a low frequency sound source in Mysticeti (baleen whales): anatomical establishment of a vocal fold homolog. The Anatomical Record, 290(6), 745-759.

Reidenberg JS, Laitman JT. 2007b. Blowing bubbles: an aquatic adaptation that risks protection of the respiratory tract in humpback whales (Megaptera novaeangliae). The Anatomical Record, 290(6), 569-580.

Reidenberg JS, Laitman JT. 2008. Sisters of the sinuses: cetacean air sacs. The Anatomical Record, 291(11), 1389-1396.

Reidenberg JS, Laitman JT. 2013. Not "The Lord of the Rings:" The Inside Story of the Trachea in Cetacea. 20th Biennial Conference on the Biology of Marine Mammals, Dunedin, New Zealand, Dec.

Reidenberg JS., Laitman JT. 2014. Trachea in Cetacea: the inside story. American Association of Anatomists: Experimental Biology, FASEB, San Diego, April.

Riede T, Lingle S, Hunter EJ, Titze IR. 2010. Cervids with different vocal behavior demonstrate different viscoelastic properties of their vocal folds. Journal of morphology, 271(1), 1-11.

Riede T, York A, Furst S, Müller R, Seelecke S. 2011. Elasticity and stress relaxation of a very small vocal fold. Journal of biomechanics, 44(10), 1936-1940.

Rossing TD. 2010. The Science of String Instruments. Springer-Verlag New-York, 470.

Roubeau B, Henrich N, Castellengo M. 2009. Laryngeal Vibratory Mechanisms: The Notion of Vocal Register Revisited. J. of Voice, 23, 425-438.

Samaran F, Gandilhon N, Doh Y, Pace F, Cazau D, Laplanche C, Lopatka M, Glotin H, White P, Zarzycki J, Motsch JF, Adam O. 2013. Detection, Classification, Localization of Marine Mammals using passive acoustics. 2003-2013 : 10 years of internayional research. Chapter 12 : Inside the sounds emitted by some cetacean species. The Bioacoustics Team.

Schoenfuss HL, Bragulla HH, Schumacher J, Henk WG, Craig George J, Hillmann DJ. 2014. The anatomy of the larynx of the bowhead whale, Balaena mysticetus, and its sound-producing functions. The Anatomical Record, 297(7), 1316-1330.

Taylor AM, Reby D. 2010. The contribution of source-filter theory to mammal vocal communication research. J. of Zoology, 280, 221-236

Titze IR. 1988. A framework for the study of vocal registers. Journal of Voice, 2(3), 183-194

Titze IR. 1992. Phonation threshold pressure: a missing link in glottal aerodynamics. The Journal of the Acoustical Society of America 91.5 (1992): 2926-2935.

Titze IR, Fitch WT, Hunter EJ, Alipour F, Montequin D, Armstrong DL, McGee J, Walsh EJ. 2010. Vocal power and pressure–flow relationships in excised tiger larynges. Journal of Experimental Biology, 213(22), 3866-3873.

Van den Berg J. 1958. Myoelastic-aerodynamic theory of voice production. Journal of Speech, Language, and Hearing Research 1.3, 227-244.

Zimmer WMX. 2011. Passive acoustic monitoring of cetaceans. Cambridge University Press.