

RESEARCH ARTICLE

Laryngeal and soft palate valving in the harbour seal (*Phoca vitulina*)

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ABSTRACT

Effective ‘valving’ in the upper aerodigestive tract (UAT) is essential to temporarily separate the digestive and respiratory pathways. Marine mammals are largely dedicated to feeding underwater, and in many cases swallowing prey whole. In seals, little work has been done to explore the anatomy and function of the UAT in the context of valving mechanisms that function to separate food and air pathways. Here we use videofluoroscopy, gross dissection, histology and computed tomography (CT) renderings to explore the anatomy of the larynx and soft palate in the harbour seal (*Phoca vitulina*), and generate models for how valving mechanisms in the head and neck may function during breathing, phonating, diving and swallowing. Harbour seals have an elevated larynx and the epiglottis may rise above the level of the soft palate, particularly in pups when sucking. In addition, the corniculate and arytenoid cartilages with associated muscles form most of the lateral margins of the laryngeal inlet and vestibule, and move independently to facilitate airway closure. The corniculate cartilages flex over the laryngeal inlet beneath the epiglottis to completely close the laryngeal vestibule and inlet. The vocal folds are thick and muscular and the medial margin of the folds contains a small vocal ligament. The soft palate has well-defined levator veli palatini muscles that probably function to elevate the palate and close the pharyngeal isthmus during feeding. Our results support the conclusion that harbour seals have evolved UAT valving mechanisms as adaptations to a marine environment that are not seen in terrestrial carnivores.

KEY WORDS: Airway, Seal, Larynx, Marine mammal, Palate, Swallowing

INTRODUCTION

The upper aerodigestive tract (UAT) in mammals consists of the nasal cavities, oral cavity, larynx, pharynx and cervical parts of the trachea and esophagus. The larynx is a highly modified part of the airway that develops at the proximal end of the laryngotracheal diverticulum where it diverges from the gut tube during development (Frazer, 1910). In mammals, the UAT performs a dual purpose supporting oral alimentation as well as breathing. As a

result, one of the primary functions of the larynx is to act as a valve that can close the lower airway and prevent food and liquid from entering the trachea and lungs. The larynx works in conjunction with the soft palate and tongue to efficiently enable the safe transport of food and liquid from the oral cavity and into the esophagus, while allowing air from the entrance of the airway (nasal and oral cavities) to move through the pharynx and into either the esophagus or the lower airway, respectively. Common ‘valving’ structures in the larynx that support the safe execution of these functions include the epiglottis, vocal folds and musculature of the supraglottis. The epiglottis moves passively to cover the laryngeal inlet, and the vocal folds and related soft tissues move laterally to open and medially close the rima glottidis and laryngeal vestibule. Both the soft palate and the larynx are re-positioned during swallowing to guide food and liquid into the esophagus (Matsuo and Palmer, 2008). Changes in position and pressure of the tongue, hyolaryngeal complex and pharyngeal walls also facilitate directing boluses of food and liquid into the esophagus.

When compared with other mammals, marine mammals face unique challenges when swallowing and breathing as they are largely dedicated to feeding underwater, often swallowing their prey whole, and are intermittent breathers. Probably the most extreme example of laryngeal adaptation in marine mammals is in odontocete cetaceans (Harrison, 1972). In these animals, the upper end of the larynx is ‘telescoped’ into the nasopharynx and held in position by a muscular palatopharyngeal sphincter (Reidenberg and Laitman, 1987), thereby completely separating the airway from the digestive tract. This modification creates a dedicated pathway from the upper airway to the lower airway, making toothed whales dedicated ‘nasal’ breathers. (Green et al., 1980; Reidenberg and Laitman, 1987). During swallowing, food moves around the larynx and into the esophagus. In mystecete cetaceans, elongated corniculate and epiglottic cartilages are also considered to project into the nasopharynx when breathing (Schoenfuss et al., 2014).

Adaptations to the UAT have also been observed in some seals (Pinnipedia). In sea lions (Otariidae), the epiglottis is short, unusually large arytenoid cartilages form prominent valves across the vestibule of the larynx, and the vocal folds are oriented more parallel to the airway than in phocid seals and terrestrial mammals (Reidenberg and Laitman, 2018). When considering adaptations in phocid seals (Phocidae), most studies on the respiratory tract and larynx have been done in connection with redistribution of and changes in volume of air during a dive (Fahlman et al., 2017) and sound production, particularly with reference to pup calls and recognition by mothers (Sauv et al., 2015; Stansbury and Janik, 2019). Only a few studies have explored airway anatomy as it relates to valving mechanisms in the UAT, and of these, most do not describe any remarkable differences between phocid seals and other mammals (Schneider, 1962; Ridgway, 1972; Reidenberg and Laitman, 2018). A study on Ross seals (*Ommatophoca rossi*)

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extensively describes the anatomy of the larynx and soft palate, but draws no connection between anatomical adaptation and valving (King, 1969). However, there is at least one study that mentions the possibility of the larynx being relatively elevated in Weddell seals (*Leptonychotes weddellii*) compared with other mammals, and further indicates that the epiglottis may actually be positioned above the soft palate (Piérard, 1969). This suggests that there may be additional features of the UAT related to valving mechanisms in other seals that remain unappreciated.

In this study, we explore the hypothesis that the larynx and soft palate in harbour seals have structural modifications that enable efficient separation of the respiratory and digestive pathways when swallowing and breathing in a marine habitat. We describe several novel and previously undescribed anatomical features of the larynx and associated structures, and we graphically illustrate how these structural adaptations are likely to participate in separating the digestive and respiratory pathways, and contribute to both sound production and airway protection during diving.

MATERIALS AND METHODS

All specimens used in this study were harbour seal pups, juveniles and adults (*Phoca vitulina richardii* Gray 1864) from local populations along the British Columbia lower mainland coastline. All seal pups and juveniles were reported abandoned by the public, Fisheries Officers, and/or biologists. After initial monitoring to assess their activity, hydration and potential for return to the dams, the animals were recovered and presented to the Vancouver Aquarium Marine Mammal Rescue Centre (MMR) for rehabilitation. Five approaches were used to investigate the structure and function of the UAT of the harbour seal: videofluoroscopy, dissection of gross specimens, histology, review of archived samples, and computed tomography (CT) scans. No animals were euthanized for the purpose of this study. All harbour seal specimens handled in this study were done under a Licence for the Transfer and Release of Marine Mammals and a Letter of Authorization from the Department of Fisheries and Oceans (DFO) Canada, in compliance with the Fisheries Act of Canada.

Videofluoroscopy

Freely behaving videofluoroscopy was completed on juvenile seals ($N=2$) while in rehabilitation using a protocol published previously (Skoretz et al., 2019). A Siemens mobile C-arm fluoroscopy unit and retrofitted tub were utilized with lossless image capture at 30 frames s^{-1} . Following their rehabilitation, these seals were successfully released back to the wild. Ethics approval was obtained in order to perform this imaging from the University of British Columbia's Animal Care Committee (A18-0252).

Dissection

Seal pup ($N=4$) and adult ($N=2$) specimens were utilized for gross dissection. The tissues obtained from pups were of those that died of natural causes in rehabilitation or were euthanized after reaching a humane endpoint as determined by the Vancouver Aquarium veterinary staff. Euthanasia was carried out by intravenous injections of sodium pentobarbital under the supervision of the veterinary staff at the Vancouver Aquarium. Fresh dead adult seals ($N=2$) were reported deceased and collected as part of the British Columbia Marine Mammal Response Program. All animals (pups and adults) were presented to the Animal Health Center for necropsy.

Most samples were fixed during necropsy for later evaluation, although two of the pup samples were dissected 'fresh'. The heads

of adult specimens ($N=2$) were removed at the atlanto-occipital joint, sagittally sectioned, then both halves were placed in 10% neutral buffered formalin at a ratio of 1:10 (v/v) tissue to fixative prior to dissection. For seal pups ($N=4$) the 'pluck' was excised and either dissected immediately ($N=2$) or placed as straight as possible in a large rectangular plastic container with 1:10 formalin to approximate *in vivo* orientation for later dissection ($N=2$).

Histology

During necropsy, UAT tissues from seal pups ($N=4$) were excised for histological analyses. The larynx and associated structures were fixed in buffered formalin. Serial transverse and longitudinal tissue blocks, which incorporated the oropharynx, segments of the nasopharynx and larynx, were prepared. Tissues were placed in cassettes and processed by conventional histological techniques through a graded series of alcohols, embedded in paraffin and sectioned at 5 μm . Tissue sections were stained by hematoxylin and eosin for initial histological evaluation, and representative tissues were further evaluated by trichrome stain to differentiate between muscle and collagen-based structures. Sections were imaged using a Panoramic MIDI (3DHistech) slide scanner.

Archived samples, CT and three-dimensional modeling

The bony landmarks related to the musculature of the soft palate were identified using skulls from the Beaty Biodiversity Museum (University of British Columbia). These skull samples ($N=2$ adult and $N=2$ pup) were from the archived collection. To create a three-dimensional (3D) seal skull model, CT scans of a live harbour seal were used (live juvenile $N=1$). The CT scans were provided courtesy of the Vancouver Aquarium. They were conducted by Vancouver Aquarium veterinary staff as part of the medical care for this seal. A helical scan was performed with a 64-slice Toshiba Aquilion using 135 kV peak and 250 mA. Axial (4 mm) and volumetric (0.5 mm) images were reconstructed using both bone and soft tissue algorithms. 3D rendering of the CT scans to generate the seal skull models was done using 3D Slicer software (NIH; <https://www.slicer.org/>) (Fedorov et al., 2012).

RESULTS

Structures superior to the arytenoids 'flex' to facilitate closing the laryngeal inlet

Videofluoroscopic analysis on harbour seals revealed the presence of prominent 'prong-like' structures on the posterior aspect of the larynx (Fig. 1). The structures projected forward or anteriorly, creating the appearance of a small depression on its posterior aspect that probably defines the position of the laryngopharynx (Fig. 1A). When the animals were presented with a fish, the prong-like structures began to flex anteriorly while independently, the vocal folds began to move towards the midline (Fig. 1B). The independent movement of these two 'valves' concludes upon mutual contact at what would be the laryngeal inlet, resulting in closure of the vestibule and inlet (Fig. 1C).

Dissections and histology

Larynx

In order to identify components of the 'prongs' and other possible anatomical and functional adaptations, we carried out several dissections in seals, focused on the UAT. A number of distinct anatomical features of the larynx were evident. The thyroid cartilage is thin and V-shaped (Fig. 2A), while the arch of the cricoid is very broad ventrally. Dissection of isolated seal pup larynges with the soft palate intact reveals that the laryngeal apparatus is high,

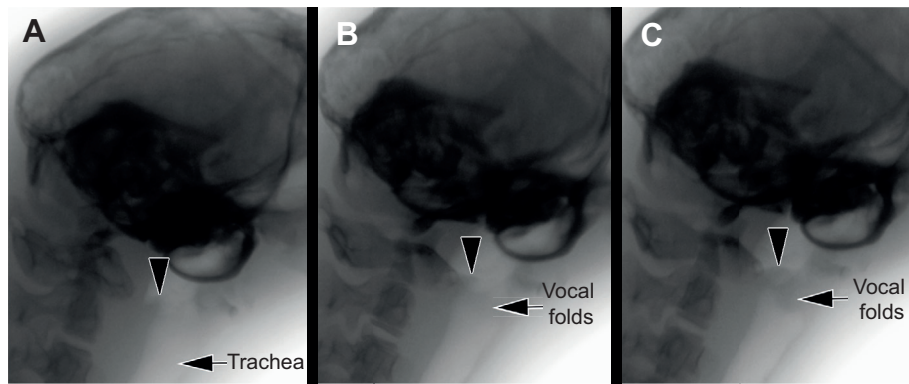


Fig. 1. A structure on the posterior wall of the larynx flexes over the laryngeal inlet during videofluoroscopy. (A) Videofluoroscopic still image of an adult seal showing a lateral view of the head and neck, focusing on the area containing the airway. A protruding structure (black arrowhead) can be seen projecting anteriorly and superiorly from the area of the laryngopharynx. (B) Still image of the same seal being presented with a fish. The tip of the protrusion (black arrowhead) begins to move anteriorly while an increase in density in the area of the vocal folds indicates that the vocal folds are beginning to meet in the midline. (C) The protruding structure (black arrowhead) has moved over the laryngeal inlet, overlapping with the posterior aspect of the vocal folds as they finish closing in the midline.

allowing the apex of the triangular-shaped epiglottis to rise over the level of the soft palate (Fig. 2B,C). In two of the four pups dissected, the apex of the epiglottis was positioned above the soft palate (see Fig. 2B,C).

On opening the pharynx posteriorly, the most obvious modification to the margin of the laryngeal inlet on each side was the presence of a large arytenoid that projected forward to define the more posterior aspects of the laryngeal inlet (Fig. 2C,D). Above each arytenoid was a prominent swelling that we interpret as the

structure seen in our videofluoroscopic analysis, and that we tentatively identify as a corniculate cartilage with overlying mucosa (Fig. 2E,F). The arytenoid and corniculate cartilages together form prong-like projections directed forward to form the posterior aspects of the lateral margins of the laryngeal inlet. Other notable features identified in the dissections were the lack of laterally projecting ventricles from the laryngeal cavity and the absence of obvious vestibular folds. The vocal folds were very prominent as broad bands on the lateral walls (Fig. 2E,F).

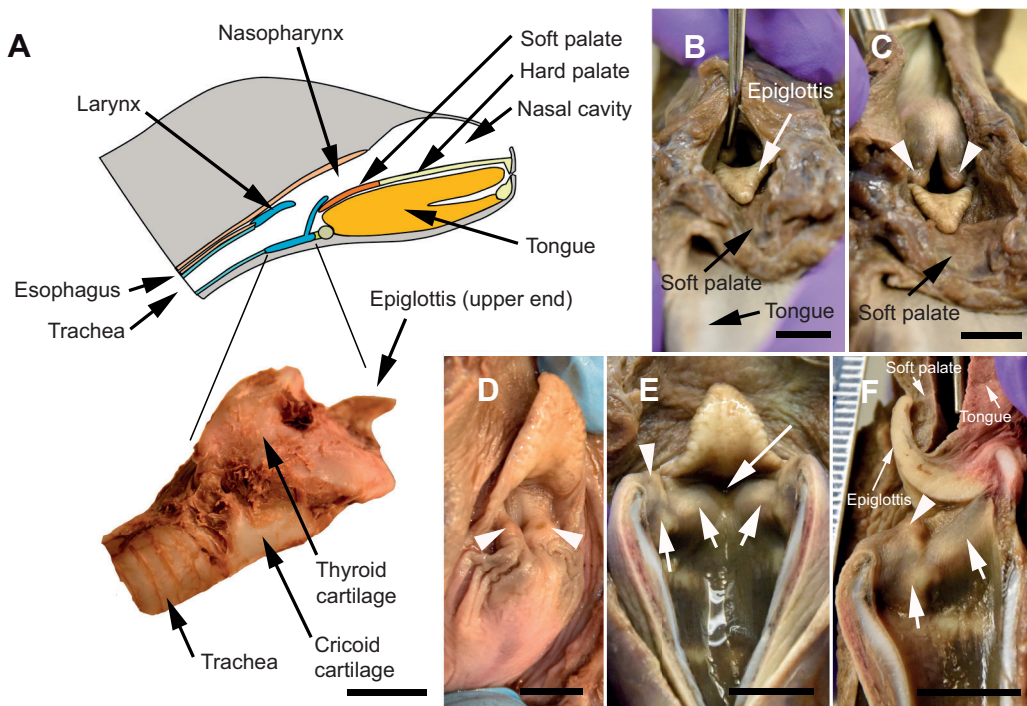


Fig. 2. The projections seen in the videofluoroscopy appear to be corniculate cartilages. Dissections of formalin-fixed seal pup larynges. (A) Isolated seal pup larynx with intact trachea, showing the thyroid and cricoid cartilages. (B,C) Isolated seal pup larynx with soft palate and tongue attached. Note that in this specimen, the epiglottis is positioned above the soft palate (B). Opening the posterior pharyngeal wall reveals two 'prong-like' structures (C; white arrowheads) forming the lateral margins of the laryngeal inlet. (D) Dorsolateral view of the 'prong-like' structures (white arrowheads), the tips of which we identify as corniculate cartilages. (E) Dorsal view of an open laryngeal vestibule showing the arytenoid cartilages and the corniculates (white arrowhead) that cap the top of the arytenoids. Thick muscular folds are visible running from the arytenoids to the apex of the thyroid cartilage. (F) Sagittal hemi-section of an isolated larynx showing the dorsally located arytenoid cartilage, corniculate cartilage (white arrowhead) and a muscular bulge running from this to the apex of the thyroid cartilage. All scale bars are 10 mm.

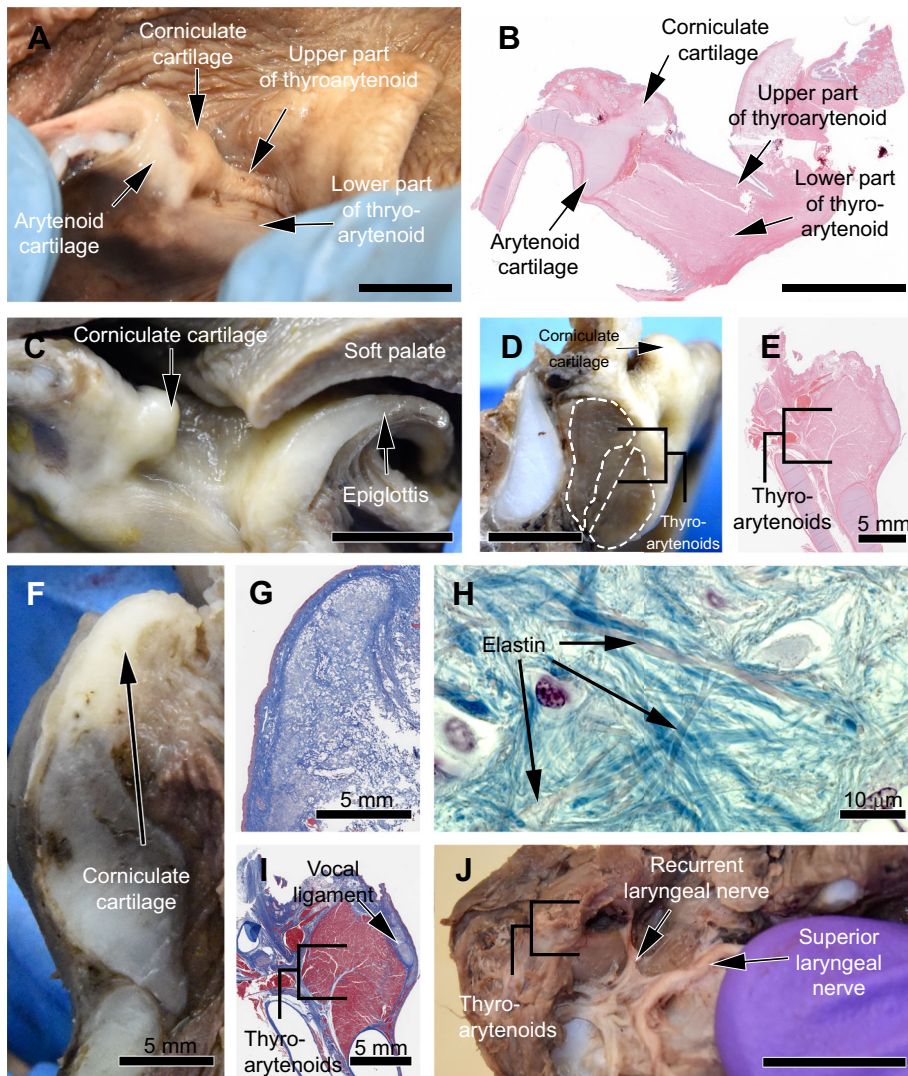


Fig. 3. The upper part of the thyroarytenoid inserts onto the corniculate cartilage, which is composed of fibro-elastic cartilage, and the thyroarytenoid is innervated by the recurrent laryngeal nerve. Dissections and histology of both pup and adult seal laryngeal structures, showing the arytenoids and corniculates with associated musculature. (A) Medial view of the laryngeal vestibule of a seal pup showing the angle of the arytenoid on the cricoid lamina, and parts of the thyroarytenoid muscle. (B) Hematoxylin and eosin-stained section of the laryngeal cartilages and associated upper and lower parts of the thyroarytenoid muscle showing their origins on the apex of the thyroid to their insertions on the corniculate and arytenoid cartilages, respectively. (C) Adult seal larynx showing the same arrangement of cartilage and muscle as seen in the seal pup. (D) Transverse dissection of the thyroarytenoid muscles in adult seal larynx further demonstrates their relative course. (E) Hematoxylin and eosin-stained transverse sections of the thyroarytenoids showing their relative size and relation to the laryngeal cartilages. (F) Transverse dissection through the arytenoid and corniculate cartilages in an adult seal larynx shows that the corniculate cartilage contributes to the tip of the 'prong-like' structure. (G) Trichome-stained histological section of the same tissue shown in panel F, demonstrating the fibro-elastic cartilage composition. (H) High magnification view of panel G showing the grey elastin components (arrows) and the collagen components (blue) of the fibro-elastic corniculate cartilage. (I) Trichome-stained cross-sections of the thyroarytenoid muscles showing a vocal ligament running on their medial border of the thyroarytenoids. (J) Dissection of a seal pup larynx showing the course of the superior and recurrent laryngeal nerves. The recurrent laryngeal nerve enters the laryngeal vestibule between the two parts of the thyroarytenoid. Scale bars are 10 mm unless otherwise indicated.

On closer examination, each arytenoid cartilage was situated on the dorsal lamina of the cricoid and was angled forward towards the anterior aspect of the larynx (Fig. 3A). The positioning of the arytenoid was obvious in sagittal histological sections of the laryngeal cartilages (Fig. 3B). A rounded corniculate cartilage was present projecting forward from the arytenoid cartilage (Fig. 3B, C). Histological sections also revealed that the medially projecting swelling on each lateral wall of the laryngeal cavity, and which we identified in dissections as the 'vocal fold', was actually formed by two muscles, both originating from the thyroid cartilage. The more inferior or caudal muscle inserted into the arytenoid, and the more superior or cranial part inserted into the corniculate cartilage (Fig. 3B). Based on their origin and insertion we believe these two muscles probably both comprise parts of the thyroarytenoid muscle. The presence of these muscles was confirmed in dissections of adult seal larynges (Fig. 3C,D). In cross-section, it was clear that the two parts of the thyroarytenoid were roughly equal in size and were situated mostly parallel to one another with the upper part slightly folded medially over the lower part (Fig. 3D). Hematoxylin and eosin-stained cross-sections of the thyroarytenoid and associated cartilages confirmed the relatively equivalent sizes of the upper and lower parts of the muscle (Fig. 3E).

Sagittal cuts through the arytenoid and corniculate cartilages showed that it is the corniculates that contribute to forming the rounded shape of the 'prong-like' structures that flex forward at the laryngeal inlet (Fig. 3F). Trichome-stained histological sections showed that each corniculate cartilage is largely composed of fibro-elastic cartilage (Fig. 3G,H). Interestingly, the sections also revealed a small vocal ligament running parallel and medial to the thyroarytenoid muscles in the area between the two muscles (Fig. 3I).

Two prominent nerves entered the laryngeal walls. One nerve, the superior laryngeal nerve, coursed ventral to the hyoid and dorsal to the thyroid cartilage and branched under the mucosa of the lateral laryngeal wall (Fig. 3J). The other nerve, the recurrent laryngeal nerve, travelled anteriorly with the trachea, passed over the cricoid cartilage and then coursed into soft tissues of the larynx. A branch of this nerve passed between the upper and lower parts of the thyroarytenoid and innervated both parts (Fig. 3J).

Anatomy of structures surrounding the laryngeal inlet were examined in unfixed seal pup larynges to better understand the possible range of movements of the arytenoid and corniculate cartilages (Fig. 4A,B). A range of positions for these cartilages were apparent, from those in which the airway was open to those where the vocal folds, laryngeal vestibule and inlet were closed. In the

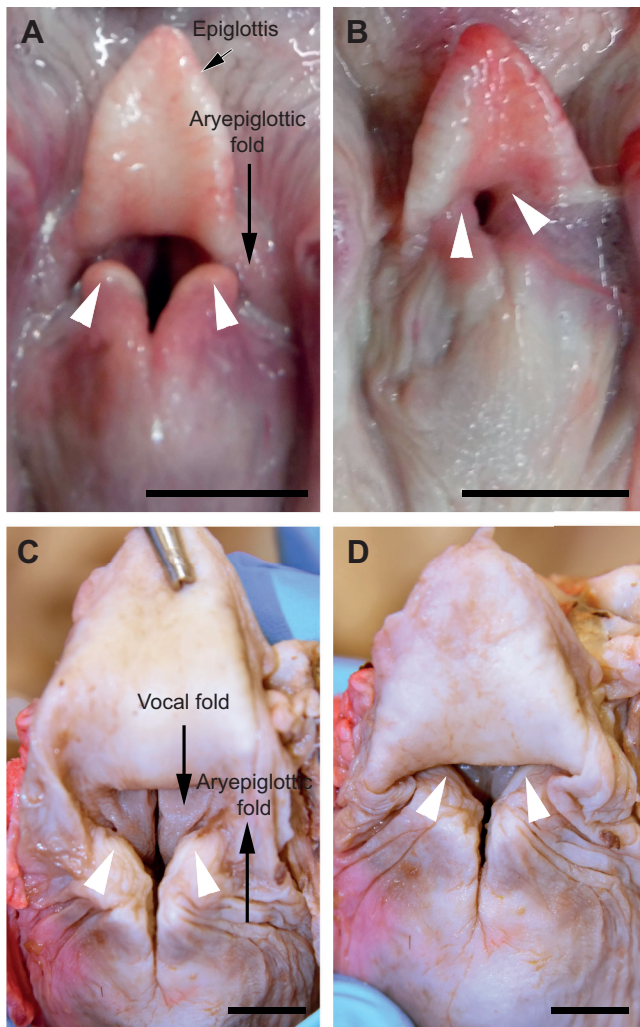


Fig. 4. The corniculate cartilages can be either in the ‘open’ or ‘closed’ position in both pups and adults. Isolated unfixed seal pup larynxes and a fixed adult seal larynx viewed dorsally. (A,B) Seal pup larynxes with the arytenoid and corniculate cartilages (white arrows) shown in both ‘open’ (A) and more ‘closed’ (B) positions. (C,D) Fixed adult seal larynx showing the ‘open’ (C) and more ‘closed’ (D) positions of the arytenoid and corniculate cartilages. The muscular vocal fold meeting together in the midline is also shown. All scale bars are 20 mm.

relaxed or ‘open’ positions, the prong-like structures containing the corniculates were several millimeters away from the epiglottis, leaving the laryngeal inlet open, and the vocal folds were separated deeper in the laryngeal cavity (Fig. 4A). In the flexed or ‘closed’ position, the corniculate-containing prongs were bent forward over the laryngeal inlet and were in close apposition to the base of the epiglottis near the midline (Fig. 4B).

Dissections of fixed adult seal larynxes revealed a similar range of ‘open’ and ‘closed’ positions seen in the unfixed pup larynxes (Fig. 4C,D). An important observation in the adult seal larynxes was that the vocal folds could be approximated together in the midline while the laryngeal inlet could remain open (Fig. 4C). The prongs containing the corniculates appeared to move independently of the arytenoids attached to the vocal folds.

Soft palate

The bony structures that support the soft palate were identified in isolated adult seal skulls (Fig. 5A). The hard palate was

characterized by a noticeable V-shaped defect caudally (Fig. 5A). A prominent pterygoid process was absent from the sphenoid bone, and the scaphoid fossa (origin for tensor veli palatini) was long, narrow and shallow, and it extended caudally from the pterygoid hamulus to the end of the sphenoid positioned just rostral and medial to the otic bulla. The pterygoid hamulus was short and blunt (Fig. 5A). In a 3D reconstruction from a CT scan of a live harbour seal pup, the pterygoid hamulus had more defined edges, suggesting the hamulus was probably damaged in the isolated skulls (Fig. 5B).

In sagittal hemi-section of an adult harbour seal head, the soft palate was in a horizontal position, and filled and projected posteriorly from the triangular-shaped gap seen in the hard palate. Its free posterior base or margin slopes downwards into the pharynx, lacks an uvula and is closely approximated to the top of epiglottis and back of the tongue (Fig. 5C).

When the mucosa was removed from the dorsal surface of the soft palate and the lateral wall of the nasopharynx, the major muscles of the soft palate were revealed (Fig. 5D). A small tensor veli palatini muscle was present and originated from the scaphoid fossa of the sphenoid bone. The muscle fibers were oriented horizontally and course rostrally. Its tendon looped from lateral to medial around the pterygoid hamulus and expanded to form the palatine aponeurosis. A large triangular-shaped levator veli palatini muscle originated from a focal attachment on the rostral border of the otic bulla. The muscle expanded as it coursed medially to insert via a broad attachment into the upper surface of the soft palate. Ventral and rostral to the soft palate, on the lateral walls of the oropharynx, prominent palatine tonsils occur in well-defined crypts between the palatopharyngeal and palatoglossal folds (Fig. 5E) overlying the same named muscles, respectively. The four muscles of the soft palate are shown in Fig. 6.

DISCUSSION

The UAT of marine mammals exhibits numerous adaptations for preventing water entry into the airway when breathing at the surface, diving and feeding while submerged. In cetaceans, these adaptations include the ability to open and close the nares or blowholes in general (Maust-Mohl et al., 2019), with more specific adaptation including: the presence of ‘nasal plugs’ in rorqual whales (Gil et al., 2020) and the telescoping of the larynx into the nasopharynx in odontocete whales (Reidenberg and Laitman, 1987). Here we report a number of structural modifications to the UAT in harbour seals that are consistent with the presence of efficient valving mechanisms for breathing at the surface and protecting the airway when diving and feeding. We have confirmed the general morphology and arrangement of the major laryngeal cartilages described in detail by others (Schneider, 1962; Piérard 1969; Ridgway, 1972). However, none of these studies describe the position of the corniculates as protruding over the laryngeal inlet. Furthermore, we show that the corniculate cartilages can flex forward to nearly approximate the base of the epiglottis and facilitate closure of the laryngeal inlet as a protective mechanism. We also provide evidence that the epiglottis may rise above the soft palate and position the laryngeal inlet in the nasopharynx to facilitate breathing, particularly in pups.

The most striking feature of laryngeal anatomy in harbour seals is the presence of forward-projecting ‘prong-shaped’ structures in the margins of the laryngeal inlet when the larynx is viewed looking down at the superior opening. Structural support for the prongs are fibro-elastic corniculate cartilages that sit upon the underlying arytenoid cartilages which in turn are angled somewhat forward on the cricoid. This anatomical arrangement starkly contrasts with that

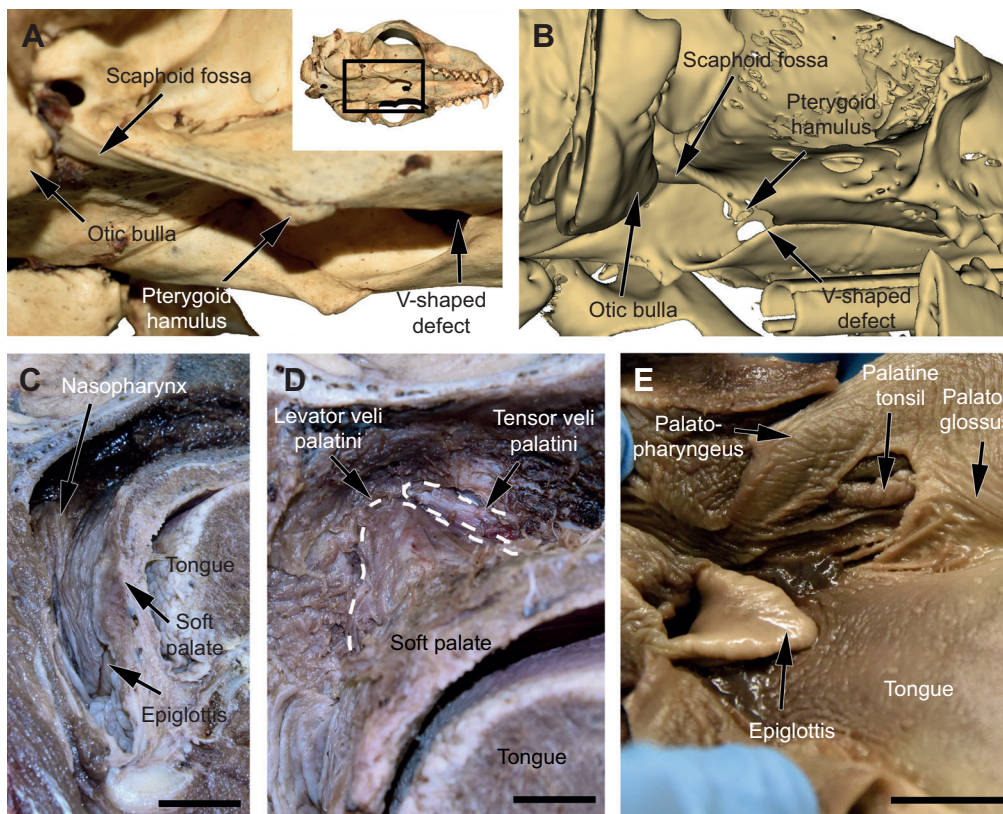


Fig. 5. Bony and soft tissue structures related to various aspects of the soft palate.

(A) Museum skull of an adult harbour seal showing the origins of the relevant soft palate musculature. A low magnification insert shows the general orientation of the skull with the region of interest outlined by the black line.

(B) 3D reconstruction from CT data of an adult harbour seal, showing similar bony architecture to the isolated skull, although the pterygoid hamuli appear more defined and intact.

(C) Sagittal dissection of an adult seal head showing the soft palate and its relationship to the epiglottis.

(D) Magnified view of the area shown in panel C, but with the mucosa removed from the superior surface of the soft palate to reveal the levator and tensor veli palatini muscles. (E) View of the lateral wall of the oropharynx of a seal pup showing prominent palatine tonsils between the palatoglossal and palatopharyngeal folds. All scale bars are 10 mm.

seen in terrestrial carnivores where the arytenoid and corniculate cartilages are often vastly under-developed compared with herbivores (Harrison, 1995). When present in carnivores, the cartilages are small and usually oriented posteriorly, such as the arrangement seen in dogs (Thrall, 2018). Videofluoroscopy revealed that the ‘prongs’ in the harbour seal larynx are able to bend anteriorly to close the laryngeal inlet, particularly when animals are presented with food and/or in the feeding context without direct presentation. Because this movement occurs during these conditions and is concurrent with vocal fold approximation, it is probably a reflexive response that aids in closing the airway before a swallow.

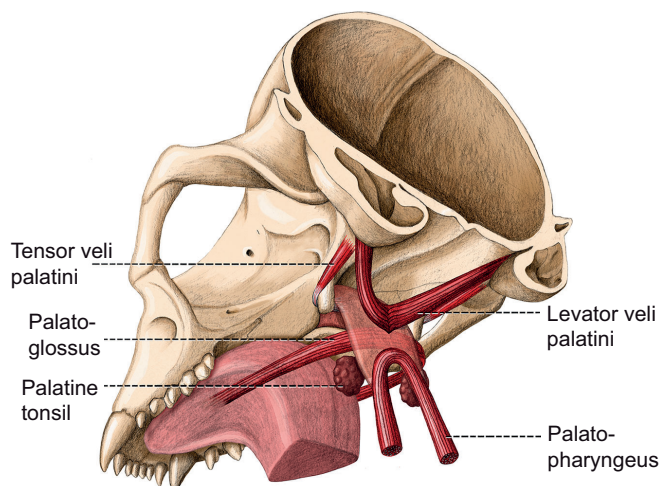


Fig. 6. Summary diagram of the seal palate musculature with relevant structures labelled. © 2019. Alex Boersma.

In many mammals, the thyroarytenoid is a singular muscle, whereas in harbour seals the muscle is separated into inferior and superior parts. The movements of the arytenoid and corniculate cartilages appear to be generated by these two parts. The more inferior or caudal of the two muscles forms a prominent bulge on the lateral wall of the laryngeal cavity and extends from the thyroid cartilage anteriorly, to the arytenoid posteriorly, and is likely to be the muscle that is mainly responsible for opposing the vocal folds and closing the glottis. Meanwhile, the superior muscle inserts into the corniculate cartilage and probably flexes the corniculate-containing ‘prongs’ to facilitate closing the laryngeal inlet. As would be predicted by homology, a branch of the recurrent laryngeal nerve courses between the two muscles and innervates both.

Our imaging indicates that vocal fold movements occur independently of the corniculates, and it is likely that the vocal folds produce sounds in these animals. During videofluoroscopy and dissections, we observed vocal fold approximation without closure of the laryngeal inlet. Moreover, during dissection, different insertions of the thyroarytenoid muscle into the arytenoids and corniculates were identified. The presence of a more rudimentary vocal ligament and the absence of an associated vocalis muscle in harbour seals is similar to Weddell seals, in which the vocal ligament is described as unremarkable, inextensible and lacks any obvious muscular insertions (Piérard, 1969).

A considerable body of work has investigated harbour seal vocalization and behavior. Mothers recognize their own pups’ calls even as the pups age (Sauvé et al., 2015), and some seals have been trained to mimic unique sounds and frequencies when trained by humans (Stansbury and Janik, 2019). The observations that the vocal ligament is both rudimentary and lacks a vocalis muscle may indicate that changes in tension of the vocal ligament is not the primary way in which calls are modulated. This hypothesis is further

supported by other works showing that vocal fold length in harbour seals does not change with body size, although vocal tract length and diameter do (Ravignani et al., 2017). These observations suggest that seals make use of other UAT structures to modulate their calls, rather than by changing tension in the vocal ligament, a conclusion supported by the anatomy. Currently, it is unclear if the corniculates and related soft tissues contribute to sound production in seals.

In seal pups, the larynx is elevated to where the epiglottis can move into a position over the soft palate, which may occur when breathing during sucking. In fact, in two pups examined post-mortem in our study, the epiglottis was positioned above the level of the soft palate. Similarly, in human infants, the epiglottis approximates the posterior aspect of the soft palate allowing infants to have nasal airflow during sucking and where milk flows around the larynx. This high position of the larynx facilitates a cyclical ‘suck–swallow–breathe pattern’ necessary for both sucking and the development of their swallow (Fucile et al., 2011).

In adult harbour seals, the epiglottis is also in a relatively high position compared with adult humans, and its contour appears to follow that of the pharyngeal surface of the tongue. The larynx is usually higher in the neck in non-human terrestrial mammals, where the epiglottis often contacts the most posterior aspect of the soft palate at rest (Harrison, 1995). In adult harbour seals, it is unclear whether the epiglottis can elevate over the soft palate when breathing at the surface and with positional changes of the head. The triangular-shaped gap seen in the hard palate would neatly accommodate the epiglottis if it were to be elevated over the soft palate. In Weddell seals, the laryngeal position is high with the epiglottis above the level of the soft palate (Piérard, 1969) while the soft palate contains a mid-sagittal notch allowing it to receive the epiglottis (Boyd, 1975). Our observations in pup and juvenile harbour seals align with these findings and support the hypothesis that similar movements occur in the adult. Specifically, we propose that in adult harbour seals, the epiglottis can rise above the soft palate and position the laryngeal inlet in the nasopharynx, allowing unobstructed airflow from the nasal cavities into the lower airway during breathing at the surface. When swallowing, the epiglottis descends and the soft palate elevates to close the pharyngeal isthmus. The observation that the levator veli palatini muscles are prominent and are well positioned to pull the soft palate towards the posterior pharyngeal wall and close the pharyngeal isthmus, suggests that they act to separate the nasopharynx from the oropharynx when swallowing.

Although there are variations in exactly how phocid seals feed (i.e. pierce feeding, grip and tear feeding, etc.), seals generally dive in order to forage in addition to swallowing without subsequent oral processing (Kienle and Berta, 2016). Both swallowing prey whole and doing so underwater suggest that airway protection would be particularly crucial for seals to avoid aspiration during a dive. Our observation that the corniculate cartilages can move forward to overlap with the vocal folds thereby closing the laryngeal vestibule provides an additional valving feature. This feature supports airway protection during swallowing and probably occurs at the initiation of a dive, or at the very least, just prior to the oral cavity opening to prehend prey.

A summary of, and predictive models for, how UAT valving may work in harbour seals during breathing, calling, diving and swallowing is summarized in Fig. 7. Major structures in the UAT are labelled in Fig. 7A. For breathing in pups while sucking (Fig. 7B), the nares are open, the epiglottis is positioned above the level of the soft palate into the nasopharynx, and the laryngeal

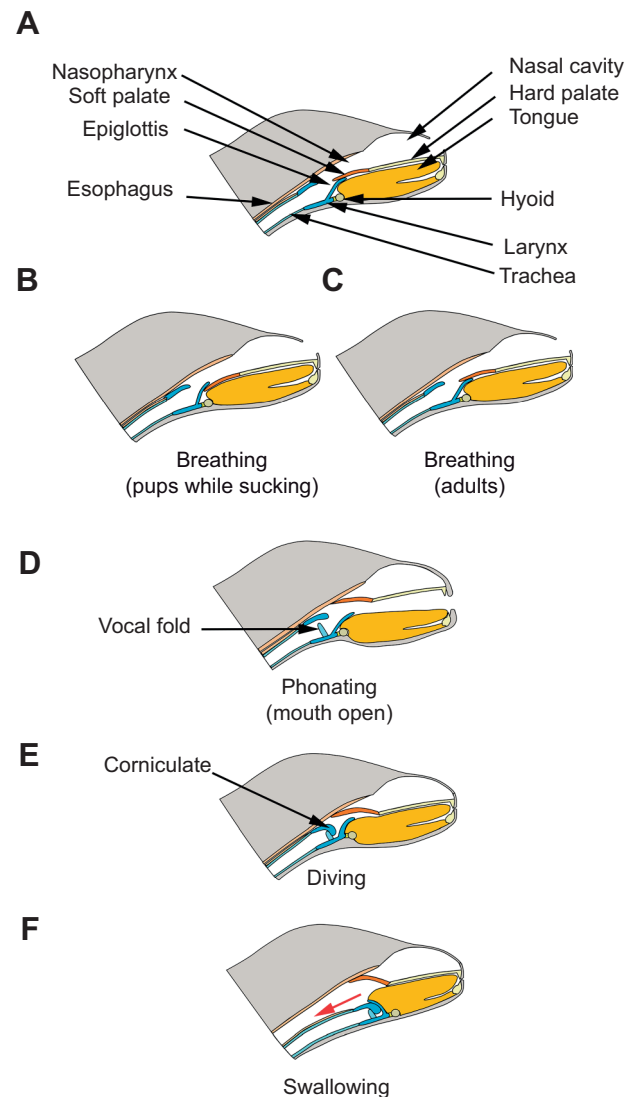


Fig. 7. Schematic diagrams of major structures in the upper aerodigestive tract and how they are predicted to function during breathing, phonating, diving and swallowing.

vestibule is cyclically open, allowing milk to move around the larynx into the esophagus. In adults (Fig. 7C), it is unclear if the epiglottis can rise above the level of the soft palate when breathing at the surface, so the epiglottis is shown here in a position immediately behind the tongue. In pups calling at the surface (Fig. 7D), the vocal folds are opposed and sounds are produced as air is forced through the closed rima glottidis. In a diving seal (Fig. 7E), the larynx is most likely to be in a position of ‘effort closure’ where the vocal folds have closed the rima glottidis and the corniculates have moved forward to overlap with a portion of the vocal folds, thereby closing the vestibule and inlet. This model does not exclude the possibility that the airway can be opened during ascent from a dive and gas exhaled, as has been observed in Antarctic fur seals (Hooker et al., 2005). Although not indicated in the figure, the pharynx is probably almost completely collapsed at depth because it has no skeletal support. During swallowing (Fig. 7F), the larynx probably moves anteriorly and superiorly, facilitating epiglottis deflection over the closed inlet, vestibule and rima glottidis as a final protective cap on the lower airway.

We have demonstrated there are several modifications to the structures in the UAT of harbour seals that may allow for more efficient underwater feeding, periodic surface breathing and feeding and swallowing patterns during early development. These include the presence of large corniculate cartilages that can move forward to close the laryngeal vestibule and inlet and an epiglottis that can rise above the level of an accommodating soft and hard palate. The anatomical modifications described here are consistent with the hypothesis that harbour seals have evolved mechanisms in their UAT for living in a marine habitat. Additional videofluoroscopic studies are needed to verify the dynamic movements of the palate, larynx and associated structures predicted by this study during breathing, vocalizing and swallowing.

Acknowledgements

We would like to thank Bob Shadwick and Margo Lillie for their invaluable feedback on our manuscript, which has resulted in much improved work. We would also like to thank Lindsay Akhurst, Emily Johnson, and the staff and volunteers at the Vancouver Aquarium's Marine Mammal Rescue Centre for their tireless efforts towards seal rehabilitation. This work would not have been possible without them.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.A., C.D., S.A.S.; Methodology: A.A., W.V., S.R., M.H., S.A.S.; Validation: A.A., W.V., S.A.S.; Formal analysis: A.A., W.V., S.A.S.; Investigation: A.A., W.V., S.R., S.A.S.; Resources: W.V., S.R., M.H., S.A.S.; Data curation: A.A.; Writing - original draft: A.A.; Writing - review & editing: A.A., W.V., C.D., S.R., M.H., S.A.S.; Supervision: S.A.S.; Project administration: S.A.S.; Funding acquisition: W.V., S.A.S.

Funding

This work was supported by a University of British Columbia (UBC) Faculty of Medicine Start-up grant to Stacey A. Skoretz and by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to A. Wayne Vogl (RGPIN-2018-03727).

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