FUNCTIONAL ANATOMY AND MECHANICS OF THE AERODIGESTIVE TRACT IN
RORQUAL WHALES

by

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the degree of    Doctor of Philosophy
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Abstract

Digestive and respiratory pathways are entwined evolutionarily, developmentally, and spatially. Adaptations of the pharynx balance breathing and swallowing, allowing food transfer while maintaining airway protection. Cetaceans have terrestrial ancestry, thus, the terrestrial framework required modifications for life underwater. Rorqual baleen whales have many adaptations related to feeding, which involves engulfing a volume of prey-laden water that can be larger than their own body. How have these adaptations affected interactions of the respiratory and digestive tract? Diving on a breath-hold requires sealing the respiratory tract to prevent water entry; however, this closure presents the risk of barotrauma in the rigid upper airways. Swallowing immense amounts of living food requires protection of upper and lower airways, and requires an effective transport mechanism to swallow large quantities of prey during limited interlunge period. I found that the nasal cavities are protected from water incursion by nasal plugs that occupy the upper half of the nasal cavities. The nasal plugs demonstrate a unique function at depth where they are pushed further into the nasal cavities with increasing ambient ocean pressure and diminishing airway volumes as dive depth increases. The heterogeneous composition of the plugs makes them effective at clearing the upper airways for breathing and filling them when diving to prevent barotrauma. I discovered a novel structure in rorquals, the oral plug, that prevents water incursion into the pharynx during engulfment. Food is prevented from entering the upper airways through elevation of the soft palate and oral plug to close the nasopharynx. Food is prevented from entering the lower airways through occlusion of the laryngeal inlet and collapse of the laryngeal sac into the laryngotracheal junction. With the pharynx dedicated entirely to the digestive tract, food is transported by a pharyngeal pump
mechanism, where food is forced through the pharynx by contraction of pharyngeal muscles, into the esophagus and on to the stomach. The actions of dynamic valve mechanisms in the pharynx and larynx allow the pharynx to function efficiently as both part of the respiratory tract and digestive tract in rorqual whales. These adaptations are imperative to achieving large body size in rorquals.
Lay Summary

Marine mammals must protect their respiratory tract from water entry, and from food and water entry during swallowing, while effectively transporting food to the stomach. Rorquals (baleen whales that lunge feed) are the largest animals and require immense quantities of food daily. The respiratory tract is protected by large nasal plugs that fill the rigid nasal cavities when diving to prevent both water entry and tissue damage associated with pressure increase at depth. During swallowing, upper and lower airways are protected by mechanisms similar to human mechanisms, where upper airways are closed by elevation of the soft palate and the inlet of the lower airway is sealed at the larynx. Food is pushed into the pharynx by the tongue, and the pharynx likely performs a single muscular contraction to push food into the esophagus, which contracts to transport food to the stomach. These adaptations balance respiratory and digestive functions.
Preface

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List of Symbols and Abbreviations

A arytenoid cartilage
CF corniculate flaps
CTJ cricotracheal region
$D$ measured stretch distance between bars
E epiglottis
$h$ wall thickness
$h_i$ inflated wall thickness
$l$ inflated length
$L$ unloaded length
LRT lower respiratory tract
LS laryngeal sac
NP nasal plugs
P pressure
$P$ inflated esophagus pressure
$P_{amb}$ ambient ocean pressure
$P_d$ pressure at depth
$P_{nasal}$ nasal cavity pressure
$P_s$ pressure at the surface
$r$ radius
$r_{ib}$ bar radius
\( r_i \) inner radius of inflated esophagus

\( R_i \) unloaded inner radius

\( r_m \) midwall radius

\( r_o \) inflated outer radius

\( R_o \) unloaded outer radius

\( \sigma_w \) circumferential wall stress

\( \sigma_m \) specific muscle tension

SPL soft palate ledge

UAT upper aerodigestive tract

URT upper respiratory tract

\( V \) volume

\( V_d \) volume at depth

VGB ventral groove blubber

\( V_s \) volume at the surface
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Chapter 1: General Introduction

1.1 Thesis aim

For approximately 300 million years, from the vertebrate land invasion in the late Devonian (~350 MYA), to the beginnings of cetacean evolution in the Eocene (~50 MYA), the cetacean lineage acquired adaptations to better suit a terrestrial existence (Long and Gordon, 2004; Thewissen et al., 2007). The return to an aquatic existence meant modifying an existing terrestrial framework to suit the needs of a life surrounded by water. The adaptations of the aerodigestive tract (encompassing the respiratory and digestive tracts from head to diaphragm) for an air-breathing animal living underwater are of interest to me, specifically in the largest animals ever to have lived – the rorqual whales. Protecting the respiratory tract from water entry is now a necessity at all times; however, the solution is not trivial. Closing the respiratory tract off from the surrounding environment and diving after inhaling a volume of air means the potential exists for pressure differentials to form between respiratory tract pressure and ocean pressure, which could lead to tissue damage, or barotrauma. Additionally, the respiratory tract must possess protective mechanisms to prevent food aspiration during swallowing. With the other bodily adaptations of cetaceans, the positions and morphology of respiratory structures have changed from those of a terrestrial mammal, so how these structures function may have changed too. Rorquals are well-known for their energetic lunge feeding, which involves engulfing innumerable small prey in a stretchy throat pouch. While the engulfment aspect of lunging has been well studied, the ability to process such large quantities of prey and transport it to the stomach has not; this must require adaptations of the digestive tract, specifically the pharynx and esophagus. Digestive and respiratory pathways are entwined evolutionarily, developmentally, and spatially. The pharynx of mammals is used by both respiratory and
digestive tracts, and adaptations of the pharynx and surrounding aerodigestive structures will impact both breathing and feeding. In this thesis I explore the relationship between respiratory and digestive structures in the aerodigestive tract of rorqual whales to assess how the aerodigestive tract has been modified to suit the needs of both breathing and feeding while living underwater.

1.2 Pharynx general introduction

The mammalian pharynx is the region of the throat behind the oral and nasal cavities that is shared by both digestive and respiratory pathways (Frisdal and Trainor, 2014). This region has been modified throughout time and across species, but in mammals it is a musculofascial half cylinder attached cranially to the base of the skull, caudally to the esophagus, and on each side to the lateral margins of the posterior openings of the nasal cavities and oral cavity, and to the lateral margins of the larynx (Fig. 1.1) (German and Palmer, 2006). The position and shared usage of the pharyngeal cavity means it is responsible for directing the flow of food and air to the appropriate channels: when food is swallowed it travels from the oral cavity, through the pharynx, and on into the esophagus, and when air is inhaled it travels from the nasal cavities, through the pharynx, and on to the larynx (German et al., 1998). The close proximity of digestive and respiratory tracts in the pharynx presents the risk of aspirating food – if the respiratory tract is not adequately protected during swallowing, then food could end up in the lower respiratory tract, resulting in injury or death. To understand the structure of the mammalian pharynx with its dangerously close proximity of digestive and respiratory tracts, it is necessary to reflect on some of the major points in the evolution of the vertebrate pharynx, and upper and lower airways, keeping in mind that the morphology of the region arose from constraint and adaptation not necessarily selection for optimal function (Graham, 2001; Hall, 2003).
1.2.1 Pharynx evolution and anatomy

The vertebrate pharynx has always been a region with dual roles in feeding and respiration (Graham, 2001). Many pharyngeal adaptations separate digestive and respiratory pathways (Crompton et al., 1997; German et al., 1998). To examine these adaptations in mammals it is useful to broadly compare the mammalian condition to the extant outgroup reptiles. Ancestral mammals and most extant reptiles do not possess a complete secondary palate or a soft palate, which means the nasal cavities open directly into the oral cavity (Smith, 1992). A secondary bony palate appeared in the Permo-Triassic (250 - 255 MYA) in the predecessors of therapsids (a group including mammals and mammal-like reptiles) at least three times (Maier et al., 2009; Smith, 1992), indicating similar constraints or pressures favoured the development of the secondary palate. The origins of the soft palate are not as clear because soft tissues are not generally preserved in the fossil record (Smith, 1992). Extant mammals possess a complete bony secondary palate and a fleshy soft palate that separates oral and nasal cavities, allowing more separation between the tasks of breathing (nasal cavities) and feeding (oral cavity) (Smith, 1992; von Arx and Lozanoff, 2017). The evolution of the soft palate also allows us to define two regions of the pharynx - the nasopharynx and oropharynx (Fig 1.1). The nasopharynx starts posterior to the nasal choanae (posterior openings of the nasal cavities) and bony palate and extends to the postero-ventral end of the soft palate, being defined solely by fleshy tissue rather than bone. The oropharynx sits directly below the nasopharynx and soft palate, posterior to the oral cavity, and superior/rostral to the larynx (Smith, 1992). The soft palate can isolate the nasopharynx and nasal cavities from the pathway of food during swallowing (von Arx and Lozanoff, 2017). The soft palate is composed of five muscles which control its movement and position (Diogo et al., 2018a; Ducharme and Cheetham, 2019; von Arx and Lozanoff, 2017;
Two of those muscles are the tensor veli palatini and the levator veli palatini (Fig. 1.2), both of which are thought to be homologous to pharynx muscles in reptiles. The tensor muscle expands the nasopharynx by pulling the soft palate slightly ventrally and ‘tenses’ it, while the levator muscle elevates the soft palate during swallowing. The musculus palatinus muscle runs longitudinally down the flap-like extension of the soft palate that separates the nasopharynx from the oropharynx and pulls the soft palate dorsally. The palatopharyngeus runs from the soft palate to the lateral walls of the pharynx and larynx and depresses the palate and closes the oropharyngeal isthmus. The last muscle that composes the soft palate is the palatoglossus muscle, and it together with the palatopharyngeus functions to block oral cavity from oropharynx by depressing the soft palate. The palatoglossus also elevates the tongue.

Below the oropharynx lies the laryngopharynx (or hypopharynx), the ventrocaudal region of the pharynx in mammals (Fig 1.1). The laryngopharynx sits posterior to the larynx and the laryngeal inlet, and its lower border extends to the caudal edge of the larynx.

Three pharyngeal constrictor muscles form the major components of the pharyngeal wall (Fig. 1.3). They help to propel a bolus through the pharynx and into the esophagus. The muscles are striated and circular in orientation (Diogo et al., 2018a; Drake et al., 2015; Moore and Dalley, 2018; Shapiro and Martin, 1996; Smith, 1992). Each of these muscles is quite broad (superiorly to inferiorly), and has landmarks generally related to the three regions of the pharynx. The superior constrictor muscle is the rostral or superior-most muscle (depending on head orientation; quadruped vs. human, respectively), and it originates generally from the oral-pharyngeal region, though the exact points of origin vary among mammals. This muscle generally corresponds to the level of the nasopharynx and upper oropharynx. The middle constrictor lies inferior to the superior constrictor and originates from the hyoid, generally related
to the oropharynx. The inferior constrictor is the inferior-most muscle and is related to the laryngopharynx. It has two parts: the more superior part which arises from the thyroid cartilage, the thyropharyngeal part, and the more inferior part which arises from the cricoid cartilage, the cricopharyngeal part. The *cricopharyngeus* is also referred to as the upper esophageal sphincter and it controls the tonicity of the esophageal opening (Mu et al., 2007). Unlike the other pharyngeal constrictors that are relaxed between swallows, the *cricopharyngeus* muscle exhibits tonic contraction and must relax to open the esophageal inlet. Extant reptiles (and presumably ancestors of mammals) do not possess a muscular pharynx, nor do they have any separation between the oral cavity and pharynx (with the exception of some crocodilians) (Diogo et al., 2018b; Ferguson, 1981; Kirchner, 1993). These distinct regions of the pharynx and the pharyngeal musculature associated with each are unique to mammals, and allow mammals to exhibit behaviours such as suckling and swallowing simultaneously (Smith, 1992).

### 1.2.2 Lower respiratory tract evolution

The lower respiratory tract develops as an out-pocketing of the gut tube (Effros, 2006). Prior to the evolution of vertebrate lungs, gills were the main respiratory structure. Early fishes that lived in environments of varying water quality and extreme shifts in oxygen availability were likely the first to develop “lungs” for air breathing (Graham, 1997). The development of these early lungs could have provided the impetus for journeys on land – where gills require water to function effectively, lungs are suited to a terrestrial environment. For air-breathing fishes that are amphibious, it is important to protect these air-based lungs from the aquatic environment. The larynx developed as a protective valve to prevent entry of water or food to the lower airways (Laitman and Reidenberg, 2013). The most primitive larynx is observed in the Bichir lungfish which possesses a simple muscular sphincter to prevent water entry (Fig. 1.4)
(Asher et al., 1996; Kirchner, 1993; Sasaki, 2000). African and Australian lungfish developed more muscular control to actively open this sphincter and “swallow” air. The innovation of air-breathing, lungs, and sphincteric control were all precursors that assisted the transition from an aquatic to a terrestrial lifestyle, and the evolution of tetrapods. Living on land required adequate ventilation and more control over the respiratory sphincter, and this is reflected in adaptions of amphibians and reptiles (Harrison, 1995; Sasaki, 2000). This was accomplished in some amphibians, including the Mexican axolotl, with lateral cartilages for dilator muscles to insert, and in reptiles, a cartilaginous ring between glottis and trachea for muscle insertion. The addition of cartilages provided more support to the laryngeal body and a surface for muscles to attach to for further control over the laryngeal opening. Thus, the secondary function of the larynx is in facilitating respiration. The larynx in most reptiles is positioned at the base of the tongue, and since most reptiles lack a secondary palate, the respiratory tract opens directly into the oral cavity and is simply controlled by the muscles acting on these laryngeal cartilages. Interestingly, crocodilians have independently acquired a secondary palate, and their “hyoid”, an epiglottis analogue, can be elevated to meet a descending flap of tissue from the secondary palate to streamline breathing while hunting in water (Kirchner, 1993; Smith, 1992).

### 1.2.3 Mammalian larynx anatomy and function

The mammalian larynx is much more refined than the ancestral or outgroup condition, with more expansive cartilages and more musculature to control the larynx (Fig. 1.5). The mammalian larynx is generally composed of nine cartilages: three paired - arytenoids, corniculates, and cuneiforms, and three unpaired - thyroid, cricoid, epiglottis (Drake et al., 2015; Moore and Dalley, 2018). These cartilages are arranged in a cylinder that is contiguous with the trachea. The thyroid cartilage is a large ventral cartilage with lateral extensions that articulate
with the cricoid cartilage. The cricoid cartilage forms a complete tube around the base of the larynx, inferior to the thyroid. The thyroid and cricoid cartilages form the main tube-like body of the larynx, with the other cartilages extending superiorly from this main structure. At the superior-most point of the cricoid cartilage, the arytenoids are attached via a synovial joint or ligaments. The base of each arytenoid sits directly below the superior-most point of the cricoid, where it is attached, with the remainder of the arytenoid body extending cranially with free edges. The corniculate cartilages sit atop the superior-most tip of the arytenoids. From the ventral aspect of the tube-like body of the larynx extends the epiglottis. The bottom of the epiglottis is attached to the internal surface of the thyroid cartilage and is connected with a ligament to the thyroid. The epiglottis extends superiorly/cranially in a leaf-like shape. The epiglottis and arytenoid cartilages are connected to one another laterally with tissue, called the aryepiglottic folds. The cuneiform cartilages are small and sit within the aryepiglottic folds. The exact morphology of the larynx varies among species, with differences in cartilage size and shape, and the loss of some cartilages in certain species.

The larynx in most mammals is generally positioned high in the neck, allowing the epiglottic cartilage to overlap with the soft palate (Fig. 1.6). This places the larynx intranarially (in the nasopharynx), and functionally separates respiratory and digestive pathways (Laitman and Reidenberg, 2013). The connection between upper and lower respiratory tracts is therefore continuous, allowing breathing to occur while food passes around either side of the epiglottis through the lateral food channels to be swallowed. This position of the larynx is especially important in all infant mammals, as it allows suckling and breathing to occur simultaneously.

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1 Orientation of the cartilages in this description refers to the human condition. The same cartilage layout exists for tetrapods, however, the larynx sits in a horizontal plane along the tetrapod neck, rather than upright as in the human.
(Crompton et al., 1997). In adult mammals, however, there are deviations from this general mammalian larynx position based on feeding style and other laryngeal functions, like sound production (Harrison, 1995). For terrestrial mammals, larynx position can be categorized quite broadly into three groups - herbivores, carnivores, and primates, though of course there are exceptions to these groupings. In herbivores the larynx is held permanently intranarial (the general mammalian position), allowing them to breathe, use olfactory senses, and swallow simultaneously (Kirchner, 1993). The morphology of the larynx reflects this: herbivores tend to have a broad elongated epiglottis, large arytenoids, and aryepiglottic folds with high walls (Harrison, 1995). The plant-based diet of herbivores that chew their food well means the food they are swallowing has a high liquid component, so the circumferential protection of the larynx is vital for swallowing safely and preventing liquid entry to the larynx. The morphology and actions of the carnivore larynx differ from the herbivore larynx. Instead of an intranarial larynx during swallowing, the non-elongated epiglottis folds down over the laryngeal inlet. The arytenoid cartilages are comparatively small, and in some species the aryepiglottic folds are absent, meaning no lateral protection. The diet of carnivores is vastly different as they are not chewing food to the same extent as herbivores, and instead swallow large chunks of meat. The folded down epiglottis allows carnivores to swallow large food items through the middle of the pharynx (Harrison, 1995). The position of the larynx dictates whether animals are capable of panting for thermoregulation or not. A “permanently” intranarial larynx negates the ability to pant (Biewener et al., 1985). The last role of the larynx is a trait only observed in mammals – sound production. The use of the larynx as a “flutter valve” for phonation requires a thoracoabdominal diaphragm, only seen in mammals. The larynx’s role of phonation is considered the least significant role (Negus, 1949; Sasaki, 2000); however, humans and primates,
along with a handful of other mammals have developed a descended larynx, in favour of more control over sound production despite potentially increasing the risk of choking by aspirating food or water (Laitman and Reidenberg, 1993; Sasaki, 2000). Humans avoid prandial aspiration with 3 laryngeal valves: closure of the vocal folds, closure of the aryepiglottic folds, and passive folding of the epiglottis. When swallowing occurs in humans, the hyoid bone, which is attached to the larynx by ligaments and muscles, moves superiorly and anteriorly tucking the larynx below the base of the tongue and passively folding the epiglottis to meet the arytenoids and cover the laryngeal inlet (similar to epiglottal folding in carnivores) (Drake et al., 2015; Massey, 2006).

1.2.4 Swallowing mechanics

Despite the close proximity of the digestive and respiratory tracts, mammals have successfully developed multiple mechanisms to prevent choking and maintain tract separation during feeding and drinking. Swallowing is an incredibly fine-tuned act that relies heavily on neurological input for coordination and involves a series of conscious and reflexive actions. Neural mechanisms are beyond the scope of this thesis, with the focus instead being the anatomical and functional mechanics of swallowing. Swallowing, or deglutition, is the process of transporting a substance from oral cavity to stomach and can be described anatomically in three phases – oral, pharyngeal, and esophageal (Goyal and Mashimo, 2006; Hiiemae and Crompton, 1985; Massey, 2006; Matsuo and Palmer, 2008; Matsuo and Palmer, 2009). Though the human is a derived model, it is most familiar to us and provides a straightforward example to demonstrate the process of swallowing. In humans eating food the oral phase involves efforts of preparing the food for transport: chewing, mixing the food with saliva, and forming the food into a bolus. The bolus is then held between the tongue and hard palate until the conscious decision is made to swallow. Once the swallow is initiated, the acts that follow are involuntary. The tongue contracts
against the hard palate and forces the bolus backwards, cradled by the tongue, until it reaches the oropharynx. The soft palate is lifted upwards and the posterior pharyngeal wall contracts and shortens with it to protect the nasopharynx from food entry. Muscles along the posterior pharyngeal wall contract, while the tongue pushes against the soft palate to move the bolus into the oropharynx (the pharyngeal phase). The tongue pushing against the soft palate simultaneously separates the oral cavity from the pharynx, preventing food from being squeezed back into the oral cavity. Contractions of the pharyngeal constrictor muscles push the bolus aborally through the pharynx. Meanwhile, the vocal cords close, the arytenoids approximate one another, and the hyoid moves superiorly and anteriorly to pull the larynx out of the way and passively fold the epiglottis down to meet the arytenoids and cover the laryngeal inlet. These actions widen and shorten the pharynx and pull the esophagus and upper esophageal sphincter upwards. In other mammals this procedure is not exactly the same, as many non-human mammals maintain an intranasal larynx during swallowing. In these mammals, the procedure is the same up until the movements of the larynx. When food is pushed back into the pharynx, it can be transiently stored in the “vallecula”, a recess rostral to the epiglottis and medial to the lateral food channels (Fig. 1.7). Instead of the larynx shifting dramatically as the human larynx does, food is forced from the vallecula, through the lateral food channels on either side of the larynx (Hiemae and Crompton, 1985). Though the larynx may not move significantly for food to pass in all mammals, the larynx is still thought to be a mobile structure in most mammals (Smith, 1992). Once food has passed the larynx, the procedure is again the same as in humans. The bolus is forced through the relaxed upper esophageal sphincter (cricopharyngeal muscle) and into the esophagus. The serial contractions of pharyngeal muscle are known as pharyngeal peristalsis. In a human, the rate of pharyngeal peristalsis can be as fast 40 cm/s, meaning a bolus can travel
through the pharynx in less than 1 second (Massey, 2006). With the pharyngeal phase complete and the bolus in the esophagus, the esophageal phase of swallowing begins.

1.2.5 Esophagus anatomy and mechanics

The esophagus is a multi-layered tube extending from the pharynx to the stomach, that transports food to the stomach through peristalsis (Kuo and Urma, 2006). In its resting state, the esophagus is dorsoventrally collapsed or flattened but expands/stretches to accommodate food. The esophageal wall is composed of four layers, from innermost to outer: mucosa, submucosa, muscularis propria, and adventitia (Fig. 1.8) (Gregersen, 2003; Oezcelik and DeMeester, 2011; Pawlina and Ross, 2016; Young et al., 2014). The mucosa surrounds the lumen and is composed of three concentric regions: the epithelium, generally a non-keratinized stratified squamous epithelium; the lamina propria, a layer of connective tissue; and the muscularis mucosae, longitudinally oriented smooth muscle. The mucosa has deep longitudinal folds down its length to allow expansion of the esophagus during swallowing. The submucosa is composed of connective tissue fibres (elastin and collagen), blood vessels, lymph vessels, and nerves. The muscularis propria is a muscular bilayer composed of an inner region of circular muscle and an outer region of longitudinal muscle. The muscularis propria is responsible for the sequential contractions that create waves of peristalsis to transport the bolus along the esophagus to the stomach. The adventitia is the outermost layer of the esophagus composed of loose connective tissue, blood and lymphatic vessels, and nerves, and attaches the esophagus to nearby structures. The esophagus spans three body regions: cervical, thoracic, and abdominal (Kuo and Urma, 2006). In the cervical region the esophagus is ventral/anterior (tetrapod/biped, respectively; notation follows throughout) to the vertebral column and dorsal/posterior to the trachea. In the thoracic region the esophagus continues ventrally/anteriorly to the vertebral column, though
close to the diaphragm the aorta comes between the esophagus and vertebral column. The esophagus is dorsal/posterior to the trachea and tracheal bifurcation, and continuing posteriorly/inferiorly, is dorsal/posterior to the aorta and heart. There is only a short segment of the esophagus that is abdominal until the esophagus widens into the stomach. This transition is not demarcated by an anatomical sphincter, but a functional lower esophageal sphincter, which constricts the passage between esophagus and stomach.

Throughout these regions of esophagus there is a transition in the muscle type of both the circular and longitudinal muscle layers from completely striated in the cervical region to smooth in the abdominal region (Kuo and Urma, 2006). This is a result of the embryology of the esophagus, where the striated muscle of the upper part of the esophagus is thought to originate from mesenchyme of pharyngeal arches 4, 5, and 6, and the smooth muscle of the lower part of the esophagus arises from splanchnic mesoderm, and the two regions meet somewhere in the middle (Kuo and Urma, 2006; Roberts, 2000; Skandalakis and Ellis, 2000). This transition zone varies across mammals. In humans, cats, pigs, horses, and opossums the rostral/upper third of the muscular bilayer is composed of striated muscle, the middle third is a transition zone, and the caudal/lower third is composed of smooth muscle. In mice, rabbits, dogs, and ruminants the entire esophagus is composed of striated muscle, with the gastroesophageal sphincter often composed of smooth muscle. The type of muscle associated with the esophagus impacts the rate of peristalsis, where esophagi with higher striated muscle content have faster peristalsis (Sukon, 2002). An average rate of peristalsis in a human esophagus is ~ 3 cm/s, whereas a dog has a rate of 6.5 cm/s, a horse 35-40 cm/s, a cow 42 cm/s, and an unspecified species of monkey from 0.7 – 3.3 cm/s (Vantrappen and Hellemans, 1974). The exact rate of peristalsis can vary along the length of esophagus (Richter, 2012) due to muscular differences, and the overall rate of
peristalsis can be influenced by factors like bolus size, viscosity, and temperature (Dooley et al., 1988; Hollis and Castell, 1975; Paterson, 2006; Ren et al., 1993; Winship et al., 1970).

1.3 Cetacean general adaptations overview

The group Cetacea includes all whales, dolphins, and porpoises. Extant cetaceans are grouped into the mysticetes – the baleen whales, and the odontocetes – the toothed whales. Cetaceans are closely related to artiodactyls (even-toed ungulates), and their closest living relative is the hippopotamus (Árnason and Gullberg, 1996; Berta et al., 2015a; Boissiere et al., 2005; Geisler and Theodor, 2009; Irwin and Úlfur Árnason, 1994; Thewissen et al., 2001). The cetacean reinvassion of an aquatic environment involved a complete overhaul of the terrestrial-adapted body systems. Extant cetaceans have a fusiform body shape with the pectoral girdle supporting flippers as the front limbs and the pelvic girdle reduced to a few small bones no longer connected to vertebrae but imbedded in abdominal muscle (Berta et al., 2015a; Rommel, 1990). The end of the tail has two lateral outgrowths called flukes that produce lift and thrust while swimming (Fish et al., 2007), and in most cetaceans the back supports a dorsal fin that prevents rolling while swimming (Fish, 2002). The genitalia of male cetaceans are held internally when in the retracted state (Slijper, 1966). The entire body is hairless, with the exception of vibrissae that are scattered along the upper jaw and mandibles. The vibrissae are innervated, suggesting a sensory role (Berta et al., 2015b; Berta et al., 2015c; Reeb et al., 2007). The size, shape, and relationships of many skull bones have changed, with a telescoping of the rostrum and shortening of the braincase, and the nasal cavities migrating to the top of the skull (Berta et al., 2015c). The cervical region is shortened as a result of the extreme flattening of the cervical vertebrae (Buchholtz, 1998; Slijper, 1962). A sacral region in the skeleton no longer exists due to the absence of a pelvic girdle (Buchholtz, 1998). Below the skin of cetaceans is a
thick layer of blubber that functions in thermoregulation, energy storage, and buoyancy control (Struntz et al., 2004). Thermoregulation is also accomplished through countercurrent heat exchange through vascular networks in the flippers, dorsal fin, tail flukes, and the mouth of some species (Berta et al., 2015d; Ekdale and Kienle, 2015; Ford and Kraus, 1992; Ford et al., 2013; Heyning and Mead, 1997; Pabst et al., 1995; Pabst et al., 1998). Changes to sensory systems are innumerable; hearing involves sound transmission through fatty pads in the jaw, vision is not important beyond the depths light penetrates, olfaction and taste are likely absent though remnants may exist in some groups, and well-developed somatosensory systems composed of many mechanoreceptors and well-innervated skin (Kremers et al., 2016a; Kremers et al., 2016b). This list is not exhaustive but provides a general overview of some of the bodily changes common to both mysticetes and odontocetes relative to terrestrial mammals.

1.3.1 Cetacean aerodigestive tract

The respiratory tract of cetaceans must be protected from water incursion at all times. This meant the development of tissue-based closure of the nasal cavities, with breathing happening periodically rather than continually as in terrestrial mammals. Most of a cetacean’s life thus happens on a breath-hold, which presents another issue: barotrauma. Taking a contained volume of air underwater, as in the air-filled respiratory tract of cetaceans, introduces the risk of tissue damage related to the development of a pressure differential. Pressure and volume in a system are inversely related to one another, according to Boyle’s Law ($P_1V_1=P_2V_2$). If a volume of air in a flexible vessel is taken to depth where pressures are high, the vessel can accommodate the necessary volume changes to allow the air volume inside to decrease. If the vessel is rigid, however, it cannot accommodate a shape/volume change and the potential exists for tissues lining the rigid vessel to become damaged as a pressure differential is formed. Cetaceans must
have mechanisms to account for the formation of a pressure differential to prevent barotrauma from occurring in the respiratory tract.

Feeding happens on a breath-hold. This limits foraging time, so cetaceans must feed efficiently to maximize energy intake. Prey must be separated from water before it is ingested, and then the standard swallowing procedure, including protecting the respiratory tract as food is transferred through the pharynx and to the esophagus, can occur. Cetaceans have a horizontal head position, with the digestive tract running horizontally from oral cavity to stomach, but the nasal cavities in a dorsal position in the skull above the food pathway. The result is that the dorsal respiratory tract at the nasal cavities must cross the food pathway nearly perpendicularly to reach the ventral lower respiratory tract. Additionally, the shortened cervical region places the larynx further rostrally in cetaceans. This changes the position of pharyngeal structures, necessitating changes to their interactions. Respiratory tract protection during swallowing must occur differently in cetaceans than in terrestrial mammals.

Prey selection and feeding style varies widely among all cetaceans, even among odontocetes and mysticetes themselves. Prey ranges from thousands of miniscule zooplankton, to incredibly large squid, to pieces of other marine mammals. These differences are not unlike the differences we see in terrestrial mammals, that feed on plant matter or large chunks of meat. There are morphological and functional differences in the aerodigestive tract among terrestrial mammals, thus, there must be differences in morphology and function among cetacean aerodigestive tracts. I address odontocetes and mysticetes separately to acknowledge these vast differences between them.
1.3.2 Odontocete general characteristics and aerodigestive tract

Odontocetes are diverse and comprise 10 extant families with at least 74 extant species, including some that inhabit freshwater (Hooker, 2018). They tend to be small to medium in size, with the exception of the sperm whale, orca and some beaked whales. This group is named for the presence of teeth, a feature that differentiates them from mysticetes, though some odontocete species exhibit reduced dentition. Odontocetes engage in single-prey capture and catch their prey through either raptorial means by grasping prey with their teeth, or by suction feeding by creating a negative pressure zone near/in the mouth, often in species with reduced dentition (Marshall, 2018). Diet is related to species, but odontocetes generally feed on individual fish, cephalopods, or other marine mammals (Trites and Spitz, 2018). Odontocetes, along with all other cetaceans, do not chew but instead swallow their food whole or in large chunks. To find their food, odontocetes rely on echolocation, a form of sound production possible through complex nasal and cranial anatomy (Pabst et al., 1999). The dorsal surface of the skull is concave and holds a fatty melon rostrally and a single blowhole leading from the bony nasal cavities posteriorly. The pathway between nasal cavities and blowhole contains multiple air sacs and fat bodies (bursae), and a pair of phonic lips that act similarly to vocal cords (Cranford et al., 1996). Air passing through the phonic lips produces vibrations that are transmitted through the melon into the environment. Sound production is important at all times and all depths for communication, foraging, and understanding environmental surroundings (Kellogg, 1958; Norris et al., 1961). Many deep diving species are capable of producing echolocating clicks at extreme depths with incredibly small air volumes in the nasal system (Foskolos et al., 2019). Air sacs for phonation lie outside the skull of odontocetes, which prevents the risk of barotrauma. Their
concave skull may shorten the length of the bony portion of the nasal pathway (i.e. the nasal cavities), decreasing dead space (or resting air volume) in the skull.

Odontocetes possess the most extreme laryngeal modifications among mammals (Fig. 1.9) (Green et al., 1980; Harrison, 1995). The larynx in odontocetes sits completely rostral to the vertebral column, placing the laryngeal inlet directly under the nasopharynx (Laitman and Reidenberg, 2013; Reidenberg and Laitman, 1987). The cartilages of the larynx have maintained their basic positions relative to one another but have changed dramatically in size, and the tissues connecting the cartilages have congruently changed. The epiglottis and corniculate/arytenoid cartilages are elongated superiorly, and the aryepiglottic folds are expansive and span the lateral margins between epiglottis and corniculate/arytenoids to form a tube called the goosebeak or laryngeal spout (Fig. 1.9A). The anterior and lateral edges of the tip of the epiglottis are enlarged into a lip or ridge. Rather than the simple overlap of epiglottis and soft palate seen in many terrestrial mammals, the laryngeal spout of odontocetes is inserted up into the nasopharynx and remains ‘permanently’ intranarial (Reidenberg & Laitman, 1987). The base of the nasopharynx has a distinct anatomical sphincter formed by the palatopharyngeal muscle that wraps around the laryngeal spout underneath the large lip on the tip of the epiglottis and holds the laryngeal spout in place. Since the laryngeal inlet is held in the nasopharynx, it is completely out of the pathway that food takes, eliminating the risk of aspirating food. When swallowing, food travels around either side of the laryngeal spout and on to the esophagus (Fig. 1.9B) Odontocetes have effectively managed to separate respiratory and digestive pathways. Though it seems to be a useful adaptation for choking prevention, the connectivity of upper and lower respiratory tracts and the ability to push air rostrally into the nasal system is indispensable for sound production to
allow odontocetes to navigate, hunt, and communicate, and at the surface, to breath (Green et al., 1980).

The esophagus of cetaceans overall is poorly studied, but there is some literature regarding the structure of odontocete esophagi, especially in freshwater species. Information about the structure of the odontocete esophagus can be used to approximate the specifics of esophageal function, such as its ability to expand, estimating peristalsis rates from muscular composition, or estimating how long it might take a bolus to get to the stomach from the length of the esophagus. Harrison et al. (1970) describes the esophagus of a young spinner dolphin (length - 160 cm long) and an adult bottlenose dolphin (length - 242 cm). In the former, the esophagus was 33 cm long and 2.5 cm wide, and in the latter 54 cm long and 5 cm wide. The esophagus is capable of much distension, providing the ability to process whole fish. This is due to longitudinal folds in its resting state, extensive submucosa, and well-developed muscularis mucosae. Unfortunately, only a single histological panel including esophagus has been published and the images are too highly magnified to glean any information about the relative size or layers of esophagus, though the muscularis propria is described as “thick”. The muscularis propria is also described as striated muscle in the upper region and smooth near the stomach, though the exact transition point is not clear. Yamasaki and Takahashi (1971) examined the esophagus of two South Asian river dolphins and found similar general characteristics – longitudinal folds of the mucosa, striated muscle orally and smooth muscle near the stomach. The muscularis mucosae was “not conspicuous”. The animals examined were 113 cm and 127 cm long. The esophagus dimensions provided are not specific to an individual but are listed as 15 cm long and 2 cm wide. The mucosal epithelium was 0.3 – 0.4 mm thick and muscularis propria was 2 mm thick. The inner circular layer of muscularis propria was 2 times thicker than the outer
longitudinal layer orally and 3 times thicker near the stomach. Yamasaki and Takahashi (1974) examined the esophagus of the La Plata dolphin and found the same patterns of longitudinal folds and composition of striated and smooth muscle. In a 171 cm long adult the esophagus was 25 cm long. Mucosal epithelium in this species measured 0.8 – 1.0 mm and muscularis propria was 3 mm thick orally and 2 mm thick near the stomach. Interestingly, the outer longitudinal layer of muscularis propria was twice as thick as the circular muscle orally, but near the stomach became thinner than the circular muscle. Perhaps more interestingly were the findings of Chaves and da Silva (1988) who described strictly striated muscle in the muscularis propria of the tucuxi, as well as a muscularis propria thinner than the mucosa.

1.3.3 Mysticete general characteristics and aerodigestive tract

Mysticetes are not as diverse as odontocetes, comprising only 14 species across 4 families - Balaenidae: right and bowhead whales, Neobalaenidae: pygmy right whales, Eschrichtiidae: gray whales, and Balaenopteridae: rorquals (Bannister, 2018). In addition to the much larger body size of mysticetes is the much larger size of their head compared to their body. The head of mysticetes can be approximately 1/4 to 1/3 of the total body length, an adaptation for bulk filter feeding and maximizing prey capture. Internal head anatomy differs greatly from odontocetes. Mysticetes possess two blowholes leading from the paired nasal cavities rather than one. The actions of the blowholes are regulated by two groups of nasolabial muscles (Berta et al., 2015e; Buono et al., 2015; Maust-Mohl et al., 2019). One group is the superficial muscles that are connected to and control the cartilaginous blowhole muscles, and the second group are paired cylindrical muscles that reside within the bony nasal cavities and extend from the rostrum to halfway down the nasal cavities. Both pairs of nasolabial muscles are thought to prevent water from entering the respiratory tract. The dorsal surface of the skull is convex, rather than concave,
to varying degrees among species with balaenids demonstrating the most curvature of the rostrum. Mysticetes do not have a melon nor do they have air sacs or bursae in their head, thus they do not produce sound in the same way as odontocetes and do not echolocate. The exact mechanism of sound production in mysticetes is still not well agreed upon, but it is well founded that sounds are produced through vibrations of laryngeal structures (Frankel, 2018; Reidenberg and Laitman, 2007a). The morphology of the mysticete larynx is vastly different from the odontocete larynx. One well-established difference between mysticetes and odontocetes is the presence of a laryngeal sac in mysticetes (Reidenberg and Laitman, 2008). The laryngeal sac is an exceedingly muscular ventral diverticulum of the larynx. The rostral-most aspect of the sac sits behind the thyroid cartilage and extends under the laryngeal body to the beginnings of the trachea. The laryngeal sac opening is formed by the caudal arytenoids, and the roof of the sac is contiguous with the floor of the larynx and cranial end of the trachea. The opening to the laryngeal sac, formed by a soft tissue fold between the two arytenoids, is shaped like the letter “u” therefore, has garnered the term the “u-fold”. The u-fold is thought to be the site of vibrations for sound production. Generally speaking, air moves between the laryngeal sac and lungs, and this air movement flowing over the u-fold produces vibrations and thus, sound. The u-fold is thought to be analogous to the vocal folds, and movements of the arytenoids to change the shape or tension of the u-fold can modify the sounds produced (Damien et al., 2019).

1.3.4 Mysticete laryngeal morphology

Differences in sound production correspond with differences in the morphology of the remainder of the larynx, for example, mysticetes do not possess an enlarged laryngeal spout (Fig. 1.10). The morphology and position of laryngeal cartilages varies among families. Only a handful of studies have examined the balaenid larynx, some from stranded animals (northern
right whales; Reidenberg and Laitman, 2007) and others from subsistence hunting (bowhead whales; Henry, 1983; Schoenfuss et al., 2014). The laryngeal inlet is narrow, with epiglottic and arytenoid cartilages held in close proximity by thick aryepiglottic folds (Fig. 1.10B). The relationship of epiglottic and arytenoid cartilages are vaguely reminiscent of the odontocete laryngeal spout, due to the distinct lateral protection creating a tube-like appearance; however, it is much shorter and stouter than its odontocete counterpart, and it lacks any distinct external ridges – the outer surface of epiglottis and arytenoids are completely smooth (Fig. 1.10C). There is a distinct pyramidal projection (pyramidal eminence) in the middle of the inner surface of the epiglottis, creating two lateral pockets that the arytenoid cartilages fit tightly into to seal the laryngeal inlet. Nasopharynx and oropharynx are separated by the ‘palatopharyngeal sphincter’, which is thought to tighten around the short laryngeal “spout” to maintain a permanently intranarial larynx, a thought attributed to Racovitza (1904), however, not aptly demonstrated in any of the balaenid literature referred to here.

Studies of the rorqual larynx focus almost entirely on sound production, however, larynx morphology is readily available from these studies (Benham, 1901; Damien et al., 2019; Reidenberg and Laitman, 2007a; Reidenberg and Laitman, 2007b). The rorqual larynx differs significantly from the balaenid larynx. The laryngeal inlet is extremely wide, with epiglottis and arytenoids not in close proximity (Fig. 1.10AB). Additionally, there is no lateral protection between epiglottis and arytenoids - no distinct aryepiglottic folds, so the laryngeal inlet is open both superiorly and laterally. The epiglottis does not possess a pyramidal eminence, but instead displays the opposite condition where it is somewhat trough shaped with the concavity on the inner surface. Again, these studies propose that the larynx is permanently intranarial and held in
place by the palatopharyngeal sphincter, as in odontocetes, but this concept is not anatomically
nor functionally demonstrated in any of these studies.

The families neobalaenidae and eschrichtiiidae are not well studied, with only one
published study on the pygmy right whale (neobalaenidae) larynx from 1999 (Reeb and Best,
1999), and likely no (or possibly no recent) studies on the gray whale (eschrichtiiidae) larynx.

1.3.5 Mysticete feeding

Mysticetes lack teeth and instead have racks of baleen along both sides of the upper jaw
used for feeding, hence the name “baleen whales”. Interestingly, tooth buds form in baleen
whales during embryonic development, but are resorbed before birth. Some extinct mysticetes
also possessed teeth as adults (Marx et al., 2015). Baleen whales engage in bulk filter feeding,
processing large amounts of water to filter out incredibly small prey (Heithaus et al., 2018).
There are a few major feeding styles with differences in baleen morphology and diet
corresponding to these techniques (Fig. 1.11).

Balaenids are well known for skim feeding, or continuous ram feeding, whereby they
open their mouths and swim slowly (< 1 m/s) through a patch of prey, commonly copepods (Fig.
1.11B) (Croll et al., 2018; George et al., 2018; Simon et al., 2009; Werth, 2001). The prey is
trapped on the lingual surface of baleen, while water is filtered out through the baleen plates and
through gaps at the posterior end of both sides of the mouth (Lambertsen et al., 2005; Werth,
2004; Werth and Potvin, 2016). Balaenids possess a well-developed muscular tongue that is
thought to help direct water flow through baleen plates and out the posterior gaps (Tarpley, 1985;
Werth, 2004). Baleen in balaenids is quite long, reaching lengths of 4 m, and is narrow and
flexible compared to other species. The rostrum of balaenids is strongly arched which allows
storage space for this lengthy baleen (Croll et al., 2018; Werth, 2001; Werth, 2004). Beyond the
mechanics of prey capture and the ecological aspects of feeding, there is no understanding of how this prey is moved from the oral cavity to the stomach. One study describes general esophagus morphology on bowhead whales collected through subsistence hunting (Tarpley, 1985). Three bowheads ~ 9 - 11 m long had esophagi no more than 7 – 10 cm in diameter. Unfortunately, distal esophagus samples were not obtained, so information gathered from this study refers solely to the oral region of the esophagus. The esophagus was described as a thick-walled muscular tube. Surprisingly, the muscularis propria appeared to have an inner longitudinal layer, a middle circular layer, and an outer longitudinal layer of muscle. Only skeletal muscle was described in the esophagus specimens, the caudal most-section of which was 16 cm caudal to the tracheal bifurcation, indicating the existence of skeletal muscle likely more than halfway down the esophagus.

Eschrichtiids have established a unique feeding mode called benthic suction feeding which is their primary feeding mode, though they have been observed skimming and lunging (Croll et al., 2018; Nerini, 1984; Werth, 2001; Young et al., 2015). This feeding mode utilizes the distinct muscular tongue of Eschrichtiids. In benthic suction feeding, the whale positions its body parallel to the ocean floor and turns its body to one side, opening its mouth and retracting the tongue in a piston like motion to create negative pressure and suck in benthic crustaceans (Fig. 1.11C). Upon closing their mouth these crustaceans are trapped on the inner (lingual) surface of baleen while water is pushed out through the baleen plates. Baleen in Eschrichtiids is short, thick/coarse, and rigid (Young et al., 2015). The heads of Eschrichtiids are short and straight (no curved rostrum), and the throat region has 3 - 5 grooves allowing for minimal expansion when suction feeding (Croll et al., 2018). There appears to be no literature on food processing and transfer to the stomach, nor esophagus morphology in eschrichtiids.
Published literature regarding Neobalaenid feeding and digestive tract morphology is sparse. They appear to eat calanoid copepods, small euphausiids, and other zooplankton, and may engage in skim and/or lunge feeding (Kemper, 2018; Werth et al., 2018; Young et al., 2015).

1.3.5.1 Rorqual feeding

Rorquals engage in lunge feeding, the “greatest biomechanical action in the animal kingdom” (Brodie, 1993). In lunge feeding a rorqual accelerates towards a patch of prey and opens its mouth to engulf a patch of prey-laden water (Fig. 1.12) (Goldbogen et al., 2007). These lunges occur multiple times during a foraging dive, with an interval between lunges as short as 30 seconds (Goldbogen et al., 2006; Goldbogen et al., 2007). After each lunge, during the interlunge interval, the whale pushes the engulfed water out through the baleen plates (Goldbogen et al., 2013), isolating prey in the buccal cavity. Krill is the preferred prey among rorquals, but they occasionally feed on small schooling fish, usually a location dependent decision (Bannister, 2018). The duration of the interlunge is related to prey handling times, likely a result of prey density or prey type (Goldbogen et al., 2013). It may also have a relationship to the amount of water that must be filtered out in each interlunge, or the ratio of prey to water. Rorquals are capable of engulfing a volume of water larger than their own body (Goldbogen, 2010; Goldbogen et al., 2007), thanks to the exceedingly expansible tissue that forms the tongue, floor of the mouth, and ventral groove blubber (Lambertsen, 1983; Orton and Brodie, 1987; Pivorunas, 1977). Engulfed water fills the buccal cavity and inverts the tongue, forcing the inverted tongue (filled with water) into an intramuscular space called the cavum ventrale that extends as far posteriorly as the umbilicus. This inflated ventral pouch gives rorquals their characteristic post-lunge tadpole shape, which is one of the factors researchers have used to
calculate the volume of water rorquals are capable of engulfing (Goldbogen et al., 2007; Goldbogen et al., 2010; Potvin et al., 2009; Potvin et al., 2010). Unfortunately, we are unable to determine how much prey is actually engulfed along with that water volume. The amount of prey captured in a rorquals mouth varies depending on prey density, patch size, and distribution (Goldbogen et al., 2011; Goldbogen et al., 2015). Measurements of krill densities in nature span across orders of magnitude depending on the time of sampling and the method used to sample (Brodie et al., 1978; Cotté and Simard, 2005; Croll et al., 2005; Goldbogen et al., 2011; Nicol, 1986; Simard and Lavoie, 1999). Prey densities that rorquals may encounter commonly used by researchers are often less than 1 kg/m$^3$ of krill, but krill surveys have produced density estimates as high as 154 kg/m$^3$ (Hazen et al., 2015; Nicol et al., 1987; Wiedenmann et al., 2011). When considering a fin whale capable of engulfing 30 m$^3$ of water per lunge this would equate to 3 kg of krill per lunge at a density of 0.1 kg/m$^3$, or 4620 kg of krill per lunge at a density of 154 kg/m$^3$. Despite these potentially huge mouthfuls of krill, rorquals tend to demonstrate a decreased interlunge interval at higher prey densities, thus increasing feeding frequency with prey density (Goldbogen et al., 2015; Hazen et al., 2015). It is not surprising that rorquals prefer high prey densities, as lunging is energetically expensive. Moreover, it is in the best interest for larger rorquals to feed on high density prey patches, as the filtering area of baleen does not increase proportionately with the increased engulfment capacity of larger rorquals, resulting in longer filtration times (Kahane-Rapport et al., 2020). There appears to be a prey density threshold for lunging, where lunging only occurs if prey density is high enough, otherwise the lunge would be too expensive to perform (Goldbogen et al., 2011; Hazen et al., 2015). Compared to other feeding types in mysticetes, lunging is the most energetically expensive (Acevedo-Gutiérrez et al., 2002; Goldbogen et al., 2008; Hazen et al., 2015).
Personal observations from necropsies show the esophagus of a ~21 m adult fin whale has an esophagus only ~10 cm in diameter, with very thick muscle. Many anecdotal references suggest the esophagus of rorquals, especially the blue whale is incredibly small, unable to stretch more than 10 inches (Scales and Smith, 2010); however, the published data to corroborate this appears non-existent. Literature regarding feeding in rorquals beyond the prey capture mechanism – i.e. processing prey and transporting it to the stomach, is also non-existent.

1.4 Thesis objectives

Cetaceans are notoriously challenging to study. Our knowledge of cetaceans is very much derived from the small, sometimes captive held, easier to dissect, odontocetes - especially regarding internal anatomy and function. As a result, the way mysticetes are studied commonly occurs through assuming the odontocete is the standard model, and that mysticetes likely look and function in the same manner. This idea ignores the millions of years of evolution and adaptation separating odontocetes and mysticetes. Despite all being cetaceans, the two groups are vastly different, and their adaptations for survival underwater likely differ. Studying the internal anatomy of baleen whales is not trivial, as the sheer size of the animal can be overwhelming, especially when trying to access the viscera. As no baleen whales are currently held in captivity, examining stranded animals has become commonplace. One issue with this is waiting for whales to strand or die before you can obtain information for your thesis. Another major issue is the condition of the tissue. Often days go by before a dead whale is discovered - occasionally dead whales sink, bloat, then float - a necropsy team assembled and the necropsy carried out, resulting in degraded tissue that usually isn’t informative anatomically nor mechanically (Moore et al., 2020). Additionally, the sheer size of baleen whales makes manipulating the body to visualize internal anatomy in context, and obtain intact internal samples extremely difficult, and often
can’t be done during a necropsy. A goal of my thesis was to assess morphology and function on fresh mysticete tissue, and the only feasible way to accomplish this was by examining tissues that are normally discarded from a legal commercial fin whale whaling operation in Hvalfjörður, Iceland (no hunting was carried out for research). The fin whale is thus the model rorqual for my thesis.

A goal of my thesis was to expand our meagre knowledge of the internal anatomy of the aerodigestive tract of whales and provide a comprehensive account of how the aerodigestive tract functions to accommodate the needs of the respiratory and digestive systems. Animals need to breathe and eat consistently throughout the day, yet we are totally unaware how these processes coexist in rorquals. How has this system, that started out as a by-product of the transition to land, changed for an aquatic existence? How is the junction between digestive and respiratory tracts suited for both feeding underwater and breathing at the surface? How has this system of intermittent breathing and nearly continuous feeding contributed to these animals becoming the largest animals ever to have lived? There appears to be a divergence in the evolutionary pathways of odontocetes and mysticetes, where odontocetes present adaptations related to sound production and mysticetes present adaptations related to feeding. Given the numerous feeding-related adaptations rorquals have, and their large body size which is inextricably linked to feeding, I expect that the aerodigestive tract of mysticetes reflects this push towards maximizing feeding ability, and that both respiratory and digestive pathways compensate for this. I address this idea in the following three chapters by questioning: 1) How is the respiratory tract protected from water entry and simultaneously protected from barotrauma? 2) How are the upper and lower respiratory tracts protected from food entry during swallowing? 3) How does esophagus morphology contribute to transporting food from pharynx to stomach?
1.4.1 How is the respiratory tract protected from water entry and simultaneously protected from barotrauma?

The air-filled respiratory tract must be protected from water entry at all times, including breathing at the surface and throughout all depths, where hydrostatic pressures can get incredibly high (increasing by ~ 1 atm every 10 m of depth). It is clear that the muscular blowhole margins and nasal plug muscles regulate opening and closing of the blowholes and nasal cavities. Why are two groups of muscles necessary to protect the respiratory tract? Are the blowhole margins alone insufficient? Viewing video footage of rorquals breathing at the surface showed water occasionally flowed over the open blowhole margins as the whale began to dive. Viewing footage of rorquals approaching the surface from underwater showed that air bubbles were released from closed blowhole margins. The blowhole margins on their own are not capable of preventing water entry, nor do they provide an airtight or watertight seal; the nasal plugs are also necessary to prevent water entry. The deep attachment of the nasal plugs is rather suspicious if just to prevent water entry, however. The plugs attach to the skull halfway down the nasal cavities (Maust-Mohl et al., 2019), which means considerable effort must be used to move these plugs to open the respiratory pathway. Hypothetically, the blowhole margins and a much smaller plug would likely be capable of preventing water entry. A problem with reducing the size of these plugs would be an increase in empty air space in the bony nasal cavities. Perhaps these plugs function in more than just prevention of water entry, and are also involved in preventing barotrauma? This question is addressed in chapter 2. I hypothesized that the deep attachment of the nasal plug muscles is a compromise to accomplish two disparate tasks – protect and control the respiratory tract opening and prevent barotrauma at depth.
1.4.2 How are the upper and lower respiratory tracts protected from food entry during swallowing?

The nasal anatomy and larynx of mysticetes are vastly different from that of odontocetes, yet the mysticete anatomy is proposed to function in the same way. This view is unfounded; functional morphology must be addressed through the lens of the animal being studied. Rorquals do not echolocate – sound is produced in the larynx; rorquals do not feed on single food items – they are bulk filter feeders. The rorqual larynx has: 1) a wide-open laryngeal inlet with splayed apart epiglottis and arytenoid cartilages, 2) no lateral protection around the laryngeal inlet, 3) no distinct elongation of epiglottis or arytenoids, and 4) smooth outer surfaces of epiglottis and arytenoids with no ridge for a sphincter to “permanently” grasp. Addressing the pharynx based simply on this morphology, I hypothesized that: 1) the larynx is not fixed in an intranarial position during swallowing, 2) protection of the lower respiratory tract is similar to a carnivore or a human during swallowing, where the laryngeal inlet is closed by contact between the epiglottis and arytenoids as food passes through the pharynx, and 3) that the upper respiratory tract is protected by elevation of the soft palate and its contact with posterior pharyngeal muscles. Pharyngeal structures shifting in this manner, when considering the position of the larynx directly under the nasopharynx (the respiratory pathway crossing the digestive pathway nearly perpendicularly), would seem to provide more protection than an intranarial larynx with no lateral protection. Further, rorquals are often ingesting thousands of krill in a slurry that could easily slip into the lateral aspect of an intranarial larynx, or swallowing live fish that could easily slide into the lateral aspect of the laryngeal inlet. This question and my hypotheses will be addressed in Chapter 3.
1.4.3 How does esophagus morphology contribute to transporting food from pharynx to stomach?

Rorquals have an incredibly small esophagus. A fin whale that is 160 times larger by weight or 5 - 10 times larger by length than a bottlenose dolphin, has an esophagus only double the diameter. Of course, rorquals and dolphins have very different feeding styles and very different prey. The esophagus of a rorqual is especially small when considering how much prey could be engulfed per lunge and the short duration between lunges. The interlunge time is composed of filtering water out, gathering prey at the back of the mouth, and then presumably transporting this prey to the stomach (though unknown if the latter occurs completely during interlunge). A large portion of the interlunge must be taken up by swallowing and it is possible that interlunge time is dictated by swallowing time. A general swallowing time could be estimated based off of the proportion of skeletal and smooth muscle along the esophagus, an unknown in rorquals. In addition to an overall small diameter, the esophageal lumen is quite small owing to the thick muscle that surrounds the lumen. Why do rorquals have such a narrow muscular esophagus and how are large volumes of prey transported to the stomach through this esophagus? I hypothesize that the morphology of the esophagus is necessary to maintain pressure for swallowing a slurry of food, and that the narrow diameter is congruent with how much a circular oriented muscle is able to contract before it no longer produces any force. These questions and hypotheses will be addressed in Chapter 4.
Figure 1.1 Anatomy of the human pharynx and surrounding structures. The pharynx is an area at the back of the throat common to both respiratory and digestive tracts. The pharynx can be divided into 3 regions: Nasopharynx (posterior to the nasal cavity), oropharynx (posterior to the oral cavity), and laryngopharynx (spanning the length of the larynx). Air is directed from the nasal cavities or oral cavity through the pharynx to the larynx, and food is directed from the oral cavity through the pharynx to the esophagus. Image modified from Terese Winslow LLC © 2012.
Figure 1.2 Posterior view of the soft palate muscles in a human. Left and right choana are part of the nasal cavities. TVP – tensor veli palatini, LVP – levator veli palatini, UvM – uvular muscle/musculus palatinus, PPM – palatopharyngeus muscle, PGM – palatoglossus muscle, PTT pharyngotympanic tube, Ham – hamulus of medial pterygoid process. The tensor veli palatini pulls the soft palate ventrally. The levator veli palatini elevates the soft palate. The uvular muscle/musculus palatinus pulls the soft palate dorsally. The palatopharyngeus pulls the pharynx walls dorsally and anteriorly and tenses the soft palate. The palatoglossus depresses the soft palate and elevates the tongue. Image from von Arx & Lozanoff, 2017.
Figure 1.3 Schematic of the pharyngeal constrictor muscles and cervical esophagus in a human in right lateral view. The superior constrictor muscle originates from the oral-pharyngeal region, and generally corresponds with the location of the nasopharynx and oropharynx. The middle constrictor originates from the hyoid and generally surrounds the oropharynx. The inferior constrictor surrounds the laryngopharynx. The inferior constrictor has two parts: the superior *thyropharyngeus* and the inferior *cricopharyngeus*. The *thyropharyngeus* arises from the thyroid cartilage, and the *cricopharyngeus* arises from the cricoid cartilage. The *cricopharyngeus* forms the upper esophageal sphincter and exhibits tonic contraction to keep the esophagus closed and must relax to open the esophageal inlet. Modified from Shapiro & Martin, 1996.
Figure 1.4 Evolution of the lower respiratory tract and larynx. (a) Respiratory diverticula exist in amphibious fishes. (b) The lower respiratory tract develops as a ventral outpocketing of the digestive tract. The most primitive larynx exists in the bichir lungfish, where the larynx is a simple muscular sphincter that contracts to prevent water entry to the lower airways. (c) African lungfish develop more muscular control of the larynx to swallow air. (d) Development of lateral cartilages for muscle insertion and structural support in the Mexican salamander. (e) Development of a cartilaginous ring between glottis and trachea for muscle insertion and structural support in the alligator. Image from Asher et al., 1996 (originally from Negus, 1949).
Figure 1.5 Human larynx and associated structures. (A) Laryngeal cartilages are colour coded according to the legend. Left image – anterior and slightly lateral view of larynx; right image – posterior view of larynx. (B) Laryngeal cartilages and the aryepiglottic fold are underlined in blue. Left image – interior view of left side of larynx with soft tissues showing position of structures relative to tongue and hyoid; right image – interior view of left side of larynx without soft tissues showing laryngeal cartilages and ligaments. Image in (A) modified from Teach me Anatomy (https://teachmeanatomy.info/neck/viscera/larynx/laryngealcartilages/); Image in (B) modified from Standring, 2021.
Figure 1.6 Midsagittal sections of mammalian heads showing the position of the larynx and soft palate. (A) adult rabbit, (B) juvenile cattle, (C) adult California sea lion, (D) adult patas monkey. E – epiglottis, S – soft palate. The larynx sits high in the neck allowing overlap of epiglottis and soft palate (i.e., and intranarial larynx), maintaining the connection between upper and lower airways during deglutition. Image from Laitman & Reidenberg, 2013.
Figure 1.7 Sagittal sketch of the opossum head and neck, showing position of vallecula, soft palate, and epiglottis. During swallowing in some herbivores the bolus can be stored transiently in the vallecula prior to being pushed into the esophagus. Image from Hiemae & Crompton, 1985.
Figure 1.8 Cross section of the human esophagus. Masson trichrome stain. E – lumen of esophagus lined by epithelium (of the mucosa), Ly – lymphoid aggregates (in the lamina propria of the mucosa), MM – muscularis mucosa (of the mucosa), SM – submucosa, CM – circular muscle (of the muscularis propria), LM – longitudinal muscle (of the muscularis propria), G – seromucous glands. The esophagus is a multilayered tube composed of connective tissues and a muscular bilayer. The connective tissues can expand to accommodate a bolus, and the muscular bilayer produces peristaltic contractions to push the bolus to the stomach. Image from Young et al., 2014.
Figure 1.9 Odontocete laryngeal spout morphology and effect of laryngeal spout on respiratory and digestive pathways compared to a dog and manatee. (A) Larynx of a striped dolphin. E – epiglottis, C – corniculate cartilage, T – thyroid cartilage, A – arytenoid cartilage, Cr – cricoid cartilage, Tr – trachea. Laryngeal lumen/inlet indicated by arrow. The rostral portion of the larynx is elongated into a spout. The epiglottis has an enlarged lip on the rostral surface.
which the palatopharyngeal sphincter grasps to keep the spout permanently intranarial. (B)

Schematic of the position of structures in the aerodigestive tract of a dog (upper left), manatee (upper right), and dolphin (bottom). In the dog and manatee, the epiglottis acts as a flap that can make contact with the soft palate to maintain a connected respiratory tract, or it can cover the laryngeal inlet to seal the larynx during swallowing. The pharynx remains an area common to both respiratory and digestive pathways. In the dolphin, the laryngeal spout completely separates the respiratory and digestive tracts eliminating the risk of choking by aspirating food. During swallowing, food travels through the lateral food channels on either side of the laryngeal spout.

Image in (A) from Reidenberg & Laitman, 1987. Image in (B) from Rommel et al., 2018.
Figure 1.10 Mysticete laryngeal morphology compared to a representative odontocete. (A) Laryngeal specimens from a white-sided dolphin (left) and a humpback whale calf (right). E – epiglottis, C – corniculates, P – palatopharyngeal sphincter. The top images are a left lateral view comparing the maximum size of the laryngeal inlet. The humpback larynx has a much larger inlet with very low aryepiglottic folds. The middle images display a dorsal view of the laryngeal inlet at rest; closed in the dolphin and open in the humpback. The bottom images show the larynx in the intranarial position. The palatopharyngeal sphincter completely encircles the...
larynx in the dolphin, yet in the whale only a portion of the epiglottis is intranarial. (B) Laryngeal specimens of 5 mysticetes with the cricoid cartilage cut dorsally down the midline and reflected laterally. (a) minke whale, (b) sei whale, (c) humpback whale, (d) fin whale, (e) northern right whale. Rostral is up in all images. The u-fold, a sound production structure formed by the arytenoid cartilages, is outlined by asterisks. The rorqual specimens, (a) to (d), all have a wide laryngeal inlet with minimal aryepiglottic folds present, and a concave epiglottis. The balaenid specimen displays a laryngeal stalk, with the median pyramidal eminence running down the middle of the epiglottis. (C) The top image displays a mid-sagittal section through a fetal bowhead whale larynx. The bottom image is a schematic of that larynx, showing the position relative to the intrapharyngeal orifice, or nasopharynx. The close proximity of the epiglottis and arytenoids are reminiscent of the odontocete larynx, however the stalk is relatively shorter than the odontocete laryngeal spout. Image in (A) from Reidenberg & Laitman 2007a. Image in (B) from Reidenberg & Laitman 2007b. Image in (C) from Schoenfuss et al. 2014.
Figure 1.11 Oral cavity morphology and feeding style in mysticetes. (A) Schematic cross sections through the oral cavity of a right whale (top), rorqual (middle), and gray whale (bottom). The shape and size of the rostrum, lower jaw and baleen vary across families in accordance with feeding style. (B) Schematic of balaenid skim feeding, whereby the whale swims through prey with its mouth open to capture prey in the oral cavity and filter water out through the baleen plates. (C) Schematic of gray whale benthic suction feeding, whereby the whale turns its body laterally along the sea floor and opens its mouth to suck sediment and prey into the oral cavity and filters the water and sediment out through the baleen. Image in (A) from Werth, 2001. Image in (B) from Croll, 2018. Image in (C) from Nerini, 1984.
Figure 1.12 Schematic of rorqual lunge feeding. The left panel shows a lateral view of a rorqual lunge feeding and the right panel shows the corresponding cross section through the oral cavity. The dashed line along the VGB represents the position of the *cavum ventrale*. The top drawing displays the whale prior to feeding. The second drawing displays the whale in the process of engulfing prey-laden water. The third drawing shows the bloated whale in the process of filtering. The fourth drawing shows the completion of filtering with prey trapped in the oral cavity. Image from Croll, 2018.
Chapter 2: Rorqual whale nasal plugs: protecting the respiratory tract against water entry and barotrauma

2.1 Introduction

Rorqual whales spend most of their lives holding their breath underwater. Expiring and inspiring at the interface of air and water and spending much of the time at depths where ambient pressures are high generate constant risks to the respiratory system. Air exits and enters the respiratory tract through the blowholes (nostrils), and the blowhole margins have been described as the first line of defense against water incursion (Maust-Mohl et al., 2019). The actions of the margins are clearly visible with aerial photography and animal cameras at the surface (Fig. 2.1): Prior to surfacing, the blowhole margins remain closed and the rostrum appears flat. Exhalation starts once the blowholes break the water surface and the blowholes open. As this occurs, the blowhole margins rise up, and a bulge is created rostrolateral to each blowhole. Inhalation follows, with a widening of the blowholes (Racovitza, 1904; Scholander, 1940) and a slight enlargement of the rostrolateral bulge. Once inhalation is complete, the blowhole margins close and the rorqual dives.

While viewing footage of rorquals surfacing and breathing, I made observations that indicate that protection of the respiratory tract involves more than just closure of the blowhole. First, rorquals occasionally submerge their rostrum before the blowhole margins are closed, allowing water to flow over and into the open blowholes (Fig. 2.2 A,B). Second, bubbles can be released from the blowholes prior to the rorqual surfacing while the blowhole margins remain closed (Fig. 2.2 C,D). These occurrences show that the blowhole margins do not always prevent water entry, nor are they airtight while submerged.
The respiratory system is protected by two groups of nasolabial muscles: those distributed superficially that link to and control the cartilaginous blowhole margins, and those on a deeper plane that form paired, cylindrical nasal plug muscles that extend from the rostrum to halfway down the nasal cavities in the skull (Maust-Mohl et al., 2019). Nasolabial muscles are described anatomically as a single functional group; however, I argue here that their different morphologies and different positions related to the skull indicate they form two, separate functional groups.

Rorqual breathing is explosive, lasting only 1 - 2 seconds. Achieving high air flow rates requires minimizing resistance through the nasal cavities and blowholes (Kooyman, 1973), but the size and tethering of the nasal plug muscles do not appear to be optimized for facilitating this. The plugs are very large. Considerable effort must be exerted to move them quickly for each breath, and their attachments deep in the nasal cavities would appear to prevent their complete removal when breathing. This incongruity raises the possibility that the nasal plug muscles are designed for another function that necessitates their large size and deep attachment in the nasal cavity. High ambient pressures expose diving mammals to two potential problems: decompression sickness from excess nitrogen absorption at depth and barotrauma from mechanical distortion of the tissue. Shifting air out of gas exchange regions prevents nitrogen absorption (Bostrom et al., 2008; Fahlman et al., 2017; Kooyman and Ponganis, 2018; Leith, 1970; Scholander, 1940), and both the high compliance of the alveoli and chest wall and the low compliance of the upper respiratory tract are essential to achieve this (Bostrom et al., 2008; Cozzi et al., 2005). The risk of nitrogen absorption ends once the lungs have collapsed and gas exchange has ceased, but the danger of barotrauma to the stiffened portions of the respiratory tract remains a problem.
I argue that the deep attachment of the nasal plug muscles is a compromise that allows them to accomplish two disparate tasks (Fig. 2.3). I hypothesize that the nasal plug muscles, rather than the blowhole margins, form the main valve controlling the opening to the respiratory tract. The blowhole margins are not competent on their own at depth, and instead they streamline the rostrum. The position of the nasal plug muscles in the nasal cavities present large masses of tissue that prevent water from being forced into the nasal cavities at high ambient ocean pressures when at depth. I suggest that the nasal plug muscles actively withdraw from their position in the nasal cavities before the blowhole margins open, and that the nasal plug muscles are back in position occluding the nasal cavities before the blowhole margins close. This timing would account for air escaping the blowhole while the margins remain closed and would also allow water to flow over the open blowhole margins without incursion into the respiratory tract. I also hypothesize that the deep attachment of the nasal plug muscles in the nasal cavities is necessary to prevent barotrauma at depth. High ambient ocean pressures force the nasal plug muscles deeper into the skull, partially filling and hence raising the pressure inside the non-collapsible nasal cavities. I investigated these hypotheses by examining videos of rorquals surfacing, morphometry of skulls, performing dissections, and making model calculations. I also examined the relevant anatomy in an MRI scan and evaluated the histology of selected regions of the nasal plug muscles.

2.2 Methods

2.2.1 Video data

Photos and videos from animal cameras and aerial cameras of blue and humpback whales were obtained from Duke Marine Robotics and Ved Chirayath (Permits: NSF IOS 1656691 and NMFS Permit 16111). I observed and described the timing of respiration, extent of blowhole
opening, anatomical features surrounding the blowhole, and general movements of tissue surrounding the blowholes.

2.2.2 Skull anatomy

Skulls from 6 rorqual whales in museums were examined in their respective locations for morphological measurements including length and diameter of the nasal cavities. Details of all skulls and heads used are listed in Table 2.1. A sub-adult minke whale skull was used to estimate nasal cavity volume by filling the nasal cavities with rice and measuring the volume of rice used.

2.2.3 MRI

MRI images of a fetal minke whale head from a previous anatomical study (Pyenson et al., 2012) were viewed on OsiriX Lite (Rosset et al., 2004). I observed the position of the nasal plug muscles in the nasal cavities and determined the volume of both the nasal cavities and nasal plug muscles from slice thickness and area using Fiji (Schindelin et al., 2012).

2.2.4 Animals and tissue samples

Fin whale specimens were collected postmortem from the commercial whaling operation at Hvalfjörður, Iceland in the summers of 2015 and 2018. All specimens were examined fresh at the station within 24 hours of death. A total of 14 animals were surveyed, with 8 examined in detail. One dissected animal was a fetus 3 m long. All others were adults with an average total length of 17 – 21 m. Tissue samples were imported to Canada under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits.

2.2.5 Gross anatomy and physical manipulation

Nasal plug muscles in fin whales were examined by removing blubber surrounding and directly dorsal to the blowhole and along the rostrum. Nasal plug muscle was revealed by progressive horizontal slicing through superficial muscle until nasal plug muscle was visible. At
various stages in the dissection, the nasal plug muscles were physically manipulated to understand how they could function in a living whale. Hooks were placed in nasal plug muscle to pull rostrally. The extent to which I was able to open the vestibule (the space created by the open blowhole) was recorded and compared to aerial photos. Likewise, the nasal plug muscles were forced ventro-caudally into the nasal cavities to determine the position the plugs could achieve under high ambient pressure while diving. In both fetal and adult specimens, sagittal and transverse slices were made through the skull to examine the attachments and location of nasal plug muscle and the proportion of the nasal cavities occupied by nasal plug tissue. Photos of transverse slices of the skull were used to estimate nasal cavity volume and nasal plug volume using Fiji.

2.2.6 Histology

Nasal plug tissue from a fin whale was sampled from 5 locations in a single nasal plug: medial and lateral aspects of the nasal fossa/vestibule region, midway between the vestibules and nasal septum attachment, and at 2 locations along the attachment to the nasal septum. Samples were fixed in 10% neutral buffered formalin, and then processed by Wax-It Histology Services Inc. (UBC), following standard techniques. Samples were sliced at 5 μm and stained with Verhoeff-Van Gieson stain to display elastin in black, collagen in pink, and muscle in yellow/beige. Slide scans were viewed using QuPath (Bankhead et al., 2017) to determine relative proportions of elastin, collagen, and muscle at each location sampled.

2.3 Results

2.3.1 Video data

Exhalation and inhalation in blue and humpback whales followed the same patterns as previously described in rorquals - exhaling upon surfacing and inhaling before diving (Fig. 2.1).
During exhalation, the superficial muscles contracted to open the blowholes and lift the blowhole margins to create a raised barrier. Additionally, a bulge was created just rostral and lateral to the blowhole (rostrolateral bulge) and appeared to divert water from the blowhole. I found that the rostrolateral bulge projected above the flat surface of the rostrum rather than solely fitting in the skull depression/nasal fossa as previously described (Maust-Mohl et al., 2019). When the blowhole margins opened, the dark epithelial lining of the vestibules became visible. During inhalation, the margins open slightly further in a buckling-like motion. Muscular contraction to open the blowholes likely further pulls on the cartilage lateral and caudal to the blowholes to cause this buckling. The rostrolateral bulge increased slightly in size during inhalation. The superficial muscles relax to close the blowholes and streamline the rostrum by smoothing its dorsal surface when submerged.

2.3.2 Anatomy

The rorqual skull possesses a large rostro-dorsal depression termed the nasal fossa (Buono et al., 2015). The nasal fossa extends from the tip of the rostrum to the external bony nares at the top of the nasal cavities. The nares are bounded caudally by the nasal bones and rostro-laterally by the premaxilla/maxilla. Below this, the nasal cavities curve caudoventrally towards the internal bony nares (choanae). The nasal cavities were elliptical in cross section and did not change significantly in size throughout their length.

In minke and fin whales, the superficial muscles formed the blowhole margins surrounding the external nares. The nasal plugs were deep to the more superficial muscle surrounding the nares, and extended caudoventrally from rostral origins in the nasal fossa into the nasal cavities (Figs. 2.4, 2.5, 2.6). The nasal plugs were attached rostrally, approximately two thirds to one half of the way down the rostrum from the anterior tip, to the mesorostral cartilage.
This cartilage lies in the midline of the nasal fossa and separates it into left and right sides (Fig. 2.5 A,C). The caudal attachment point of each nasal plug was also medial, on the nasal septum, approximately halfway down the nasal cavities (Figs 2.4, 2.5). Each side of the nasal fossa was filled by a cylindrical nasal plug. The nasal plugs enlarged towards the blowholes, acquiring a bulbous appearance and filled the vestibules. From this position, the nasal plugs extended down into and completely filled the upper half of the nasal cavities. The plugs narrowed toward their attachment on the nasal septum leaving some unoccupied air space lateral to the plugs when relaxed (Figs 2.4, 2.6).

Removing the skin, blubber, and superficial muscle surrounding the blowholes in fin whales revealed the nasal plugs. I was able to pull the nasal plugs rostrally (Fig. 2.5 B,D), a maneuver that probably mimics the creation of the rostrolateral bulge visible in videos of rorquals breathing. The plugs are attached to the skull at both ends; it was the fatty bulbous portion in the vestibule that I pulled rostrally from the upper nasal cavities to create the bulge. Shifting the plug formed an open space through the dorsal portion of the nasal cavity and revealed the black epithelial lining of the nasal cavities that was visible in videos. With more force, the entire bulbous portion could be cleared from the nasal cavity to maintain an unobstructed airway for breathing.

Sagittal slices of the adult fin whale skulls made slightly off the midline and through one of the nasal cavities revealed the position of nasal plug tissue in the nasal cavity and the remaining air space below this (Fig. 2.6). Transverse slices of multiple skulls also yielded an average estimate of nasal plug volume and air space volume. For an average 19 m adult fin whale, the volume of both nasal plugs combined within the nasal cavities was 16 L, and the air space volume in the nasal cavities was 16 L with the plugs in place and 32 L with the plugs
removed. The total length of the plugs from attachment in the rostrum to attachment in the nasal cavities was approximately 1.3 m. The total length of the nasal cavities alone was approximately 1m.

There were visibly distinct differences in the composition of the fin whale nasal plugs along their length (Fig. 2.6). The rostral nasal fossa region was almost entirely skeletal muscle. Near the blowhole and vestibules this transitioned to adipose tissue (fat) with much less muscle. A slice through the plugs in the vestibule revealed there was some regionality to the muscle and fat distribution, laterally presenting more muscle, while medially there was more fat. Collagen fibers were visible throughout the fat and muscle in this region (Fig. 2.7 A,B). There was very little muscle beyond the vestibules as the plugs descended towards their attachment points. Histology confirmed this difference in tissue composition along the fin whale nasal plugs (Fig. 2.7). No samples were collected from rostral to the blowhole, but my gross observations demonstrate that this region is almost strictly muscle, clearly making it the contractile region of the nasal plugs (Figs 2.5-2.7). The bulbous vestibule region and the upper nasal cavity region was mainly fat with only some muscle (Fig. 2.7, A-D). Since fat is incompressible but easily deformed, this region of the plug can readily seal the nasal cavity. Collagen was distributed around the muscle bundles and throughout the adipose tissue (Fig. 2.7 C,D). There was almost no muscle present caudal to this region, except for a few small “islands” of muscle near the rostral attachment region to the nasal septum (Fig. 2.7E). Along the attachment region to the nasal septum, the plug was composed almost completely of elastin and collagen, especially at the caudal-most part (Fig. 2.7 F,G). I thus refer to this region as the “tendon”, due to its composition and its role in attaching the nasal plug muscle, through collagen, to the bony nasal septum at the posterior of the vomer bone. The very high elastin content of the tendon would allow it to readily
stretch and store elastic energy. Due to the wide distribution of tissue types seen along their
length, I have chosen to use the term “nasal plugs” to refer to the entire structure rather than
“nasal plug muscles” as it more accurately reflects their heterogenous composition and specific
function(s). The heterogenous composition of the plugs is summarized in Fig. 2.8.

2.4 Discussion

Actions of the blowhole margins are easily observable in breathing rorquals, so it is not
surprising that the blowhole margins are currently regarded as the primary mechanism
preventing water incursion. However, based on observations of videos and photographs from
directly above living rorquals at the surface and the anatomy of fresh specimens, my results
indicate that the nasal plugs are the primary valves preventing water incursion. Closing the
respiratory tract and carrying a volume of air into a high-pressure environment poses challenges
to marine mammals: preventing water entry, preventing decompression sickness, and preventing
the formation of damaging pressure differentials. The nasal plugs are a novel protective feature
in rorquals that are the final step in the graded compressibility chain of the respiratory tract and
respond to the creation of pressure differentials to prevent barotrauma. Much of my data was
collected from fin and minke whales, but I believe my results are representative of all rorquals as
they possess similar skull structure, and exhibit similar diving patterns with explosive breathing.
I propose the following model (Figs. 2.8, 2.9) for how the nasal plugs function at the surface in
preventing water incursion and at depth in preventing barotrauma.

2.4.1 At the surface

The superficial muscles and nasal plugs are activated asynchronously throughout the
breathing cycle and remain in a relaxed state while submerged. General sensory innervation of
the plugs is likely by the maxillary division of the trigeminal nerve (CN V2) and motor control of
the muscle is likely by the facial nerve (CN VII), as nasolabial muscles are modified muscles of facial expression. When surfacing, before the blowholes have breached the water surface, air bubbles visibly escape the passively closed blowhole margins, indicating the muscular nasal plugs have begun to contract to allow passage of air through the nasal cavities (Fig 2.2). Beginning exhalation while still underwater minimizes time spent at or near the surface where drag forces can be substantially higher (Hertel, 1966). As a rorqual’s blowholes break the water surface, the nasal plugs are pulled fully rostrally and the superficial muscles spread the blowhole margins. This withdraws the bulk of the plug tissue from the nasal cavities, clearing the passages for nearly unobstructed air flow. The rostral movement of the nasal plugs stretches the elastin-dominated tendons that attach the plugs to the nasal septum. After inhalation, but just before the rostrum dips underwater, the nasal plug muscles relax, releasing the tension on the tendons which then pull the nasal plugs back into their rest position through elastic recoil. Given the high elastin composition of the tendon (Fig. 2.7), this recoil is likely rapid, which minimizes the time that the upper respiratory tract is open and at risk of water incursion. Importantly, the plugs are at rest when they are within the nasal cavities and therefore require no work to keep them in the “plugged” or default position. In this position, the nasal cavities are occluded by the fatty bulbous part of the nasal plugs (Fig. 2.6). Occasionally, when near the end of a breathing cycle, the whale’s rostrum dips below the water surface before the superficial muscles have relaxed to close the blowhole margins, and water flows into the vestibules (Fig. 2.2). This demonstrates that the superficial muscles relax after the nasal plug muscles. The relaxed nasal plug muscles prevent water from entering the respiratory tract beyond the vestibules during this interval. The regional heterogenous composition of the nasal plugs is necessary for them to be withdrawn from the nasal cavities during breathing. The nasal plugs have been referred to as “nasal plug
muscles”; however, if these structures were entirely composed of muscle, it would be impossible to remove them from the nasal cavities. When muscles contract, they increase in cross-sectional area; when fat is pulled on, it decreases in area. If the plugs were strictly muscular and sealed the nasal cavities in their relaxed state, then muscular contraction could only further plug the nasal cavities. Instead, the plugs must have a contractile region to withdraw them from the nasal cavities; an elastic region to anchor them, allow them to be withdrawn, and pull them back into place; and a fatty region that lengthens when pulled, and settles to fill out and seal the nasal cavities as the tendon recoils.

2.4.2 At depth

The air-filled respiratory tract is subject to ambient ocean pressures (P_amb) that increase by ~ 1 atm (~ 101.3 kPa) for every 10 m of depth. Depending on the compliance of the surrounding structures, these external pressures progressively reduce the air volume and consequently raise the pressure inside the respiratory tract. The resultant internal pressure can be calculated from Boyle’s law: P_d = P_s V_s / V_d, where P_d and V_d are the pressure and volume at depth, and P_s and V_s the values at the surface. If the surrounding structures are stiff and resist compression, there will be little reduction in volume and hence little rise in internal pressure. This will result in a pressure differential between the respiratory tract and P_amb. A pressure differential may not damage the walls of the structure if they are strong, as in submarines or the bones of the skull that surround the nasal cavity, but the soft tissue lining the bony nasal cavities or in contact with the cavities could swell and rupture, leading to hemorrhaging. To avoid barotrauma, the respiratory tract must be sufficiently compliant to prevent pressure differentials from developing at any depth to which the animal dives.
The cetacean respiratory tract is composed of upper and lower components, defined by developmental origins. The upper respiratory tract (URT) resides in the skull and is composed of the bony nasal cavities and nasopharynx. Below this, the larynx, trachea, bronchi, and lungs form the lower respiratory tract (LRT). The main structural component of the URT is bone, whereas cartilage dominates the LRT. The different structural materials of these regions determine their response to pressure. “Lung collapse”, the compression and reduction of the volume of the lungs and thoracic cavity, requires the relatively compliant tissue of the LRT. Estimates of lung collapse depth in marine mammals range from 70 m – 225 m, with the deeper estimates often corresponding to deep diving pinnipeds (Kooyman, 1973; McDonald and Ponganis, 2012; Moore et al., 2014; Scholander, 1940). There is a wealth of knowledge on how the compressible LRT responds to pressures at depth, but no equivalent information on how the URT responds, or what happens to the URT once the LRT reaches lung collapse and is no longer capable of deforming under pressure.

I considered two scenarios for how the URT and LRT might work together at depth to accommodate pressure and volume changes to avoid barotrauma in the URT. The first follows the ideas of Scholander (1940), that the entire respiratory tract remains connected allowing air to be forced rostrally into the bony nasal cavities during a dive. The second integrates the movement of laryngeal cartilages during sound production and the larynx’s potential to compress under pressure, effectively dividing the respiratory tract into its anatomically defined upper and lower regions. All of the morphological measurements provided here are representative of an average 19m long adult fin whale.
2.4.2.1 Scenario 1 – continuous respiratory tract

Scholander (1940) stated that during a dive air from the lungs is forced rostrally into the rigid airways and nasal cavities, with the air “safely stored” in these rigid spaces until surfacing. My underlying assumption of the term “safely stored” is not only that the air has a place to go once lung compression occurs, but also that the air fills any incompressible space and prevents barotrauma. This assumption follows from the graded compliance of the respiratory tract whereby the distal-most structures (alveoli) are most compliant and lead proximally to decreasingly compliant structures (bronchi, trachea, larynx) and eventually the rigid nasal cavities (Bagnoli et al., 2011; Davenport et al., 2013; Scholander, 1940). With depth, as a pressure differential begins to form between the respiratory tract and $P_{amb}$, the thorax (including the diaphragm) responds first and forces air rostrally. The response of the respiratory tract essentially follows Boyle’s law - air volumes in compressible spaces decrease as ambient pressure increases up to the point where further tissue deformation is no longer possible.

I found the total volume of the two nasal cavities combined was ~ 32 L. Half of this (~ 16 L) is occupied by the nasal plugs. The other half (~ 16 L) is air space below the nasal plugs. Total lung capacity of an average adult fin whale is around 1440 L (Scholander, 1940). Assuming the air in the semi-rigid trachea is largely diminished (Bostrom et al., 2008; Cozzi et al., 2005), a fin whale could dive to ~ 900 m before the volume of respiratory tract air compresses down to 16 L. Feeding depths for fin whales average 300 m (Croll et al., 2001), and the deepest recorded dive is 470 m (Panigada et al., 1999). Therefore, in this scenario, a fin whale can compensate for a pressure differential at all depths encountered while diving, and barotrauma would not occur.
2.4.2.2 Scenario 2 – physical separation of URT and LRT

Scholander’s assumption does not account for the action of the larynx in rorquals. The larynx acts as a valve for breathing and has been established as the sound producing organ in rorquals. Sound production requires movement of the laryngeal cartilages and inflation/deflation of the laryngeal sac (Aroyan et al., 2000; Damien et al., 2019; Gandilhon et al., 2015; Reidenberg and Laitman, 2007b; Reidenberg and Laitman, 2008). These movements can isolate portions of the respiratory tract. When the blowholes and larynx are closed, the respiratory tract is functionally separated into two sections – the lungs and trachea form one section, and the laryngeal sac, larynx, and nasal cavities form another (Damien et al., 2019; Gandilhon et al., 2015). The laryngeal sac can also be pushed upward and into the caudal portion of the larynx and rostral portion of the trachea, and this physically separates the URT and LRT (Reidenberg and Laitman, 2008). If separation occurs during a dive, it would prevent any further shift of air into the URT, and alter the pressures in the now isolated URT.

Separation of the respiratory tract by the larynx introduces a potentially damaging situation where the compressible parts of the respiratory tract (i.e., lungs, bronchi, trachea) are no longer connected to the incompressible parts (i.e., nasal cavities). In this scenario, I assume that the larynx collapses when the lungs collapse and, therefore, lung collapse dictates the separation depth. Prior to separation, the respiratory tract response follows Scholander, with the graded compliance accounting for pressure and volume changes that prevent the development of pressure differentials (Fig. 9). After separation, I am concerned only with the rigid URT. If no URT tissue can deform, air volume and hence internal pressure will remain at the value at separation, and a pressure differential between $P_{\text{amb}}$ and nasal cavity pressure ($P_{\text{nasal}}$) will form and grow progressively with greater depth. Although ambient ocean pressure pushes in on the
nasal plugs at all depths up to the point of separation, the internal pressure has been maintained at ambient by thorax deformation. Thus, up to the point of separation, there is no pressure differential across the plugs. Only after separation does a pressure differential form across the nasal plugs if depth continues to increase. Ambient ocean pressure pushes on the muscular region of the nasal plugs in the rostrum, perpendicular to the surface, forcing the plugs into the only available space - the nasal cavities. Below separation depths, the nasal plugs are the last remaining compliant tissues in the URT that can respond to $P_{\text{amb}}$. Movement of the nasal plugs decreases the volume of air space in the nasal cavities to equilibrate pressure between $P_{\text{amb}}$ and $P_{\text{nasal}}$. From my assessment, the fatty caudal region of the plugs can be easily forced deeper into the nasal cavities to account for half of the air space. Thus, for my estimate of 16L total air space in an average adult fin whale skull, perhaps 8 L could be accounted for by the forced inward movement of the plugs. The remaining space under the nasal plugs would be 8 L. Inward movement of the nasal plugs raises the internal pressure within the nasal cavities and prevents the formation of a significant pressure differential up to the point where they can effectively move no farther. Assuming a lung collapse depth of 150 m, with the nasal plugs responding to pressure beyond this depth, a fin whale could dive to 300 m without pressure differential formation. This places a fin whale within normal feeding depths (Croll et al., 2001). Importantly, the depth at which the nasal plugs can help alleviate pressure differentials depends on the depth of lung collapse. My determination that the nasal plugs can decrease air space volume by half means that, from Boyle’s Law, they can double the internal pressure; this allows the nasal plugs to manage pressure in the nasal cavities between the collapse depth and double the collapse depth (150 m and 300 m in this scenario). Lung and larynx collapse depth is thus an important
variable for determining the maximum depth to which the nasal plugs contribute to pressure management.

I don’t know the depth at which nasal plugs stop moving, but it appears likely that fin whales can descend below that limit. Other anatomical structures must compensate for the increase in pressure required to reach depths below 300 m. Although not observed in my own study, swelling of vascular tissue (such as retia) in the nasal cavities could decrease air volumes, as in the Southern right whale (Buono et al., 2015). Beyond 300 m, the remaining air volumes are small (8 L) and require minimal further volume changes to effect large changes in pressure (Fig. 9b).

2.4.3 Other cetaceans

Although the term “nasal plug” is used to describe anatomical structures in other cetaceans, these plugs do not share the same morphology or proposed barotrauma function as the rorqual nasal plugs. Odontocetes possess a single nasal plug directly ventral to the singular blowhole opening and dorsal to the external nares (Maust-Mohl et al., 2019). The odontocete nasal plug resides completely above the skull with no extensions into the nasal cavities. It is contiguous with the dorsal surface of the premaxillary air sacs used in sound production (Reidenberg and Laitman, 2008). The position of the plug implies it could block the dorsal-most portion of the fleshy nasal passage. Southern right whales possess a bulbous protrusion in each vestibular lumen termed the nasal plug (Buono et al., 2015), but the plugs do not extend into the bony nasal cavities. Bowhead whales possess a valvular mass that arises rostrolaterally from the vestibular walls and passively occludes the blowholes by relaxing medially (Henry, 1983). It is unclear how deep these valvular masses extend as the nasal cavity was not examined. Nasal plug anatomy was described for a fin whale calf and minke whale calf (Maust-Mohl et al., 2019);
however, the descriptions were based on older tissue that was detached from the skull, which may have led to an incomplete assessment of the size and composition of the nasal plugs.

2.5 Summary

Nasal plugs are a unique innovation in rorqual whales, and I argue that the plugs are the true valves protecting the respiratory tract both from water incursion and from barotrauma. Protecting the respiratory tract requires a compromise between two opposing actions – opening the respiratory tract at the surface and filling it when diving. These functions are made possible by the plugs’ heterogenous composition – muscle at the rostral end, fat in the part that fills the nasal cavity, and elastin and collagen in the tendon that attaches each plug to the nasal septum (Fig. 2.8). The deep attachments of the nasal plugs in the nasal cavities – seemingly confounding when considering the function of the plugs at the surface, is explained by their function at depth. This study demonstrates the necessity of the compliant nasal plug tissue in protecting rorqual nasal cavities from water entry and barotrauma in a high-pressure environment. The nasal plugs demonstrate their importance and novelty during diving, where pressure becomes as important an issue as the danger of water entry.
Figure 2.1 Blowhole activity from aerial photography in a blue whale (top row) and video stills from an animal camera on a humpback whale (bottom row). (A,D) Submerged. The blowhole margins remain closed and the rostrum is flat. (B,E) Exhalation. The blowhole margins open once the rostrum breaks the water surface. A bulge rostrolateral to the blowholes is formed. (C,F) Inhalation. The blowhole margins widen and the rostrolateral bulge enlarges. Photographs used with permission from Duke Marine Robotics and Ved Chirayath; NMFS Permit 16111.
Figure 2.2 Water flowing over the open blowhole margins (A,B) and air bubbles escaping from the closed blowhole margins (C,D) in two humpback whales. (A,B) After inhalation and upon beginning to dive, the blowhole margins remained open and water flowed into the vestibule. Video stills from aerial photography. (C,D) Before the blowhole has broken the water surface, air bubbles are released from the closed blowhole margins, indicating exhalation has begun while underwater. Video stills from an animal camera. The reflection of the whale on the water surface can be seen at the top of the images in (C,D). Photographs used with permission from Duke Marine Robotics and Ved Chirayath; NMFS Permit 16111.
Figure 2.3 Proposed nasal plug position while submerged near surface (A), during breathing (B), and at depth (C). Superficial nasal muscles in blue and nasal plugs muscles in red. (A) Superficial nasal muscles and nasal plug muscles are passively closed at rest, maintaining closure of the respiratory tract to prevent water incursion. (B) Muscular contraction of the nasal plug muscle withdraws it from the nasal cavity. The superficial muscles contract and open the blowhole margins. (C) Ambient ocean pressure at depth pushes the nasal plug muscles deeper into the nasal cavity. Illustration by Alex Boersma.
Figure 2.4 Transverse sections from MRI scan of a fetal minke whale head showing nasal plug morphology and position in the nasal cavities. The images have been cropped to remove mandibular regions. Inset line drawing shows position of panels along the skull. Arrows show left nasal plug. (A) Nasal plug tissue in in the rostrum. The nasal plugs fill the nasal fossa and merge dorsally with soft tissue (B) Nasal plugs at the blowholes. The top/margins of the blowholes are visible at the top of the image between the plugs. (C) The nasal plugs occlude the nasal cavities just caudal to the blowhole. (D) The nasal plugs narrow caudally and appear as semicircles that attach medially to the nasal septum. Air space is visible below the plugs. (E) The nasal plugs diminish in size. They remain connected medially and air space is visible on all other sides.
Figure 2.5 Dorsal view of nasal plugs of fetal (AB) and adult (CD) fin whales, showing the position when relaxed (A,C) and pulled rostrally (B,D). Rostral is to the left in all panels. Skin, blubber, and superficial muscles were removed to show the nasal plug muscles. (A,C) Inward curving striations of the muscles attach to the mesorostral cartilage at the midline of the rostrum. (B,D) When the plugs are pulled rostrally the vestibule is opened, and the black epithelial lining of the nasal cavity is revealed. The black epithelial lining is not complete in the fetus (B). Green arrows indicate hooks used to pull adult nasal plug (D). The rostral portion of skull was removed in the adult prior to pulling the plugs rostrally (D). Scale bars: AB – 2 cm, CD – 10 cm.
Figure 2.6 The nasal plugs in an adult fin whale differ in morphology in different regions of the nasal cavities. Transverse (ABC) and sagittal (DEF) slices. Rostral is to the left. (A) Nasal plug muscles at the blowholes. The plugs occupy the nasal fossa entirely and extend upwards towards the blowhole margins. The blowhole margins and superficial muscle above the plugs
were removed. The plugs in this region are composed largely of adipose tissue with muscle is
distributed throughout. (B) The cylindrical nasal plug muscles completely occupy the nasal
cavities caudal to the blowholes (similar to Fig. 4C). The plugs are largely composed of adipose
tissue. Black epithelial tissue can be seen surrounding the plugs. The plugs attach medially and
ventrally to the nasal septum. (C) Caudal attachment of nasal plugs. The plugs attach medially to
the nasal septum. Arrow shows white attachment tissue, revealed by cutting through the black
epithelial layer that encases the plugs. (D) Sagittal slice through the head just to the left of the
midline showing left nasal cavity. Upper portion of the nasal cavity with black and white
epithelium, at left, continues behind the left lateral wall of the nasal cavity (middle) down to the
nasopharynx (bottom right, indicated by white triangle). Parts of the cranial cavity are visible in
the upper right. The front of the rostrum was removed, as indicated by the grey box in the inset.
The left nasal plug was largely removed, but some is visible in the lower left. White arrows
indicate path of airflow. (E) Left nasal plug in left nasal cavity, slightly displaced ventrally.
Some of the plug was detached while cutting the skull. The plug is composed largely of adipose
tissue, with some muscle. The plug narrows caudally into the tendon, shown by the arrow. (F)
Tendon/caudal attachment of plug, with nasal plug tissue displaced across nasal cavity. Air space
ventral to the plug leads to the nasopharynx (white triangle) in the lower right. The nasal septum
is covered in black and white epithelial tissue. Part of the cranial cavity is visible in the top right.
Coloured shapes show relative position of all panels. Red square – blowhole; green circle – mid-
nasal cavity; blue star – caudal attachment of nasal plug muscle; white triangle – nasopharynx.
Scale bars: ABC – 10 cm, DE (ruler) – 30 cm, F (blue bar on ruler) – 10 cm.
**Figure 2.7 Regional differences in nasal plug tissue composition.** Histology from four locations in the right nasal plug. (ABCD) from the right nasal plug near the blowhole. (A) Cross-section of muscle tissue with small pockets of adipose tissue surrounded by collagen fibres on left. Representative of the rostral nasal plug tissue. (B) Slice into right nasal plug filling the vestibule (same tissue from Fig. 5D). High adipose tissue content with muscular projections throughout. (C) Detail of B, showing muscle bundles amongst fat, with collagen fibres distributed throughout. Distinct thick collagen fibre in bottom right. (D) Cross-section representing region from (B,C), showing collagen distributed throughout the extensive adipose tissue. Muscle tissue on right, surrounded by collagen. (E) Longitudinal section just rostral to the tendon showing high elastin and collagen content. Elastin fibres have multiple orientations throughout the slice. Some muscle is visible along the bottom. (F) Longitudinal section of tendon showing strictly elastin and collagen. (G) Cross section of tendon. Elastin in cross section visible throughout. Stained with Verhoeff-Van Gieson stain. Elastin in black, collagen in pink/purple, muscle in beige and fat white/clear. Scale bars: A – 1 mm B – 5cm, C – 1 cm, D – 1 mm, EFG – 100 um.
Figure 2.8 Illustration showing the predominant tissue types and position of the nasal plugs. The nasal plugs possess three main tissue types along their length. The rostral-most portion of the nasal plug is almost entirely muscle, making it the contractile region of the plug. The midsection of the plug is adipose tissue dense, allowing it to settle into and seal the nasal cavities. The caudal-most tendon region is elastin and collagen dense, defining it as the stretchy region of the plug. Illustration by Alex Boersma.
Figure 2.9 Schematic illustration of changes in nasal cavity volume (A) and pressure differential between ambient ocean pressure and the nasal cavities (B) with depth. Grey boxes display the status of the larynx at depth. (A) Nasal cavity/airspace below plugs volume. Solid line is the actual response of the nasal plugs. Dashed line is the hypothetical response of nasal plugs. (B) Solid line is actual pressure differential. Dashed line is a hypothetical pressure differential. In the first depth zone (0 - 150 m), thorax volume decreases, allowing $P_{\text{nasal}}$ to match ambient ocean pressure. The nasal plugs do not move. In the second depth zone (150 – 300 m), closure of the larynx has separated the URT and LRT. The thorax is no longer responsible for pressures in the nasal cavities. The nasal plugs now respond to $P_{\text{amb}}$ and get pushed inward, decreasing the volume of the nasal cavities and maintaining pressure equilibrium between $P_{\text{amb}}$ and $P_{\text{nasal}}$. In the third depth zone (310 - 500 m), the nasal plugs cannot be pushed further inward (A, solid line). If no other tissue responds to decrease volume and maintain pressure equilibrium, the potential exists for a pressure differential to form (B, solid line). If the plugs continued to move further inward (A, dotted line) then the creation of a pressure differential would be
dissipated (B, dashed line). The difference between the dashed and solid lines in (A) shows the total remaining volume (3L) across both nasal cavities that must be accounted for to dissipate a pressure differential. Changes in the schematic are shown as abrupt, however, they would occur more gradually in a diving rorqual.
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Skull = bones only; head = soft tissue and bones. Hvalfjörður = Hvalfjörður commercial catch operations, Iceland. BBM = Beaty Biodiversity Museum, UBC, British Columbia, Canada. AERL = Aquatic Ecosystems Research Laboratory, UBC, British Columbia, Canada. WIC = The Whale Interpretive Centre, Telegraph Cove, British Columbia, Canada.
Chapter 3: Protection of upper and lower airways in the fin whale during deglutition

3.1 Introduction

The upper aerodigestive tract (UAT) is composed of the nasal cavities, oral cavity, larynx, pharynx, and cervical regions of the trachea and esophagus (Adams et al., 2020). Separation of respiratory and digestive tracts in the mammalian pharynx is critical for survival. Food must be kept out of the respiratory tract and directed into the digestive tract when swallowing, while air must be directed into the respiratory tract and not the digestive tract when breathing (German et al., 1998). The evolution of a lung-based respiratory tract has made the seemingly simple task of directing food and air to the appropriate tracts a challenging one. The respiratory tract develops as an out-pocketing of the gut tube, which places the openings to both respiratory and digestive tracts beside one another at the base of the pharynx, presenting the risk of choking by aspirating food (Effros, 2006). This risk is mitigated by the actions of the larynx.

The larynx is a musculo-ligamentous structure composed of three paired cartilages (arytenoid, corniculate, cuneiform) and three unpaired cartilages (cricoid, thyroid, epiglottis), that sits atop the lower respiratory tract (Drake et al., 2015; Moore and Dalley, 2018). Its primary function is to act as a valve (Asher et al., 1996). Terrestrial mammals seem to have established three main patterns of ‘valving’ to protect the respiratory tract during deglutition, or swallowing: an intranarial larynx (ex. herbivores), breaking the intranarial connection (ex. carnivores), and a descended larynx (ex. humans). In most mammals the larynx sits high in the neck, allowing the epiglottis to overlap with the soft palate to functionally separate respiratory and digestive pathways, i.e. an intranarial larynx (Harrison, 1995; Laitman and Reidenberg, 1993). In
herbivores, the position of epiglottis and soft palate is maintained during breathing and swallowing, allowing them to maintain olfactory senses to detect predators while grazing. Herbivores tend to have a broad elongated epiglottis, large arytenoids, and high aryepiglottic folds, making it easy to maintain an intranarial larynx and protect the respiratory tract during deglutition (Harrison, 1995). Carnivores, on the other hand, occasionally break this connection between epiglottis and soft palate to swallow large chunks of flesh. The arytenoid cartilages of carnivores are comparatively small, the epiglottis is not elongated, and in some species, there are no aryepiglottic folds. To maintain protection while swallowing, the epiglottis is folded down and makes contact with the arytenoids to cover the laryngeal inlet during the passage of food (Harrison, 1995). Humans are an exception to an intranarial larynx, and instead have a larynx that descends in position during growth to reside in the neck by adulthood (Laitman and Reidenberg, 1993; Nishimura, 2018; Reidenberg and Laitman, 2005). In infants, the larynx is high in the neck and the epiglottis and soft palate overlap, allowing sucking and breathing to occur simultaneously. In adult humans, where the larynx has descended into the neck, breathing and deglutition cannot occur simultaneously as the position of the larynx does not permit overlap of epiglottis and soft palate. Instead, during deglutition the vocal folds and aryepiglottic folds are approximated, and the hyoid and larynx are pulled forward and upward, simultaneously shifting the larynx below the base of the tongue and pushing the epiglottis downwards to close the laryngeal inlet. The vocal folds inside the laryngeal body come together in the midline to seal the glottis (Matsuo and Palmer, 2008).

Valving patterns in marine mammals somewhat vary in accordance with their clade. The morphology and actions of the pinniped larynx align well with their diet and semi-aquatic lifestyle. Sea lions possess a short epiglottis and large arytenoids (Reidenberg and Laitman,
2010), Weddell seals may have an elevated larynx with an epiglottis that rises above the soft palate, though the position during deglutition is unclear (Piérard, 1969), and harbour seals have distinct valvular closure of the laryngeal inlet by actions of the arytenoids and corniculates during swallowing (Adams et al., 2020). The positional flexibility of the larynx in pinnipeds allows it to be intranarial during terrestrial sojourns, but not intranarial and instead closed during swallowing – similar to terrestrial carnivores, to protect the respiratory tract from water and food entry during swallowing. Cetaceans, by contrast, are obligate aquatic mammals that only breathe periodically at the surface and have many structural adaptations to survive underwater that have affected the functional morphology of the aerodigestive tract. The nasal cavities have moved to the top of the head to form the blowhole(s) (Berta et al., 2015c), the cervical region is shortened owing to flattened cervical vertebrae (Buchholtz, 1998; Slijper, 1962), and the head is in a horizontal position in the same plane as the body. This morphology has impacted the intersection of respiratory and digestive tracts in the pharynx. The larynx in cetaceans sits far rostrally, with the laryngeal inlet directly under the nasopharynx. This presents the issue of the respiratory tract passing perpendicularly through the pathway that food travels during swallowing. Odontocetes, or toothed whales, have dealt with this through extreme laryngeal modification where the larynx is “permanently” inserted into the nasopharynx (Harrison, 1995; Reidenberg and Laitman, 1987). The epiglottis and arytenoid cartilages are elongated and arise from the laryngeal body in close proximity to one another. There are high-walled aryepiglottic folds that connect these cartilages laterally, forming a tubular structure called the laryngeal spout. The epiglottis has a large ridge around its dorsal-most aspect. The palatopharyngeal sphincter of the nasopharynx surrounds the laryngeal spout below this epiglottic ridge, holding the larynx in place (Reidenberg and Laitman, 1987; Reidenberg and Laitman, 1994; Reidenberg and Laitman, 2007a). With the laryngeal inlet
held permanently in the nasopharynx, the respiratory tract is completely separated from the digestive tract, and there is virtually no risk of choking by aspirating food. Food simply travels through the lateral food channels on either side of the laryngeal spout (Reidenberg and Laitman, 1987; Rommel et al., 2018) and on to the esophagus. The odontocete larynx is immobile and does not move during deglutition.

Mysticetes do not share the same morphology as odontocetes, and additionally show variation in laryngeal anatomy between the families Balaenidae and Balaenopteridae (rorquals). Balaenids, the skim-feeding baleen whales, possess a laryngeal stalk similar to but relatively shorter than the odontocete laryngeal spout (Fig 1.10). The laryngeal stalk is also thought to be held permanently in the nasopharynx by the palatopharyngeus muscle (Henry, 1983; Reidenberg and Laitman, 2007a; Schoenfuss et al., 2014). The epiglottic and arytenoid cartilages arise closely together from the body of the larynx and there are distinct aryepiglottic folds, forming a short tube. The epiglottis does not have an enlarged ridge as in odontocetes, but instead has a triangular peak - the median pyramidal eminence, that runs down the midline of the inner dorsal surface of the epiglottis. There are two trough-shaped grooves on either side of the eminence that the corniculate processes of the arytenoids fit tightly into during deglutition (Henry, 1983), to seal the laryngeal inlet.

Rorquals, the lunge feeding baleen whales, do not possess a tubular larynx. Instead, the larynx of some rorquals has been likened to the human larynx (Hosokawa, 1950). In dissected specimens, the tongue-shaped epiglottis extends anteriorly in an almost horizontal plane, while the corniculate processes/arytenoids project dorsally, leaving a large splayed-open laryngeal inlet. The aryepiglottic folds are minimal and do not rise up laterally at all, leaving the lateral edges of the laryngeal inlet from epiglottis to arytenoids unprotected. The outer surfaces of these
cartilages are smooth with no obvious enlarged ridges. There is no median pyramidal eminence on the epiglottis; conversely, the epiglottis appears concave and trough-like on its inner dorsal surface.

Despite the differences in morphology between odontocetes and mysticetes, it is thought that the mysticete larynx functions in the same way as the odontocete larynx; that is, the larynx is permanently intranarial and during deglutition food passes through the lateral food channels (Laitman and Reidenberg, 1993). This assessment may be valid for the balaenids, which have a laryngeal stalk, but I question whether it applies to rorquals: The lack of lateral protection or a specialized tube-like morphology would risk food aspiration through the lateral margins. What then, is the position of the mysticete larynx during swallowing? How is the respiratory tract protected during swallowing?

The primary function of the larynx is to act as a valve that protects of the respiratory tract; however, the larynx has a secondary function of respiration (controlling airflow through the larynx), and a tertiary function of sound production (Bartlett, 1989; Reidenberg and Laitman, 2005; Shapiro and Martin, 1996). These functions also have an impact on pharynx morphology. For example, humans risk the protective function of the larynx in favour of a descended larynx for sound production (Laitman and Reidenberg, 1993; Sasaki, 2000). The permanently intranarial larynx of odontocetes not only protects the respiratory tract from food but maintains the connection between lower and upper respiratory tracts for echolocation. Echolocation is required for odontocetes to interact with their environment, and it requires air from the lungs being able to reach the extracranial sound producing structures atop the skull. Without a laryngeal spout providing this connection, odontocetes would quite literally be lost. Mysticetes do not echolocate and sound production instead occurs in the larynx, so they would not require an intranarial larynx.
for sound production as odontocetes do. In addition to sound production, feeding style differs between odontocetes and mysticetes. Odontocetes feed on whole single prey items such as fish or cephalopods, or large chunks of meat torn away from prey, while mysticetes are bulk filter feeders processing large amounts of water to filter out innumerable small prey. Humpback whales engage in bubble-net feeding, which involves releasing air bubbles generally from the blowholes or the mouth to encircle prey. For bubbles to be released from the mouth, the larynx must move from an intranarial breathing position to place the laryngeal inlet in the oral cavity - the humpback whale larynx cannot be permanently intranarial. Reidenberg (2007b) suggests this intraoral position is attained by pushing the epiglottis into the oral cavity to allow air to flow from the larynx into the oral cavity. This repositioning requires mobility of the larynx and aryepiglottic folds that do not form high lateral walls, or else the epiglottis alone could not be forced intraorally. Since a splayed-out laryngeal inlet and minimal aryepiglottic folds are characteristic of the morphology found in all rorquals, it suggests that the larynx of rorquals is not permanently intranarial. The differences in anatomy and behaviour between odontocetes and mysticetes, especially rorquals, suggest profound differences in how the larynx operates.

In this chapter, I investigate how rorquals protect their respiratory tract during deglutition. I argue that the larynx cannot remain intranarial during deglutition without risking food aspiration through the lateral edges of the laryngeal inlet. If the entirety of the epiglottis and corniculate/arytenoids were forced intranariaily to eliminate the laterally exposed edges, then the space in the pharynx would be so diminished that food could not be easily transported through to the esophagus. Rather, I hypothesize that the rorqual larynx functions similarly to the human larynx during deglutition, whereby movements of the larynx close the laryngeal inlet at the base of the pharynx to protect the lower respiratory tract. Specifically, I predict that the laryngeal
inlet, and thus, the lower respiratory tract, is closed by contact between the epiglottis and corniculate/arytenoids. I hypothesize that the upper airway is protected by the soft palate being elevated dorsally and posteriorly, as in humans. I suggest that this arrangement of structures protects the upper and lower airways while maintaining space in the pharynx for transferring bulk quantities food to the esophagus. I explored these hypotheses by performing dissections of adult and fetal fin whales, and physically manipulating the larynx and pharyngeal structures. I also dissected a Risso’s dolphin fetus as a representative odontocete for comparison of pharyngeal anatomy with that of the fin whale.

3.2 Methods

3.2.1 Animals and tissue samples

Fin whale specimens were collected postmortem from the commercial whaling operation at Hvalfjörður, Iceland, in the summers of 2015 and 2018. All specimens were examined fresh at the station within 36 hours of death. A total of 19 specimens were surveyed, with 5 examined in detail. One dissected animal was a fetus, 2.94 m long. All others were adults with a total length of 16.4 – 20.9 m. Tissue samples were imported to Canada under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits. A Risso’s dolphin fetus was collected at a necropsy of animals stranded and recovered along the coast of British Columbia, Canada, by Fisheries and Oceans Canada.

3.2.2 Gross anatomy and physical manipulation

The soft palate of all specimens was first examined by opening the mouth. In adult fin whales this was accomplished by laying the animal on its left side and pulling the mandibles ventrally with powered winches associated with the whaling station. For both the fin whale fetus and the Risso’s dolphin fetus the animal was laid on its ventral surface and the upper jaw and
head were pulled up dorsally to open the mouth. In the fetal fin whale the position of all pharyngeal structures was examined further by making a sagittal slice down the midline of the head.

The pharynx of adult fin whales could only be examined by removing the entire viscera from the body with powered winches, and dissecting away the pharynx from surrounding structures. A mass including nasopharynx muscle (detached from the skull), soft palate, hyoid, larynx, and trachea was removed in a single piece from the viscera for examination. Nasopharyngeal muscle was sliced sagittally down the dorso-caudal aspect to reveal the interior of the nasopharynx and the portion of the pharynx ventral to nasopharynx, the soft palate, and the epiglottic and arytenoid cartilages. Epiglottic and arytenoid cartilages were manipulated to determine their flexibility, interaction with the soft palate, and potential positions during deglutition. The epiglottis was moved above and below the soft palate and was pushed rostrally and caudally. The corniculates/arytenoids were pushed rostrally and caudally, and adducted and abducted. One pharynx specimen was suspended off of the ground by placing the forks of a forklift under the hyoid so that the larynx specimen was not collapsed on itself while manipulating the cartilages. Sagittal and transverse slices were made through the larynx at varying levels to determine the relative positions of all parts of the larynx. One larynx specimen was dissected down to just the cartilages by the progressive removal of nasopharyngeal muscles, extrinsic laryngeal muscles, and the hyoid. The trachea was removed from this specimen at the junction between the cricoid cartilage and tracheal rings. The position of epiglottic and arytenoid cartilages were again manipulated to determine the interaction of these movements with the laryngeal sac. The laryngeal sac was removed, and the cartilages were once again manipulated. The entire dissection procedure was photographed and/or videotaped for each specimen.
3.3 Results

3.3.1 Soft palate

Opening the mouth of the adult fin whale exposed the soft palate originating from the hard palate and extending ventrally to meet the dorsal surface of the posterior-most aspect of the tongue (Fig. 3.1AB). This region of soft palate directly above the tongue was bulbous and completely plugged the oropharyngeal channel, held in place by the surrounding palatoglossal arch, (muscle identity assumed based on position). I termed this bulbous region of the soft palate the “oral plug”. The oral plug closed the oropharyngeal channel in the animal’s dead state, and this presumably indicates the plug’s resting (passive) position in a live animal - muscular activation would be required to clear the oropharyngeal channel. I attempted to displace the oral plug by pushing or pulling on it, but was unsuccessful; I hardly managed to fit my hand between the plug and the tissue surrounding it, demonstrating how tightly it was held in place in its relaxed state. A slice through the oral plug revealed it was composed of a combination of adipose and muscle tissues. The position of the oral plug meant I was unable to see any part of the larynx from the oral cavity.

In the fin whale fetus the hard palate was fully ossified, and the tongue was distinct and muscular, unlike the adult (Fig. 3.1CD). There was not a distinct oropharyngeal plug held in place by the palatoglossus muscle as in the adult, but a partially developed bulbous region was visible when the mouth was opened and more-so when the soft palate was sliced sagittally (Fig. 3.2).

In contrast, the Risso’s dolphin fetus showed no such oral plug, nor any evidence of the development of a bulging region (Fig. 3.1EF). The palatoglossal arch extended from the roof of the mouth to the back of the tongue. The base of the laryngeal spout was visible beyond the
palatoglossal arch when the mouth was opened. The morphology I observed in the fetus matched what has been published for adult odontocetes.

3.3.2 Fin whale fetus pharynx

The fin whale fetus, though not fully to term, was well-developed allowing me to easily identify structures (Fig. 3.2). The skull and skeleton were not ossified, and cartilaginous structures were not completely solid. Additionally, muscle tissue was obvious but mostly lacking distinct orientation. In the process of sagittally slicing the head, some of the structures became displaced, but by manipulating the structures I was able to assess their normal position. The nasopharynx was extensive. The anterior-most wall of the nasopharynx, the soft palate, descended from the caudal aspect of the vomer into an enlarged developing oral plug. Caudal to the oral plug, the soft palate continued horizontally forming a sheet-like ledge of tissue. At the junction between the soft palate ledge and the lateral walls of the nasopharynx was an anterolateral diverticulum, and above this the walls of the nasopharynx were covered in pits – both structures similar to those described by Kienle et al. (2015) in a neonate gray whale. The oropharyngeal inlet was partially intact below the soft palate ledge. The arytenoids and epiglottis were at the level of the first two cervical vertebrae. The corniculate/arytenoid cartilages were well-developed and rather stiff compared to the equally well-developed but floppy epiglottis. The arytenoids were directed superiorly, and the epiglottis was directed rostrally. A very small portion of the epiglottis made contact with the soft palate ledge. There were no aryepiglottic folds whatsoever. The esophagus, trachea, and laryngeal sac were all easily identifiable.

3.3.3 Nasopharynx, soft palate, and oropharynx in adult fin whales

The remainder of the soft palate that was not visible through the open mouth extended back to form part of the anteroventral wall of the nasopharynx and separated the nasopharynx
from what I will refer to as the oropharynx, the region of the pharynx ventral to the soft palate through which food travels to reach the esophagus (Fig. 3.3). The nasopharynx and oropharynx were both composed of extensive muscle. Unfortunately, due to the removal process I was unable to definitively define the muscle groups composing all of the pharyngeal musculature. The nasopharynx contained many pits and two anterolateral diverticula at the junction of what was assumed to be the palatoglossal and palatopharyngeal arches (Fig. 3.3), similar to those described in a neonate gray whale (Kienle et al., 2015). There was no anatomical sphincteric muscle ring at the base of the nasopharynx. The region of the soft palate that was presumed to be dorsocaudal (in the dissecting position) to the oral plug instead formed a ledge-like sheet of tissue medially between the lateral nasopharynx walls (Fig. 3.3AD). The rostral tip of the epiglottis rested on the soft palate ledge, with no more than a few centimeters of overlap. The epiglottis was easily moved from this position in the nasopharynx and placed under the soft palate ledge and in the oropharynx (Fig. 3.3B,C,E). The overall size or volume of the nasopharynx appeared to be larger than that of the oropharynx. The posterior portion of the oropharynx was composed of what was likely the inferior constrictor muscle (Fig. 3.4). This muscle is contiguous with the “caudal pharynx muscle” that was sliced sagittally in some dissections to reveal nasopharynx, oropharynx, epiglottis, and arytenoids (Fig. 3.3). The esophagus was positioned a short distance behind the arytenoids, under the inferior constrictor. The inferior constrictor was thick and wrapped tightly around the dorsal aspect of the larynx to meet the thyroid cartilage laterally, leaving little space for food transfer in the oropharynx.

3.3.4 Larynx and laryngeal sac in adult fin whales

During the dissections of the larynges, I found no distinct connection or fusion point between the arytenoid and corniculate cartilages; therefore, I refer to the main dorsally projecting
structure of cartilage as an “arytenoid”, with the rostral projecting tissue flap referred to as the “corniculate flap”. The larynx was positioned directly posterior and attached to the hyoid apparatus with the epiglottis projecting craniodorsally, and the epiglottic base at the level of the ceratohyal bones of the hyoid apparatus (Fig. 3.5A). The epiglottic and arytenoid cartilages projected up through the base of the pharynx (Fig. 3.3). The epiglottis projected rostrally in a “V” shape, with a dorsal concavity. The arytenoids projected dorsally and were connected to one another posteriorly, with free flap-like edges in the shape of a semicircle directed anteriorly, the corniculate flaps. The lateral faces of the corniculate flaps bulged outward in a convexity, while the medial faces that opposed one another were flat. The arytenoid cartilages rose slightly higher than the epiglottis, as they were directed dorsally, versus the rostrally directed epiglottis. The epiglottis and arytenoids were connected with tissue at the base of the pharynx, but there were no lateral-walled aryepiglottic folds – the cartilages had distinct free edges and did not form a tube or spout. The laryngeal inlet, extending between epiglottis and arytenoids was rather long, varying in length depending on the position of epiglottis and arytenoids (Fig. 3.3A,C,D). Manipulating the epiglottis and arytenoids demonstrated they could completely occlude the laryngeal inlet (Fig. 3.3F). The flat medial faces of the corniculate flaps and arytenoids allowed both sides to come together in the midline, and when pushed together in this manner the corniculate flaps and arytenoids easily fit in the dorsal trough of the epiglottis, with the lateral bulging faces of the corniculate flaps filling out the trough. The entirety of the outer surface of both the epiglottis and arytenoids was smooth with no large ridges or lips as in the odontocete epiglottis.

The arytenoids led caudoventrally to form the u-fold, which controls access between the laryngeal sac and trachea, as it forms part of the roof of the laryngeal sac and part of the floor of
the trachea (Fig. 3.6). The arytenoids have a hinging point between their rostral and caudal aspects, where they are attached to the cricoid cartilage at the cricoarytenoid joints. The caudal-most point of the u-fold was about halfway down the length of the cricoid. The laryngeal sac underneath the u-fold extended beyond the body of the larynx, traversing the laryngotracheal junction, with the caudal tip of the sac at the level of the bronchus trachealis (Fig. 3.5B). The walls of the laryngeal sac were composed of dense muscle, especially the ventral aspect of the laryngeal sac (Fig. 3.6B). The laryngeal sac can be pushed dorsally into the larynx and trachea to completely occlude the lumen of both (Fig. 3.7); the thick muscular walls of the laryngeal sac fill out the lumen (Fig. 3.7A), with the u-fold pushed against the cricoid and the roof of the trachea. This position of the u-fold and lumen completely plugs the respiratory tract, blocking communication between the trachea and the rostral-most laryngeal cartilages and oropharynx. When the u-fold and laryngeal sac are pushed against the cricotracheal roof, lateral tracheal ridges appear, running obliquely from the lateral cricoid walls towards the u-fold (Fig. 3.7B), similar to those described by Damien et al. (2019).

Dissection of the larynx down to the cartilages allowed for easier manipulation of each of the cartilages (Fig. 3.8). Pushing the rostral arytenoids (superior to the cricoarytenoid joint) together medially and then rostroventrally closed the u-fold and forced the u-fold dorsally to touch the cricoid cartilage. This forced the roof of the laryngeal sac upward as u-fold and sac roof are contiguous (Fig. 3.8B). This movement of the arytenoids effectively blocks the lumen of the larynx. To open the laryngeal inlet and trachea but close the u-fold and laryngeal sac (as likely occurs during inhalation), the caudal arytenoids must be adducted but the rostral arytenoids abducted. To open the laryngeal inlet, the trachea, and the u-fold (laryngeal sac), the entire length of the arytenoids must be abducted (Fig. 3.8D). The cricoarytenoid joints are an
important hinging point to enable movement of rostral and caudal regions of the arytenoids to produce different positions.

3.4 Discussion

Mammalian larynx morphology varies widely across species. However, despite differences in morphology, mammals have converged on a few protective configurations of laryngeal cartilages and larynx positions during deglutition. My dissections and manipulations of fin whale larynges did not provide sufficient evidence to suggest that the fin whale, and more broadly the rorqual larynx remains permanently intranarial during deglutition. The fin whale and rorqual larynx must function similarly to the carnivore or human larynx during deglutition to maintain respiratory tract protection. I propose the following models for the position of laryngeal and pharyngeal structures during breathing versus swallowing (Fig. 3.9).

3.4.1 Breathing

To breathe, the upper and lower airways must be connected. During periodic breathing, the larynx is pushed intranariaIly to connect the lower and upper airways (Fig 3.9A). The rostroventral surface of the epiglottis rests against the soft palate and the caudal surface of the arytenoids rest against the posterior pharynx muscle. To maximize airway diameter for exhalation, the entirety of the arytenoids are abducted, potentially leaving the u-fold slightly open which would not inhibit outward airflow. During the shorter duration inhalation, however, the caudal arytenoids are adducted to close the u-fold so that air can only be directed into the trachea and not the laryngeal sac.

3.4.2 Feeding and swallowing

Lunge feeding involves massive forces acting on and in the mouth. During a lunge the ventral pouch is filled with water that accelerates as it is engulfed, while the whale decelerates
(Goldbogen et al., 2006), indicating that a significant amount of force is transferred into the mouth with this water. Protecting the oropharyngeal inlet during lunging is the first step in protecting the respiratory tract. The oral plug is an evolutionarily novel feature, not previously reported, in fin whales and likely rorquals, that prevents water from entering the oropharyngeal inlet. The oral plug withstands the force of water entering the mouth to protect the oropharynx and subsequently the airway from water entry during this forceful stage of lunge feeding. No muscular activation is required for this protection; the oral plug in its relaxed state blocks the oropharyngeal channel. The dorsal position of the oral plug, at the top of the oral cavity behind the hard palate, places it out of the main trajectory of water entry into the oral cavity. The force of water entering the mouth inverts the tongue and inflates the ventral pouch. Inversion of the tongue places tension on the palatoglossus, pulling it caudoventrally against the bulbous oral plug to create a tight seal. Once the lunge has been completed, water is filtered out of the mouth, the tension is released, and the food is swallowed.

To swallow, the oropharyngeal inlet must be opened, and the upper and lower airways must be protected. The oropharyngeal inlet is opened by shifting the oral plug out of the way (Fig. 3.9B). Since the oral plug is a part of the soft palate, it can only be shifted caudally into the oropharynx. Activation of the muscles that control the position of the palate elevate the soft palate dorsocaudally. As in humans, contact between the soft palate and the posterior pharyngeal muscles plugs the nasopharynx with muscle, effectively eliminating it as an empty pharyngeal space. The soft palate and oral plug form the roof of the oropharynx, and the upper airway is now protected from food entry. The larynx must be withdrawn ventrally out of its intranarial breathing position to make space for the soft palate and oral plug to effectively seal and protect the upper airways.
The lower airway must be protected from food entry by valvular actions of the larynx. The first valvular action of the larynx is adducting the corniculate flaps and arytenoids, and forcing the adducted corniculates and arytenoids rostroventrally into the trough-like epiglottis. This seals the laryngeal inlet. Adducting the arytenoids also closes the u-fold, and forcing the arytenoids rostroventrally brings the u-fold dorsally into contact with the cricoid cartilage.

Movement of the u-fold draws the roof and the entirety of the muscular mass of the laryngeal sac up into the cricotracheal region, occluding the lower airway. This is the second valvular action of the larynx that protects the respiratory tract. This occurs passively at depth, with high ambient ocean pressure causing collapse of the laryngeal sac at likely similar depths to lung collapse. The close proximity and connectivity of all of these structures - the epiglottis, arytenoids, u-fold, and laryngeal sac - when performing their protective functions completely plug the entire lumen of the larynx. The lower airway, from epiglottis to approximately the bronchus trachealis, is filled with tissue, making it nearly impossible for food or water to penetrate the lower airway.

3.4.3 Summary

Prior to beginning this study, there appeared to be little evidence to suggest the larynx remained intranarial during deglutition in rorquals. From my observations and the data collected throughout this study, I contend there is no anatomical evidence to suggest an intranarial larynx during deglutition. The epiglottis and arytenoids have no ridges or lips for a sphincter to grasp, and no anatomical sphincter nor any structure capable of grasping or tightening around the epiglottis or arytenoids was present at the base of the nasopharynx. There is no lateral protection between epiglottis and arytenoids, so if the cartilages were held in the positions I often observed during dissection, i.e., just a few centimeters of overlap between epiglottis and soft palate, the lateral aspects of the laryngeal inlet would remain open to the oropharynx. If the entirety of the
epiglottis and rostral arytenoids were inserted into the nasopharynx, this could protect the lateral aspects of the laryngeal inlet. However, this would diminish the already limited size of the oropharynx (Fig. 3.4) and hinder the flow of food through to the esophagus. If the larynx were held intranarially, the oral plug and soft palate could not be shifted to clear the oropharyngeal inlet. By retracting the larynx ventrally and the soft palate dorsally, the oropharynx is opened for food transfer and can funnel food from the mouth to the esophagus, unobstructed. Sealing the upper airway through movement of the soft palate, and the lower airway through movements of the larynx negates the possibility of an intranarial larynx during deglutition.

It is likely that the hyoid and larynx move in conjunction to position the larynx for breathing and swallowing, though I was unable to demonstrate the process due to the size of the specimens. Movements of the hyoid would place the larynx intranarially during breathing, and during swallowing, pull the larynx ventrally and rostrally to assist in closing the laryngeal inlet and placing the closed laryngeal inlet behind the base of the tongue. The hyoid pulling the larynx ventrally and rostrally are the same movements the human hyoid and larynx undergo during swallowing (Massey, 2006).

Protecting the respiratory tract in rorquals is a combination of passive and active responses. The default or resting position of respiratory structures is likely the position that confers protection, thus saving energy by not having to actively protect the respiratory tract. Breathing in rorquals would require the active response of positioning the larynx intranarially. This movement would coincide with other large anatomical shifts described in rorquals, like the movement of the nasal plugs to open the top of the respiratory tract (Gil et al., 2020). The nasal plugs are withdrawn from the nasal cavities as exhalation begins and replaced as inhalation ends. When the plugs are relaxed, they completely occlude the upper half of the nasal cavities. Another
example of passive protection is the oral plug, that in its relaxed state protects the rostral opening of the pharynx to prevent water or prey from entering the pharynx during lunging. Passive protection is also demonstrated in the laryngeal sac at depth. At depths where respiratory tract collapse occurs it is likely that the easily deformable laryngeal sac will be pushed into the cricotracheal region as a pressure differential forms between ambient ocean pressure and respiratory tract pressure. Not only would this protect the more rigid laryngeal cartilages from barotrauma (Reidenberg and Laitman, 2008), but this passive protection could also help save energy as rorquals commonly feed at depth.

The lifestyle of rorquals is very different from their terrestrial relatives, yet the protective mechanisms of the larynx are more similar to those terrestrial relatives than they are to odontocetes. Modifications of the pharyngeal region in mammals reflect the major evolutionary trends of different mammalian groups. Humans compromise respiratory tract protection with a descended larynx, but are capable of making a diverse range of sounds and rely on speech for communication (Laitman and Reidenberg, 1993; Sasaki, 2000). Despite the risk, the morphology of the larynx still provides protection to the respiratory tract in the vast majority of cases. An intranarial larynx in herbivores allows them to take an extended amount of time to graze and use olfactory senses simultaneously so they can detect predators. Carnivores require a flexible epiglottis to enable them to swallow large chunks of meat (Harrison, 1995). Dogs and a few other mammals require a mobile larynx rather than a permanently intranarial larynx to allow panting for thermoregulation (Biewener et al., 1985). An intranarial larynx in odontocetes maintains a continuous respiratory tract to allow echolocation for interacting with the environment, and communication for the social nature of many odontocetes. The larynx of odontocetes is immobile and permanently intranarial, completely separating respiratory and
digestive tracts while maintaining effective use of both tracts and negating the possibility of
depth by aspirating food. Surprisingly, although the vast majority of times a single prey item
easily passes the laryngeal spout through a lateral food channel, death by suffocation can occur if
prey manages to displace the laryngeal spout from its intranarial position. Numerous studies have
demonstrated an odontocete attempting to swallow a fish that’s too large (Byard et al., 2003;
Elliser et al., 2020; Mignucci-Giannoni et al., 2009; Stolen et al., 2013; Watson and Gee, 2005),
a fish with spines (Byard et al., 2010), or a living octopus trying to escape (Stephens et al.,
2017), all unsuccessfully. It appears that like humans, odontocetes also risk respiratory tract
protection, risking asphyxia for the more important ability to echolocate. The pharynx is used by
both respiratory and digestive tracts, but unfortunately is not optimized for both.

Rorquals do not produce sound in the same way as odontocetes, nor do they echolocate.
They lunge feed on bulk small prey that are swallowed as a large volume of concentrated slurry,
rather than single food items. Unlike terrestrial mammals, the head position of rorquals (and all
cetaceans) relative to the body is completely horizontal, and the cervical vertebrae are shortened.
While this places the larynx far rostrally, it increases the angle of crossover between respiratory
and digestive tracts. Some terrestrial mammals have valleculae (Fig. 1.7) to store food prior to
swallowing (Hiiemae and Crompton, 1985), but the head and body position of rorquals precludes
valleculae from existing. Like other mammals, rorquals require a larynx and pharynx that are
suited to their feeding behaviour. The prey of rorquals is tiny and still alive, so protection of the
lower respiratory tract requires completely sealing the larynx to prevent the tiny prey from
infiltrating the lower airways. Protection of the upper respiratory tract requires movement of the
soft palate, which simultaneously seals access to the upper airways and opens the oropharyngeal
channel for food to pass. The oral plug is a unique structure on the ventral surface of the soft
palate that confers passive protection during lunging in rorquals. The passive protection provided by the laryngeal sac is a novel mechanism that does not occur in other mammals, despite the existence of potentially homologous laryngeal sacs in some artiodactyls such as reindeer or takin (Reidenberg and Laitman, 2008). The larynx as a whole, along with its individual parts, must be mobile in rorquals to allow different positions for breathing and swallowing, to optimize both functions. For humpback whales, a mobile larynx is necessary for bubble-net feeding (Reidenberg and Laitman, 2007b). Humpback whales have also been observed trap feeding, which involves opening the mouth at the water surface and using the pectoral fins to direct prey into the open mouth while remaining stationary (McMillan et al., 2019). An oral plug would be crucial to protect the pharynx while feeding in this manner.

Adaptations of the pharynx and larynx balance respiratory and digestive functions. This work on pharyngeal morphology in rorquals contributes insight on feeding adaptations and maintaining respiratory tract protection while feeding. For rorquals, bulk filter feeding by lunge feeding is possible due to multiple protective mechanisms and anatomical novelties. Rorquals are the largest animals ever to have lived, and lunge feeding is commonly touted as one of the reasons rorquals have attained such large body sizes. The anatomical novelties of the upper aerodigestive tract in rorquals must have been central to the evolution of lunge feeding, thus, central to the evolution of their large body sizes.
Figure 3.1 Open mouths of adult fin whale (left), fetal fin whale (middle), and fetal Risso’s dolphin (right) displaying the soft palate and muscles associated with the pharynx and tongue. (A) Adult fin whale laying on its left side. The mandibles are on the left side with partially intact VGB tissue, and the skull is on the right side with two baleen racks and the vomer bone in between. The vomer leads caudally to the oropharyngeal inlet which is blocked by the oral plug. (B) Close up of an adult fin whale oral plug descending from the caudal end of the vomer and making contact with the dorsal surface of the tongue (cut away). The tissue
surrounding the oral plug and appearing to hold it in place is likely the palatoglossus muscle. (C) Fetal fin whale with an open mouth displaying the tongue and oropharyngeal inlet caudal and slightly dorsal to the tongue. The vomer is not well developed. (D) Close up of caudal end of the tongue leading to the oropharyngeal inlet in the fetal fin whale. The palatoglossal arch surrounds the oropharyngeal inlet, leading from the palate and inserting ventral to the tongue. The oral plug is not completely developed but appears as a slight downward bulge from the soft palate between the lateral margins of the palatoglossal arch. (E) Open mouth of a Risso’s dolphin fetus showing the tongue leading back to the open oropharyngeal inlet. The palatoglossal arch surrounds the oropharyngeal inlet, leading from the palate and inserting ventral to the tongue. The laryngeal spout is visible in the oropharynx behind the tongue and palatoglossal arch. (F) Close-up of the Risso’s dolphin fetus displaying the base of the laryngeal spout and a shadow to the right of this which is the animal’s left lateral food channel. Scale bar in A: 50 cm, B,C: 10 cm, D,E,F: 5 cm.
Figure 3.2 Fetal fin whale head sliced sagittally. Rostral is to the left. Dissecting tool indicates upper airway. At the caudal end of the vomer the soft palate descends ventrally and enlarges into the developing oral plug. Caudal to this bulge the soft palate extends in an almost horizontal plane as a ledge-like sheet of soft palate. The partially intact oropharyngeal inlet is indicated by the balled-up blue glove immediately under the ledge-like soft palate. An anterolateral diverticulum exists in the intersection between the soft palate ledge and lateral nasopharynx tissue, indicated by the downward directed arrow. The upward directed arrow indicates pits that line the nasopharynx. The larynx is on the lower right, with prominent arytenoids projecting superiorly and the epiglottis projecting rostrally, ventral to the arytenoids. The laryngeal sac, trachea, and esophagus are all visible but not fully developed. Scale bar: 5 cm.
Figure 3.3 Nasopharynx, oropharynx, and larynx manipulation in two adult fin whales.

Top row and bottom row show two different specimens. A – arytenoids, CF – corniculate flaps, E – epiglottis, SPL – soft palate ledge. Rostral is directed downwards in all panels except (B) and (C), where rostral is towards the top left corner. Anterolateral diverticula at junction between soft palate ledge and nasopharynx tissue visible in all panels and indicated by white arrows. Nasopharynx pits visible in all panels, distributed across nasopharynx tissue. (A) and (D) Resting position of the structures during dissection. The epiglottis is directed rostrally and rests on the
soft palate ledge with minimal overlap. (A) Odd morphology of epiglottis where the lateral proportions of soft tissue surrounding the cartilage varied. (B) Pulling the epiglottis caudally displaced it from its position atop the soft palate. (C) The epiglottis is easily directed into a resting position below the soft palate ledge and into the oropharynx. (D) Normal morphology of the larynx where the cartilages are symmetrical. (E) Epiglottis under the soft palate ledge in the oropharynx. (F) Adducting the corniculate flaps and arytenoids, and pushing the epiglottis caudally completely seals the laryngeal inlet.
Figure 3.4 Inferior constrictor muscle wrapped around the body of the larynx and inserting laterally on the thyroid cartilage. Arytenoid cartilages are sliced through transversely and dangling downward with the cornicate flaps. Mucosa is visible lateral to the arytenoids and is the floor of the lateral food channels of the oropharynx. The esophagus is visible as a mound extending caudal to the arytenoids and under the inferior constrictor, indicated by white arrows in (A). (A) Distant view showing the viscera caudal to the pharynx, including the lungs. (B) Close-up view of inferior constrictor wrapping around the oropharynx.
Figure 3.5 Hyoid and larynx of an adult fin whale. (A) Rostral face of the hyoid with epiglottis and arytenoids resting atop the hyoid, and the body of the larynx extending caudally. Dorsal is up. (B) Ventral view of hyoid and larynx complex, with laryngeal sac on top of the larynx. The laryngeal sac extends to the *bronchus trachealis*. 
Figure 3.6 Dorsal view of the u-fold exposed by cutting through the caudal aspect of the arytenoids and sagittally through the cricoid cartilage. Rostral is towards the top of the panels. The trough of the epiglottis is easily visible, however, obscured slightly by the odd morphology of this slightly asymmetric specimen. (A) The u-fold regulates the junction between laryngeal sac lumen and trachea lumen. The floor of the trachea bulges upwards slightly where the muscular laryngeal sac lies underneath. The cricoarytenoid joints are visible between rostral and caudal aspects of the arytenoids. The cricotracheal junction (CTJ) exists where the sheet of cricoid cartilage transitions into tracheal cartilage rings. (B) The muscular mass of the laryngeal sac lies caudal to the u-fold and ventral to the trachea. Cutting through the floor of the trachea and roof of the laryngeal sac exposed the laryngeal sac lumen. Cutting into the floor of the laryngeal sac revealed very thick muscle. Tracheal cartilages are incomplete ventrally until the level of the bronchus trachealis, which allows the laryngeal sac to bulge upwards into the trachea.
Figure 3.7 Transverse cuts through the larynx demonstrating the U-fold and laryngeal sac pushed dorsally into and occluding the cricotracheal region. Inner mucosal lining of the laryngeal sac is visible in both panels. (A) View facing caudally from the rostral half of the cricoid, about halfway through the u-fold. The esophagus is marked by a tube placed inside the esophageal lumen. The arytenoid cartilages forming the u-fold rest against the cricoid cartilage. The laryngeal sac ventral to the arytenoids fills out the larynx, completely occluding the lumen. (B) View facing rostrally from the caudal end of the laryngeal sac, at the cricotracheal junction. The u-fold is in contact with the cricoid cartilage, occluding the larynx. Lateral tracheal ridges are visible on both sides of the cricotracheal region and indicated on the right side by a black arrow.
**Figure 3.8 Physical manipulation of the dissected larynx.** (A) & (B) dorsal is up, (C) & (D) ventral is up. (A) The larynx is held in a neutral state to observe the position of all cartilages. (B) The arytenoid cartilages are forced rostroventrally, pushing the u-fold to make contact with the cricoid cartilage, and occluding the trachea. (C) All laryngeal sac muscle was removed revealing the full extent of the arytenoid cartilages. (D) The arytenoids are pulled apart opening both the trachea and laryngeal sac lumen.
Figure 3.9 Positions of laryngeal and pharyngeal structures during breathing (A) and swallowing (B) in a fin whale. (A) During breathing the larynx is pushed intranarially, with the epiglottis resting against the soft palate, and the arytenoids in contact with the posterior pharynx muscle. The laryngeal inlet is opened by abduction of the arytenoids and corniculate flaps. During exhalation the u-fold may remain open slightly, but during inhalation the u-fold must be closed to direct air into the trachea. (B) During swallowing the soft palate is elevated dorsocaudally, displacing the oral plug from the oropharyngeal inlet. The soft palate makes
contact with the posterior pharynx muscle, completely occluding the upper airway and effectively removing the nasopharynx as an empty pharyngeal space. The epiglottis and arytenoids come together to seal the laryngeal inlet. The corniculate flaps and arytenoids are adducted and pushed rostroventrally into the trough of the epiglottis. Bringing the arytenoids together in the midline closes the u-fold, and pushing the arytenoids rostroventrally pushes the u-fold against the cricoid cartilage. This brings the laryngeal sac up into the cricotracheal region and completely occludes the cricotracheal region, protecting the lower airway. The muscle composing the laryngeal sac is not depicted in this schematic, but would be collapsed and occluding the lumen of the sac. The label ‘vomer’ is synonymous with the hard palate. Illustration by Alex Boersma.
Chapter 4: Morphology and mechanics of the fin whale esophagus

4.1 Introduction

Mysticetes, or baleen whales, are the largest filter feeding animals. Bulk feeding on small aggregating prey enables access to large amounts of energy at lower trophic levels, which is required for mysticetes to maintain large body sizes (Goldbogen and Madsen, 2018; Goldbogen et al., 2019). In mysticetes, three modes of bulk filter feeding are observed: benthic suction feeding in Esrichtiidae (gray whales), skim feeding in Balaenidae (right and bowhead whales), and lunge feeding in Balaenopteridae (rorquals - blue, fin, humpback, etc.). Lunge feeding in rorqual whales has been described as the largest biomechanical event on the planet (Brodie, 1993), and involves a rorqual accelerating towards a patch of prey and opening its mouth to engulf a volume of prey-laden water that can be larger than its own body volume (Goldbogen, 2010; Goldbogen et al., 2007). This process is repeated successively during a foraging dive, with each lunge averaging 16 seconds and the interval between lunges averaging 30 seconds in fin whales (Goldbogen et al., 2006; Goldbogen et al., 2007). During the interlunge interval, the engulfed water is filtered out through the baleen plates to isolate the prey in the mouth, with the duration of the interlunge interval dependent on prey density (Goldbogen et al., 2013). What has been neglected when considering the interlunge interval is the necessity of the whale swallowing the prey and emptying the mouth – a process that must be completed before the whale can open its mouth for the next lunge. Therefore, the interlunge interval must consist of both filtration time and swallowing time, despite studies consistently using the entirety of the interlunge interval to calculate filtration times and volumetric flow rates (Goldbogen et al., 2013). Since swallowing remains unstudied in rorquals we are unaware of the time that it takes, and as such, swallowing rather than filtering could be the stage of interlunge that imposes limitations on lunge timing and
frequency. The dimensions and actions of the esophagus dictate how fast food travels to the stomach.

The esophagus is a hollow muscular tube responsible for transporting food from the pharynx to the stomach, thus spanning three body regions: cervical, thoracic, and abdominal. At rest, the esophagus is dorsoventrally flattened, but it expands/stretches to accommodate food. The esophageal wall is composed of four tissue layers: mucosa, submucosa, muscularis propria, and adventitia, from the lumen outwards (Gregersen, 2003; Oezcelik and DeMeester, 2011; Pawlina and Ross, 2016; Young et al., 2014). The mucosa is composed of epithelium, the lamina propria (connective tissue), and the muscularis mucosae (smooth muscle fibres), and it possesses longitudinal folds along the inner lumen surface to allow expansion during food transport. The submucosa is composed of blood vessels, lymphatics, and connective and nerve tissues. The muscularis propria is a muscular bilayer, with an inner layer with circular muscle fibres and an outer layer of longitudinal muscle fibres. The adventitia is the outermost layer of the esophagus and is a connective tissue layer that wraps around the muscularis propria and connects the esophagus to surrounding structures.

The muscularis propria is responsible for the sequential contractions that create waves of peristalsis to transport a bolus along the esophagus to the stomach, and thus, it dictates the time it takes to swallow food. The muscularis propria, in addition to its differently oriented muscle fibres (inner circular, outer longitudinal) is composed of striated muscle in the cervical region and smooth muscle in the abdominal region, with the transition zone commonly occurring in the thoracic region. In humans, cats, pigs, horses, and opossums the rostral/upper third of the muscular bilayer is composed of striated muscle, the middle third is a transition zone, and the caudal/lower third is composed of smooth muscle. In mice, rabbits, dogs, and ruminants the
entire esophagus is composed of striated muscle, with the gastroesophageal sphincter often composed of smooth muscle. The type of muscle associated with the esophagus impacts the rate of peristalsis, where esophagi with higher striated muscle content have faster peristalsis (Sukon, 2002).

The esophagus of cetaceans overall is not well studied, especially the mysticete esophagus. The bowhead whale (balaenid) esophagus has striated muscle from the cervical region past the point of the tracheal bifurcation, but how far caudally the striated muscle extends is unknown (Tarpley, 1985). Esophagus width (horizontal axis) is important when considering food transport rates. In bowhead whales that ranged from 9 – 11 m in length, the outer width of the esophagi were no more than 7 – 10 cm (Tarpley, 1985). Anecdotal references suggest that the rorqual esophagus is also rather small in width. For example, in a blue whale that can be up to 29 m long (and engulf a volume of water larger than its own body) the esophagus is “unable to stretch more than 10 inches” (Scales and Smith, 2010). Personal observations from necropsies show the esophagus of a ~ 21 m adult fin whale has an esophagus only ~ 10 cm in outer width, with very thick muscle. The esophagus of rorquals seems incredibly small relative to body size when compared to terrestrial mammal body size and esophagus width: human < 1.5 cm, llama ~ 3.2 cm, sheep ~ 2.15 cm, and cow ~ 4.7 cm (Kuo and Urma, 2006; Sukon, 2002). Even when compared to odontocetes the rorqual esophagus seems small: spinner dolphin ~ 2.5 cm and bottlenose dolphin ~ 5 cm. A rorqual that is at least 170 times heavier and nearly 10 times longer than the above-mentioned dolphins has only double the esophagus width. Esophageal widths similar to those above were measured for odontocetes that I have assisted in performing necropsies on. When these outer esophageal widths (from literature and necropsies) are plotted against body mass it is clear the baleen whale esophagus is smaller than expected from isometric
scaling, with the effect being even more pronounced when esophageal lumen width is considered instead (Fig. 4.1). With such a narrow esophagus that is smaller than expected for an animal so big, it is unknown how large volumes of food are transported to the stomach during the short period of interlunge after filtering.

Mathematical models of fin whale buccal cavity and ventral groove blubber (VGB) inflation have produced estimates of the amount of water engulfed per lunge (Goldbogen et al., 2006; Goldbogen et al., 2007; Potvin et al., 2009); however, the amount of prey captured in a lunge varies depending on prey density, patch size, and distribution in the environment (Goldbogen et al., 2015). Additionally, measurements of krill density span across orders of magnitude depending on the time of sampling and the method used to sample (Goldbogen et al., 2011). Krill densities that rorquals may encounter and feed on vary, with lower estimates around 0.1 kg/m$^3$, but higher estimates of krill densities, from studies unrelated to whale presence, are as high as 154 kg/m$^3$ (Goldbogen et al., 2011; Goldbogen et al., 2019; Nicol et al., 1987). This wide range of densities means an average adult fin whale could hypothetically engulf anywhere between 1.5 kg of krill/lunge (at 0.05 kg/m$^3$ density) or as much as 4000 kg of krill/lunge (at 154 kg/m$^3$ density). While the latter estimate seems highly unlikely, there appears to be a minimum prey density threshold for lunging, where lunging only occurs if krill density is high enough, otherwise the lunge itself is not worth the energy exerted to do so. Along with this, feeding frequency in rorquals increases with prey density to maximize energy intake (Goldbogen et al., 2015; Hazen et al., 2015). The potential thus exists for rorquals to engulf large quantities of prey should they encounter them in nature, as it is most energetically advantageous.

Food transport down the esophagus is similar across mammals, but the distinct morphology of the rorqual esophagus with its comparatively small lumen, and the fact that these
whales are bulk feeders suggests the esophagus is not just a scaled-up version of an average mammalian esophagus. Despite its small appearance, the esophagus of rorquals must be optimized to allow rapid transport of large quantities of food from the pharynx to the stomach. Filtering takes up a large portion of the interlunge period (from videos of rorquals engulfing food and the time it takes for the VGB to deflate), so I suspect that swallowing, from oral cavity to stomach, occurs rapidly. From necropsies, I know the esophagus of fin whales has a small width and appears quite muscular. I hypothesize that the small width of the esophagus and the thick muscular walls exist to generate and withstand large pressures for swallowing rapidly. In order to swallow quickly, I predict that the esophagus is composed of more striated than smooth muscle, for faster peristalsis, and that differences in muscle type will correspond with differences in mechanics. With peristalsis occurring fast, I suspect that a fin whale would swallow multiple boluses of food during the interlunge, and that the interlunge duration is dictated by how fast swallowing can occur. I suggest that the morphology of the esophagus is related to the type of food that rorquals are ingesting – a slurry of prey in bulk, rather than a single prey item, requires a pressurized system to be transported to the stomach. To test these predictions, I examined the gross and microscopic morphology and performed mechanical tests including inflation and uniaxial stretch tests on the esophagus of fin whales. I combined the morphological and mechanical data with fin whale feeding data from the literature to propose a model for feeding and swallowing. I also examined the microscopic morphology of a Pacific white-sided dolphin esophagus as a representative odontocete for comparison of esophageal anatomy with that of the fin whale.
4.2 Methods

4.2.1 Animals and tissue samples

Fin whale specimens were collected postmortem in the summers of 2015 and 2018 as part of the commercial catch operation at Hvalfjörður, Iceland. Whales were dissected and mechanical tests were performed within 36 hours of death. All animals examined were adults. A total of 17 esophagi were examined with 5 of those contributing to the mechanical data and 4 contributing to histology. Tissue samples were imported to Canada under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits.

A Pacific white-sided dolphin was collected at a necropsy of animals stranded and recovered along the coast of British Columbia, Canada, by Fisheries and Oceans Canada. Histological samples were collected within 48 hours of the necropsy that was performed shortly after the animal’s death.

4.2.2 Gross anatomy

In fin whales, the viscera of the whale from the pharynx to anus was removed in a single piece. The esophagus was revealed by cutting through thick fascia in the midline dorsally between the lungs. The esophagus was then removed whole - rostrally from the posterior end of the pharynx at the level of the inferior pharyngeal constrictor (cricopharyngeus), and caudally from directly thoracic to the diaphragm. Length was measured. Photos were taken throughout the revealing and removal process. A single esophagus was sliced down its length longitudinally to examine changes in muscle type and thickness.

In the Pacific white-sided dolphin, the viscera from tongue to stomach was removed. The esophagus was removed from the surrounding tissue. Photos were taken throughout the removal and of the esophagus afterwards.
4.2.3 Inflation tests

The esophagi of 5 fin whales were inflated with water. Water was run through the esophagus prior to inflation to ensure the esophagus was clean and empty. A pipe fitting attached to a water hose was inserted into the caudal end of the esophagus and secured with a metal hose clamp around the esophagus. The rostral end of the esophagus was plugged with a pipe fitting attached to a simple tube manometer, and a metal hose clamp was tightened around the esophagus. The esophagus was filled with water to condition the tissue and was drained until the manometer had a “0” pressure reading. The length of the esophagus was measured. The esophagus was then inflated to an estimated “max inflation/pressure” which corresponded with no further visible width increase. Photos were taken before inflation and at max pressure. Max pressure and the inflated length were recorded. After inflation the water in the esophagus was drained into a bucket down to the 0 pressure mark and weighed for a volume estimate. The remaining water to empty the esophagus was drained and weighed to determine total esophageal volume.

Outer dimensional changes and the percentage of expansion along the esophagus were measured from photos using Fiji (Schindelin et al., 2012). Landmarks on the esophagus ensured the same location was consistently measured for width. Changes in wall thickness before inflation and at max inflation were calculated. The inner radius of the inflated esophagus, $r_i$, for each segment (anterior, middle, posterior) was calculated with:

$$r_i = \sqrt{r_o^2 - (R_o^2 - R_i^2)L/l},$$

assuming constant wall volume (Lillie et al., 2013), where $r_o$ is the inflated outer radius, $R_o$ is the unloaded outer radius, $R_i$ is the unloaded inner radius, $L$ is the unloaded length, and $l$ is the
inflated length. Circumferential wall stress, $\sigma_w$, in the inflated esophagus was calculated with $r_i$ for each segment using the Law of Laplace formula:

$$\sigma_w = P_i r_i / h_i,$$

where $P_i$ is the inflated esophagus pressure and $h_i$ is the inflated wall thickness. Wall stress relative to esophageal position during inflation was calculated for each segment of the esophagus.

Esophageal volume was determined from the calculated inner radius by applying the inner radius of each segment to $1/3^{rd}$ of the total esophagus length and summing the values. The calculated esophageal volume was compared to the measured esophageal volume from the inflation to confirm the accuracy of the inner radius calculation.

To determine if the esophageal muscle at max inflation would be able to overcome the inflation pressure to produce a peristaltic contraction, I calculated the stress, or pressure, that the circular muscle could produce at full inflation/expansion for each segment tested, using the calculated wall thickness and inner radius at maximum inflation. I assumed that each wall layer (mucosa/submucosa, circular muscle, longitudinal muscle) decreased in thickness equally, so divided the wall thickness by 3 to account for just the circular muscle layer. A reasonable average specific muscle tension (i.e. stress, or force per unit area) for vertebrate striated muscle is 200 kPa (Rospars and Meyer-Vernet, 2016). This same value was used for the entire esophagus, even the smooth muscle region, which means the values produced for smooth muscle may be an overestimate of the force production that the smooth muscle is actually capable of.

The formula used to calculate the pressure the muscle could generate, $P$, was:

$$P = \sigma_m h / r$$

where $\sigma_m$ is specific muscle tension, $h$ is wall thickness, and $r$ is radius.
4.2.4 Uniaxial stretch tests

Uniaxial stretch tests were performed on the esophagi of 5 fin whales. Segments of esophagus ~20 cm long were cut from the anterior, middle, and posterior regions, with an attempt to cut the segments from the same approximate location in each esophagus. One piece at a time was tested. The section of esophagus was placed on a wet table to reduce friction and two 2.5 cm wide bars were placed through the lumen of the esophagus. One of the bars was fastened to a metal cable with eye bolts and cable clamps at either end of the bar, with the esophagus between these attachment points. The metal cable was hooked around a force transducer connected to DataQ hardware (Dataq DA Convert DI-205; Dataq Instruments Inc. Akron, Ohio, USA; sampling rate 10 Hz) synced with WinDaq software (version 1.37 Dataq Instruments Inc.) to record force measurements. The other bar was manually pulled away steadily from the bar affixed to the force transducer, opening and stretching the esophageal lumen. A video camera was fixed above the set-up to record the extensibility of the esophagus. The force recording and video were synced up by tapping the force transducer and providing an auditory cue at the same time. A video camera beside the set-up provided a side view of the lumen being stretched. Each esophageal section was stretched 3 times, allowing for conditioning of the tissue with the final stretch for analysis. During the last stretch the bar was pulled on until no further stretch was obtainable ("max" stretch was reached). All esophagus pieces were photographed to measure dimensions. Video and photo data were analyzed in Fiji (Schindelin et al., 2012). The stretched distance was measured at one frame per second up to the maximum distance which was the final measurement. The mechanical length of the tissue was calculated as:

\[
\text{length} = \pi r_m + 2r_{ib} + D ,
\]
where $D$ is the measured stretch distance between bars, $r_m$ is the midwall radius and $r_{ib}$ is the bar radius, to account for the tissue wrapped around the bars that is also being stretched (Lillie et al., 1994). Midwall in this case denotes the outer edge of the circular muscle layer, calculated as $2/3$ of the total wall thickness. The longitudinal muscle layer does not restrict expansion of the esophagus; therefore, only the circular muscle was considered. Stress vs. stretch ratio graphs were produced from these data. To combine and compare all of the fin whale data, linear interpolation was used on the stress and stretch data from each segment to determine the stress at 0.05 stretch ratio intervals, and new graphs were plotted. Inflated wall stress and the corresponding stretch ratio, calculated from width expansion in inflation photos, was plotted on the stress vs stretch ratio graphs to compare the two mechanical tests. The dimensions of all esophagus segments used in uniaxial tests were measured from photos using Fiji (Schindelin et al., 2012) and compiled to determine trends in morphology along the length of the esophagus.

4.2.5 Histology

Esophagus tissue was sampled from the anterior (cranial), middle, and posterior (caudal) regions (thoracic) of 2 fin whale esophagi and processed for histology. These sampling locations corresponded with the segments tested in the uniaxial stretch tests. The Pacific white-sided dolphin esophagus tissue was sampled from anterior and posterior thoracic regions, and one abdominal region. All samples were fixed in 10% neutral buffered formalin, and then processed by Wax-It Histology Services Inc. (University of British Columbia), following standard techniques. Samples were sliced at 5 μm thickness and stained with Verhoeff–Van Gieson stain to display elastin in black, collagen in pink and muscle in yellow/beige. The relative proportions of muscle in each region were established using light microscopy.
4.3 Results

4.3.1 Gross and microscopic anatomy

The esophagus of fin whales extended from the pharyngeal constrictor at the posterior end of the pharynx to just past the diaphragm where it met the stomach. Whales ranged from 15.55 – 19.42 m with an esophagus length range from 1.29 – 1.90 m from the pharyngeal constrictor to the thoracic side of the diaphragm (Fig 4.2). The esophagus was a flattened hollow cylinder and was oval in cross section at both anterior and posterior ends, and occasionally more circular in the middle (Fig. 4.3). The average outer esophageal width across all fin whales and all segments of esophagus was 11.8 cm across (largest axis of the oval). However, width varied slightly along the length of the esophagus with the smallest width in the middle region, corresponding with the change in shape from oval to circular (Table 4.1). Lumen width increased along the length of the esophagus to its widest at the posterior end, with a corresponding decrease in wall thickness. Thickness of the individual muscle layers decreased overall, and the mucosa/submucosa layer was thickest in the middle. The edge of the esophageal lumen was quite wavy with deep folds, so it was challenging to determine what an “accurate” thickness measurement of the esophageal layers was. The degree of deep folding was less pronounced in the posterior segments (Fig 4.3). The shape changes of the esophagus, varying from oval to round, made it challenging to maintain consistency in measuring dimensions along the esophagus.

The anterior (cranial) ~ 3/4 of the esophageal muscle was thick, stiff and deep red in colour. The posterior 1/4 of the esophagus was much more compliant than the first 3/4 and ranged from pink to white in colour (Figs. 4.2, 4.3). Cross sections along the esophagus confirmed a muscular transition from completely striated muscle in both muscle layers at the
anterior end to a combination of striated and smooth at the posterior end. The transition of muscle type began around 2/3rds of the way along the esophagus and was a gradual transition - there was no distinct singular point where a switch in muscle type occurred. Additionally, the two muscle layers didn’t transition at the same location; the inner circular layer was often solely smooth muscle in the posterior-most esophagus, but the outer longitudinal layer often had a combination of striated and smooth muscle well into the posterior-most esophagus (Fig. 4.3). The longitudinally sliced esophagus confirmed these muscular transitions from anterior to posterior.

Histology confirmed this transition of muscle type through the esophagus and displayed additional morphological changes along esophagus (Figs. 4.4, 4.5). The mucosa was thick in all regions sampled. In the anterior region, the lamina propria of the mucosa was characterized by a thick layer of dense collagen fibres along with a layer of diffuse adipose tissue supported by a framework of collagen (Fig. 4.5A). Elastin was more prevalent closer to the muscularis mucosae. The muscularis mucosae was extensive and composed of a relatively thick layer of striated muscle. A few smooth muscle islands and many striated muscle islands were scattered along the innermost edge of the muscularis mucosae (Fig. 4.5B). The submucosa was thin, and was composed of dense collagen and elastin fibres, lymphatics (lymphoid tissue), and blood vessels. The muscularis propria surrounding the submucosa did not always show distinct layers in the cross sections (Fig. 4.4AB), but these layers were clear in the longitudinal sections (Fig. 4.4C). The muscularis propria of the esophagus was thicker than the other esophageal layers, forming the largest component of the esophageal wall. Both the circular and longitudinal muscle layers were about the same thickness. Within the muscle layers were many large bands of dense collagen and elastin fibres (Fig. 4.5C).
The middle region showed different proportions of the esophageal layers. The lamina propria collagen layer was thinner, with a thicker adipose layer extending closer towards the epithelium (Fig. 4.5D). The muscularis mucosae was again extensive and composed mostly of striated muscle, however, there were patches of mixed striated and smooth muscle (Fig. 4.5E). The islands of muscle on the lamina propria side of the muscularis mucosae were composed of smooth or striated muscle, but striated muscle islands were still more numerous. The submucosa again was thin and composed of dense collagen and elastin fibres, and blood vessels. As in the anterior, the muscularis propria was the thickest portion of the esophageal wall, and the two muscle layers were about the same thickness. The muscularis propria was composed mostly of striated muscle, but bands of smooth muscle were visible throughout both circular and longitudinal layers (Fig. 4.5F). Collagen and elastin fibres were woven throughout the muscularis propria.

The posterior (caudal) region again showed a change in proportions of the esophageal layers. The lamina propria collagen layer was almost non-existent and hugged the edge of the epithelium, while the adipose layer was much thicker, and extended nearly to the epithelium (Fig. 4.5G). Smooth muscle dominated this region, with no visible striated muscle in histological samples. The adipose layer of the lamina propria was replete with smooth muscle islands (Fig. 4.5H). The islands extended to the muscularis mucosae and were larger closer to the muscularis mucosae. It was not possible to tell the difference between the lamina propria layer and the muscularis mucosae. The muscle layer of the muscularis mucosae was less extensive than in the regions anterior to this. The submucosa was very thin. The muscularis propria was composed entirely of smooth muscle. Collagen and elastin fibres were present in the muscularis propria in thinner bundles than in anterior and middle regions, though a layer of collagen and elastin
between the circular and longitudinal muscle layers appeared more distinctive but less dense than in the anterior and middle regions (Fig. 4.5I). The two muscle layers of the muscularis propria again had similar thicknesses.

The Pacific white-sided dolphin esophagus had similar tissue arrangements but differing proportions and thicknesses of tissue compared to the fin whale (Fig. 4.6). Three major differences were noted. First, there was no diffuse adipose tissue in any region of the esophagus. Instead, the lamina propria of the mucosa was composed almost entirely of dense collagen fibres. Second, the muscularis mucosae did not have a distinct muscle layer but was composed only of islands of muscle that were comparatively less abundant than in the fin whale. Lastly, the submucosa was relatively much thicker in the Pacific white-sided dolphin compared to the fin whale.

In the anterior region of the dolphin esophagus, the muscularis mucosae was composed of sparse striated muscle islands that appeared to be localized to certain regions along the circumference of the esophagus. The submucosa was relatively thicker than in the fin whale. The muscularis propria was composed of striated muscle, and both the inner circular and outer longitudinal layer were a similar thickness. Collagen and elastin fibres were woven throughout the striated muscle, as in the fin whale.

The posterior region of the dolphin esophagus was similar in morphology to the anterior, the main difference being muscle type. The muscularis mucosae muscle islands were composed of smooth muscle rather than striated, and they were more abundant and more evenly distributed than in the anterior. The submucosa was thicker in the posterior region compared to anterior. The muscularis propria was composed entirely of smooth muscle. There appeared to be more collagen and especially more elastin fibres running through the muscle bilayer in the posterior
region compared to the anterior region. The inner circular layer was at least double the thickness of the outer longitudinal muscle layer.

The abdominal esophagus of the dolphin did not differ much in morphology from the posterior region. The main difference was a decreased size but an increased abundance of the muscularis mucosa smooth muscle islands. These islands were also distributed more throughout the lamina propria, making it difficult to distinguish the two layers of mucosa. The submucosa was thickest in the abdominal region. The proportions of circular and longitudinal muscles in the muscularis propria remained the same as in the posterior region.

4.3.2 Esophagus mechanics

4.3.2.1 Inflation

One esophagus was not included in the inflation data as the procedure differed for photographing and inflating the esophagus, and the data were not comparable. Thus, a total of 4 esophagi were used in comparative inflation data.

The esophagi inflated quite easily and expanded in all directions, taking on a circular shape in cross section along the length, compared to the flattened resting state (Fig. 4.2). The extracted esophagus lengths varied from 1.3 - 1.9 m. During the inflation test esophagi were inflated until there was no more visible increase in width, which resulted in inflation pressures from 6.9 - 8.8 kPa (Table 4.2). The esophagi increased in length by 6 - 25%, and width increase varied widely: anterior 26 - 34%, middle 25 - 70%, posterior 6 - 51%. The volumes of water to deflate the esophagi back to 0 pressure ranged from 16-24 L, and the total volume to empty the esophagi varied from 18 - 27 L. The estimated esophageal volumes calculated from wall thickness and inner (lumen) radius matched well with the measured esophageal volumes, varying from 15 - 29 L.
Calculated wall stress in inflated esophagi increased from the anterior to the posterior end (Fig. 4.7). This corresponds with the change in muscle type from striated to smooth, the increase in the lumen width, and the decrease in wall thickness from anterior to posterior ends of the esophagus. The pressure that the circular muscle layer can produce as a cylinder to overcome the inflation pressure was determined from the calculated muscle thickness at max inflation. The results are presented in Table 4.3. The ability for circular muscle to contract against the inflation pressure decreases posteriorly, corresponding again with the changes listed above. For all esophagi, the anterior region can overcome the inflation pressure. In the middle and posterior regions, however, there were 3 segments that appeared unable to overcome the inflation pressure. The change in muscle type, morphology, and ability to generate force along the esophagus all indicate that the anterior region is built for and important for the force generation required for peristalsis.

4.3.2.2 Uniaxial

Segments for uniaxial stretches (Fig. 4.3) were cut from the previously inflated esophagi. Table 4.4 provides the distance along the esophagus that segments were cut from. These data are useful for linking inflation and uniaxial tests and checking the consistency of mechanics but do not refer to the entire length of the esophagus. While all esophagi were extracted from the viscera at the same anatomical landmarks, the position of the esophagus relative to the diaphragm can change, due to things like stomach fullness or the position of the diaphragm when the specimen is laid out for dissection. Thus, the data in Table 4.4 are not representative of the entire esophagus in the specimen, but the length of the inflated esophagus that was extracted from the specimen. This is also a reason for some variation and outliers in the data, along with individual variation.
Stress vs. stretch ratio graphs showed little difference across all esophageal regions at low stresses with the exception of three outliers (Fig 4.8), indicating that regardless of differences in muscle type along the esophagus, the passive ability to restrict expansion is similar. Connective tissue, primarily collagen, controls the restriction of the esophagus rather than muscle tissue; the uniaxial stretches demonstrated the passive mechanics of the esophagus, and the increasing slope of the curves at higher stretch values shows where collagen acts to resist further expansion and prevent tissue damage. The lower stretch values, before collagen kicks in, demonstrate the biologically relevant zone, and are likely representative of the stress and stretch that the esophagus will experience during bolus transport. Inflated wall stress and stretch ratio plotted on the uniaxial graphs fell in the low stress region (< 100 kPa, with the exception of one outlier) close to the corresponding uniaxial curves (Fig. 4.9), corroborating the biologically relevant zone.

Outliers existed in three esophagi tested (esophagi 1, 2, and 4). The anterior region of esophagus 1 had extra lateral muscle bands along approximately half the total length of the segment (Fig. 4.3A). These bands of muscle are likely pharyngeal muscle from the cricopharyngeus that could be a part of the upper esophageal sphincter. The anterior region was cut in approximately the same location for all esophagi, so the extra muscle in anterior esophagus 1 may be the result of individual variation. The mechanical response may also be due to rigor mortis. The posterior region of esophagus 2 showed a narrowing just cranial to the diaphragm (Fig 4.2A). There was limited expansion in this region in inflation tests (~ 6%), indicating there was some difference in this specimen that affected the mechanics and extensibility. There were no obvious morphological indications of why this region behaved differently, but again, rigor mortis was a possibility. In esophagus 4 the posterior region was sampled further caudally than
the other esophagi; the lumen had rugae rather than longitudinal folds, indicative of a transition to the forestomach rather than being a strictly esophageal sample (Fig. 4.3D). The gastroesophageal sphincter is a physiological sphincter rather than an anatomical sphincter, meaning there was no morphological evidence that I had sampled beyond the esophagus until the cut had already been made. Additionally, for esophagus 4 more than 3 segments were sampled, which limited my ability to recut the esophagus to get an esophageal sample of similar dimensions to the other segments.

4.4 Discussion

The rorqual esophagus is a muscular thick-walled cylinder with a small lumen, built to transport a slurry of food to the stomach. While the appearance of the esophagus seems counterintuitive based on rorqual body size and the immense mouthfuls of food they are engulfing, the morphology and mechanics of the esophagus are intricately linked to feeding/food type and are optimized for transporting fluid-like content. The variation in muscle type, proportion, and thickness, and the small yet extensible lumen are the keys to the function of the esophagus.

The fin whale esophagus has a variable morphology along its length (Fig. 4.3). The anterior region is characterized by a small lumen and thick walls composed of thick striated muscle and abundant collagen. These components provide the anterior esophagus with its stiffer shape, and the thick striated muscle allows the esophagus to generate large forces and pressures for peristalsis. The middle region shows some deviation from these characteristics: an increase in lumen width, decrease in wall thickness, similar striated muscle content, but appears to have less collagen. The posterior region continues with the trend of increasing lumen width, decreasing wall thickness, and decreasing collagen content. Additionally, there is a large increase in adipose
tissue, and a shift to smooth muscle rather than striated, giving the posterior esophagus its more compliant structure. Based on these varying characteristics along the esophagus, the anterior 2/3 of the esophagus is the main force generating and propulsive region. Striated muscle extends along the anterior 2/3 of the esophagus. The muscle in this region is thick and can produce high pressures (Table 4.3), and because the muscle is striated, it can produce rapid peristaltic contractions (Sukon, 2002). In a dog, which has almost entirely striated muscle in its esophagus, peristalsis speeds are as fast as 10 cm/s (Sukon, 2002). Therefore, I would expect a similar peristalsis speed in a rorqual.

As a bolus is pushed into the esophagus, the small lumen expands, and the esophageal muscles relax to accommodate the bolus. A small esophageal lumen is a necessity for transporting a slurry of food, owing to the limited ability for muscle to shorten and decrease lumen size in a circular orientation. The consistency of the krill (and likely some water) being swallowed is likely amorphous and in order for the amorphous bolus to be pushed along the esophagus, the esophagus must be able to contract down behind the bolus to push it towards the stomach. The ability for muscle to contract and decrease the lumen circumference behind a bolus is limited by a muscle’s ability to shorten (~ 25 - 30%). A large lumen means a bolus, especially an amorphous one, may not expand the lumen very much, and the muscle would be unlikely to shorten enough to significantly decrease lumen size to push a bolus forward. A small lumen is needed so that a fluid-like bolus can force the lumen open, and the muscle can contract to decrease lumen circumference and push the bolus forward. Additionally, if the esophagus in its resting state had a large opened/floppy lumen, then much more muscle would be needed to generate the same pressures (following Laplace) required for swallowing. With the morphology and mechanics of the esophagus as described above, the anterior region of the esophagus then
acts as a pressure head to continually pump food down the esophagus. The thick walls and high collagen content of the esophagus enable it to resist the pressures associated with peristalsis. The estimates of wall thickness during inflation, while useful for estimating capacity of the esophagus and the potential for expansion, do not account for the active mechanics of the tissue. In a living mammal, the longitudinal muscle layer also contracts during peristalsis. In combined ultrasound and manometry studies it has been demonstrated that the maximum pressure during peristalsis coincides with maximum wall thickness as a result of both circular and longitudinal muscle layers contracting simultaneously. This means an increase in wall thickness relative to lumen width, which, according to Laplace’s law (stress = pressure × radius/thickness) maintains a constant wall stress and prevents an esophageal diverticulum, the equivalent of an aneurysm, in the esophagus (Mittal et al., 2006; Pehlivanov et al., 2001; Puckett et al., 2005).

Combining the esophageal mechanics with krill densities from the literature and active muscle mechanics from other mammals allows me to produce a simple model for swallowing in a fin whale. A fin whale is capable of engulfing 60 - 82 m³ (average 71 m³) in a single lunge (Goldbogen et al., 2007). To account for fin whales not always completely filling their mouths during a lunge (Arnold et al., 2005) I will include an additional estimate of 30 m³ in a single lunge (Goldbogen et al., 2006). These volumes include water and krill. Water is then filtered out, but an unknown volume of water is likely swallowed along with the krill. I estimate that an additional volume equivalent to 10% of the total krill volume is swallowed water – if 100 L of krill are being swallowed then 10 L of water will be swallowed with the krill meaning a total volume swallowed of 110 L (specific gravity of krill – 1.025 g/ml, assuming krill are isosmotic to seawater). Using krill densities from the literature I can estimate how much krill and water might be swallowed with each lunge (Tables 4.5, 4.6). Unfortunately, bolus size is not a value
that I can predict. Instead, if I assume an average esophageal capacity of 20 L, I can then determine how many swallows at maximum esophagus capacity would be required to get the mouthful to the stomach. This would of course be an underestimate of how many swallows would actually be required, as I believe the esophagus would not be completely full of food and instead would propel food in boluses as in other mammals.

This brings about the first limitation in rorqual feeding frequency – transferring food from the mouth to the stomach, with limited esophagus capacity, in order to clear the mouth for the next lunge. It is unknown how food is transferred from the oral cavity to the esophagus in rorquals. Pharyngeal structures differ in position in rorquals, owing to their shortened cervical region and horizontal head and body position (Berta et al., 2015c), meaning there is no posture-related downward transfer of food from head to neck, and instead food must be pushed directly posteriorly into the pharynx and again directly posteriorly into the esophagus. Mammals generally push food back into the oropharynx with their tongue, where subsequent pharyngeal contractions push it down into the esophagus (Massey, 2006). Some mammals, usually herbivores, possess valleculae – a space between tongue and epiglottis that food can be stored prior to being pushed into the esophagus as a bolus (Hiemae and Crompton, 1985) (see Fig 1.7). In all of these cases, the food taken into the mouth does not exceed the capacity of the esophagus. In rorquals, however, it is likely common that the food volume in the mouth exceeds the esophageal capacity, and potentially (but unlikely) the pharyngeal capacity, meaning multiple boluses must be swallowed. After a lunge, water is purged/filtered out of the mouth by the VGB and tongue retracting back to their resting states. As this happens, space in the mouth is diminishing, and food is likely concentrated at the back of the mouth on the tongue. The tongue then, as in other mammals, can push food back into the pharynx. Unlike other mammals,
however, the oral plug must first be displaced from its position occluding the oropharyngeal channel in order to push food into the pharynx. The oral plug is elevated posterodorsally to block the nasopharynx and protect the upper airways from food incursion, and clear the oropharyngeal channel.

The scenarios for how food is transferred to the esophagus are limited: food is either held in the mouth and pushed directly through the pharynx into the esophagus, or food is stored transiently in the pharynx prior to being swallowed. If the volume of food in the mouth exceeds esophagus (or pharynx) capacity, then food may be held in the mouth, with only “bolus-sized” volumes of food being transferred through the pharynx sequentially. The tongue would be responsible for holding the entire food volume, with muscular wave-like actions of the tongue pushing some of the food to the back of the mouth to be pushed through the oropharyngeal channel. The problem with this scenario is the distinct lack of musculature in the rorqual tongue (Lambertsen et al., 2005). The tongue is composed largely of adipose and connective tissues, so it’s ability to produce wavelike contractions is limited. Alternately, the entire volume of food from the mouth may be pushed into and held in the pharynx and parsed out into boluses by actions/muscular contractions of the pharynx. The first potential problem with this scenario is storing food in the pharynx for long periods of time could risk respiratory tract protection with the absence of valleculae in rorquals. A second issue with this scenario is that there doesn’t appear to be any structures or mechanisms that could be used to parse out boluses to be pushed into the esophagus. Additionally, a problem common to both of these scenarios is the time that it would take to swallow multiple boluses in order to clear the mouth and/or pharynx of food in preparation for the next lunge. Even with a rapid peristalsis rate like 10 cm/s as in a dog, it would take 19 seconds for a single bolus to travel through the longest esophagus (1.9 m) examined in
this study. Multiple boluses would likely need to be swallowed. Individual primary peristaltic
contractions, the “normal” peristaltic contractions that transport food, take time to travel all the
way along the esophagus, and a refractory period follows this contraction. Secondary peristaltic
contractions occur unrelated to pharyngeal contractions for swallowing and are triggered by
distension of the esophagus. The inhibitory effects after a primary peristaltic contraction prevent
an esophageal contraction from happening during the primary wave, preventing multiple
unsynchronized contractions from occurring in the esophagus. This phenomenon, termed
‘deglutitive inhibition’, could allow for rapid successive swallows by inhibiting the esophagus
from completing an entire peristaltic contraction until the last swallow in the series (Mashimo
and Goyal, 2006; Shaker et al., 2013). Deglutitive inhibition is the phenomenon that allows
animals to drink fluids. Regardless of this ability, 19 seconds is about 2/3rds of the average
interlunge time in a fin whale (30 seconds), and filtering appears to take up most of the
interlunge interval in whales (Goldbogen et al., 2013), so, the likelihood of a fin whale purging
water from the mouth and then swallowing multiple boluses in an average 30 second interlunge
interval is almost non-existent. A property of swallowing not yet considered in these calculations
is the creation of pressure differentials in the digestive tract that can speed up the transit time of
the bolus (Standring, 2021). Opening a previously collapsed space, such as the pharynx once the
oral plug has shifted (Chapter 3), would create a zone of lower pressure than the mouth, drawing
the bolus into the pharynx. Likewise, relaxation of the cricopharyngeus to open the esophageal
inlet would also produce a zone of decreased pressure, assisting in moving the bolus into the
esophagus. A scenario that does not rely solely on peristalsis must be considered.

The muscular pharynx could act as a pump that transports a slurry of food through the
esophagus, like viscous fluid flow through a pipe, with a peristaltic wave at the end, like in
deglutitive inhibition, to completely clear the esophagus. The VGB retracting during the filtration stage of interlunge brings the floor of the mouth back into its resting position, mechanically filling the oral cavity with the fatty tongue and increasing pressure in the mouth. This forces the entire food volume through the oropharyngeal channel into the pharynx, a zone of lower pressure. The tongue filling the oral cavity in its resting position provides a barrier between oral cavity and pharynx. The protective configuration of pharyngeal structures during swallowing - with the soft palate and oral plug occluding the upper airways, and the closed and anteroventrally tucked larynx with collapsed laryngeal sac occluding the lower airways (Fig. 3.9) - would inhibit food from entering the respiratory tract. The pharynx would contract, increasing pharyngeal pressure; meanwhile, the cricopharyngeus would relax to open the esophageal inlet, decreasing pressure downstream. This combination would continuously push a stream of food from the pharynx, down the esophagus and into the stomach until the pharynx was empty (in this case, the esophagus would indeed be filled with food, contrary to my earlier objection to this, and opposite to my prediction of multiple boluses). The thick skeletal muscle at anterior end of the esophagus, along with the connective tissue, would reinforce the esophageal wall to resist any damaging expansion under the pressure produced by the pharyngeal muscle. Then, similar to deglutitive inhibition, a peristaltic contraction would commence as the end of the food stream was pushed into the esophagus (following pharyngeal contraction), completely clearing the esophagus of food by providing a final wave-like push along the esophagus to the stomach. The other previously discussed assessments of morphology relating to peristaltic actions of the esophagus in the fin whale would hold true for this peristaltic contraction. A pharyngeal pump with a peristaltic wave would provide a rapid way to transfer food from mouth to stomach, so that the next lunge can commence.
Once food makes it to the forestomach, another limitation is presented – the capacity of the forestomach. The average capacity of a fin whale forestomach is ~ 754 L (Víkingsson, 1997). Based on the krill densities and fin whale feeding kinematics, I can estimate how many lunges and dives can take place before the forestomach is filled (Tables 4.5, 4.6). Fin whales average 4 lunges per dive, with each dive with surface recovery time lasting 9 minutes (Goldbogen et al., 2007). This allows an estimate of the time it would take to fill the forestomach, or, the longest feeding bout a fin whale can engage in before it is full. When compared with observations of fin whale feeding bouts, the data match incredibly well. A study on the feeding habits of 5 fin whales found on average they performed 19 foraging dives lasting 2.7 hours (Irvine et al., 2019). My calculations suggest at a krill density of 0.15 kg/m$^3$ a fin whale would fill its forestomach after 16.5 dives, in a total of 2.5 hours. There are multiple sources of variation that can explain the difference between observed and calculated feeding times – the actual volume of engulfment, varying krill densities across lunges, the time it takes swallowed water to pass through the forestomach compared to krill, or how much water is actually being consumed with krill. With all of these variables the calculated value is impressively close to the observed, suggesting that forestomach capacity limits foraging bout duration. Víkingsson (1997) suggests a food transit time of 3-6 hours from forestomach to fundic chamber, so feeding again within 3 hours of a feeding bout is likely somewhat limited. Filling the forestomach does not necessarily coincide with meeting metabolic demands, so multiple feeding bouts must occur to meet metabolic demands. A 20 m long fin whale requires approximately 901 kg of krill per day to meet metabolic demands (Croll et al., 2006). Based on krill densities, the time it takes to meet metabolic demands can be calculated in the same manner (Tables 4.5, 4.6). This aspect of the model has been investigated previously (Goldbogen et al., 2007), but without considering
stomach capacity or varying krill densities, both of which would impact the length of an actual feeding bout. From these data, we can see how many more foraging dives are required to meet metabolic demands once food processing in the stomach has commenced.

Feeding style and prey type between rorquals (generally baleen whales) and odontocetes are vastly different, and the anatomy of the esophagus reflects this. Baleen whales appear to be the odd ones out in terms of esophagus morphology with such a small lumen. Rorquals are even more unusual with the high collagen content in the first 2/3\textsuperscript{rd}s of the esophagus, incredibly high adipose content in the last 1/3\textsuperscript{rd}, and the presence of a third muscle layer that is likely part of the submucosa. Histology of normal human esophagus morphology (Pawlina and Ross, 2016; Young et al., 2014) does not show adipose content even close to what is observed in the fin whale. Likewise, the odontocete esophagus showed no adipose tissue along its length. The adipose tissue in the fin whale must have importance mechanically. Adipose tissue is deformable, and this may play allow expansion of the esophagus by the entry of a bolus, increasing lumen size. To push a bolus forward, contraction of the muscular propria surrounding the adipose tissue would force the adipose inwards, closing the lumen behind the bolus to push the bolus along. The muscularis propria cannot be stretched very much - perhaps compressing this adipose layer increases lumen size enough for bolus transport to occur without stretching the muscularis propria. The muscularis mucosae muscle layer (in addition to islands of muscle) in fin whales seems to exist in place of just the islands of muscle as in the odontocete. This increase in muscle content could provide an increased ability to tonically resist damaging expansion forces, as well as assist with increasing force production or speed for the final peristaltic wave to clear the esophagus. Additionally, the fin whale esophagus has striated muscle quite far posteriorly, likely meaning a rapid rate of peristalsis. Unfortunately, I am unaware at what point
the transition to smooth muscle occurs in odontocetes. I would assume that the fin whale has striated muscle further posteriorly to decrease food transport time for the time-limited feeding events of fin whales.

Rorquals may be the only mammals, possibly the only animals, that capture a volume of food that is too large to be swallowed as a single distinct bolus. This requires an ability to process and transport large volumes of food to the stomach while on a time-limited dive; thus, performing these tasks rapidly is energetically advantageous. The rorqual esophagus should be adapted to meet transport requirements, and the morphology and mechanics revealed in this study support that. The rorqual esophagus has a small width lumen surrounded by thick muscular walls, capable of expanding around a fluid-like bolus and generating and withstanding forceful peristaltic pressures. The volume of food a rorqual engulfs is dictated by krill densities in nature, while the timing of lunging is dictated by the morphology and mechanics of the esophagus. Rorquals are incredibly successful at feeding considering that they include the largest animals on the planet. Their adaptations for engulfing immense volumes of prey are largely credited for their large body size, but, adaptations of the digestive tract are also imperative to their success and cannot be overlooked.
Figure 4.1 Scaling plots of terrestrial mammal, odontocete, and mysticete esophagi outer width vs. body mass (A), and lumen width vs. body mass (B) plotted on log axes. The far right points on both graphs are for mysticetes. The red trendlines and equations are for terrestrial mammals, odontocetes, and mysticetes plotted together. The blue trendlines and equations are for terrestrial mammals and odontocetes, with the equation from those mammals applied to mysticete body masses to plot where mysticete data would fall if they scaled in the same way, indicated by triangle markers and circled in black. For a fin whale (50000 kg), actual esophageal outer width is 0.118 m and predicted outer esophageal width is 0.226 m (A); actual esophageal lumen width is 0.069 m and predicted is 0.884 m (B). For a bowhead whale (55000 kg), actual esophageal outer width is 0.08 m and predicted outer
Esophageal width is 0.241 m (A); actual esophageal lumen width is 0.04 m and predicted is 0.993 m (B). Esophageal widths were collected from literature or measured by me. In most cases an average body mass was identified in literature and used. Animals used in both outer diameter and lumen diameter plots include human, South Asian river dolphin, bowhead whale, fin whale, harbour porpoise, Pacific white-sided dolphin*, common dolphin, Dall’s porpoise, Risso’s dolphin, Beluga*, and false killer whale*. Additionally, the outer diameter plot included llama, sheep, cow, spinner dolphin*, and bottlenose dolphin*. * = measured specimen body mass.
Figure 4.2 Uninflated (top of panels) and inflated (bottom of panels) esophagi of 4 fin whales. (A) - esophagus 2, (B) - esophagus 3, (C) - esophagus 4, (D) – esophagus 5. Posterior (stomach) is to the left, anterior (pharynx) to the right. The difference in overall tissue colour (white posteriorly and red anteriorly) demonstrates a difference in muscle type, where whiter tissue is smooth muscle and redder tissue is striated muscle. However, not all colours are indicative of differences in muscle type; the esophagus is covered with a layer of adventitia that obscures some of the tissue colours but displays a darker red in the anterior region as it connects the esophagus to other tissues and organs. The bottom left of (D) shows interdigitation of the two muscle types along the outer layer. Not all esophagi inflated equally. In (A) there is a restriction
in the posterior region and in (C) there is a restriction between middle and posterior regions.

Scale bar - 30 cm.
Figure 4.3 Esophageal segments from 5 fin whales used in uniaxial stretches. (A) - esophagus 1, (B) - esophagus 2, (C) - esophagus 3, (D) - esophagus 4, (E) - esophagus 5. Esophageal segments arranged from left to right are anterior, middle, and posterior. In (D) the second segment from the right was an additional sample; the far right segment is the posterior segment. Left
column – rostral faces of segments, right column – caudal faces of segments. The esophageal lumen is wavy and folded to varying degrees. The anterior segments show deep folds in addition to the wavy epithelium lining. Middle segments are generally similar in appearance to the anterior segments with more deep folds. Posterior segments show fewer deep folds but consistent epithelial waviness. In (D) the posterior segment shows rugae indicative of a transition to forestomach rather than being strictly esophageal tissue. The tissues directly surrounding the inner lumen are the mucosa and the submucosa; the boundary between them is difficult to distinguish from gross anatomy. The mucosa appears to contain a lot of adipose tissue, especially in some of the posterior segments, for example in panel (E). Surrounding the mucosa and submucosa is the muscular bilayer. The two muscle layers are easily distinguished in most segments. Deep red coloured muscle is striated muscle, and pink/white coloured muscle is smooth muscle. There are varying amounts of striated muscle extending into the posterior region, especially in the outer longitudinal muscle layer. There is variation in the shape of the esophageal segments. Scale bar - 5 cm.
Figure 4.4 Esophageal histology from two fin whales. Top of panels – anterior region, middle of panels – middle region, bottom of panels – posterior region. (A) and (B) cross sections of esophageal tissue, (C) longitudinal sections from same locations and specimen as (B). Collagen stains pink, elastin stains black, muscle stains brown/tan, other tissues (epithelium, lymphatic) stain brown or grey, adipose appears white (unstained). The esophageal lumen is to the left. The epithelium is visible as a brown/tan band running down the left side of all of the images. Immediately to the right of this is the lamina propria, composed of collagen, adipose, islands of muscle, and in the anterior and middle regions a distinct band of muscle. The submucosa is not large or obvious in these specimen, but exists as a small band of connective tissue between the muscularis mucosae and the muscular bilayer of the muscularis propria. The muscularis propria consists of an inner layer of circular muscle and outer layer of longitudinal muscle. The regions of muscle
(including muscularis mucosae) are most easily distinguished in the longitudinal sections (C). From anterior to posterior there is a decrease in thickness of the collagen in the lamina propria and a corresponding increase in the thickness of adipose tissue. The anterior region of the muscularis propria is striated tissue, the middle is mostly striated tissue with scattered bands of smooth muscle, and the posterior is smooth muscle. The muscularis mucosae is more band-like in appearance in anterior and middle sections but appears as more diffuse islands in the posterior region. Specimen (B)/(C) has very little muscularis mucosae present in the posterior region. Both layers of the muscularis propria decrease in thickness posteriorly, and this change is more evident in the longitudinal muscle layer.

Lymphatic nodules are visible in the middle image of panel (B) as grey ovals in the lamina propria. Scale bars – 2 mm.
Figure 4.5 Esophageal tissue at higher magnification from the anterior (ABC), middle (DEF), and posterior (GHI) regions of a fin whale esophagus. The top row of images shows the corresponding locations that the colour-coded panels display. Collagen stains pink, elastin stains black, muscle stains brown/tan, other tissues (epithelium, lymphatic) stain brown or grey, adipose appears as white (unstained). (A) The left side of the image shows a dense and thick layer of collagen, and the right side shows adipose tissue, both in the lamina propria. (B) Lamina propria is visible as the collagen and adipose tissue on the right side. The region indicated by the square is the muscularis mucosae, the star is the submucosa, and the circle is part of the circular muscle layer of the muscularis propria. (C) Dense collagen and elastin band running through striated muscle of the muscularis propria. (D) A thinner and less dense layer of collagen, but thicker layer of adipose tissue than in the anterior region. (E) The square indicates the muscularis mucosae. Though most of the muscularis mucosae is striated muscle, this image highlights part of the muscularis mucosae that has a high smooth muscle content with pockets of striated muscle indicated by the arrows. The star indicates the submucosa, which in this region is dense collagen and elastin fibres. The circle indicates the circular muscle layer of the muscularis propria. (F) A combination of striated and smooth muscle form the muscularis propria. Striated muscle is dominant, and smooth muscle regions are indicated by arrows. (G) A thin layer of collagen and a diffuse extensive layer of adipose composes the lamina propria. (H) The lamina propria and muscularis mucosae are visible in this image, though the boundary between the two is not clear. Smooth muscle islands exist in the adipose layer of the lamina propria. The smooth muscle band along the right side is the muscularis mucosae. (I) The muscularis
propria is composed solely of smooth muscle. A comparatively less dense band of collagen and elastin fibres define the boundary between the two muscle layers of the muscularis propria. Scale bars - 500 μm.
Figure 4.6 Comparison of esophageal histology from a Pacific white sided dolphin (A) and fin whale (B). Side by side images do not correspond with equivalent sampling locations. In (A) top image – anterior region, middle image – posterior region, bottom image
– abdominal region. In (B) panels and description follow as in Figs. 4.4 and 4.5, top image – anterior region, middle image – middle region, bottom image – posterior region. Collagen stains pink, elastin stains black, muscle stains brown/tan, other tissues (epithelium, lymphatic) stain brown or grey, adipose appears white (unstained). Both (A) and (B) demonstrate similar tissue arrangement, however, vary in abundances of tissue and thickness of the layers. (A) The mucosa is substantial; the lamina propria is replete with collagen fibres, but adipose tissue is not present. The muscularis mucosae does not form a distinct muscle band and instead forms islands of striated muscle in the anterior region and islands of smooth muscle in the posterior and abdominal regions. The islands of muscle increase in abundance through the posterior and abdominal sections, and the boundary between lamina propria and muscularis mucosae becomes unclear; the islands of muscle penetrate the collagen layer of the lamina propria. The submucosa is relatively thicker compared to (B) and increases in thickness towards the abdominal section. Elastin fibres (black) and blood vessels (blood in gold/yellow) are visible in the submucosa and increase in abundance abdominally. The muscularis propria is composed of striated muscle in the anterior region and smooth muscle in the posterior and abdominal regions. The inner circular layer and outer longitudinal layer are about the same thickness in the anterior region. There is an increase in the thickness of the circular layer and a decrease in the thickness of the longitudinal layer in both posterior and abdominal regions. Scale bars: A - 500 μm, B - 2 mm.
Figure 4.7 Wall Stress vs. position along the esophagus. Anterior is towards 0, posterior is towards 100. Wall stress is the product of pressure and inner radius divided by wall thickness. The increase in stress towards the posterior end is the result of the decreasing wall thickness and increasing lumen diameter towards the posterior region.
Figure 4.8 Stress–stretch ratio plots of all esophageal segments tested in uniaxial stretch tests. Colours represent individual esophagus segments. (A) Complete data set of uniaxial stretches. Stresses remain low for the first half of the total stretch and gradually increase as the segments become stiffer in the latter half of the total stretch. The low stress and stretch zone is likely the biologically relevant zone, before the segments increase in stiffness as collagen restricts further expansion. The red box indicates this area, expanded in (B). (B) The biologically relevant portion of the graph, where stresses are low, is indicated by the grey box. There is little difference across all esophageal segments in this region, indicating the passive mechanics of the esophagi are all similar, despite differences in muscle or adipose content. Three outliers exist, which can be explained by examining the morphology of the segments. Outlier 1 (esophagus 1, anterior) had lateral muscle bands, likely extensions of pharyngeal muscle, that could have increased the
stiffness of the esophagus (see Fig. 4.3A). Despite working with fresh tissue, this segment could also have been in rigor mortis, increasing the stiffness of the segment. Outlier 2 (esophagus 2, posterior) did not have any obvious morphological indications of why it was so stiff (see Fig. 4.3B). Inflation tests showed little inflation in the posterior region (Fig. 4.2A). Rigor mortis could have affected this segment. Outlier 3 (esophagus 4, posterior) was much less stiff than the other segments as it included a portion of the stomach (Fig. 4.3D).
Figure 4.9 Stress-stretch ratio curves with inflation data (points) for 4 fin whale esophagi. (A) - esophagus 2, (B) - esophagus 3, (C) - esophagus 4, (D) – esophagus 5. Red – anterior, purple – middle, blue – posterior. Inflation data indicated by the points on the graphs matched the uniaxial data well. Inflation stresses all fell under the 100 kPa mark (most under 50 kPa), with the exception of the posterior of esophagus 4 (C, addressed in Fig. 4.8). Another exception is the posterior region of esophagus 2 (A) which showed a low inflation stress and stretch (also addressed in Fig. 4.8). Combined inflation and uniaxial data corroborate the biologically relevant zone being the low stress zone, with the esophagi generally stretching less than 60%.
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Anterior</th>
<th>Middle</th>
<th>Posterior</th>
<th>Change along length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer width (m)</td>
<td>0.127</td>
<td>0.106</td>
<td>0.122</td>
<td>Narrows at middle</td>
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<td>Lumen width (m)</td>
<td>0.063</td>
<td>0.064</td>
<td>0.078</td>
<td>Increase</td>
</tr>
<tr>
<td>Wall thickness (m)</td>
<td>0.032</td>
<td>0.030</td>
<td>0.027</td>
<td>Decrease</td>
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<td>Longitudinal muscle thickness (m)</td>
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<td>0.007</td>
<td>0.006</td>
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<td>Circular muscle thickness (m)</td>
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<td>0.008</td>
<td>Decrease then holds</td>
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<tr>
<td>Mucosa &amp; submucosa thickness (m)</td>
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<td>0.013</td>
<td>Thickest at middle</td>
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<tr>
<td>Midwall radius (m)</td>
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<tr>
<td>Wall area (m²)</td>
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<td>0.012</td>
<td>0.012</td>
<td>Decrease then holds</td>
</tr>
<tr>
<td>Circular muscle area (m²)</td>
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<td>0.003</td>
<td>0.004</td>
<td>Smallest at middle</td>
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Table 4.2: Esophageal inflation data (n = 4)

<table>
<thead>
<tr>
<th>Esophagus</th>
<th>Inflation P (kPa)</th>
<th>Volume to 0P (L)</th>
<th>Total volume (L)</th>
<th>Length increase (%)</th>
<th>Anterior width increase (%)</th>
<th>Middle width increase (%)</th>
<th>Posterior width increase (%)</th>
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</thead>
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<tr>
<td>2</td>
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<td>22.8</td>
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<td>6</td>
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<td>3</td>
<td>7.4</td>
<td>15.6</td>
<td>18.4</td>
<td>21</td>
<td>37</td>
<td>70</td>
<td>51</td>
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<tr>
<td>4</td>
<td>6.9</td>
<td>16.5</td>
<td>21.9</td>
<td>12</td>
<td>26</td>
<td>25</td>
<td>43</td>
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<tr>
<td>5</td>
<td>7.3</td>
<td>23.5</td>
<td>27.2</td>
<td>25</td>
<td>34</td>
<td>48</td>
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<tr>
<td>Average</td>
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<td>19.2</td>
<td>22.6</td>
<td>16</td>
<td>31</td>
<td>43.8</td>
<td>33.3</td>
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Table 4.3: Measured inflation pressure and calculated pressure muscle can generate as a cylinder (n = 4)

<table>
<thead>
<tr>
<th>Esophagus</th>
<th>Inflation P (kPa)</th>
<th>Calculated anterior P (kPa)</th>
<th>Calculated middle P (kPa)</th>
<th>Calculated posterior P (kPa)</th>
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</thead>
<tbody>
<tr>
<td>2</td>
<td>8.8</td>
<td>21.3</td>
<td>17.8</td>
<td>17.6</td>
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<tr>
<td>3</td>
<td>7.4</td>
<td>17.3</td>
<td>7.3</td>
<td>6.9</td>
</tr>
<tr>
<td>4</td>
<td>6.9</td>
<td>17.8</td>
<td>14.0</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>7.3</td>
<td>12.6</td>
<td>8.8</td>
<td>11.6</td>
</tr>
<tr>
<td>Average</td>
<td>7.6</td>
<td>17.3</td>
<td>12.0</td>
<td>10.1</td>
</tr>
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</table>
Table 4.4: Percentage along extracted esophagus length that segments for uniaxial stretch were sampled from (n = 5)

<table>
<thead>
<tr>
<th>Esophagus</th>
<th>Anterior (%)</th>
<th>Middle (%)</th>
<th>Posterior (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>50</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>45</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>49</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>49</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>48</td>
<td>88</td>
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</table>
Table 4.5: Feeding requirements of fin whales engulfing 71 m$^3$ per lunge to fill forestomach (754 L) and meet metabolic demands (901 kg krill/day) at varying krill densities

<table>
<thead>
<tr>
<th>Krill density (kg/m$^3$)</th>
<th>154$^a$</th>
<th>4.5$^b$</th>
<th>1.65$^b$</th>
<th>0.5$^b$</th>
<th>0.15$^c$</th>
<th>0.05$^d$</th>
<th>0.01$^d$</th>
<th>0.003$^d$</th>
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<tbody>
<tr>
<td>Krill per lunge (L)</td>
<td>10667.3</td>
<td>311.7</td>
<td>114.3</td>
<td>34.6</td>
<td>10.4</td>
<td>3.5</td>
<td>0.69</td>
<td>0.21</td>
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<tr>
<td>Water per lunge (L)</td>
<td>1066.7</td>
<td>31.2</td>
<td>11.4</td>
<td>3.5</td>
<td>1</td>
<td>0.35</td>
<td>0.07</td>
<td>0.02</td>
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<tr>
<td>Krill + water (L)</td>
<td>11734</td>
<td>342.9</td>
<td>125.7</td>
<td>38.1</td>
<td>11.4</td>
<td>3.8</td>
<td>0.76</td>
<td>0.23</td>
</tr>
<tr>
<td>Times esophagus capacity</td>
<td>586.7</td>
<td>17.1</td>
<td>6.3</td>
<td>1.9</td>
<td>0.57</td>
<td>0.19</td>
<td>0.04</td>
<td>0.01</td>
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<tr>
<td>Lunges to fill FS</td>
<td>0.1</td>
<td>2.2</td>
<td>6.0</td>
<td>19.8</td>
<td>66</td>
<td>197.9</td>
<td>989.6</td>
<td>3298.5</td>
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<tr>
<td>Dives to fill FS</td>
<td>0.02</td>
<td>0.5</td>
<td>1.5</td>
<td>4.9</td>
<td>16.5</td>
<td>49.5</td>
<td>247.4</td>
<td>824.6</td>
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<tr>
<td>Time to fill FS (hrs)</td>
<td>0.002</td>
<td>0.1</td>
<td>0.2</td>
<td>0.7</td>
<td>2.5</td>
<td>7.4</td>
<td>37.1</td>
<td>123.7</td>
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<tr>
<td>Lunges to meet MD</td>
<td>0.1</td>
<td>2.8</td>
<td>7.7</td>
<td>25.4</td>
<td>84.6</td>
<td>253.8</td>
<td>1269</td>
<td>4230</td>
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<tr>
<td>Dives to meet MD</td>
<td>0.02</td>
<td>0.7</td>
<td>1.9</td>
<td>6.3</td>
<td>21.2</td>
<td>63.5</td>
<td>317.3</td>
<td>1057.5</td>
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<tr>
<td>Time to meet MD (hrs)</td>
<td>0.003</td>
<td>0.1</td>
<td>0.3</td>
<td>1.0</td>
<td>3.2</td>
<td>9.5</td>
<td>47.6</td>
<td>158.6</td>
</tr>
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</table>

FS – forestomach, MD – metabolic demand; a - (Nicol, 1986), b - (Goldbogen et al., 2011), c - (Croll et al., 2005), d – (Goldbogen et al., 2015)
<table>
<thead>
<tr>
<th>Krill density (kg/m³)</th>
<th>154&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4.5&lt;sup&gt;b&lt;/sup&gt;</th>
<th>1.65&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.5&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0.15&lt;sup&gt;c&lt;/sup&gt;</th>
<th>0.05&lt;sup&gt;d&lt;/sup&gt;</th>
<th>0.01&lt;sup&gt;d&lt;/sup&gt;</th>
<th>0.003&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
<td>Krill per lunge (L)</td>
<td>4507</td>
<td>131.7</td>
<td>48.3</td>
<td>14.6</td>
<td>4.4</td>
<td>1.5</td>
<td>0.29</td>
<td>0.09</td>
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<tr>
<td>Water per lunge (L)</td>
<td>450.7</td>
<td>13.2</td>
<td>4.8</td>
<td>1.5</td>
<td>0.44</td>
<td>0.15</td>
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<td>0.01</td>
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<tr>
<td>Krill + water (L)</td>
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<td>144.9</td>
<td>53.1</td>
<td>16.1</td>
<td>4.8</td>
<td>1.6</td>
<td>0.32</td>
<td>0.1</td>
</tr>
<tr>
<td>Times esophagus capacity</td>
<td>247.9</td>
<td>7.2</td>
<td>2.7</td>
<td>0.81</td>
<td>0.24</td>
<td>0.08</td>
<td>0.02</td>
<td>0.005</td>
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<tr>
<td>Lunges to fill FS</td>
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<td>5.2</td>
<td>14.2</td>
<td>46.8</td>
<td>156.1</td>
<td>468.4</td>
<td>2342</td>
<td>7806.6</td>
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<td>Dives to fill FS</td>
<td>0.04</td>
<td>1.3</td>
<td>3.5</td>
<td>11.7</td>
<td>39.0</td>
<td>117.1</td>
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<td>1951.6</td>
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<td>Time to fill FS (hrs)</td>
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<td>0.5</td>
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<td>5.9</td>
<td>17.6</td>
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<td>292.7</td>
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<tr>
<td>Lunges to meet MD</td>
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<td>18.2</td>
<td>60.1</td>
<td>200.2</td>
<td>600.7</td>
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<td>10011.1</td>
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<tr>
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<td>50.1</td>
<td>150.2</td>
<td>750.8</td>
<td>2502.8</td>
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<tr>
<td>Time to meet MD (hrs)</td>
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<td>0.3</td>
<td>0.7</td>
<td>2.3</td>
<td>7.5</td>
<td>22.5</td>
<td>112.6</td>
<td>375.4</td>
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</table>

FS – forestomach, MD – metabolic demand; a - (Nicol, 1986), b - (Goldbogen et al., 2011), c - (Croll et al., 2005), d – (Goldbogen et al., 2015)
Chapter 5: Conclusion

5.1 Thesis overview and major contributions

My objective for this thesis was to investigate the relationship between two profoundly linked systems, respiratory and digestive, in rorqual whales, to understand how the needs of both of these systems are fulfilled and balanced in an aquatic environment, and how the relationship of these two systems differs in rorquals compared to other aquatic and terrestrial mammals. The crossover point of these two systems, the pharynx, was the crux of this research, with my focus directed dorsally to the nasal cavities, ventrally to the larynx, and caudally to the esophagus. I expected to see adaptations for maximizing feeding ability that did not hinder the function, nor compromise the protection, of the respiratory tract. I addressed these ideas with the questions: 1) How is the respiratory tract protected from water entry and simultaneously protected from barotrauma? 2) How are the upper and lower respiratory tracts protected from food entry during swallowing? 3) How does esophagus morphology contribute to transporting food from pharynx to stomach?

In researching the first question through morphological studies, video analysis, and model calculations, I demonstrated that the nasal plugs of rorqual whales perform the dual function of protecting the respiratory tract from water incursion and preventing barotrauma in the upper respiratory tract at depth. At the surface the high adipose content nasal plugs act as self-sealing valves that require muscular effort to open the nasal cavities, and close the nasal cavities through elastic recoil. The nasal plugs of rorquals are unique and their passive movements during diving, where ambient ocean pressures increase, is a novel mechanism for preventing barotrauma (Chapter 2).
I addressed the second question through dissections and physical manipulations of the rorqual pharynx and larynx, and determined that despite the larynx being positioned far rostrally the rorqual pharynx has a similar configuration to a terrestrial carnivore or human, and protection of upper and lower respiratory tracts is achieved by breaking contact between the two tracts during swallowing. I also described a novel structure extending from the soft palate, the oral plug, that passively protects the rorqual pharynx during feeding (Chapter 3).

The third question was addressed through morphological examination and mechanical tests of the fin whale esophagus, which showed that the small lumen and thick muscular walls of the esophagus allow it to withstand the forces associated with swallowing by a pharyngeal pump mechanism (Chapter 4). The adaptations described in this thesis enhance feeding abilities in rorquals while simultaneously providing respiratory tract protection.

5.2 Chapter specific conclusions

5.2.1 Protecting the respiratory tract from water entry and barotrauma through movements of the novel nasal plugs in rorquals

Protection of the respiratory tract involves both preventing water incursion and avoiding barotrauma. Closing the blowholes to prevent water entry seals the upper airways, enclosing a volume of air in the rigid upper airways below the nasal plugs and presenting the risk of barotrauma. The nasal plugs are a novel structure utilized for both preventing water entry and avoiding barotrauma. While previously described simply as muscles (Buono et al., 2015; Maust-Mohl et al., 2019), I demonstrated that the nasal plugs are composed of muscle, adipose tissue, and elastin (Fig. 2.8), and that the regional heterogenous composition of these plugs is necessary for their function. When breathing, the muscular portion of the plugs in the rostrum contracts, pulling the adipose dense region from its position of occluding the nasal cavities. The elastin
dense tether that attaches the plugs halfway down the nasal cavities stretches to accommodate this movement. The plugs are displaced from their protective position, narrowed, and the respiratory tract is cleared for breathing. After inhalation, the muscular region of the nasal plugs relax, the elastin region pulls the plugs back into the nasal cavities through elastic recoil, and the adipose region settles into position occluding the upper half of the nasal cavities. The passive self-sealing aspect of the nasal plugs renders rorquals unable to drown, completely opposite to the terrestrial mammalian condition. Prior to this study, the composition of the nasal plugs was not established, how they are cleared from the nasal cavities was unknown, and the full extent of their protective mechanism that relies on malleable adipose tissue was not understood.

The regional heterogeneous composition of the plugs is also important in preventing a pressure differential from forming between the rigid nasal cavities and ambient ocean pressure as hydrostatic pressures increase with depth during a dive (Fig. 2.9). The nasal plugs are the only compliant tissue in the upper airways, thus, the only tissue that can shift in response to the formation of a pressure differential. When feeding, the upper and lower airways are disconnected for protection during food transfer through the pharynx, separating upper and lower airways (Fig. 3.9). As a pressure differential forms in the rigid upper airways, ambient ocean pressure pushes on the muscular region of the plugs perpendicular to the surface, forcing the compliant adipose region further into the nasal cavities, reducing the volume of the nasal cavities, and equilibrating pressure between ambient and the nasal cavities. While the issue of barotrauma in the lower respiratory tract has been raised many times, no study prior to this has assessed the risk of barotrauma in the upper respiratory tract. This study has both raised that issue and proposed a mechanism used in rorquals to avoid barotrauma.
5.2.2  Protecting the respiratory tract from food entry during swallowing in rorquals

Terrestrial mammals and rorquals are vastly different, yet the position of the pharyngeal structures conferring protection during swallowing in rorquals is remarkably similar to the human pattern, as I demonstrated through an assessment of morphology and physical manipulation of structures. The lower respiratory tract of rorquals is protected through closure of the larynx, achieved by bringing together the arytenoids and epiglottis to seal the laryngeal inlet (Fig. 3.9). The caudal portion of the arytenoids are lifted towards the roof of the larynx, bringing the laryngeal sac into the body of the laryngotraheal junction, completely occluding the larynx and rostral trachea. The top of the lower airway is completely plugged, preventing any chance of food incursion. The upper airways are also protected through complete obstruction by actions of the soft palate – actions which are also a necessity to allow food into the pharynx. The boundary between oral cavity and pharynx is blocked by the oral plug, a novel structure in rorquals that confers passive protection of the pharynx during a lunge, when larges forces are imposed upon the oral cavity. The oral plug blocks the oropharyngeal channel and must be displaced from this position during swallowing to allow food to travel from mouth to pharynx. The oral plug is an extension of the soft palate, so when the soft palate is lifted to block the nasopharynx during swallowing the oral plug is shifted with it, clearing the oropharyngeal channel for food to pass through, and simultaneously occluding the entrance to the upper airways. Entirely plugging the upper and lower airways prevents the rorqual’s tiny still-living prey from entering the respiratory tract. Rorquals have maximized feeding ability by dedicating the entire pharyngeal space to the digestive tract during swallowing, in contrast to odontocetes who possess a permanently intranarial larynx and have thus permanently dedicated a portion of the pharynx to the respiratory tract.
5.2.3 Transporting food from mouth to stomach during a time-limited foraging dive

Rorquals may be the only mammals that engulf a volume of food that can be too large to swallow as a single bolus. The adaptations used during prey collection are matched with a pharyngeal pump mechanism that allows to rorquals do exactly this – rapidly swallow a mouthful of food all at once. My morphological examinations and mechanical tests of the fin whale esophagus combined with fin whale feeding data allowed me to create a simple mathematical model for swallowing in rorquals. The rorqual esophagus has a small outer width, thick muscular walls, and a small lumen width that is smaller than expected for an animal so large. These characteristics of the esophagus allow it to withstand pressures associated with swallowing. The changes in muscular composition and abundance, and changes in adipose abundance along the esophagus indicate the first 2/3rds of the esophagus is the main force generating or propulsive region as well as the pressure or inflation resisting region. Through mechanical tests I established the capacity of the fin whale esophagus and the extent to which it can be stretched uniaxially, allowing me to determine the biologically relevant portion of the mechanical response and the maximum volume that could be transferred during a single swallow.

These data, when combined with the morphology of the pharynx, demonstrated that the pharynx and anterior esophagus are the capable of producing and withstanding large forces. The pharynx is filled with food from the oral cavity, and contraction of the pharyngeal musculature pushes the food into the esophagus in a single stream down to the stomach. A peristaltic wave follows this to clear the esophagus, similar to the process of deglutitive inhibition. The upper and lower airways are protected through tissue occlusion, therefore establishing the pharyngeal space for use by the digestive tract. Swallowing individual boluses of food does not match the timing
of the interlunge; it would take more time than exists in the interlunge itself to swallow multiple boluses. A system of swallowing involving a pump mechanism with a final wave of peristalsis to clear the esophagus provides a rapid way to transport food that fits into the short interlunge interval.

5.3 Summary

The research presented in this thesis provides a novel view of the anatomy and mechanics of the aerodigestive tract of rorqual whales, and the novel structures and mechanisms that are responsible for rorquals becoming the largest animals on the planet. Studying the internal anatomy of rorqual whales is notoriously challenging as a direct result of these adaptations! The opportunity to work on fresh tissue at a facility capable of manipulating these large animals was invaluable for this thesis, and the scope of research would not have been possible otherwise. Despite being well adored charismatic megafauna, there remain so many unknowns about cetaceans, especially regarding their internal anatomy, a fact that was obvious to me upon examining numerous unique structures and mechanisms that rorquals rely on for feeding or breathing alone. Contextualizing the work in this thesis has been challenging at times, as similar mechanical studies don’t exist, anatomical studies generally rely on tissue from necropsies which is usually much less informative, and some aspects of the functional morphology of rorquals that I have considered in this thesis have not been examined prior to this thesis.

Rorquals require immense amounts of energy daily (Croll et al., 2005), a feat that is manageable through numerous adaptations of the feeding apparatus and digestive tract. Immense amounts of food can be captured in a single gulp, and after filtering water from the mouth, the food can be rapidly transported to the stomach, which is important for animals that feed on a breath-hold and are time-limited on their dives. Gaining energy is important, but so is saving it.
Rorquals have established both behavioural mechanisms - like a short interlunge interval, or not lunging unless food densities are high enough, and anatomical mechanisms – like the default passive protection conferred by the nasal plugs, the oral plug, and the laryngeal sac at depth, to save energy.

The crossover of respiratory and digestive tracts in the pharynx necessitates cooperation between the tracts, and a series of active and passive mechanisms facilitate this. Breathing only happens periodically, so the upper and lower airways only need to be connected during the brief surface interval when breathing occurs. This frees up the pharyngeal space for use by the digestive tract, which is a necessity when swallowing large volumes of food in a short time. During a foraging dive, the rigid upper airways are safe from barotrauma, owing to the nasal plugs. The cartilaginous larynx and trachea are also safe from barotrauma, as well as food incursion, owing to the laryngeal sac. The actions of pharyngeal and surrounding structures are incredibly well-balanced to optimize the pharyngeal space for its use as part of the respiratory tract and the digestive tract.
References


