A Comparative Analysis of Marine Mammal Tracheas

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Summary

In 1940, Scholander suggested that stiffened upper airways remained open and received air from highly compressible alveoli during marine mammal diving. There are little data available on the structural and functional adaptations of the marine mammal respiratory system. The aim of this research was to investigate the anatomical (gross) and structural (compliance) characteristics of excised marine mammal tracheas. Here we defined different types of tracheal structures, categorizing pinniped tracheas by varying degrees of continuity of cartilage (categories 1-4) and cetacean tracheas by varying compliance values (categories 5*A* and 5*B*). Some tracheas fell into more than one category, along their length, for example, the harbor seal (*Phoca vitulina*) demonstrated complete rings cranially, and as the trachea progressed caudally tracheal rings changed morphology. Dolphins and porpoises had less stiff, more compliant spiraling rings while beaked whales had very stiff, less compliant spiraling rings. The pressure-volume (P-V) relationships of isolated tracheas from different species were measured to assess structural differences between species. These findings lend evidence for pressure-induced collapse and re-inflation of lungs, perhaps influencing variability in dive depth or ventilation rates of the species investigated.

Keywords

Diving, Lung collapse, Pressure-volume, Compliance, Diving physiology, Alveolar compression

1. Introduction

The unique distribution of cartilage in the trachea and bronchi of marine mammals was noted by Scholander (1940), detailing that the extent and length of cartilage varied between species. Scholander noted that dolphins, porpoises, and sea lions (*Zalophus californianus*) all have cartilage extending far to the alveolar sac, thus supporting the hypothesis that the cartilaginous trachea may play a role in alveolar compression and collapse (Kooyman and Sinnett, 1979), acting as either rigid reinforced space for respiratory air or compliant collapsible tissue during compression. Scholander (1940) suggested that the anatomy of the respiratory system of marine mammals would allow alveolar collapse and cessation of gas exchange upon deep dives. The model proposed by Scholander suggested compression of the respiratory system would force air from the alveoli and alveolar ducts into the rigid, cartilaginous bronchi and trachea. Depending on the mechanism of alveolar compression, this may reduce the ventilated alveolar surface area and increase the thickness of the alveolar membrane, thereby reducing the gas diffusion rate (Bostrom et al., 2008). This would eventually result in atelectasis, or alveolar collapse (commonly referred to as lung collapse), as all alveolar air is pushed into the upper airways (bronchi and trachea), thus terminating gas exchange. Upon ascent, the alveoli are reinflated, an apparently effortless action for marine mammals, yet problematic in humans (Fahlman, 2008).

Tracheal rigidity facilitates the rapid and more complete emptying of the lungs as compared with terrestrial mammals (Denison et al., 1971; Bostrom et al., 2008). The modified trachea also allows for high ventilation rates during the surface interval, and tidal volumes that are close to the vital capacity, resulting in efficient gas exchange and faster replenishment of O_2 stores and removal of CO_2 at the surface (Scholander, 1940; Olsen et al., 1969; Kooyman and Sinnett, 1979; Kooyman and Cornell, 1981).

Recent theoretical models suggest that, in addition to the relative volume between the upper and lower respiratory system, the compliance of the trachea is important in determining the lung collapse depth (Bostrom et al., 2008) and subsequent levels of gas exchange at pressure (Fahlman et al., 2009). Lung diffusion measurements in harbor seals and California sea lions concurred that the diffusion rate is directly related to the diving lung volume and the ambient pressure (Kooyman and Sinnett, 1982). Given the predicted lung volumes and diffusion rates, theoretical models (Bostrom et al., 2008) allow predictions as to how pressure affects the volume in the various compartments of the respiratory system and theoretically how pulmonary shunts might develop. However, these models are influenced by compliance estimates for the various portions of the respiratory system and little mechanical information currently exists for the upper airways of marine mammals (Sokolov et al., 1968; Cozzi et al., 2005; Bagnoli et al., 2011), making predictions for respiratory changes uncertain.

The relationship between pressure and volume gives an estimate of the compliance of the respiratory tract and has been successfully performed on excised lungs from terrestrial mammals (Bachofen et al., 1970), with few data documenting compliance of marine mammal lungs (Denison et al., 1971; Piscitelli et al., 2010; Fahlman et al., 2011). The compliance of the trachea has been suggested to affect the amount of air displaced from the lungs (Bostrom et al., 2008), and thereby the depth where the alveoli collapse and gas exchange ceases. The alveolar collapse depth and cessation of gas exchange should occur at a shallower depth for a mammal with a more rigid trachea (Bostrom et al., 2008). Our aim was to assess the airway compliance of several species of marine mammals as compared to gross morphological observations, encompassing both shallow and deep diving cetaceans and pinnipeds, in an attempt to link form and function and provide more detail on the role of the trachea during diving.

2. Material and methods

2.1 Animals

By-caught marine mammals were recovered in association with fishing gear from the Northeast Fisheries Observer Program (NEFOP). Animals were kept on ice when available and transported to the Woods Hole Oceanographic Institution (WHOI), Marine Research Facility, within 24 hours of landing. Animals were stored in a 4ºC chiller prior to necropsy. Stranded animals were collected by the International Fund for Animal Welfare (IFAW) staff and volunteers and transported to WHOI as soon as possible, and also stored at 4ºC prior to necropsy. The California sea lions in this study were collected by the Marine Mammal Center (Sausalito, CA) and the excised tracheas were shipped chilled to WHOI for compliance studies. Both the True's beaked whale (*Mesoplodon* *mirus*) and Gervais' beaked whale (*Mesoplodon europaeus*) were stranded in northern North Carolina, and transported to the North Carolina State Veterinary School in Raleigh. By-caught and stranded marine mammals used for compliance measurements were recovered at a decomposition code of 2. In addition, three species of terrestrial mammals were utilized in compliance and morphology data collection (dog, cattle and pig).

2.2 Morphometrics

Upon arrival, the sex was determined and each animal was weighed (± 0.2 kg) using a Tri-coastal, class III scale (model # LPC-4) (Table 1). Routine morphometric measurements were also completed. These included standard length, girth, flipper length, dorsal fin (dolphin and porpoise) or hind flipper length (seals). During the necropsy, the trachea was removed as detailed below.

2.3 Protocol

The entire respiratory system was excised (lungs, bronchi and trachea) from each carcass. The structural properties of the lungs were measured and have been discussed in a separate publication (Fahlman et al., 2011). The trachea was separated from the lungs and excess associated tissue was removed. Removal of associated tissue could affect compliance data, and that was not accounted for in this study. The trachea included all tissue caudal to the larynx and just cranial to the first bronchial bifurcation. Because the relative length of the odontocete trachea is much shorter than that of the pinniped and was found in association with an accessory bronchus, the conducting airway was excised closer to the secondary bronchial bifurcation (Fig. 1).

Tracheal gross morphology was determined by examination of the entire length of each trachea as well as cross sectional views at cranial, mid and caudal areas. Five tracheal categories were assigned to describe major differences in gross morphology (Figs 2-5; Table 2). Tracheal categories were determined by continuity of cartilage for pinnipeds and compliance values for cetaceans.

For pinnipeds specifically, if complete rings were present, a portion of the trachea was designated as category 1 (Fig. 2). If an overlay of cartilage, or "slip", was present in cross sections, where during compression one side of cartilage can easily slide over the other, reducing internal volume, the trachea was categorized as 2 (Fig. 3). When a "slip" was accompanied by a large gap in cartilage, it was defined as category as 3 (Fig. 4). The 4th tracheal category (Fig. 5) consisted of a "horseshoe" shaped tracheal ring: incomplete cartilage rings separated by one "gap". Tracheal category 5, consisted solely of cetacean tracheas, comprised of spiraling rings divided into two subcategories, *A* and *B* (Fig. 6; Table 2), because of similar gross morphology with only compliance differences (*A* was less stiff/complaint, and *B* was rigid).

To estimate compliance, excised tracheas were placed on a tray and intubated using a human (Hudson RCI, Teleflex Medical Inc.) or veterinary (Equine Nasotracheal Tube, Jorgensen Laboratories) endotracheal tube of suitable size. The endotracheal tube was attached to a system of 3-way valves with a volumetric syringe of suitable size (3-60 ml). Ends of the trachea were sealed shut with a Twixit clip (Linden Sweden, Inc.). The compression at the end of the trachea by the Twixit clip, as well as the addition of the endotracheal tube, affected the volume measurement slightly. This was accounted for during measurements of the floodable volume by assuming that both instruments are not collapsible, measuring additional volume and subtracting that volume from the end measurements.

The compliance of the trachea, or in some cases sections of the trachea, was estimated by determining the P-V relationship. The trans-luminal pressure difference ($\Delta P = P_{trach} - P_{amb}$, cmH_2O), the differential pressure between the inside of the trachea (P_{trach}) and the ambient pressure (P_{amb}), was measured using a differential pressure transducer (MPX type 339/2, Harvard Apparatus) connected to an amplifier (Tam-A, Harvard Apparatus) and the data collected on a laptop using an A/D card (USB 1208LS, Measurement Computing) sampling at 2 Hz. All compliance measurements were performed laying the trachea horizontally on a tray in air. The total volume injected or removed from the trachea to reach a ΔP of 50 cmH2O or -50 cmH2O, respectively, was divided in 4-5 equal increments. The trachea was then inflated or deflated with these volumes using a volumetric syringe. A minimum of three leak-free inflation/deflation curves was recorded for each sample. After an injection or removal of a bolus of air, the volume was held constant until the pressure stabilized before the next step-wise change in tracheal volume. The tracheal compliance appeared to change along the length of the trachea in some species, e.g. harbor seal. In those species, the tracheal compliance was separately measured at the upper and lower section of the trachea (Table 3).

The volume change (ΔV) was expressed as a fractional change from the internal (floodable) volume of the relaxed trachea (i.e. ΔP=0). For inflation and deflation experiments, ΔP was expressed as the pressure change between P_{amb} and P_{trach}. As a convention, ΔP and the volume used for inflation (ΔV) was expressed as a positive value while both ΔP and ΔV were negative during deflation trials. As the animal dives two forces will balance P_{amb}; 1) the pressure in the airways (e.g., P_{trach}) and 2) the structural properties of the trachea and alveolar space (Bostrom et al., 2008). Thus, changes in the internal volume of the respiratory system will depend on the balance between these pressures. In the current study, we investigated the structural properties by measuring the P-V relationship of the trachea. The transpulmonary pressures in the mammalian lung seldom exceed 30 cmH_2O (2.93 kPa). Thus, we only exposed the tracheas to transtracheal (ΔP) pressures up to about 4 kPa, as those pressures were within the physiological range, and much higher differential pressures would result in trauma (Brown and Butler, 2000).

The volume of the relaxed trachea (floodable volume) was measured by immersing the trachea horizontally in a water bath to avoid tissue expansion or change in length (Table 1). One end was sealed with a twixit clip, the trachea flooded with water, and the total volume of water measured. Floodable volume was measured in triplicate by weighing a filled volumetric flask.

2.4 Data processing and statistical analysis

The room temperature, and the ambient pressure were used to convert all volumes to standard temperature pressure dry (STPD). It was assumed that air used to inflate the excised trachea was completely saturated with water vapor.

The relationship between pressure and volume was determined using a Generalized Linear Model (GLM), with relative volume as the dependent variable, ΔP, body mass, and species as independent variables, and animal ID as a random variable (Littell et al., 1998). The slope of each P-V relationship represented the elastic properties of the trachea, with increasing slope representing a more compliant tissue. To compare compliance among species, the 95% confidence limit (CL) was estimated for the slope.

The standard error of the mean (SEM) for each parameter was used to determine the 95% CL and differences among species were assessed by determining which samples had overlapping CL's. Parameters were fitted using R (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, version 2.5.1, 2007).

In this study P-values ≤ 0.05 were considered significant. Data were presented as mean values ± standard deviation (SD) unless otherwise stated.

3. Results

3.1 Tracheal category types

We described five pinniped species, four cetacean species and three species of terrestrial mammals in total during this study (n=32, Table 1). All tracheas were fresh with the exception of one cetacean trachea (Gervais' beaked whale) that was fixed in formalin and thus was used for morphology descriptions only (not included in Table 1 or Fig. 7A,B). The fin whale (*Balaenoptera physalus*) was too large for compliance measurements and was also used for morphology descriptions only (not included in Table 1 or Fig.7A,B). Four distinct gross tracheal structures, or categories, were observed for the pinniped species and one for cetaceans (Table 2). As such, five tracheal structures were designated in marine mammals (category 1 through 5; Figs 2-6; Table 2).

3.2 Overview of P-V relationships

The P-V relationship was plotted for each species and the results are detailed in Fig. 7A,B. There was good reproducibility in the P-V relationship between tracheas for the California sea lion (data not shown). As the size of the trachea varied with body mass, the volume was expressed as a percentage of the floodable volume. In this study we compared the compliance during inflation and deflation for each species (Fig. 7A,B). Compliance is a numerical value for the slope, therefore the numerical value for the compliance of inflation and deflation of each trachea was considered here (Fig. 7A,B).

3.3 Form and function of pinnipeds

Harbor seal and gray seal

The harbor seal and gray seal (*Halichoerus grypus*) were grouped together morphologically, as they had similar external tracheal anatomy, where there appeared to be two distinct areas lengthwise of rigidity which determined the areas of measured compliance in this study (Fig. 8). Further histology would be needed to determine the type and amount of tissue present between rings lengthwise (versus cross sectional), thus potentially indicating differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue separating the cartilage rings (Fig. 8). However, the middle and caudal sections were "accordion"-like in structure, suggesting a lengthwise change in morphology and compliance, as the trachea progressed (Fig. 8; Table 3).

Specific to the harbor seal, three different cross sectional tracheal categories were revealed based on macroscopic differences in cartilage continuity (Figs 2-4, 8; Table 2), despite the two distinct regions (lengthwise) of differing rigidity confirmed by compliance measurements (Table 3). A cross section of the cranial portion revealed that the cartilaginous rings of the harbor seal trachea were complete throughout their circumference, thus forming a complete circle of cartilage with no connective tissue bridges (Fig. 2). We categorized this cranial section of complete rings as category 1 (Fig. 2). Mid sections of the harbor seal trachea were incomplete, forming a gap as well as a "slip" (Category 3; Fig. 4). Caudal cross-sections of the harbor seal trachea conformed to category 2 morphologically (Fig. 3).

As previously mentioned, the gray seal trachea also displayed (external lengthwise) a distinct cranial area of relative rigidity, becoming more "accordion"-like in structure moving towards the lungs (Fig. 8). Cross sectional examination revealed complete cartilage rings cranially with a distinct change in morphology as the trachea progressed towards the lungs, specifically demonstrated by a "slip" (Fig. 2,8). Thus, the gray seal began cranially as category 1 and changed morphologically to category 2 in mid and caudal regions (Fig. 2,8; Table 2).

For both the harbor seal and gray seal, the "slip" allowed for variation in volume over a ΔP in the physiological range (up to 4 kPa). At higher differential pressures, the trachea stiffened, specifically when deflated (data not shown). The compliance in the gray seal trachea varied with inflation and deflation and also demonstrated age related changes (Fig. 7A,B). Therefore, the gray seals were divided into three age groups (Hg1, Hg2 and Hg3). Interestingly, older gray seals (Hg1: Fig. 7A,B) demonstrated higher compliance values on both inflation and deflation, as compared to the younger gray seals (Hg2 and Hg3: Fig. 7A,B). One gray seal (DO7662) trachea was frozen and compliance measurements were taken pre and post-freeze. Compliance results were identical to the other gray seal in its age class (DO6322), indicating that freezing the tracheas prior to measurements appeared not to affect the validity of the data, although longer freezing intervals may affect the results.

Overall, both the harbor seal and gray seal demonstrated similar rigidity in tracheal compliance (Fig. 7A,B). In addition, both species also demonstrated a change in morphology as the trachea approached the lungs, both beginning cranially with a complete cartilage ring and developing a break in cartilage in more caudal regions (Fig. 8). Further compliance measurements in all three cross sectional areas of the harbor seal, as opposed to the two areas measured, may have revealed slightly different compliance measurements, however this could also have proved more difficult with smaller tracheal pieces to measure.

Harp seal

The cartilaginous rings of the harp seal (*Pagophilus groenlandica*) were discontinuous around the circumference of the trachea along the entire length of the trachea (Category 2; Table 2). During compression, cartilage sides were able to “slip” over each other reducing the circumference of the airway.

The harp seal showed no distinct variation in tracheal compliance along its entire length. The overlapping tracheal structure allowed expansion or compression as the connective tissue stretched, and alternatively as one side was able to “slip” over the other. During inflations, the connective tissue separating the ends of the incomplete cartilage circumference stretched (Fig. 7B). In other words, the harp seal trachea was comparatively very compliant when inflated. Similarly, the trachea was also very compliant during deflations, but at very low volumes, or at differential pressures > 4 kPa, it became rigid and non-compliant (data not shown, out of physiological range). During an inflation of the harp seal trachea, ΔP initially increased sharply but then gradually declined to a stable value within 10-15 sec. This was not seen in the gray seal, where the ΔP stabilized immediately following inflation. This was probably due to the elastic structure of the harp seal trachea that kept the discontinuous rings together. Thus, the excised trachea of the harp seal acted as an elastic buffer, dampening the pressure changes.

Northern elephant seal

Elephant seals (*Mirounga angustirostris*) were classified as category 4 (Table 2). The trachea was “horseshoe” shaped, where cartilage rings were incomplete but not overlapping. In all cases, this incomplete morphology continued the length of the trachea as it progressed caudally.

During both inflation and deflation experiments, the elephant seal tracheal compliance data values (Fig 7A,B) were between those of other pinniped species. In other words, compliance values for the elephant seal were neither the highest (harp seal) nor the lowest (harbor seal) among pinnipeds (Fig 7A,B).

California sea lion

California sea lions demonstrated a cylinder of overlapping discontinuous cartilage rings fused by connective tissue, progressing from the larynx to the first bronchial bifurcation (Fig. 9; Table 2). The sea lion tracheal morphology was classified as category 2, as discontinuous cartilaginous rings formed a “slip” where sides overlapped one another (Table 2).

During compression the lateral sides of the sea lion trachea overlapped throughout the tracheal length. The sea lion trachea exhibited tissue involution cranially which continued in the mid-section until the caudal regions where the trachea separated into two fused primary bronchi that traveled towards the lungs (Fig. 9). This was indicative of the thoracic inlet region in otariids. Both sea lion bronchi displayed overlaying cartilage, or “slip” features as well, allowing both tubes to compress separately. Thus, each bronchial tube was also considered to be in category 2, although not measured separately or as part of the true trachea.

The discontinuous cartilaginous rings of the sea lion allowed for medium compliance relative to other species in this study (Fig. 7A,B), perhaps due to the primary bifurcation being located more cranially. In other words, either the two bronchi were elongated or the true trachea was shortened affecting overall compliance (Fig. 7A,B). In addition, similar compliance values were found for both the inflation and deflation measurements (Fig. 7A,B).

3.4 Form and function of odontocetes

The trachea of the harbor porpoise (*Phocoena phocoena*) was conical and wide (Fig. 1), similar to other odontocetes investigated in this study. An accessory bronchus was observed cranial to the main tracheal bifurcation, and joined to the cranial section of the right lung in all odontocete species studied (Fig. 6) as well as one baleen whale, the fin whale. All cetacean species examined were classified as category 5 due to overall similarity in gross structure. Compliance values revealed two subcategories for cetaceans: ones with (*A*) compliant and (*B*) rigid/less complaint spiraling rings (Fig. 6; Table 2). Tracheal rigidity was observed in one beaked whale species: True's beaked whale (Category *5B;* Table 2), where as harbor porpoise and dolphin species demonstrated greater compliance, or were less rigid overall (Category *5A;* Fig. 6; Table 2). The Gervais' beaked whale trachea was not analyzed for compliance, just morphology, as it was in a fixed state of preservation (Fig. 6). Any microscopic discontinuities in the cartilage of cetacean tracheas were not distinguished here, and further histology would be needed to confirm any microscopic discontinuity suggested by the observed compliance differences between subcategories. This is a future aim for further study.

The common dolphin (*Delphinus delphis*) and white-sided dolphin (*Lagenorhynchus acutus*) tracheas were more compliant during deflation (Fig. 7A) than when inflated (Fig. 7B). The harbor porpoise demonstrated the opposite, being more compliant on inflation than deflation (Fig. 7A,B). The True's beaked whale had lower compliance values on inflation and deflation than any of the other odontocete species investigated (Fig. 7A,B).

3.5 Form and function of terrestrial animals

The dog (*Canis lupis familiaris*) trachea was determined to be category 4 as cartilaginous rings were incomplete and a gap was present between ends of the cartilage, forming a “horseshoe” (Table 2). The gap was present through the entire length of the trachea. Dogs, although not the closest land relative to pinnipeds, showed similar tracheal morphology to the elephant seal (Table 2). Compliance values for both inflation and deflation for the dogs did not vary much amongst themselves (Fig. 7A,B).

The pig (*Sus scrofa*) trachea was grouped in category 2 (Fig. 3; Table 2), as there were incomplete cartilaginous rings with distinct overlapping of cartilaginous sides, forming the characteristic “slip” feature seen in some pinniped species. Examined species sharing this category were distal portions of the harbor seal trachea and the entire length of the sea lion and harp seal tracheas. The pig trachea demonstrated the highest values of compliance on inflation, although deflation data were not measured (Fig. 7A,B).

The cattle (*Bos primigenius*) tracheas had incomplete rings with a common dorsal ridge unique to this species in this study. Thus, cattle were grouped in category 2 (Table 2), as it was the closest categorical group for this morphology. Compliance values for cattle indicated similar compliance values on inflation and deflation (Fig. 7A,B). The most compliant trachea for terrestrial mammals was the pig followed by similar compliance values for the dog and cows (Fig. 7A,B).

4. Discussion

In this study, we provided detailed descriptions and categorically classified the varied respiratory anatomy of some marine mammal species with different diving behaviors and lineages. Specifically, we described gross and structural properties of several pinniped and cetacean tracheas. Results suggested that there was great variety in tracheal morphology and physical properties among pinniped species. For example, tracheas were found to have complete, incomplete and/or changing cartilage rings throughout the trachea. However, less variation based on compliance data and morphology was demonstrated in the odontocete trachea, even given their equally diverse diving behavior. For example, all cetaceans demonstrated similar spiraling cartilage rings and the addition of an accessory bronchus. Although microscopic differences may exist, cetacean gross morphology was similar. This finding suggested that evolutionary pressures other than diving adaptation could be involved.

Our experimental approach had limitations, specifically regarding decomposition of the collected tissues and the longitudinal change in tracheal length during the compliance measurements that proved difficult to quantify. Thus, the tracheal compliance in live animals may prove to be different. We suggest that future experiments attempt *in situ* inflations and deflations in order to account for the dynamics of the thoracic wall, visceral mass, etc. Although we did not find inter-specific variation in tracheal rings apart from age-related changes, further histology and greater sample size may have indicated otherwise. Our study was different from the approach by Cozzi et al. (2005), where strain curves were used to assess the compliance of the trachea in the striped dolphin (*Stenella coeruleoalba*). Our approach allowed a simultaneous assessment of both compression and expansion of the trachea. Despite these limitations, there was good reproducibility between P-V curves from different individuals within the same species and our method proved to be useful for indicating functional differences in tracheal samples, especially when matched to gross anatomy. In addition, we categorized tracheas based on visual observation. Undiscovered microscopic differences in pinniped and cetacean tracheas may be revealed with future histology, which may help to improve category descriptions. Given the limited data pertaining to marine mammal respiratory behavior, this work can be considered an addition to the growing field of knowledge in respiratory adaptations for marine mammals.

4.1 Tracheal form and function

Previous studies have published accounts of the anatomical characteristics of the trachea in marine mammals (Scholander, 1940; Slijper, 1962; Sokolov et al., 1968; Kooyman and Sinnett, 1979; Bagnoli et al., 2001; Cozzi et al., 2005). In this study, we separated the various structural characteristics observed into five distinct categories depending on the continuity of cartilage throughout the circumference of the tracheal rings and compliance values, thus correlating form and function in a more complete descriptive manner. For all phocid seals in this study (harbor seal, gray seal, harp seal and elephant seal), the P-V curve matched well to observable morphology, and illustrated the efficiency of P-V calculations when describing morphology.

Pinnipeds – Phocidae

The harbor, gray, bearded (Sokolov et al., 1968) and ribbon (Sokolov et al., 1968) seals all demonstrated changing tracheal morphology as the trachea progressed towards the lungs (Table 2). However, other pinniped species (California sea lion, elephant and harp seal) had a consistent morphology the entire length of their trachea. One hypothesis for a changing morphology may be that “slips” present caudally result in a stiffer region cranially and more compliant region caudally. The caudal incomplete rings add compliance to an overall rigid trachea, thus allowing a small degree of deeper diving behavior.

Harbor and gray seals are often sympatric in their North Atlantic range and reports have indicated that average dive depths for both species are relatively shallow, however they sometimes perform deep dives (mean depth: 12-40 m and 48-51 m respectively) (Thompson and Fedak, 1993; Boness et al., 1994; Thompson et al., 1996; Lesage et al., 1999; Gjertz et al., 2001;Vincent et al., 2002; Moore et al., 2009, Table 4). If morphological traits limit physiology we would expect these species to express similar tracheal anatomy and compliance. The compliance indicated a rather stiff trachea during deflation (compression) in young animals of both species (Figure 7A,B). In the gray seal, the compliance increased with age. The trachea of the harp seal had even greater compliance as compared with the harbor and gray seal (Fig. 7A,B) and was classified in category 2 (Table 2). Although harp seals tend to maintain the majority of dives in shallow water, they are capable of dives to even greater depth as compared with the harbor and gray seals (Lydersen and Kovacs, 1993; Folkow et al., 2004, Table 4).

The tracheal morphology of the Northern elephant seal was “horseshoe” shaped throughout the length of the trachea (Table 2). The morphology was different as compared with other pinnipeds in the current study (Table 2), but similar to that of the bearded and ribbon seals (Sokolov et al., 1968), the dog and the human (Netter, 2011). Despite the morphological differences, the tracheal compliance was high in this deep diving species (LeBoeuf et al., 2000; Kuhn et al., 2009; Robinson et al., 2012, Table 4).

The relative lack of cartilage throughout the circumference of the trachea may have aided in the higher compliance, as compared with the harbor and gray seals. In addition, the greater thickness and prominence of connective tissue in the gap between cartilaginous rings as compared to the harp seal's thin connective tissue bridge, may explain its lower compliance values as compared to the harp seal (Fig. 7A,B).

If tracheal compliance has important consequences for alveolar collapse (Bostrom et al., 2008), the results in the phocids suggest that the alveolar collapse depth should occur at higher pressure in deep diving species. There are several possible explanations for this unexpected result. Theoretical models suggest access to the lung helps to lower the end-dive N_2 levels during deep dives, and thereby reduce the risk of bubbles to form (Fahlman et al., 2009; Hooker et al., 2010). Additionally, the compliance of the excised trachea may not accurately describe the compliance *in situ* in a live animal.

Pinnipeds- Otariidae

The sea lion trachea has been previously described by Sokolov et al. (1968) and was shown to remain consistent in length regardless of neck length, whereas the longer neck length was correlated with longer bronchial tubes (Sokolov et al., 1968). The sea lion trachea was equally compliant during inflation and deflation, and had properties that fell in between those of the phocids (Fig. 7A,B). The dive depth of the California sea lion is deeper than the harbor and gray seals but shallower than the elephant seal (Feldkamp et al., 1989; Weise et al., 2006, Table 4).

Odontocete tracheal categories

Gross anatomical features of all the cetacean tracheas were very similar. Tracheas maintained the same general shape and all cetacean tracheas also displayed an accessory bronchus leading to the upper right lobe of the lung (Fig. 6). The tracheas of all cetaceans sampled appeared to be composed of a relatively short, single cylinder of tightly compact, spiraling, irregularly shaped tracheal rings, which fuse with adjacent cranial and caudal rings at one or more locations. Although the two ends of each ring may not meet to form a complete ring, the firm fusion to adjacent rings forms a seemingly more robust structure than the generic gap or "slip" type of discontinuous tracheal rings seen in other mammals. Since all cetacean tracheas examined showed this type of morphology, a single category was used for cetaceans in this paper (Fig. 6; Table 2).

The P-V relationships were very different for the white-sided dolphin as compared with the other odontocetes, especially the True's beaked whale (Fig. 7A,B). Therefore, we divided the cetacean tracheas into two subcategories based on the compliance (*A* and *B*). Variation in compliance may be due to microscopic differences in tracheal morphology, specifically continuity, thickness and longitudinal area of cartilage. Any discontinuities in cartilage rings likely aided in ease of compression under pressure and may explain the differences in compliance values. However, histology is required to support that claim.

Beaked whales are known deep divers (Hooker and Baird, 1999; Baird et al., 2006; Tyack et al., 2006), yet have relatively rigid tracheas. The stiffer trachea would cause the alveoli to collapse at a shallower depth and reduce gas exchange during deeper dives (Bostrom et al., 2008). This was the opposite of what was seen in the pinniped tracheas, where deeper diving animals had a more compliant upper airway and shallow diving animals tended to have more rigid tracheas. However, marine mammals may also alter alveolar collapse by altering the respiratory volume during diving and dive with either a partially or completely filled lung (Scholander 1940; Kooyman et al., 1970; Hooker et al., 2005) or exhale during ascent (Hooker et al., 2009).

The role of an accessory bronchus in diving

All cetaceans examined in this study shared a common characteristic: an accessory bronchus. This anatomical feature is not found in the pinniped, but has been described in Artiodactyl species (Nickel et al., 1979). Pigs, although not the closest terrestrial relative, are related to cetaceans and similarly demonstrated an accessory bronchus. Its presence could be significant to lung function, as an additional bronchus may allow for altered ventilatory flow rates, and/or alterations in internal tracheal and lung pressures during inhalation and exhalation.

In humans, the presence of this additional bronchus is an anomaly and associated with respiratory disease (Manjunatha and Gupta, 2010). There is an increased prevalence of disease when the tracheal bronchus is associated with the right lobe (Barat and

Konrad, 1987). However, the accessory bronchus invests a distinct lung lobe in humans and pigs, yet cetaceans lack lung lobes (Berta et al., 2006), perhaps limiting the detrimental effects associated with the human clinical condition. Given the universal prevalence of the accessory bronchus in cetacean species, this anatomical feature may have an important function. For example, it has been shown that cetaceans have strong musculature, among them the scalenus, intercostals and rectus abdominus to power respiratory flow rates up to 160 l sec^{-1} (Kooyman and Cornell, 1981; Cotton et al., 2008). The accessory bronchus may aid the generation of these flow rates. In addition, cetaceans have cartilage extending down to the alveolar sac (Kooyman and Sinnett, 1979) as well as additional elastic fibers and musculature in the conducting airways (Denison et al., 1971; Kooyman and Sinnett, 1982) that allow extreme respiratory flow rates (Kooyman et al., 1975) while at the surface. Future imaging work to look at the airway dynamics may help elucidate if the accessory bronchus has an important function and if it acts to further enhance the respiratory adaptations of cetaceans. Finally, it is also possible that although this anatomical feature coincidentally aids in compression and recruitment of the alveoli, its presence arises as a conserved evolutionary trait.

In an evolutionary context, the smaller odontocete species (harbor porpoise, white-sided dolphin, and common dolphin) in this study shared the same basic gross tracheal structure as the fin whale, a baleen whale and of the mysticete lineage of cetacean species. Thus, the accessory bronchus likely was conserved in cetacean evolutionary history, and was maintained on both sides of the odontocete-mysticete evolutionary tree.

4.2 The trachea's role in lung function and as a diving adaptation

While Scholander (1940) suggested that the trachea of marine mammals was a rigid structure, his observations were mainly based on the gray seal and bottlenose whale (*Hyperoodon ampullatus*). While this appears to be valid for the gray seal and possibly the bottlenose whale (which is in the beaked whale family) we suggest that not all tracheas of marine mammals can be considered incompressible. That the trachea is in fact compressed during diving has been shown in live submerged Weddell (*Leptonychotes weddelli*) and elephant seals (Kooyman et al., 1970). We further propose that there may be great variation between species and that this variation may affect compression of the respiratory system, the depth of alveolar collapse, and gas dynamics during diving (Bostrom et al., 2008; Fahlman et al., 2009).

There are inconsistent data with regards to hypothetical modeled depth of alveolar collapse for marine mammals. Ridgway and Howard (1979) estimated collapse and termination of gas exchange at 70 m, whereas a more recent calculation, which accounted for the dive response, suggested that the alveolar collapse depth might occur at depths greater than 70 m (Fahlman et al., 2006). Pulmonary shunt measurements in the harbor seal and California sea lion infer that complete alveolar collapse may not occur until depths greater than 150 m even at diving lung volumes as low as ~20% of the total lung capacity (Kooyman and Sinnett, 1982). More recent studies, measuring changes in arterial partial pressure of O_2 during a dive, have suggested alveolar collapse for sea lions to be approximately 225 m (McDonald and Ponganis, 2012). It should be noted that the degree of pulmonary filling at the onset of a dive might vary considerably between pinnipeds and cetaceans; consequently, the depth of alveolar collapse may similarly vary considerably within and between species.

Recent studies suggest that the structural characteristics of the respiratory tract are important in determining the alveolar collapse depth in marine mammals (Bostrom et al., 2008). With all other variables equal, a more compliant trachea pushes the collapse depth deeper. The reason is that the upper respiratory system compresses concurrently with the lower sections of the respiratory system, reducing the available volume of alveolar air circulation (Bostrom et al., 2008). This may seem counterintuitive as an adaptive advantage, as a faster alveolar collapse could limit N_2 absorption, however there may also be occasions where gas exchange at a deeper depth could actually be beneficial. One such example being that a deeper depth of collapse has been modeled to reflect lower end dive N_2 levels (Fahlman et al. 2009). Regardless, the diving lung volume and the relative size of the conducting airways (trachea and bronchi) and alveolar space are important in determining the collapse depth, as well as behavioral adjustments while the animal is diving. Thus, anatomical differences in tracheal structure may correlate with life history and diving ability.

There were important differences in tracheal compliance between shallow diving species of pinniped (harbor and gray seal) versus deeper diving species (elephant seal), lending more evidence to the notion that although diving ability is not entirely dictated by tracheal morphology, anatomy may play an important role in lung function during a dive. As an animal dives, air from collapsing alveoli is pushed up into the trachea, but if the trachea is also compliant, like the alveoli, and they both compress under pressure, this will alter the alveolar collapse depth (Bostrom et al., 2008). For example, the tracheal compliance for compression was significantly higher in the deeper diving elephant seal as compared with the comparatively shallow diving gray and harbor seals. This suggests that the alveolar collapse depth may be deeper in the elephant seal. It is possible that this unexpected result represents tracheal compliance of juvenile animals, or that the *in vivo* compliance, when the trachea is surrounded by tissue, is different. However, theoretical results suggest that a deeper alveolar collapse depth during deep dives reduces end-dive venous N_2 tension (P_{N_2}) and thereby the risk for bubbles to form during ascent (Fahlman et al., 2009).

Caution should be taken when correlating dive ability to tracheal morphology, as dive data may show great variability among individuals and may be dependent on particular range, animal behavior, size and age. Simple morphological comparisons between shallow diving species of cetacean and deep diving cetaceans (beaked whales) may not be appropriate, as they have demonstrated vastly different dive behavior. In addition, other anatomical features of the trachea may also affect compliance. For example, connective tissue or the endotracheal venous plexus, which may fill and displace the intra-tracheal air volume (Cozzi et al., 2005; Costidis and Rommel, 2012). It would make sense that associated tissues as well as engorgement would affect compliance in measurements taken *in situ* rather than in excised tracheas. Furthermore, different tissue types may affect compliance values differently. This is a limitation of this study that could be further addressed in future work by repeating measurements *in situ* and carefully examining the associated tissues.

Our data suggested that in the pinniped model, deeper divers had a more compliant trachea, while the opposite was true for the cetacean. As far as gas exchange is concerned, this suggests different strategies for pinnipeds as compared with cetaceans.

Differences could also be linked to diverging behaviors such as exhalation versus inhalation before a dive (Snyder, 1983). It is possible that this reflects the need to limit N_2 absorption in deep-diving cetaceans diving on inspiration as compared with deep-diving pinnipeds that commonly exhale before diving (Snyder, 1983).

Unlike most terrestrial species, the marine mammal respiratory system and specifically its behavior under pressure, remains obscured from general knowledge (Bagnoli et al., 2011). Future work is aimed at expanding these data to encompass many more species of marine mammals, with specific concentration on histology. Results may lend more insight into lung mechanics during deep dives. In addition, a wider range of ages will likely reveal more detail pertaining to the degree of tracheal compliance as related to ontogeny of diving in these species. It is hopeful that further investigation into the compliance and microscopic anatomical characteristics of the respiratory tract of these divers will lead to a more comprehensive knowledge of their respiratory system.

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Figure Legends.

Fig. 1. Excised harbor porpoise (DO8803) trachea, cut cranially at larynx and caudally after first bifurcation.

Fig. 2. Category 1 tracheal model- complete cartilage rings at the cranial cut (arrow indicates cross sectional area taken) found in both gray and harbor seals.

Fig. 3. Category 2 tracheal model- slip feature present in cartilage ring at the caudal cut (arrow indicates cross sectional area taken).

Fig. 4. Category 3 tracheal model- slip feature and gap present in cartilage rings at the mid cut (arrow indicates cross sectional area taken).

Fig. 5. Category 4 tracheal model- a gap of connective tissue/musculature, i.e. “horseshoe” shaped cartilage rings.

Fig. 6. Category 5 cetacean tracheal model- blue arrow indicates location of the accessory bronchus found in all cetacean species examined (Odontocete and Mysticete).

Sub category *A* refers to the compliant/less rigid spiraling cartilage rings found in dolphin species and the harbor porpoise. Sub category *B* refers to the less compliant/rigid spiraling cartilage rings found in beaked whale species.

Fig. 7. A (Deflation) and **B** (Inflation): Elastic properties (compliance) of marine mammal tracheas. The compliance was determined as the slope of the relationship between pressure and volume by fitting the data with a GLM using individual animal as a random factor. Pressure was expressed as the absolute trans-mural pressure and volume as a percent change from the internal volume when the trans-mural pressure was 0 kPa. Numbers above the error bars indicate tracheal category as defined by Table 2. Species Pv = *Phoca vitulina*, Hg= *Halichoerus grypus,* Pg = *Pagophilus groenlandicus*, Ma= *Mirounga angustirostris*, , Zc=*Zalophus californianus*, Dd = *Delphinus delphis*, La = *Lagenorhynchus acutus*, Pp = *Phocoena phocoena,* Mm= *Mesoplodon mirus* Clf= *Canis lupis familiaris***,** Ss=*Sus scrofa*, Bp=*Bos primigenius*. Hg1 (adult) includes DO7662Hg and DO6322Hg, Hg2 (juvenile) is DO5257Hg and Hg3 (YOY) is H-0066Hg (Table 1). Hg represents an average of all gray seals analyzed. Bp1 is an adult cow, Bp2 a calf and Bp the average value.

Fig. 8. Lengthwise change in morphology of harbor seal and gray seal tracheas. Horizontal arrows indicate 3 different cross sectional morphologies (cranial, mid and caudal) in the harbor seal trachea and 2 in the gray seal trachea. Vertical arrows indicate two regions of differing rigidity lengthwise for both harbor and gray seals.

Fig. 9. California sea lion trachea and representative graphic. Arrows indicate location of trachea and elongated fused bronchial tubes.