ABSTRACT
In 1940, Scholander suggested that stiffened upper airways remained open and received air from highly compressible alveoli during marine mammal diving. There are few 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 5A and 5B). 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.

KEY WORDS: Diving, Lung collapse, Pressure–volume, Compliance, Diving physiology, Alveolar compression

INTRODUCTION
The unique distribution of cartilage in the trachea and bronchi of marine mammals was noted by Scholander (Scholander, 1940), who detailed that the extent, continuity and length of cartilage varied between species. Scholander (Scholander, 1940) noted that some marine mammals have cartilage extending far to the alveolar sac. The morphology supports the hypothesis that the cartilaginous trachea may play a role in alveolar compression and collapse (Kooymen and Sinnett, 1979), acting as a reinforced space for respiratory air expelled from the compliant alveolar space. Scholander (Scholander, 1940) suggested that the anatomy of the respiratory system of marine mammals would allow alveolar compression and collapse, resulting in cessation of gas exchange as pressure increases during dives. Depending on the mechanism of alveolar compression, this may reduce the alveolar respiratory 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 a significant clinical problem in humans (Fahlman, 2008).

Tracheal rigidity facilitates the rapid and more complete emptying of the lungs of marine mammals as compared to 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 O2 stores and removal of CO2 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 (Zalophus californianus) concurred that the diffusion rate is 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 how physiologic 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), but will also depend on the volume of inspired air at the beginning of a dive. 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.
RESULTS

Tracheal category types

We examined 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, *Mesoplodon europaeus*) that was fixed in formalin and thus was used for morphological descriptions only (not included in Table 1 or Fig. 1). The fin whale (*Balaenoptera physalus* [Linnaeus 1758]) was too large for compliance measurements and was also used for morphological descriptions only (not included in Table 1 or Fig. 1). 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 (categories 1–5; Figs 2–6; Table 2).

Overview of pressure–volume relationships

The compliance estimates were plotted for each species and the results are detailed in Fig. 1. There was good reproducibility in the pressure–volume (*P–V*) relationship between tracheas (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. 1). 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. 1).

Form and function of pinnipeds

Harbor seal and gray seal

The harbor seal (*Phoca vitulina*) 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. 7). Further histology would be needed.

### Table 1. Animal data

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Species</th>
<th>Group</th>
<th>M₀ (kg)</th>
<th>Age class</th>
<th>Trachea length (cm)</th>
<th>Trachea floodable volume (ml)</th>
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<tbody>
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<td>Ph</td>
<td>54</td>
<td>J</td>
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<tr>
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<td>A</td>
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<td>A</td>
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<td>Terr</td>
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<td>A</td>
<td>53.2</td>
<td>37</td>
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<td>Ss (pig)</td>
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<td>Terr</td>
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<td>–</td>
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<td>Bp 1 (cow)</td>
<td>Bp</td>
<td>Terr</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8.7</td>
</tr>
<tr>
<td>Bp 2 (cow)</td>
<td>Bp</td>
<td>Terr</td>
<td>–</td>
<td>J</td>
<td>–</td>
<td>70</td>
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</table>

Species: Pv, *Phoca vitulina* Linnaeus 1758; Hg, *Halichoerus grypus* (Fabricius 1791); Pg, *Pagophilus groenlandicus* Erxleben 1777; Ma, *Mirounga angustirostris* (Gill 1866); Zc, *Zalophus californianus* (Lesson 1828); Dd, *Delphinus delphis* Linnaeus 1758; La, *Lagenorhynchus acutus* (Gray 1828); Pp, *Phocoena phocoena* (Linnaeus 1758); Mm, *Mesoplodon mirus* True 1913; Clf, *Canis lupus familiaris* Linnaeus 1758; Ss, *Sus scrofa* Linnaeus 1758; Bp, *Bos primigenius* (Bojanus 1827).

Group: Ph, phocid; Ot, otariid; Od, odontocete; Terr, terrestrial mammals.

Age class: YOY, young of the year; J, juvenile; I, immature; A, adult.

M₀, body mass. Trachea length and floodable volume were used for compliance measurements.

*Tracheas frozen prior to compliance measurement.
to determine the type and amount of tissue present between rings lengthwise (versus cross-sectionally), 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 differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions. The cranial end to the middle section appeared to be a stiff, rigid area, with minimal tissue differing flexibility between the two regions.

As previously mentioned, the gray seal trachea also displayed (external lengthwise) a distinct cranial section of complete rings as category 1 (Fig. 7; 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). Midsections 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 section of relative rigidity, becoming more accordion-like in structure moving towards the lungs (Fig. 7). 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 (Figs 2, 7). Thus, the gray seal trachea began cranially as category 1 and changed morphologically to category 2 in middle and caudal regions (Figs 2, 7; 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. 1). Therefore, the gray seals were divided into three age groups (Hg1, Hg2 and Hg3). Interestingly, older gray seals (Hg1: Fig. 1) demonstrated higher compliance values on both inflation and deflation, as compared with younger gray seals (Hg2 and Hg3: Fig. 1). One gray seal (DO7662Hg) trachea was frozen and compliance measurements were taken pre- and post-freezing. Compliance results were identical to those of the other gray seal in its age class (DO6322), indicating that freezing the tracheae prior to measurements did not affect the validity of the data, although longer freezing intervals may affect the results.

Overall, the harbor seal and gray seal demonstrated similar rigidity in tracheal compliance (Fig. 1). In addition, both species also demonstrated a change in morphology as the trachea approached the lungs, beginning cranially with a complete cartilage ring and developing a break in cartilage in more caudal regions (Fig. 7). 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 groenlandicus*) 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. 1). 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

Table 2. Marine mammal tracheal categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Morphology</th>
<th>Description</th>
<th>Species</th>
<th>Location</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>Complete ring: stiff/less compliant</td>
<td>Harbor seal</td>
<td>Cranial</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gray seal</td>
<td>Cranial</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Walrus (Sokolov et al., 1968)</td>
<td>Entire tracheal length</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Bearded seal (Sokolov et al., 1968)</td>
<td>Cranial</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ribbon seal (Sokolov et al., 1968)</td>
<td>Cranial</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>‘Slip’ feature: less stiff/compliant</td>
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<td>Caudal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gray seal</td>
<td>Mid and caudal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Harp seal</td>
<td>Entire tracheal length</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>California sea lion</td>
<td>Entire tracheal length</td>
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<tr>
<td></td>
<td></td>
<td>Pig</td>
<td>Entire tracheal length</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>Cow</td>
<td>Entire tracheal length (with dorsal ridge)</td>
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<td>3</td>
<td>‘Slip’ and ‘gap’: less stiff/compliant</td>
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<td>Large ‘gap’: less stiff/compliant</td>
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<td></td>
<td></td>
<td>Human (Netter, 2011)</td>
<td>Entire tracheal length</td>
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<td>Northern elephant seal</td>
<td>Entire tracheal length</td>
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<tr>
<td></td>
<td></td>
<td>Bearded seal (Sokolov et al., 1968)</td>
<td>Mid and caudal sections</td>
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<tr>
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<td></td>
<td>Ribbon seal (Sokolov et al., 1968)</td>
<td>Mid and caudal sections</td>
<td></td>
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<td>5a</td>
<td>Spiraling rings: compliant</td>
<td>Harbor porpoise, white-sided dolphin, common dolphin</td>
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<tr>
<td>5b</td>
<td>Spiraling rings: rigid/less compliant</td>
<td>True’s and Gervais’ beaked whales</td>
<td>Entire tracheal length</td>
<td></td>
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</tbody>
</table>

Fig. 1 tracheal model. Complete cartilage rings were found at the cranial cut (arrow indicates cross-sectional area taken) in both gray and harbor seals.
During an inflation of the harp seal trachea, $\Delta P$ initially increased sharply but then gradually declined to a stable value within 10–15 s. This was not seen in the gray seal, where $\Delta P$ 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. 1) 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. 1).

**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. 8; 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. 8). This was indicative of the thoracic inlet region in otariids. Both sea lion bronchi displayed overlaying cartilage, or slip features as well, allowing the two 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. 1), 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. 1). In addition, similar compliance values were found for the inflation and deflation measurements (Fig. 1).

Form and function of odontocetes
The trachea of the harbor porpoise (*Phocoena phocoena*) was conical and wide (Fig. 9), similar to other odontocetes investigated in this study. An accessory right 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 because of their overall similarity in gross structure. Compliance values revealed two subcategories for cetaceans: (A) ones with compliant and (B) ones with rigid/less compliant spiraling rings (Fig. 6; Table 2). Tracheal rigidity was observed in one beaked whale species: True’s beaked whale (*Mesoplodon mirus*) (category 5B; Table 2), whereas 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

### Table 3. Compliance estimate along the length of the trachea in three different species

<table>
<thead>
<tr>
<th>Animal ID</th>
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<th>Inflation (% kPa⁻¹)</th>
<th>Deflation (% kPa⁻¹)</th>
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<td>Caudal</td>
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<td>6.3</td>
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<tr>
<td>DO8032</td>
<td><em>H. grypus</em></td>
<td>8.4</td>
<td>14.4</td>
</tr>
<tr>
<td>CSL10328</td>
<td><em>Z. californianus</em></td>
<td>1.9</td>
<td>6.8</td>
</tr>
</tbody>
</table>

The trachea was cut in the middle and the upper (from pharynx to the middle) and lower (from the middle to the bifurcation) portions tested separately.
during deflation (Fig. 1A) than when inflated (Fig. 1B). The harbor porpoise demonstrated the opposite, being more compliant on inflation than deflation (Fig. 1A,B). The True’s beaked whale had lower compliance values on inflation and deflation than any of the other odontocete species investigated (Fig. 1A,B).

**Form and function of terrestrial animals**

The dog (*Canis lupus 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 shape (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. 1).

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. 1).

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. 1). The most compliant trachea for terrestrial mammals was the pig followed by similar compliance values for the dog and cows (Fig. 1).

**DISCUSSION**

In this study, we have provided detailed descriptions and categorically classified the varied respiratory anatomy of some marine mammal species with different diving behaviors and lineages. Specifically, we have described gross and structural properties of several pinniped and cetacean tracheas. The results suggested that there is 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 an accessory bronchus. It should be noted that details such as number, distribution and pattern of fusion of spiraling cartilages...
were not assessed. 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. Additionally, the contribution of peritracheal and endotracheal tissue properties (e.g. engorgement of endotracheal venous plexus) was not investigated (Cozzi et al., 2005; Costidis and Rommel, 2012). 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. (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 a 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.

**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., 2011; 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 compliance matched well to observable morphology, and illustrated the efficiency of the structural properties (compliance) when describing morphology.

**Pinnipeds – Phocidae**

The harbor, gray, bearded (Erignathus barbatus) (Sokolov et al., 1968) and ribbon (Histriophoca fasciata) (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 a 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 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 (Fig. 1). In the gray seal, the compliance increased with age. The trachea of the harp seal had even greater compliance compared with that of the harbor and gray seal (Fig. 1) 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 from that of 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 trachea was compliant (Fig. 1) 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, compared with that of the harbor and gray seals. In addition, the greater thickness and prominence of connective tissue in the gap between cartilaginous rings, as compared with the harp seal’s thin connective tissue bridge, may

**Table 4. Approximate pinniped dive depths and citations from the literature**

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Max. depth</th>
<th>Mean depth</th>
<th>Depth range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vitulina</em></td>
<td>Harbor seal</td>
<td>122 m</td>
<td>Female: 12–40 m</td>
<td>45% below 4 m</td>
<td>Moore et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50% below 40 m</td>
<td>Boness et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lesage et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gjertz et al., 2001</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moore et al., 2009</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Beck et al., 2000</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Vincent et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thompson and Fedak, 1993</td>
</tr>
<tr>
<td><em>H. grypus</em></td>
<td>Gray seal</td>
<td>122 m</td>
<td>Post-molt: 48 m</td>
<td>80–100 m</td>
<td>Lydersen and Kovacs, 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-breeding: 51 m</td>
<td>&gt;100 m</td>
<td>Folkow et al., 2004</td>
</tr>
<tr>
<td><em>P. groenlandi</em></td>
<td>Harp seal</td>
<td>90 m</td>
<td>Female: 106 m</td>
<td>100–400 m</td>
<td>LeBoeuf et al., 2000</td>
</tr>
<tr>
<td><em>M. angustirostris</em></td>
<td>Elephant seal</td>
<td>1735 m</td>
<td>Female: 456 m</td>
<td>304.9–614 m</td>
<td>Kuhn et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male: 312 m</td>
<td></td>
<td>Robinson et al., 2012</td>
</tr>
<tr>
<td><em>Z. californi</em></td>
<td>Sea lion</td>
<td>274 m</td>
<td>33 m</td>
<td>80 m</td>
<td>Feldkamp et al., 1989</td>
</tr>
</tbody>
</table>

The Journal of Experimental Biology
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 their 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 is 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). The accessory bronchus also invests the right lung in cetaceans, although cetaceans lack lung lobes, which are distinct in humans (Berta et al., 2006). 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 1601s\(^{-1}\) (Kooyman and Cornell, 1981; Cotten 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 Simnett, 1979) as well as additional elastic fibers and musculature in the conducting airways (Denison et al., 1971; Kooyman and Simnett, 1982) that allow extreme respiratory flow rates while at the surface (Kooyman et al., 1975). Future imaging work looking at the airway dynamics may help elucidate the function of the accessory bronchus. If this anatomical feature aids in alveolar compression, its presence may have persisted 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 mysticete. Thus, the accessory bronchus is likely a conserved trait in cetacean evolutionary history, and has persisted on both sides of the odontocete–mysticete evolutionary tree.

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

While Scholander (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 the gray seal, and possibly the bottlenose whale (which is in the beaked whale family), have rigid upper airways, 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 weddellii) and elephant seals (Kooyman et al., 1970). We propose that there is great variation in the morphology and structural properties of the conducting airways between species. 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 (Ridgway and Howard, 1979) estimated alveolar 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 might occur at depths greater than 70 m (Fahlman et al., 2006). Pulmonary shunt measurements in the
harbor seal and California sea lion imply 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 O2 in diving sea lions suggested that the alveolar collapse depth could be as deep as 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, and also individuals.

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 alveolar air circulation volume (Bostrom et al., 2008). This may seem counterintuitive as an adaptive advantage, as a faster alveolar collapse could limit N2 absorption. However, there may also be occasions where gas exchange at a deeper depth during ascent could actually be beneficial, as this helps to remove the gas from the tissues (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 pinniped species (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 compressed under pressure, this will alter the alveolar collapse depth (Bostrom et al., 2008). For example, the tracheal compliance during deflation (compression) was significantly higher in the deeper diving elephant seal as compared with the comparatively shallow diving gray and harbor seals (Fig. 1). 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 N2 tension ($P_{N2}$) and thereby the risk of bubbles forming 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 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 diving behaviors such as exhalation versus inhalation before a dive (Snyder, 1983). It is possible that this reflects the need to limit N2 absorption in deep-diving cetaceans diving on inspiration as compared with deep-diving pinnipeds, which commonly exhale before diving (Snyder, 1983).

Unlike most terrestrial species, the respiratory physiology of marine mammals is poorly understood (Bagnoli et al., 2011). Future work is aimed at expanding these data to encompass many more species of marine mammals and include histological data to confirm the visual observations presented in the current study. In addition, a wider range of ages will likely reveal more detail pertaining to the degree of tracheal compliance as related to the ontogeny of diving in these species. Future results may lend more insight into lung mechanics during deep dives.

**MATERIALS AND METHODS**

**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 h of landing. Animals were stored in a 4°C 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 4°C prior to necropsy. The California sea lions in this study were collected by the Marine Mammal Center (Sausalito, CA, USA) and the excised tracheas were shipped chilled to WHOI for compliance studies. Both the True’s beaked whale and Gervais’ beaked whale 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).

**Morphometrics**

Upon arrival, sex was determined and each animal was weighed (±0.2 kg) using a Tri-coastal, class III scale (model no. LPC-4, Snohomish, WA, USA) (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.

**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 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. 9).

Tracheal gross morphology was determined by examination of the entire length of each trachea as well as cross-sectional views at cranial, middle 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 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 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/compliant, and B was rigid).

To estimate compliance, excised tracheas were placed on a tray and intubated using a human (Hudson RCI, Teleflex Medical Inc., Research Triangle Park, NC, USA) or veterinary (Equine Nasotracheal Tube, Jorgensen Laboratories, Loveland, CO, USA) 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). The ends of the trachea were sealed shut with a Twixit clip (Linden Sweden, Inc., Edina, MN, USA). 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 the two 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 (ΔP=Ptuch–Pamb cmH2O), the differential pressure between the inside of the trachea (Ptuch) and the ambient pressure (Pamb), was measured using a differential pressure transducer (MPX type 339/2, Jorgensen Laboratories, Loveland, CO, USA) connected to an amplifier (Tamb, A, Harvard Apparatus) and the data were collected on a laptop using an A/D card (USB 1208LS, Measurement Computing, Norton, MA, USA) sampling at 2 Hz. Compliance measurements were performed through laying the trachea horizontally on a tray in room air. The total air volume injected or removed from the trachea to reach a ΔP of 50 cmH2O or ~50 cmH2O, respectively, was divided into four to five 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 were 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 sections 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. ΔV=0). For inflation and deflation experiments, ΔP was expressed as the pressure change between Pamb and Ptuch. As a convention, ΔP and the volume used for inflation (ΔV) were expressed as positive values while both ΔP and ΔV were negative during deflation trials. As the animal divers, two forces will balance Pamb: (1) the pressure in the airways (e.g. Pamb) 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 (compliance) of the trachea. The trans-pulmonary pressures in the mammalian lung seldom exceed 30 cmH2O (2.93 kPa). Thus, we only exposed the tracheas to trans-tracheal (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 twixt 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.


