

RESEARCH ARTICLE

Structure, material characteristics and function of the upper respiratory tract of the pygmy sperm whale

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SUMMARY

Cetaceans are neckless, so the trachea is very short. The upper respiratory tract is separate from the mouth and pharynx, and the dorsal blowhole connects, *via* the vestibular and nasopalatine cavities, directly to the larynx. Toothed cetaceans (Odontoceti) are capable of producing sounds at depth, either for locating prey or for communication. It has been suggested that during dives, air from the lungs and upper respiratory tract can be moved to the vestibular and nasal cavities to permit sound generation to continue when air volume within these cavities decreases as ambient pressure rises. The pygmy sperm whale, *Kogia breviceps*, is a deep diver (500–1000 m) that is known to produce hunting clicks. Our study of an immature female shows that the upper respiratory tract is highly asymmetrical: the trachea and bronchi are extremely compressible, whereas the larynx is much more rigid. Laryngeal and tracheal volumes were established. Calculations based on Boyle's Law imply that all air from the lungs and bronchi would be transferred to the larynx and trachea by a depth of 270 m and that the larynx itself could not accommodate all respiratory air mass at a depth of 1000 m. This suggests that no respiratory air would be available for vocalisation. However, the bronchi, trachea and part of the larynx have a thick vascular lining featuring large, thin-walled vessels. We propose that these vessels may become dilated during dives to reduce the volume of the upper respiratory tract, permitting forward transfer of air through the larynx.

Key words: pygmy sperm whale, diving, Boyle's Law, trachea, larynx, vascular lining, cetaceans.

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INTRODUCTION

The respiratory systems of all air-breathing vertebrate lineages have been constrained by the nature of structures evolved primarily for a terrestrial existence. These consist of lungs of varying degrees of complexity, plus the upper respiratory system: the larynx and the trachea, together with bronchi that connect the trachea to the lungs (in some groups *via* a subdividing bronchial tree). Lungs have little resistance to compression, while the tracheae and bronchi of terrestrial reptiles, birds and mammals have a common structure designed to maintain a patent airway, yet provide flexibility, especially in the neck region. They are all composed of a series of circular, semi-rigid cartilaginous rings (complete in some groups, incomplete in others), interspersed with sections of thin and stretchable connective tissue (e.g. Romer and Parsons, 1977).

Diving air-breathing vertebrates encounter a number of problems that are exacerbated by increasing depth and duration of dives (Kooyman, 1989). First, they require adequate oxygen supplies for each dive, but all diving groups feature enhanced blood and tissue storage of oxygen, and generally have rather smaller lungs than do related groups of terrestrial vertebrates [but see Piscitelli et al. (Piscitelli et al., 2010) for a contrary view], though their lungs feature extremely high tidal volumes [$<90\%$ of lung volume (Wartzok, 2002)]. Second, the air spaces of the respiratory system become compressed and this decreases the buoyancy of the animal, increasing locomotor costs. Third, the possibility of highly compressed air being in close proximity to moving blood has the

potential to cause N_2 accumulation and decompression sickness (DCS). Scholander (Scholander, 1940) developed a simple 'balloon and pipe' model that addressed the DCS problem. He envisaged that the rib cage and lungs of a diving mammal would be compressed, and the latter would finally collapse at relatively shallow depths (30–50 m), displacing air into the relatively rigid (and poorly vascularised) tubes of the upper respiratory system, where little gaseous exchange would be possible and the threat of DCS would be eliminated [but see Costidis and Rommel (Costidis and Rommel, 2012), who demonstrate that bottlenose dolphins, *Tursiops truncatus*, can suffer from DCS in some circumstances]. In support of this concept, it was observed that the tracheae of diving mammals tended to have much wider tracheal rings, with far less connective tissue between them than terrestrial mammals, and that the rings were composed of more rigid materials, sometimes calcified (Tarasoff and Kooyman, 1973).

Subsequently this model has become extensively modified as understanding of the complexity of diving physiology has improved. Some deep divers that exceed depths of 1000 m (e.g. Weddell seals, *Leptonychotes weddellii*, and northern elephant seals, *Mirounga angustirostris*) exhale before they dive, apparently to minimise lung volume and nitrogen accumulation (Leboeuf et al., 1986; Ponganis et al., 1993; Sato et al., 2003). However, other deep divers such as sperm whales (*Physeter macrocephalus*) and leatherback turtles (*Dermochelys coriacea*) inhale before diving (Sato et al., 2002; Miller et al., 2004), presumably to maintain buoyancy and delay

alveolar collapse to greater depths. Evidence indicates that lung collapse and gaseous exchange can be delayed to depths of well over 100 m in some seals (Kooyman and Sinnott, 1982; Moore et al., 2011b) and in the leatherback turtle (Murphy et al., 2012). Whereas some shallow divers (e.g. marine otters and extinct mosasaurs) feature calcified tracheal rings that help to provide the rigid pipes envisaged by Scholander (Scholander, 1940), this appears not to be the case in deep divers such as Weddell seals and northern elephant seals. They show reduced tracheal volume when compressed in hyperbaric chambers (Kooyman et al., 1970), and it is now clear that this is caused by 'slipping' of overlapping cartilaginous rings (Moore et al., 2011a). Other researchers (Bostrom et al., 2008; Murphy et al., 2012) have demonstrated that a trachea with flexible rather than rigid walls causes increased depth of alveolar collapse and a gradual rather than abrupt collapse of the upper respiratory tract.

Cetaceans' upper respiratory tracts are additionally constrained by other factors. First, they are essentially neckless and consequently have much shortened tracheae (e.g. Romer and Parsons, 1977). Second, the upper respiratory tract is entirely separate from the mouth and pharynx. The blowhole connects, *via* the vestibular and nasopalatine cavities, directly to the larynx, which is held by circular muscles (palatopharyngeal sphincter) in the wall of the nasopalatine cavity. Studies of the structure of cetacean tracheae have been rare. A much-quoted study of the deepest of all air-breathing divers, the sperm whale, which can dive to depths exceeding 2000 m, suggested that the short, wide trachea of the sperm whale is rigid (Berzin, 1972). However, Berzin's concept of rigidity (actually incompressibility) is simply based on observations that the cartilaginous tracheal rings of the sperm whale are (a) complete, (b) overlapped to some extent and (c) have interdigitating tracheal ring processes that are partially fused to form a cartilaginous tube; there appear to have been no studies of the structure's mechanical properties. Recently, Cozzi et al. (Cozzi et al., 2005) and Bagnoli et al. (Bagnoli et al., 2011) have made observations on sections of the tracheae of the striped dolphin (*Stenella coeruleoalba*) and the bottlenose dolphin (*T. truncatus*) that indicated that their tracheae were compressible; both of these species are relatively shallow divers normally, but can reach depths of 200 m (*S. coeruleoalba*) and 390 m (*T. truncatus*).

Finally, odontocete (toothed) cetaceans employ ultrasound in communication and the detection of prey. The sound-producing apparatus is located dorsal to the skull between the anterior opening of the larynx and the blowhole, and consists of various nasal passages, sacs and vocal/phonic valves (e.g. Clarke, 2003; Cranford et al., 2008; Reidenberg and Laitman, 2008). Phonic valves that produce the sound are located between the nasal passage and the vestibular sac (Huggenberger et al., 2009; Prah et al., 2009). Both sides of the valves are connected to extensive soft-walled sacs and it is clear that sound could in principle be produced by air travelling in either direction. Hence, although the vestibular sac is also connected to the blowhole [guarded by nostril alae (Reidenberg and Laitman, 2007)], there is no requirement for air to be released to the environment when sounds are produced, though bubble emission appears to be correlated with whistle production in bottlenose dolphins (McCowan and Reiss, 1995). The larynx can be closed off anteriorly from this apparatus by action of the arytenoid cartilages, but if the sound-producing apparatus is to function at depth, it must contain adequate quantities of air (Clarke, 2003). Because air is compressed by 1 atm for every 10 m depth increment, which will reduce the volume of the soft-walled sacs of the sound-producing apparatus (by 90% at a depth of 1000 m), this suggests that air may need to be transported between the lungs and the vocal apparatus

during dives to support the air flows required to emit sounds of appropriate frequency and loudness (Clarke, 2003). Image analysis suggests that such transfer of air definitely takes place in the bottlenose dolphin (Houser et al., 2004).

The pygmy sperm whale, *Kogia breviceps* (Blaineville 1838) (Cetacea, Odontoceti, Kogiidae), is a dolphin-sized (adult length <3.5 m; mass 315–450 kg) relative of the far larger sperm whale [16.5 m males have a mass of approximately 41,000 kg (Berzin, 1972)]. Pygmy sperm whales have a worldwide distribution in temperate and tropical waters, but have been relatively little studied because they are of minimal commercial interest and live alone or in small groups, usually seaward of the continental shelf edge over depths of 400–1000 m. They are known to feed by suction feeding (Bloodworth and Marshall, 2007), predominantly on cephalopods, including bioluminescent species characteristic of midwater levels. Pygmy sperm whales are believed to be a deep-diving species [500–1000 m according to Clarke (Clarke, 2003)] that is particularly characteristic of productive upwelling areas (for a review, see Bloodworth and Odell, 2008).

Here we present novel information on the structure and function of the larynx, trachea and bronchi of an immature pygmy sperm whale. Though not large enough to be reproductively active, it was nevertheless well beyond the suckling stage and several years old (see Plön, 2004); it was also larger than the specimens studied by Clarke (Clarke, 2003). The investigation combined macroscopic and microscopic investigations with a study of the mechanical properties of the trachea.

MATERIALS AND METHODS

Collection and autopsy

The specimen studied was a 216-cm-long immature female beached (live) in southern Ireland at Dungarvon (52°03'59"N, 7°35'19"W) and transferred to cold storage shortly after death. The cetacean was in good condition and the stomach contained cephalopod beaks and crustacean parts, indicating relatively recent feeding. It was not feasible to measure its body mass as necropsy (including collection of pathological samples) was conducted as soon as possible. However, from length (L_b ; cm) and body mass (M_b ; kg) values for female pygmy sperm whales presented by Plön (Plön, 2004), we can derive the following relationship: $\log M_b = -1.78 + 1.75 \log L_b$ ($r^2 = 0.93$, $P = 0.001$). This yields a predicted body mass for the specimen of 202 kg. This indicates that it was approximately two-thirds of adult size, and therefore several years old.

Macroscopic anatomy

The upper respiratory tract was harvested during necropsy and gross dissection of the trachea was performed on the fresh material. Photographs were taken using a Casio Exilim 10.1 megapixel digital camera (Shibuyaku, Tokyo, Japan). The material was then frozen at -20°C for later investigation. The volume of the trachea was calculated from internal diameters and lengths; the volume of the larynx was established by filling it with water.

Subsequent treatment of material

The material was thawed several weeks later. Histological samples and the whole of the larynx were initially fixed in 10% formalin and then placed in storage solution (20% glycerine, 15% E + OH, 65% H_2O , 5–7 g thymol per 25 l). The remaining tracheal material (with attached bronchi) was refrozen (without fixation) at -20°C . The preserved larynx was subject to further gross dissection and photography. Later the trachea plus bronchi were thawed again and two tracheal rings (denoted T_1 , T_2) removed from the laryngeal end

of the trachea were fixed, transferred to storage solution and subjected to biomechanical analysis (see below). The rest of the material was refrozen. Because of concerns about the possible effects of fixation on tracheal elasticity, the results for T_1 and T_2 were discarded. The frozen material was again thawed and two further rings (denoted T_3 , T_4 and unpreserved) were subject to biomechanical analysis.

Microscopic anatomy

Samples of the trachea and bronchi were removed for histological examination. These were dehydrated, embedded in paraffin and then sectioned longitudinally and transversely at a thickness of $7\ \mu\text{m}$. Staining was with haematoxylin and eosin. Sections were examined using a Leica ICC50HD microscope (Leica Microsystems GmbH, Wetzlar, Germany) connected to a Dell workstation.

Biomechanical investigation

Tracheal rings T_3 and T_4 from the anterior (laryngeal) end of the trachea were subjected to classical engineering tensile and compressive material examinations in order to assess the behaviour of the rings under external loading. These rings were frozen (unpreserved) at -20°C following necropsy and thawed before testing in a refrigerator at 4°C . All tests were carried out at a room temperature of 16°C and the samples were kept hydrated throughout using isotonic saline solution. All material tests were performed with a Tinius Olsen Benchtop Tester H5KS-UTM (Tinius Olsen, Salford, Surrey, UK) using a 50 N load cell, with the applied displacement and corresponding load being recorded continuously during all testing cycles. Testing of the rings was in accordance with all standard biomechanical standards and procedures. The characteristic stress–strain relationships were evaluated for each material testing using Eqns 1 and 2, respectively, in the same manner as Cozzi et al. (Cozzi et al., 2005):

$$\sigma = \frac{F}{A}, \quad (1)$$

$$\varepsilon = \frac{\Delta L}{L_0}. \quad (2)$$

In these equations, σ and ε denote the stress and strain, respectively, in the material at a given applied displacement, F is the applied load, A is the area over which the load is applied ($A=2th$, where t

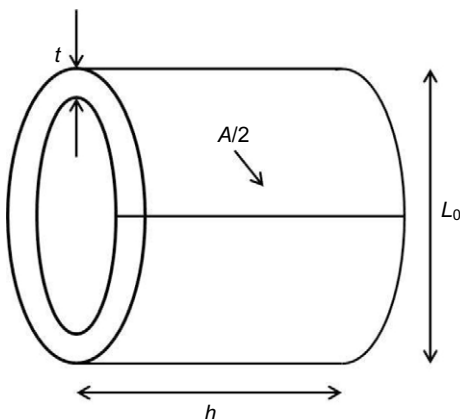


Fig. 1. Structure of the morphometric parameters of a cylindrical tracheal sample. A , area over which load is applied during testing; L_0 , original length in the direction of loading; t , wall thickness; h , height in the direction perpendicular to applied loading.

is wall thickness and h is height; Fig. 1), ΔL is the change in length of the material during the testing and L_0 is the original length of the material in the direction of loading.

Compressive testing

Non-destructive cyclical testing was performed on both tracheal rings by placing each ring between flat plates on the benchtop tester and applying a compressive displacement. Preconditioning of each ring was required in order to reduce the inherent viscoelastic properties that are common to most biological soft tissues (Fung, 1993). Both rings were subjected to 10 continuous loading and unloading cycles using a constant velocity of $3\ \text{mm}\ \text{min}^{-1}$.

Tensile testing

The same two tracheal rings were individually placed between two triangular thin wires, which were clamped in position at the machine grips. It has been shown previously that tracheal rings that experience both compression and tensile testing do not show statistical deviations from those rings that undergo only one series of testing (Cozzi et al., 2005). The tracheal rings T_3 and T_4 were subjected to non-destructive preconditioning cyclical testing using a speed of $3\ \text{mm}\ \text{min}^{-1}$, followed by a stretch to near-complete failure.

RESULTS

Anatomy

As in other toothed cetaceans, the pygmy whale larynx is elongate, while the trachea itself is short. Fig. 2A shows the structure of the

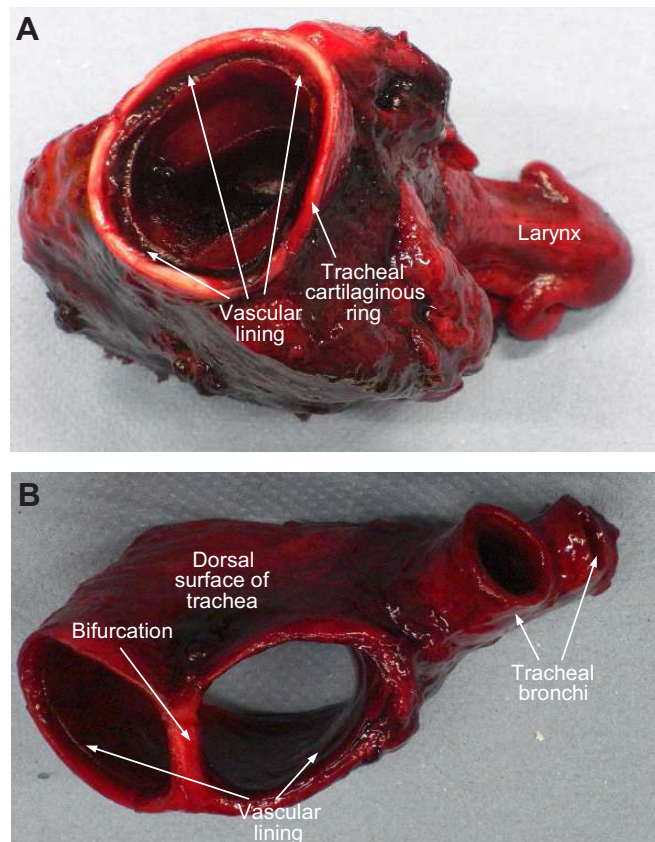


Fig. 2. (A) Cut end of *Kogia breviceps* trachea close to junction with larynx. The vascular lining is continuous with that of the larynx. (B) Posterior view of trachea with larynx and two main bronchi removed. The tracheal bronchi are on the right-hand side. The continuous vascular lining of the trachea is evident.



Fig. 3. Transverse section of *K. breviceps* trachea showing the lumen; the mucosa, which is comprised of the epithelium (ep) and lamina propria (LP); the sub-mucosa, which is highly vascularised (bv, blood vessel) and has an abundance of adipose tissue (adi); the perichondrium (pc); and the cartilage of a tracheal ring.

isolated trachea, approximately 60 mm in length. It is roughly ellipsoidal in cross-section (approximately 39×49 mm in internal diameter), though somewhat asymmetrical. The tracheal wall is dominated by thin (2.5–3.5 mm thick) cartilaginous rings lined with thick vascular tissue that has a smooth luminal surface. The arrangement of bronchi is asymmetrical, with two ‘extra’ small (tracheal) bronchi leading off the right-hand side of the trachea (Fig. 2B), anterior to the bifurcation of the trachea into two main bronchi.

The tracheal rings are irregular, overlapping and with interdigitating processes; there is little connective tissue between them. All four bronchi have a similar basic structure to the trachea, being composed of closely applied cartilaginous rings and with a vascular lining continuous with that of the trachea.

Fig. 3 shows a histological cross-section of the tracheal wall. This shows that the bulk of the wall is composed of cartilage, bounded by perichondrial connective tissue. However, underlying the luminal mucosa is a thick submucosa that contains large, thin-walled venous blood vessels, as well as lipid deposits.

Fig. 4 illustrates elements of the larynx. The posterior (tracheal) opening is approximately circular (38 mm diameter)

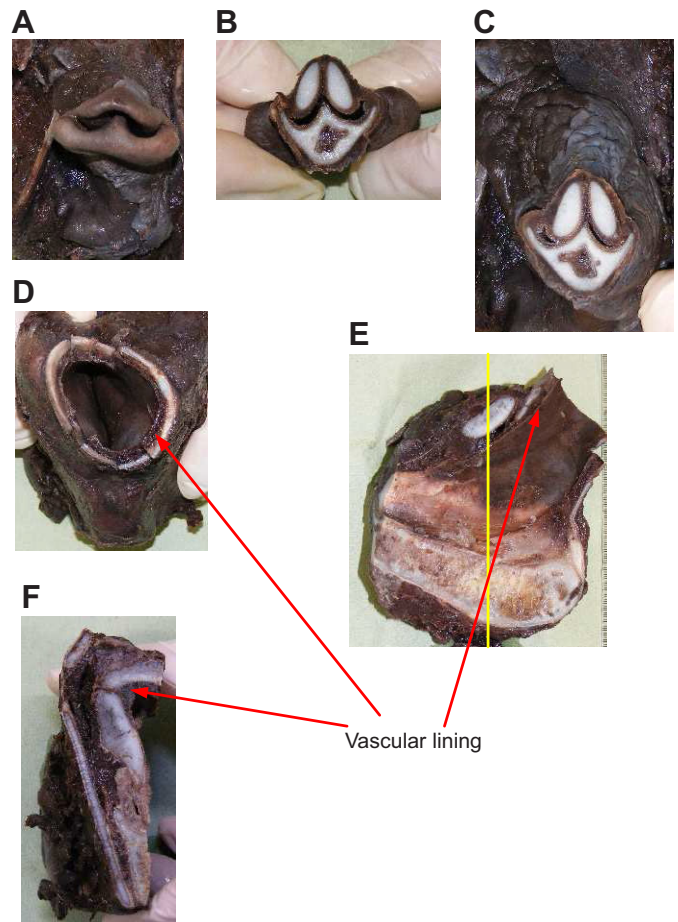


Fig. 4. Structure of pygmy sperm whale larynx. In all cases dorsal surfaces are uppermost. (A) Anterior view of larynx, showing opening bounded by arytenoid cartilages. (B) Cross-section of tubular portion of larynx; note minimal luminal volume. (C) Cross-section of tubular portion of larynx close to posterior end. (D) Posterior view of main cavity of larynx (trachea removed as close as possible to larynx). (E) Lateral view of main cavity of larynx (visualised by dividing larynx in midline). (F) Anterior view of right half of main cavity, sectioned at level corresponding to the yellow line shown in E.

and opens into a chamber (80 mm long) that has a flattened triangular pyramidal shape, tapering markedly anteriorly. This chamber is surrounded by 12-mm-thick cartilage (thyroid cartilage) laterally and 8- to 10.5-mm-thick cartilage dorsally (ventrally the two sides of the chamber meet; there is no separate ventral wall); the cartilage is stiff and apparently lightly calcified. The posterior and dorsal part of this chamber has a vascular lining, but the rest does not. Anteriorly, the chamber joins with a tubular section that is directed anteriorly and dorsally; when the arytenoid cartilages are closed its lumen is of negligible volume. During measurement of the laryngeal volume it was found that the tubular section was watertight unless the arytenoid cartilages were prised apart.

Fig. 5 illustrates the upper respiratory tract diagrammatically. The trachea had a volume of 118 ml, the larynx a volume of 45 ml. From Piscitelli et al. (Piscitelli et al., 2010) it may be calculated that a pygmy sperm whale of this size (202 kg) will have a lung plus bronchial tree volume of approximately 4200 ml, so the whole respiratory tract (excluding the vocal apparatus) would have a volume of 4363 ml.

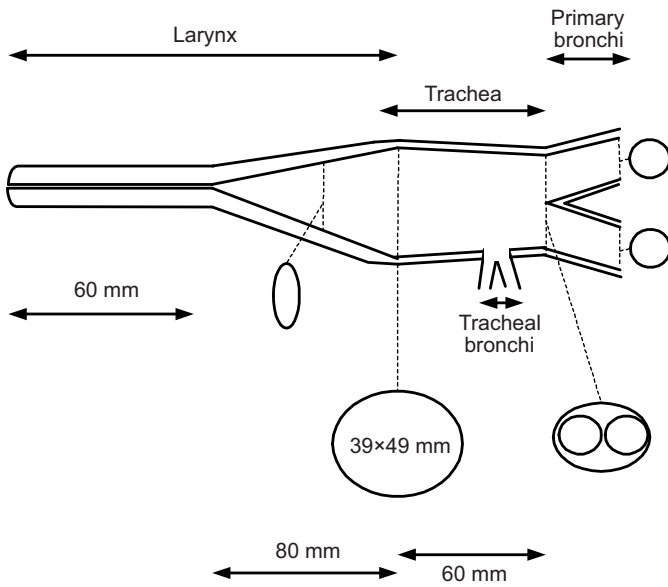


Fig. 5. Diagram of larynx and trachea of pygmy sperm whale (not to scale).

Biomechanical investigation

Simple palpation of the trachea and bronchii established that they were easily compressible; finger pressure alone could collapse their walls together (occlusion). Referring to Fig. 1, the first ring (T_3) was measured as having a mean (\pm s.d.) thickness (t) of 1.88 ± 0.42 mm, a mean height in the direction perpendicular to the applied loading (h) of 7.84 ± 1.32 mm and an original length in the direction of loading (L_0) of 33.0 mm. Corresponding measurements for the second ring, T_4 , were $t = 1.72 \pm 0.30$ mm, $h = 6.61 \pm 0.88$ mm and $L_0 = 29.0$ mm. The stress-strain relationships obtained for the two tracheal ring specimens, T_3 and T_4 , are shown in Fig. 6A and 6B, respectively. A similar result may be noted for all curves, with an increase in strain causing a

corresponding increase in stress. Slight discrepancies in stress values at a given strain were evident between the two samples, which are presumably reflective of anatomical and geometrical variations. The viscoelastic nature of the rings was also apparent from the classic hysteresis loops shown in Fig. 6A. The ‘shift’ between adjacent curvatures becomes less noticeable with the increasing number of preconditioning cycles. Estimates for the slope of the linear segment of the stress-strain relationship for each ring, E_c , were evaluated [see fig. 2 in Cozzi et al. (Cozzi et al., 2005)] and are stated in Table 1.

Fig. 6C,D conveys the stress-strain relationships obtained from the tensile examinations of T_3 and T_4 , respectively. The material ruptured at an ultimate stress of 0.51 MPa for T_4 . The stress-strain relationships evaluated are comparable with those reported in the compressive material testing and, as before, an increase in strain results in a related increase in stress. Fig. 6C again displays hysteresis, consistent with the material being viscoelastic. The slopes of both the linear portions, E_{t3} and E_{t4} , in each relationship were computed [see fig. 2 in Cozzi et al. (Cozzi et al., 2005)] and are detailed in Table 1.

DISCUSSION

This study is limited because of its reliance upon a single specimen that was immature. However, the pygmy sperm whale rarely strands (unlike some other cetaceans), so the prospect of other specimens becoming available for study was remote. The specimen studied was approximately two-thirds of adult size, so was several years old (see Plön, 2004). Pygmy sperm whales are often seen in small groups (Bloodworth and Odell, 2008), so it seems probable that such a large youngster would dive with adults to substantial depths (Clarke, 2003).

The asymmetry of the structure of the pygmy whale trachea and bronchi was expected as it has been reported from other cetacean species [e.g. the beluga, *Delphinapterus leucas* (Jackson, 1845), and the minke whale, *Balaenoptera acutorostrata* (Kida, 1998)]. A right ‘tracheal’ bronchus leading off the trachea anterior to its bifurcation into two primary bronchi is regarded as diagnostic of cetaceans as

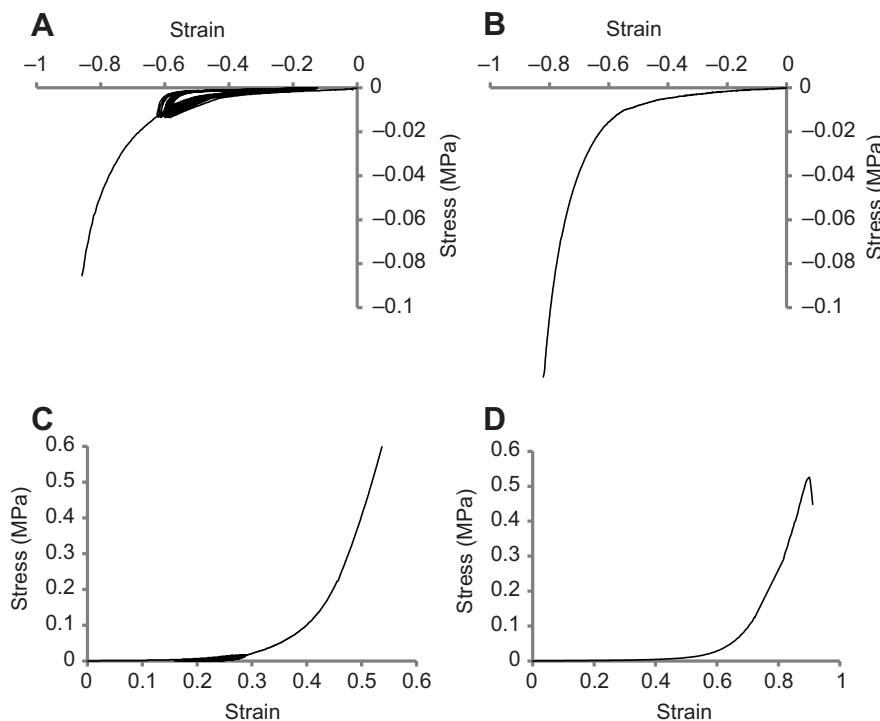


Fig. 6. Results of biomechanical testing of two tracheal rings (T_3 , T_4) of a pygmy sperm whale. (A) T_3 stress-strain relationship during compressive testing showing hysteresis during pre-conditioning. (B) T_4 stress-strain relationship during compressive testing and omitting hysteresis for clarity. (C) T_3 stress-strain relationship during tensile testing showing hysteresis during pre-conditioning. (D) T_4 stress-strain relationship during tensile testing to failure and omitting hysteresis for clarity.

Table 1. Characteristic parameter values of the stress–strain curves for pygmy sperm whale and striped dolphin

Trachea	S_{MAX} (MPa)	E_{I3} (MPa)	E_{I4} (MPa)	E_c (MPa)
Pygmy sperm whale				
Ring T ₃	N/A	0.028	0.158	0.007
Ring T ₄	0.51	0.007	0.364	0.007
Striped dolphin	2.44±0.771	0.065±0.058	13.389±4.230	0.044±0.019

Striped dolphin values are means ± s.d. (from Cozzi et al., 2005).

S_{MAX} , ultimate tensile stress; E_{I3} and E_{I4} , slopes of the stress–strain tensile curve in the linear segments; E_c , slope of the stress–strain compression curve in the linear segment.

well as the terrestrial Artiodactyla (even-toed ungulates: e.g. pigs, hippopotamuses, cattle), that are known from molecular evidence to be close relatives (Kida, 1998). In the related sperm whale *P. macrocephalus*, there is only a single tracheal bronchus [supplying the apical lobe of the right lung (Berzin, 1972)]. Kuo et al. (Kuo et al., 2002) prepared latex casts of the lungs and upper respiratory system of three stranded specimens of the even more closely related dwarf sperm whale (*Kogia sima*); they also reported a single tracheal bronchus. Clearly the immature specimen of pygmy sperm whale reported on here had two such tracheal bronchi, but whether this is a species characteristic or an anomaly of this particular specimen [cf. human tracheal abnormalities (Barat and Konrad, 1987)] is presently unknown. There appears to be no adaptive significance in this asymmetry; it is contingent on embryological history. The nature of the tracheal ring structure of the pygmy sperm whale is very similar to that described for the sperm whale by Berzin (Berzin, 1972). The overlapping and irregular rings form a tubular structure with no connective tissue gaps.

The posterodorsal part of the larynx, the whole of the trachea and the portions of bronchi harvested in this specimen of *K. breviceps* were all seen to be lined with thick vascular tissue, featuring multiple thin-walled vessels. Such a lining is not seen in terrestrial mammals, or in pinnipeds. A tracheal vascular lining was also identified in striped dolphins by Cozzi et al. (Cozzi et al., 2005), and interpreted by them as being an erectile tissue that helped to reinflate a collapsed trachea during the ascent phase of dives. Birds and terrestrial mammals control the humidity and temperature of the inspired air delivered to the lungs by the action of bony nasal turbinates and their associated vascular networks, which also regulate heat and water loss in expired air. Cetaceans do not have

turbinates (Chinsamy-Turan, 2011). Another aquatic endotherm that does not have turbinates is the leatherback turtle, which has a thick vascular laryngeal and tracheal lining. In leatherbacks, the vascular lining was interpreted as having a turbinate-like function (Davenport et al., 2009) to control inspired air temperature and humidity. Because the elastic nature of tracheal and bronchial cartilage reported here, combined with expanding air from the lungs, will ensure reinflation of the upper airways during ascents from dives, we believe that it is unnecessary to assign a reinflation function to the vascular lining as suggested by Cozzi et al. (Cozzi et al., 2005). It is possible that the lining has a thermoregulatory function in the pygmy sperm whale during inspiration and expiration, but the shortness of the vascular lining (stemming from the shortness of the trachea and bronchi) indicates that this is unlikely to be important, as the residence time of moving air within this part of the respiratory tract would be low, especially because the high tidal volume of cetaceans means that air velocities are extremely high (Wartzok, 2002). It is also the case that much heat exchange with (and humidification of) inspired air presumably takes place in the passages between the blowhole and the larynx.

The closed and semi-closed anatomy of the tracheal cartilaginous rings has the potential to provide the trachea of *K. breviceps* with increased stiffness and structural rigidity beyond that of the conventional horseshoe (C-shaped) arrangement of terrestrial vertebrate tracheae. However, it is evident from palpation and the measurements presented here that the trachea of *K. breviceps* is easily compressible – as are the bronchi, with characteristic E_c values comparable with those of the striped dolphin and terrestrial mammals. However, the tensile testing yielded much lower values for E_{I1} and E_{I2} for both rings tested than the published values for

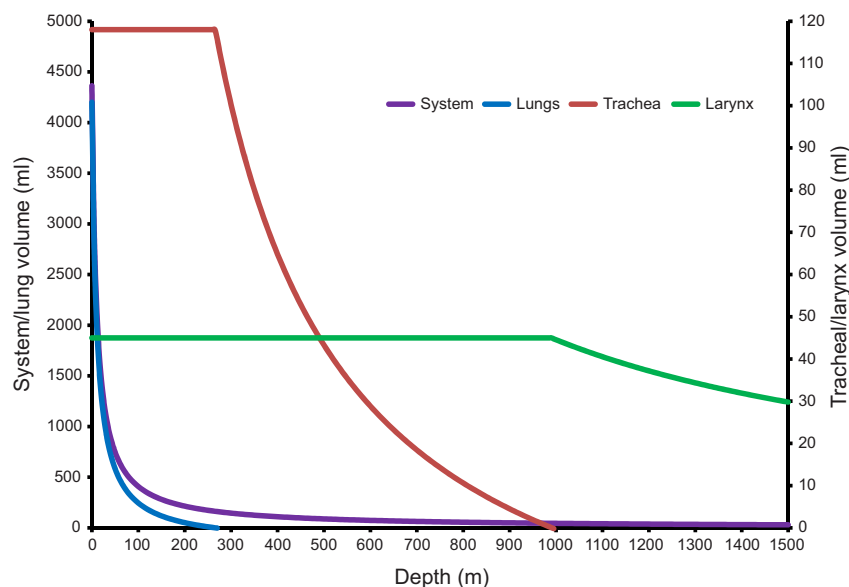


Fig. 7. Calculated effects of depth on air volume within elements of the respiratory system of the pygmy sperm whale.

S. coerulealba (Table 1). Although both rings were near complete (overlapping and near-fused cartilage), there were significant variations in the thickness and the height along the circumference of each ring. Eqns 1 and 2 form the fundamentals of structural analysis and are used in many engineering problems to obtain indicative values for stress and strain acting over a uniform area. However, the morphometric measurements of the tracheal rings of *K. breviceps* examined here were not consistent, therefore leading to the possibility of localised stress accumulation when loaded. This, consequently, could produce a lower ultimate stress value as the ring would be likely to rupture at these locations.

Though the biomechanical analysis confirms that the cartilaginous tracheal rings are composed of a viscoelastic material that has the potential to deliver a degree of rigidity (as well as permitting the repeated distortions in response to repeated dives), the rings have a geometry that promotes compressibility; they are effectively tubular (the two rings studied were ~30 mm long), but very thin-walled (~1.8 mm thick). To make an analogy, a broad, thin plank of wood constrained at both ends will take a considerable load without significant distortion if that loading is edge-on. In contrast, the same load applied to the broad face will cause the plank to bend easily; the same material characteristics, but with two different outcomes. The trachea and bronchi have a geometry that promotes repeated collapse; their elliptical rather than circular nature also favours this (see Murphy et al., 2012).

The trachea and bronchi are located in the anterior part of the thoracic cavity, so they are not constrained by attached tissues (i.e. they are not surrounded by solid tissue that might minimize/prevent changes in their shape or volume). In contrast, the larynx is more rigid and is embedded in supportive muscle tissue in the live animal. Murphy et al. (Murphy et al., 2012) have demonstrated that a trachea with a flexible wall (as in the leatherback turtle) can remain fully inflated until the lungs are completely collapsed – it does not need to be rigid to retain its shape. However, in their study they were able to treat the larynx–trachea–lungs as a closed system and make inferences about the depth of lung collapse and the depth at which compression of the trachea would be initiated. The situation in the pygmy sperm whale is different. Throughout a dive there is the possibility of transfer of air between the lungs and the vocal apparatus, *via* the trachea (see Clarke, 2003), though it is equally true (from the present study) that sustained closure of the arytenoid cartilages can provide a tight seal to prevent such transfer.

If we assume a closed system plus graded compressibility of the wall (lungs>bronchi>trachea>larynx), and the volumes of larynx, trachea, bronchi and lungs presented earlier, it is possible to calculate, using Boyle's Law (after Murphy et al., 2012), the depths of potential respiratory system collapse (see Fig. 7). Air in the lungs/bronchi would be expected to have left them completely at a depth of approximately 270 m. For the trachea, complete expulsion of air into the larynx would be expected at 995 m. These collapse depths are much shallower than those predicted for the leatherback turtle by Murphy et al. (Murphy et al., 2012), and stem largely from the relatively small lungs and trachea of the pygmy sperm whale. If we assume that the volume of the whole respiratory system from larynx to lungs at the sea surface will be 4363 ml, then this falls to 86 ml at a depth of 500 m and only 43 ml at 1000 m. The latter value implies that the respiratory air mass would not completely fill the larynx (volume 45 ml) at 1000 m, implying that the larynx itself would have to collapse to some extent at such a depth (see Fig. 7). These calculations strongly imply that significant transfer of air from the lungs to the vocal apparatus during deep dives would not be possible.

We believe, however, that there is an alternative scenario. Our macroscopic and microscopic investigations revealed that the pygmy sperm whale's trachea and bronchi have a thick vascular layer, with substantial numbers of thin-walled, venous vessels within it. This layer also continues into the upper posterior portion of the larynx, where it lines the largest part of the laryngeal chamber. If this layer becomes substantially thicker by virtue of distended vessels during dives, then it is feasible that air from the collapsing lungs is not stored in the bronchial tree, trachea and larynx, but is allowed to move anteriorly and dorsally into the vocal apparatus, thereby augmenting the air already present in that part of the respiratory tract and helping to provide sound production, as envisaged by Clarke (Clarke, 2003). The presence of a vascular lining should not pose significant DCS problems: the luminal surface area of the lining is relatively low, limiting gaseous exchange, while blood flow through the lining during dives is presumably limited.

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AUTHOR CONTRIBUTIONS

Dissections were carried out by J.D. and E.R. C.M. and D.K. carried out biomechanical analysis. L.C. conducted histological investigations. J.D. wrote the first draft with significant intellectual input from all authors, who also contributed to final revisions of the paper.

COMPETING INTERESTS

No competing interests declared.

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