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Unique characteristics of the trachea of the juvenile leatherback turtle facilitate feeding, diving and endothermy

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ABSTRACT

The adult leatherback turtle *Dermochelys coriacea* overlaps in body size (300–500 kg) with many marine mammals, yet develops from a 50 g hatchling. Adults can dive deeper than 1200 m and have core body temperatures of 25 °C; hatchlings are near-surface dwellers. Juvenile leatherbacks have rarely been studied; here we present anatomical information for the upper respiratory tract of 3 turtles (66.7–83.0 cm straight carapace length; 33.2–53.4 kg body mass) incidentally captured by long-line fisheries. Combined with existing information from adults and hatchlings, our data show that there is an ontogenic shift in tracheal structure, with cartilaginous rings becoming broader and eventually fusing anteriorly. This ontogenic shift during independent existence is unique among extant deep-diving air breathing vertebrates. Tract wall thickness is graded, becoming progressively thinner from larynx to bronchi. In addition, cross-sectional shape becomes increasingly dorsoventrally flattened (more elliptical) from anterior to posterior. These characteristics ensure that the tract will collapse from posterior to anterior during dives. This study contains the first report of a double (=internally bifurcated) posterior section of the trachea; it is suggested that this allows continuous food movement along the esophagus without tracheal collapse. The whole upper respiratory tract (from larynx to lungs) has a vascular lining (thicker anteriorly than posteriorly) that appears to be a simple analog of the complex turbinates of birds and mammals. Our study confirmed that the leatherback tracheal structure represents a distinctive way of dealing with the challenges of diving in deep, cold sea water.

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1. Introduction

The leatherback sea turtle, *Dermochelys coriacea* (Vandelli 1761), the sole living species of the Family Dermochelyidae, is a very unusual turtle. Dermochelyidae diverged from other (non-marine) chelonians 100–150 million years ago (MYA; Wood et al., 1996) and represent a separate evolution of sea turtles from all other living species (which form the hard shelled Family Cheloniidae). Adult leatherbacks are large animals (typically 300–500 kg), overlapping in size with many marine pinniped and cetacean species. However, in contrast to marine mammals, they start their aquatic life as 40–50 g hatchlings, so undergo a 10,000-fold increase in body mass during independent existence, much of which is still enigmatic as post hatchlings and juveniles are rarely seen in the wild (c.f. Grant, 1994).

The leatherback is a strange chelonian anatomically, with a highly modified and reduced shell structure that, with the thick leathery skin, allows the animals to change shape considerably and escape

some of the limitations of the turtle shell (Davenport et al., 2011). It is also highly unusual in terms of its feeding ecology, biogeography and physiology. *Dermochelys* feeds mainly on cnidarians and tunicates throughout its life (den Hartog and van Nierop, 1984; Davenport and Balazs, 1991; see Jones and Seminoff, 2003 for review). Its diet is therefore of poor quality for a carnivore (Doyle et al., 2007), especially as the leatherback is probably the fastest-growing living reptile species (Jones et al., 2011; Rhodin, 1985). It consequently has to eat great quantities of food (Jones et al., 2012). Although most observers have suggested that leatherbacks eat large prey items such as the jellyfish *Rhizostoma octopus* (Hays et al., 2003; Houghton et al., 2006), Fossette et al. (2012) have recently shown that they can also gain adequate quantities of food from dense patches of jellyfish individuals as small as 4g. The prey densities of adult leatherbacks are geographically patchy and leatherbacks migrate over long distances (thousands of kilometers) between breeding areas in the tropics and feeding areas in cool temperate coastal waters (e.g. Hays et al., 2006).

Adult *Dermochelys* are well known to have core body temperatures elevated substantially above ambient (to around 25 °C) when in cool water (e.g. off Nova Scotia) by virtue of their large size ('gigantothermy'), exercise, thick subcutaneous blubber (unique in living reptiles), plus vascular countercurrent heat exchangers in the flippers (Bostrom and

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Jones, 2007; Davenport et al., 1990; Frair et al., 1972; Greer et al., 1973; James and Mrosovsky, 2004; Paladino et al., 1990). They even have extensive intracranial insulation to keep brain and salt glands warm (Davenport et al., 2009a). A recently published review (Wallace and Jones, 2008) considers the gigantothermic/endothermic status of the species in the context of metabolic rate, concluding that they are true endotherms (though see Bradshaw et al., 2007). There is good paleoecological evidence that the link between the distribution of Dermochelyidae into cool waters (and hence of gigantothermy/endothermy) is of great antiquity (>40 MYA; Albright et al., 2003). Bostrom et al. (2010) have recently shown that even relatively small juvenile leatherback turtles (16–37 kg) can sustain significant temperature gradients between the body and the external environment.

Adult leatherbacks are also accomplished divers and can be regarded as the living deep-diving vertebrate with the longest evolutionary history. They forage regularly to depths of several hundred meters, making tens of thousands of dives annually (Houghton et al., 2008). Dives beyond 1000 m were first inferred (Eckert et al., 1986, 1989) and subsequently confirmed (to levels deeper than 1200 m) by satellite tags (Houghton et al., 2008), though such deep dives are rare.

In terms of the respiratory system, 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 branching bronchial tree). Lungs have little resistance to compression, while the tracheae and bronchi of terrestrial reptiles, birds and mammals have a series of complete-to-incomplete circular, semi-rigid cartilaginous rings interspersed with elastic connective tissue designed to maintain a patent airway and provide flexibility in the neck region.

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 blood and tissue oxygen supplies for each dive. Second, the air spaces of the respiratory system become compressed and this decreases buoyancy which affects descent and ascent phases of dives. Third, the possibility of highly compressed air being in close proximity to moving blood has the potential for causing N₂ accumulation and decompression sickness (DCS). 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 finally collapse, at relatively shallow depths (30–50 m), displacing air into the rigid (and poorly vascularized) tubes of the upper respiratory system, where little gaseous exchange would be possible and the threat of DCS eliminated. 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. Marine mammals also had cartilage support much further down the bronchiolar tree than terrestrial mammals. Such bronchiolar trees are absent in marine reptiles.

Subsequently this model has become extensively modified as understanding of the complexity of diving physiology and biochemistry has improved. Evidence indicates that lung collapse and gaseous exchange can be delayed to depths of well over 100 m in some seals (Kooyman and Sinnett, 1982; Moore et al., 2011b). A recent comprehensive review (Hooker et al., 2012) argues that diving mammals "manage DCS" rather than eliminate it. Whereas several shallow divers (e.g. marine otters and extinct mosasaurs) feature calcified tracheal rings that help to provide the rigid pipes envisaged by Scholander (1940), this appears not to be the case in deep divers such as Weddell seals and northern elephant seals (both of which exceed 1000 m depth on many dives). 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). Bostrom et al. (2008) demonstrated that a

compliant trachea causes increased depth of alveolar collapse and a gradual rather than abrupt collapse.

Davenport et al. (2009b) and Murphy et al. (2012) showed that adult *Dermochelys* (but not hatchlings) have a specialized tracheal structure that facilitates collapse of the respiratory tract during dives. As in other reptiles, the larynx and the tracheal structure begins at the anterior of the floor of the mouth. The adult larynx has a somewhat rectangular cross section and is thick-walled. At the rear of the hyoid plate (Fraher et al., 2010) it joins the trachea, which is a continuous long tube, consisting of fused, irregularly shaped, tracheal rings, with minimal amounts of connective tissue between them (Davenport et al., 2009b). The cross section of the trachea is near-elliptical in shape, but thinner-walled dorsally than ventrally. The material of this nearly-elliptical tube is predominantly uninterrupted, uncalcified cartilage. Davenport et al. (2009b) reported that the trachea could easily be compressed between the fingers, suggesting that it should collapse progressively given small pressure differences between lumen and exterior; this was confirmed by the biomechanical study of Murphy et al. (2012) who showed that the trachea had elastic properties ideally suited to repeated collapse and dilation. However, Murphy et al. (2012) also demonstrated mathematically that collapse of the upper respiratory tract would not be initiated until the lungs themselves had collapsed; they recognized that air moving from the collapsing lungs into the trachea and larynx would keep the latter two structures inflated until depths of several hundred meters had been attained.

Davenport et al. (2009b) also showed that the adult leatherback trachea was lined with an extensive, dense vascular plexus that should warm and humidify cold inspired air and possibly retain heat on expiration. They suggested that this structure was functionally equivalent to the nasal turbinates of birds and mammals and similarly diagnostic of endothermy.

All of the investigations of leatherback tracheal structure described above had the following shortcomings. First, all of the adult studies were conducted on sections of the upper respiratory tract between buccal cavity and the base of the turtle neck; no data were available from the more posterior parts of the trachea and bronchi between neck and lungs. Second, although some details of the structure in hatchling leatherbacks were given by Davenport et al. (2009a), who showed that their trachea was circular in cross section and dominated by connective tissue rather than cartilaginous rings (which were narrow), no information about juvenile leatherback turtles has ever been published. Here we present data derived from three juvenile turtles (70.9–87.3 cm curved carapace length; 33.2–53.4 kg mass) that shed light on ontogenic changes in respiratory tract structure and also yield novel information about likely functionality. Turtles of this size have rarely been encountered, have unknown diving capabilities, but are now known to be capable of several degrees of endothermy (Bostrom et al., 2010).

2. Methods

The three study specimens of juvenile leatherbacks (see Table 1 for their basic dimensions and body masses) were collected as bycatch by observers (NOAA Fisheries Service, Pacific Islands Regional Office, Observer Program) on longline fishing vessels operating in the equatorial Pacific. All were immediately frozen after death and so were in good post-mortem condition. Turtles were thawed 24 h before necropsy, which entailed a complete external and internal examination with procurement of tissues for routine histopathology in attempts to confirm cause of death (drowning) (c.f. Work and Balazs, 2002, 2010). Note that previous work has indicated that drowning damages the lungs (Work and Balazs, 2010); there is no evidence that drowning damages the larynx or trachea. Histological study of the tracheal lining of a drowned adult leatherback (Davenport et al., 2009b) exhibited no obvious damage. The upper respiratory tracts (from larynx to anterior of bronchi) were harvested and refrozen before further investigation. After thawing,

Table 1

Details of three juvenile leatherback turtles studied (all drowned in N. Pacific oceanic longline fisheries and were immediately frozen). The guts of all three turtles contained food; no plastic was present. SCL = straight carapace length; SCW = straight carapace width; CCL = curved carapace length; CCW = curved carapace length.

Turtle ID	Sex	SCL (cm)	SCW (cm)	CCL (cm)	CCW (cm)	Body mass (kg)	Year and site of collection
LL2103	Male	66.7	43.0	70.9	51.8	33.2	2006; 4°49.5' N; 163°43.0' W.
LL3596	Female	74.1	45.1	83.6	58.6	46.2	2010; 14° 32.2' N; 167°07.3' W.
AS0088	Male	83.0	47.6	87.3	59.6	53.4	2011; 11° 20.2' S; 169°44.5' W.

the lengths of the larynx and trachea were established and the wall thickness measured with vernier calipers. Scalpels were used to open the different parts of the upper respiratory tract and to cut cross sections. In the case of Specimen LL3596, the posterior part of the right bronchus was opened to inspect its structure and connection with the corresponding lung. A single hatchling leatherback (Museum code NHM 1969.2804) had been dissected by one of us (JD) at the Natural History Museum, London some years earlier (2008) and photographs of the tracheal system collected; these were revisited for comparison with the juveniles. The hatchling had originally been collected in French Guiana in 1969, so was from the Atlantic population of leatherbacks. Throughout the study photographs of dissections were taken with a Casio Exlim 10.1 megapixel digital camera.

3. Results

Fig. 1 exemplifies the gross anatomy of the upper respiratory tract of the juvenile leatherback turtles studied. As in adults and hatchlings, the larynx and anterior portion of the trachea are bound to the hyoid plate (see Fraher et al., 2010 for details of hyoid plate structure). From Fig. 2 it can be seen that, although tracheal rings (including the most anterior part associated with the hyoid plate) are very broad, with limited intervening connective tissue, they do not form the continuous tube reported for the anterior trachea of adults by Davenport et al. (2009b).

From Figs. 1–3 (plus Table 2) it can be seen that much of the length of the trachea (33–54% in the 3 specimens) had a posterior double

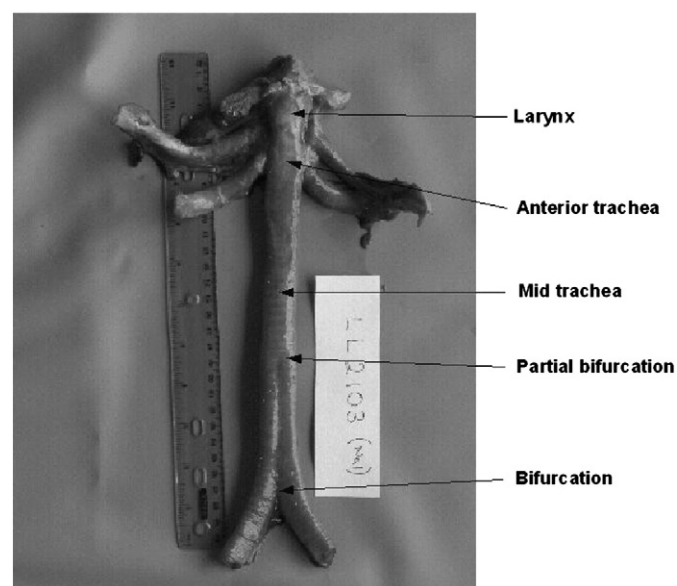


Fig. 1. Gross anatomy of upper respiratory tract of leatherback specimen LL2103 (from dorsal aspect).

structure which can be described as a partial or internal bifurcation. Externally this is evident in faint dorsal and ventral grooves (Figs. 1, 2, 3C). Internally (Fig. 3C), the double section had a central septum of cartilage of similar thickness to the lateral walls of the trachea.

Cross sections of the anterior ends of the bronchi (see Fig. 3D for example) had a rounded rectangular shape, with widths (16–18 mm) being much greater than dorsoventral height (about 8–9 mm). The dimensions were difficult to measure precisely as their walls were extremely thin (about 0.5 mm) and yielded to the lightest touch of the vernier calipers. Table 2 demonstrates that the wall thickness of the upper respiratory tract declines markedly from the larynx to the tracheal bifurcation. Mean thickness of the laryngeal walls was 3.2 mm, of the tracheal wall in the anterior undivided portion 1.6 mm (about half that of adults; Davenport et al., 2009b), and in the double, internally-bifurcated portion mean wall thickness was 1.0 mm. Taken with the thickness of the bronchial walls, it is evident that the upper respiratory tract becomes thinner-walled from anterior to posterior. This is associated with progressively increased compressibility, evident in the resistance to pinching to closure of the different sections of the tract.

From Fig. 3 and Table 2 it may be seen that the tract also becomes more dorsoventrally flattened from anterior to posterior too. In the larynx the mean ratio between external width and external dorsoventral height was about 1.3, in the anterior trachea 1.8 and in the bronchi about 2.0. Circular pipes withstand greater pressure differences across their walls without distortion than do elliptical pipes (e.g. Wild et al., 1977), so the progressively increasing flattening of the tract also implies increased compressibility. However, from Fig. 3C it can be seen that the posterior, double part of the trachea effectively consists of two tubes, each with a much more circular cross section (width:height ratios of about 0.8). This indicates that this part of the trachea is less compressible than the anterior trachea or bronchi; this was also evident when the different sections were pinched between finger and thumb.

Fig. 4 shows that the bronchus ends deep in the lung structure where it connects with the lung tissues by a series of openings. This is similar to the images shown by Wyneken (2001) and is general for chelonians; the bronchial wall in this region was extremely flexible.

From the respiratory tract cross sections (Fig. 3), together with longitudinal sections (not shown) it was evident that the entire juvenile respiratory tract had a mucosal vascular lining, as reported for the adult anterior trachea by Davenport et al. (2009b). The lining was thicker in the larynx than in the trachea, where it was in turn thicker than in the bronchi.

4. Discussion

The results of our study demonstrate that there are significant structural differences between the upper respiratory tracts of hatchling, juvenile and adult leatherback turtles. There are ontogenic increases in the quantity of cartilage within the tracheal and bronchial walls, with the narrow cartilaginous rings present in the hatchlings becoming proportionally broader in the juveniles and (at least anteriorly) becoming essentially fused in the adult. The ring structure of the anterior trachea, still clearly defined by intervening connective tissue in the juveniles (this study), essentially disappears in the adult (Davenport et al., 2009b). It is also evident that increased proportions of cartilage (by comparison with hatchlings) are present in the posterior trachea and bronchi of juveniles, so that the progressive ontogenic development of a cartilaginous tubular structure affects the whole of the upper respiratory tract (Fig. 5).

These changes in longitudinal wall structure are also accompanied by changes in cross-sectional shape and a graded wall thickness. Murphy et al. (2012) established that the adult tracheal cartilage has hysteresis properties that facilitate repeated collapse and expansion. Here we show a decreased thickness of the cartilage of the wall of the

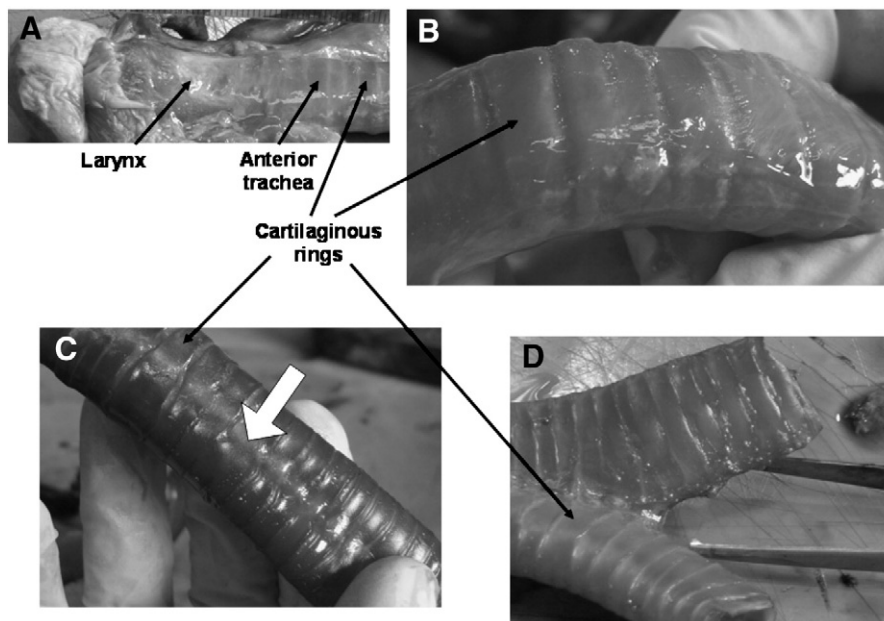


Fig. 2. Cartilaginous ring structure of upper respiratory tract of leatherback specimen LL3596. A. Solid larynx and ringed anterior trachea. B. Middle portion of trachea (bent manually), showing broad rings separated by narrow connective tissue. C. Ring structure in region of partial (internal) bifurcation. White arrow indicates anterior limit of partial bifurcation. D. Ring structure of bifurcation and anterior sections of bronchi.

upper respiratory tract from larynx to bronchi, combined with an increasingly elliptical (dorsoventrally flattened) cross section. This means that, during a dive, the posterior part of the tract will collapse

earlier than the anterior part. Thus, air displaced from the highly compressible lungs will be progressively squeezed anteriorly as depth increases. At present the diving capabilities of juvenile leatherbacks

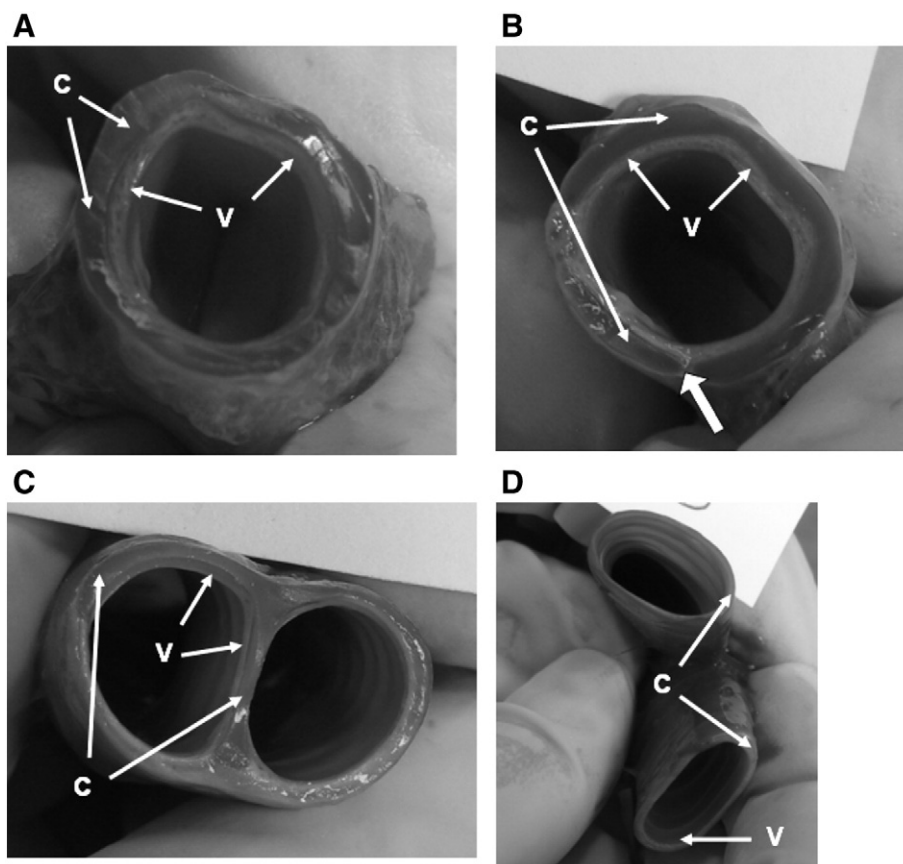


Fig. 3. Cross sections of upper respiratory tract of leatherback specimen LL2103, each viewed from posterior aspect. In each section, C indicates cartilage, V indicates luminal vascular layer. A. Larynx. Note thick, continuous cartilage. B. Anterior trachea. White arrow indicates apparent break in cartilaginous ring – this is an artifact of cutting a section. C. Partial (internal) bifurcation section of trachea. Note that medial wall is lined with vascular layer. D. Cut ends of bronchi. Note rounded rectangular section, thin cartilaginous rings and very thin luminal vascular layer.

Table 2
Measurements (mm) of larynx and trachea in three juvenile leatherback turtles.

Specimen ID#	Larynx				Trachea					
	Length	External width	External height ^a	Wall thickness	Length to external bifurcation	Length to internal bifurcation	External width	External height ^a	Wall thickness 1 ^b	Wall thickness 2 ^c
LL2103	31.0	15.8	10.9	3.2	201	125	20.2	11.2	1.2	0.7
AS0088	49.0	19.5	15.2	3.1	255	170	21.4	10.7	2.1	1.3
LL3596	36.0	17.3	14.3	3.2	227	105	21.5	12.8	1.4	1.0

^a Dorsoventral measurement.

^b Measured in anterior undivided region of trachea.

^c In posterior internally bifurcated region of trachea.

are unknown, so the adaptive significance to them of this structure is unclear. However, this graded compliance of the respiratory tract will obviously be advantageous to deep-diving adults (c.f. Bostrom et al., 2008).

The extensive double portion (internal or partial bifurcation) of leatherback turtles' tracheae appears not to have been remarked upon before beyond an extremely brief comment by Dunlap (1955), although Wyneken (2001), in a seminal study of general sea turtle anatomy stated (not in the context of the leatherback turtle) that "the bifurcation begins internally, anterior to the external division to form the bronchi". Wyneken (2001) did not specify the species concerned, or the anterior limit of internal bifurcation; associated illustrations were mostly for the cheloniid loggerhead turtle *Caretta caretta* though a figure of the airways of a Kemp's ridley turtle *Lepidochelys kempii* (visualized by a C-T scan) indicated a small amount of internal bifurcation (not remarked upon by the author). Lavín et al. (2007) present a C-T scan of a loggerhead turtle; although the trachea was not a major objective of their study, there is no sign of significant internal bifurcation.

Some internal bifurcation in the form of a carina (a forwardly-directed projection of the rearmost tracheal ring) is normal in a variety of air breathers including humans, but the internal bifurcation of leatherbacks is far more extensive than this, involving numerous tracheal rings. Double tracheae appear to be extremely unusual among vertebrates as Zeek (1951) remarked when reporting them for penguins and California sealions (*Zalophus californianus*). She assigned no especial function to these, and it appears that no other author has considered this either. She illustrated the penguin trachea, which appears to be double throughout, but did not provide an illustration of the sealion trachea. In Website 1 (2013), the (undivided) trachea of the sealion is described as dividing into two bronchi at the top of the chest, and that the bronchi run parallel to each other for some distance; no biological significance of this layout is known. This description implies that the trachea is not in fact internally bifurcated and this has been confirmed by recent X-radiographs presented by Dennison et al (2009). It is also the case

that not all penguin species possess a medial tracheal septum. Emperor and king penguins (*Aptenodytes forsteri* and *Aptenodytes patagonicus* respectively) appear to have undivided tracheae, while in the rock hopper penguin (*Eudyptes chrysocome*) the septum projects only 5 mm anteriorly from the carina (King and McLelland, 1989). The yellow-eyed penguin (*Megadyptes antipode*) has the lower third of the trachea divided (Hocken, 2002), but the African (or jackass) penguin (*Spheniscus demersus*) septum almost reaches the larynx (King and McLelland, 1989). All other published reports concern occasional instances of a central septum or double trachea in humans: these can cause medical problems, particularly for anesthesia (e.g. Fitzmaurice et al., 2010). This could also be the case for leatherbacks of this size class that are intubated for anesthesia. The septum, together with the progressive thinning of cartilage in the distal bronchus would prompt caution when intubating these animals to avoid trauma to the upper respiratory tract (a problem also for veterinary treatment of sealions; Website 1, 2013).

We believe that the double portion of the leatherback trachea is of adaptive significance. It coincides with the part of the trachea where it emerges from the neck region and is curved dorsally in the body cavity towards the dorsally-located lungs. The central septum and the near-circular tubular cross sections mean that this section of the trachea will not collapse as readily as the bronchi posteriorly. Why might this be important? The trachea of leatherbacks is closely associated with an unusually long, muscular and capacious esophagus. Leatherbacks eat huge quantities of gelatinous food at all stages of their life cycle (Fossette et al., 2012; Jones et al., 2012). Bels et al. (1998) demonstrated that juvenile *Dermochelys* have the ability to simultaneously catch prey and swallow material already in the rear part of the buccal cavity; they have a conveyor-like browsing and swallowing feeding strategy. This means that, when it breathes at the surface, a leatherback feeding in a rich patch of jellyfish will have a full, dilated esophagus that will press ventrally onto the trachea, especially where the latter curves dorsally into the anterior part of the body cavity. The double structure with a central septum will oppose occlusion of the trachea during inhalation and exhalation. This approach is in total contrast to that shown in the anatomy of the leopard seal (*Hydrurga leptonyx*), which also swallows large prey (penguins) whole, albeit intermittently, not continuously. The leopard seal has a trachea in which tracheal rings are replaced with transverse cartilaginous bars, so that the trachea collapses totally during feeding (Murphy, 1913).

Finally, this study confirms that the entire upper respiratory tract of juveniles has a vascular lining of the type reported for the adult anterior trachea by Davenport et al. (2009b). Their histological investigations revealed that the vascular plexus within the lining was characterized by longitudinal blood vessels (both arteries and veins, but predominantly large-bore venous vessels) with many cross connections, and links (via connective tissue between cartilaginous plates) with blood vessels outside the trachea. Our study provides further evidence of a counter-current exchange structure that will warm and humidify inhaled air, so that it will approach core body temperature and 100% relative humidity by the time it enters the lungs. There may also be some heat recovery too during exhalation. Unlike turbinates of birds and mammals, where a highly convoluted vascular structure with a great surface area exchanges heat and water over a short linear distance (e.g. Geist, 2000),

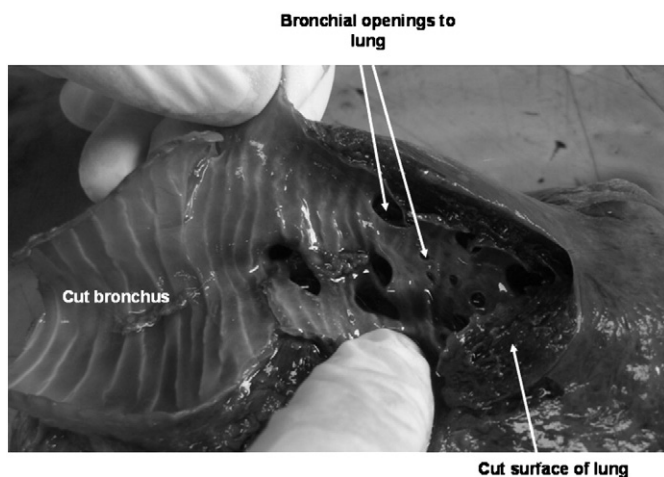


Fig. 4. Junction of bronchus and lung in leatherback specimen LL3596.

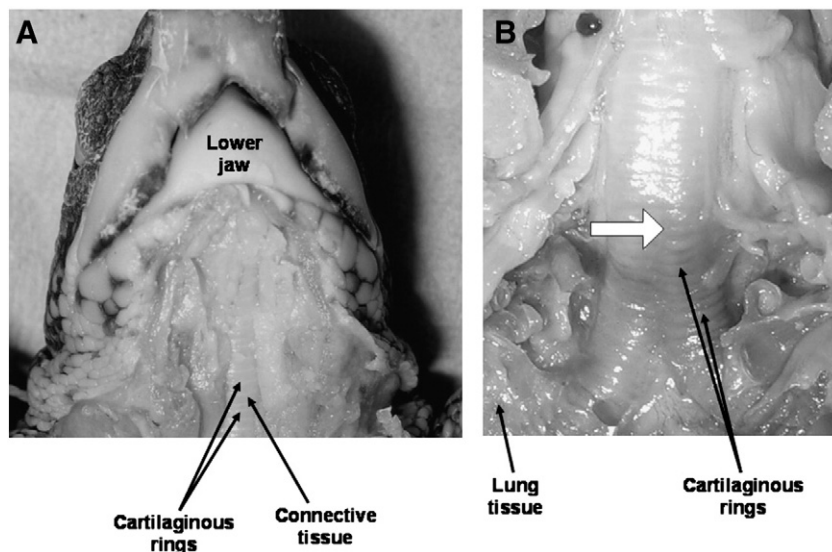


Fig. 5. Trachea of hatchling leatherback turtle. A. Ventral view of anterior trachea. Note narrow cartilaginous rings and broad intervening connective tissue. B. Ventral view of posterior part of trachea and bifurcation into bronchi. White arrow indicates ventral groove of partially-bifurcated section of trachea. Note that cartilaginous rings are broader than in anterior portion of trachea.

the heat exchangers of the leatherback upper respiratory tract are long and simple. Geist (2000) associates turbinate evolution with the high tidal respiratory volume and high metabolic rate of birds and mammals, which have metabolic rates around an order of magnitude higher than reptiles of comparable body mass. The metabolic rate of leatherbacks, although controversial (Bradshaw et al., 2007; Wallace and Jones, 2008) is undoubtedly lower than in marine mammals of comparable size, so simple heat exchangers presumably suffice. Leatherback turtles show some physiological convergence (e.g. high blood hematocrit, high tissue myoglobin) with deep diving mammals, but have much lower tidal volumes (Lutcavage et al., 1990), despite having more complex lungs than other sea turtles (Wyneken, 2001). The only other deep divers known to have vascular tracheal linings are some cetaceans (Cozzi et al., 2005; Bagnoli et al., 2011; Davenport et al., in press), but their tracheae are extremely short (because cetaceans effectively are neckless, and the trachea is not connected to the buccal cavity). As cetacean tidal volumes are very high (<90% of lung volume; Wartzok, 2002), the velocity of air passing over these short lengths of vascular tissue must be considerable, making a turbinate-like function improbable as there would be insufficient time for significant heat exchange (Davenport et al., in press). In contrast, the long trachea and relatively low tidal volume of the leatherback should provide plenty of time for effective heat transfer.

A laryngeal–bronchial vascular lining has the theoretical potential to create problems of N₂ accumulation (and hence DCS) during dives when the upper respiratory tract will be full of air under pressure. If blood flowed freely through this lining during a dive, nitrogen from the tracheal air could be transferred elsewhere within the body, causing DCS on ascent. However, blood is presumably either displaced from the mucosal plexus to the blood vessels surrounding the trachea during dives (when the warming function is not required) (c.f. Davenport et al., 2009b), or the blood remains in the lining and flow is restricted.

This study confirms that the upper respiratory tract of the leatherback turtle, apparently uniquely among living deep-diving air breathers, shows marked changes during growth from hatchling to adult. These culminate in the development of a complex collapsible and highly-vascularized structure that provides a distinctive approach to dealing with the challenges of diving into deep, cold sea water. In other deep divers (penguins, pinnipeds and cetaceans), there is no life history equivalent of the long period of inability to dive that is characteristic of leatherbacks after hatching; they are probably surface

foragers for several years. In contrast, young penguins, seals and cetaceans have to be able to dive within weeks/months of either entering the water or weaning (in the case of mammals). Our study suggests that there is considerable scope for further investigation of tracheal function and development. The double trachea of some penguin species merits further study, particularly as birds have proportionately larger tracheal and bronchial volumes (and much more efficient lung ventilation by virtue of air sacs and one-way airflow through the parabronchi than those of mammals of similar size (Schmidt-Nielsen, 1997)). The development of tracheal rings (including the calcification exhibited by some shallow-diving species; Tarasoff and Kooyman, 1973) of aquatic mammals before and after birth would also be a fruitful area for future investigation.

Authors' contributions

The work presented here was carried out in close collaboration between all authors. JD wrote the first draft of the paper and the final revision; all other authors contributed to refining and finalizing the manuscript.

Disclosure statement

We wish to confirm that there are no known conflicts of interest associated with this publication and that there has been no financial support for this work that could have influenced its outcome. The manuscript has been read and approved by all named authors.

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References

- Albright III, L.B., Woodburne, M.O., Case, J.A., Chaney, D.S., 2003. A leatherback sea turtle from the Eocene of Antarctica: implications for antiquity of gigantothermy in Dermochelyidae. *J. Vertebr. Paleontol.* 23, 945–949.
- Bagnoli, P., Cozzi, B., Zaffora, A., Acocella, F., Fumero, R., Costantino, M.L., 2011. Experimental and computational biomechanical characterisation of the tracheo-bronchial tree of the bottlenose dolphin (*Tursiops truncatus*) during diving. *J. Biomech.* 44, 1040–1045.
- Bels, V.L., Davenport, J., Renous, S., 1998. Food ingestion in the estuarine turtle *Malaclemys terrapin*: comparison with the marine leatherback turtle *Dermochelys coriacea*. *J. Mar. Biol. Assoc. UK* 78, 953–972.
- Bostrom, B.L., Jones, D.R., 2007. Exercise warms adult leatherback turtles. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 147, 323–331.
- Bostrom, B.L., Fahlman, A., Jones, D.R., 2008. Tracheal compression delays alveolar collapse during deep diving in marine mammals. *Respir. Physiol. Neurobiol.* 161, 298–305.
- Bostrom, B.L., Jones, T.T., Hastings, M., Jones, D.R., 2010. Behaviour and physiology: the thermal strategy of leatherback turtles. *PLoS ONE* 5, e13925.
- Bradshaw, C.J.A., McMahon, C.R., Hays, G.C., 2007. Behavioral inference of diving metabolic rate in free-ranging leatherback turtles. *Physiol. Biochem. Zool.* 80, 209–219.
- Cozzi, B., Bagnoli, P., Acocella, F., Costantino, M.L., 2005. Structure and biomechanical properties of the trachea of the striped dolphin *Stenella coeruleoalba*: evidence for evolutionary adaptations to diving. *Anat. Rec.* 284A, 500–510.
- Davenport, J., Balazs, G.H., 1991. 'Fiery bodies': are pyrosomas important items in the diet of leatherback turtles? *Br. Herpetol. Soc. Bull.* 37, 33–38.
- Davenport, J., Holland, D.L., East, J., 1990. Thermal and biochemical characteristics of the fat of the leatherback turtle *Dermochelys coriacea* (L.): evidence of endothermy. *J. Mar. Biol. Assoc. UK* 70, 33–41.
- Davenport, J., Fraher, J., Fitzgerald, E., McLaughlin, P., Doyle, T., Harman, L., Cuffe, T., 2009a. Fat head: an analysis of head and neck insulation in the leatherback turtle (*Dermochelys coriacea*). *J. Exp. Biol.* 212, 2753–2759.
- Davenport, J., Fraher, J., Fitzgerald, E., McLaughlin, P., Doyle, T., Harman, L., Cuffe, T., Dockery, P., 2009b. Ontogenetic changes in tracheal structure facilitate deep dives and cold water foraging in adult leatherback sea turtles. *J. Exp. Biol.* 212, 3440–3447.
- Davenport, J., Plot, V., Georges, J.V., Doyle, T.K., James, M.C., 2011. Pleated turtle escapes the box – shape changes in *Dermochelys coriacea*. *J. Exp. Biol.* 214, 3474–3479.
- Davenport, J., Cotter, L., Rogan, E., Kelliher, D., Murphy, C., 2013. Structure, material characteristics and function of the upper respiratory tract of the pygmy sperm whale. *J. Exp. Biol.* (in press).
- den Hartog, J.C., van Nierop, M.M., 1984. A study on the gut contents of six leathery turtles, *Dermochelys coriacea* (Linnaeus) (Reptilia: Testudines: Dermochelyidae) from British waters and from the Netherlands. *Zool. Verh.* 200, 1–36.
- Dennison, S.E., Forrest, L., Gulland, F.M.D., 2009. Normal thoracic radiographic anatomy of immature California sea lions (*Zalophus californianus*) and immature northern elephant seals (*Mirounga angustirostris*). *Aquat. Mamm.* 35, 36–42.
- Doyle, T.K., Houghton, J.D.R., McDevitt, R., Davenport, J., Hays, G.C., 2007. The energy density of jellyfish: estimates from bomb calorimetry and proximate composition. *J. Exp. Mar. Biol. Ecol.* 34, 239–252.
- Dunlap, C.E., 1955. Notes on the visceral anatomy of the giant leatherback turtle (*Dermochelys coriacea*, L.). *Bull. T. Med. Fac.* 14, 55–69.
- Eckert, S.A., Nellis, D.W., Eckert, K.L., Kooyman, G.L., 1986. Diving patterns of two leatherback sea turtles (*Dermochelys coriacea*) during interesting intervals at Sandy Point, St. Croix, US Virgin Islands. *Herpetologica* 42, 381–388.
- Eckert, S.A., Eckert, K.L., Ponganis, P., Kooyman, G.L., 1989. Diving and foraging behaviour of leatherback sea turtles (*Dermochelys coriacea*). *Can. J. Zool.* 67, 2834–2840.
- Fitzmaurice, G.J., Nasir, A., MacGowan, S., 2010. A tracheal septum during routine pre-operative work-up. *Ann. Card. Anaesth.* 13, 69.
- Fossette, S., Gleiss, A.C., Casey, J.P., Lewis, A.R., Hays, G.C., 2012. Does prey size matter? Novel observations of feeding in the leatherback turtle (*Dermochelys coriacea*) allow a test of predator–prey size relationships. *Biol. Lett.* 8, 351–354.
- Fraher, J., Davenport, J., Fitzgerald, E., McLaughlin, P., Doyle, T., Harman, L., Cuffe, T., 2010. Opening and closing mechanisms of the leatherback sea turtle larynx: a crucial role for the tongue. *J. Exp. Biol.* 213, 4137–4145.
- Frair, W., Ackman, R.G., Mrosovsky, N., 1972. Body temperature of *Dermochelys coriacea*: warm turtle from cold water. *Science* 177, 791–793.
- Geist, N.R., 2000. Nasal respiratory turbinate function in birds. *Physiol. Biochem. Zool.* 73, 581–589.
- Grant, G.S., 1994. Juvenile leatherback turtle caught by longline fishing in American Samoa. *Mar. Turt. Newslett.* 66, 3–5.
- Greer, A.E., Lazell Jr., J.D., Wright, R.M., 1973. Anatomical evidence for a counter-current heat exchanger in the leatherback turtle (*Dermochelys coriacea*). *Nature* 214, 181.
- Hays, G.C., Houghton, J.D.R., Doyle, T., Davenport, J., 2003. Aircraft give a new view of jellyfish behaviour. *Nature* 426, 383.
- Hays, G.C., Hobson, V.J., Metcalfe, J.D., Righton, D., Sims, D.W., 2006. Flexible foraging movements of leatherback turtles across the North Atlantic Ocean. *Ecology* 87, 2647–2656.
- Hocken, A.G., 2002. Post-mortem Examination of Penguins. DOC Science Internal Series 65. Department of Conservation, Wellington, New Zealand.
- Hooker, S.K., Fahlman, A., Moore, M.J., Aguilar de Soto, N., de Quirós, Y.B., Brubakk, A.O., Costa, D.P., Costidis, A.M., Dennison, S., Falke, K.J., et al., 2012. Deadly diving? Physiological and behavioural management of decompression stress in diving mammals. *Proc. R. Soc. B* 279, 1041–1050.
- Houghton, J.D.R., Doyle, T.K., Wilson, M.W., Davenport, J., Hays, G.C., 2006. Jellyfish aggregations and leatherback turtle foraging patterns in a temperate coastal environment. *Ecology* 87, 1967–1972.
- Houghton, J.D.R., Doyle, T.K., Davenport, J., Wilson, R.P., Hays, G.C., 2008. The role of infrequent and extraordinary deep dives in leatherback turtles (*Dermochelys coriacea*). *J. Exp. Biol.* 211, 2566–2575.
- James, M.C., Mrosovsky, N., 2004. Body temperatures of leatherback turtles (*Dermochelys coriacea*) in temperate waters off Nova Scotia, Canada. *Can. J. Zool.* 82, 1302–1306.
- Jones, T.T., Seminoff, J.A., 2003. Feeding biology: advances from field-based observations, physiological studies, and molecular techniques. In: Wynneken, J., Lohmann, K., Musick, J. (Eds.), *The Biology of Sea Turtles*, vol. III. CRC Press, Boca Raton.
- Jones, T.T., Hastings, M.D., Bostrom, B.L., Pauly, D., Jones, D.R., 2011. Growth of captive leatherback turtles, *Dermochelys coriacea*, with inferences on growth in the wild: implications for population decline and recovery. *J. Exp. Mar. Biol. Ecol.* 399, 84–92.
- Jones, T.T., Bostrom, B.L., Hastings, M.D., Van Houtan, K.S., Pauly, D., Jones, D.R., 2012. Resource requirements of the Pacific leatherback turtle population. *PLoS ONE* 7 (10), e45447. <http://dx.doi.org/10.1371/journal.pone.0045447>.
- King, A.S., McLelland, J. (Eds.), 1989. *Form and Function in Birds*. vol.4. Academic Press, New York.
- Kooyman, G.L., 1989. *Diverse Divers: Physiology and Behaviour*. Springer-Verlag, Berlin, Germany.
- Kooyman, G.L., Sinnott, E.E., 1982. Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* 55, 105–111.
- Kooyman, G.L., Hammond, D.D., Schroeder, J.P., 1970. Bronchograms and tracheograms of seals under pressure. *Science* 169, 82–84.
- Lavín, S., Alegre, F., y Marco, I., 2007. Computed tomography of the vertebral column and coelomic structures in the normal loggerhead sea turtle (*Caretta caretta*). *Vet. J.* 174, 362–370.
- Lutcavage, M.E., Bushnell, P.G., Jones, D.R., 1990. Oxygen transport in the leatherback sea turtle *Dermochelys coriacea*. *Physiol. Zool.* 63, 1012–1024.
- Moore, M.J., Hammar, T., Arruda, J., Cramer, S., Dennison, S., Montie, E., Fahlman, A., 2011a. Hyperbaric computed tomographic measurement of lung compression in seals and dolphins. *J. Exp. Biol.* 214, 2390–2397.
- Moore, C., Fahlman, A., Moore, M., Niemeyer, M., Lentell, B., Oakes, S., Trumble, S., 2011b. Histological investigation of the 'slip' in marine mammal tracheas. Society for Marine Mammalogy 19th Biennial Conference on the Biology of Marine Mammals, November 27–December 2, 2011. Tampa, Florida.
- Murphy, R.C., 1913. The trachea of *Ogmorhinus*. With notes on other soft parts. *Bull. Am. Mus. Nat. Hist.* 32, 505–506.
- Murphy, C., Kelliher, D., Davenport, J., 2012. Shape and material characteristics of the trachea in the leatherback sea turtle promote progressive collapse and reinflation during dives. *J. Exp. Biol.* 215, 3064–3071.
- Paladino, F.V., O'Connor, M.P., Spotila, J.R., 1990. Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* 344, 858–860.
- Rhodin, R.G.J., 1985. Comparative chondro-osseous development and growth of marine turtles. *Copeia* 1985, 752–771.
- Schmidt-Nielsen, K., 1997. *Animal Physiology: Adaptation and Environment*. Cambridge University Press, Cambridge, U.K.
- Scholander, P.F., 1940. Experimental investigations on the respiratory function in diving mammals and birds. *Hvalradets Skr.* 22, 1–131.
- Tarasoff, F.J., Kooyman, G.L., 1973. Observations on the anatomy of the respiratory system of the river otter, sea otter, and harp seal. II. The trachea and bronchial tree. *Can. J. Zool.* 51, 171–177.
- Wallace, B.P., Jones, T.T., 2008. What makes marine turtles go: a review of metabolic rates and their consequences. *J. Exp. Mar. Biol. Ecol.* 356, 8–24.
- Wartzok, D., 2002. Breathing. In: Perrin, W.F., Würsig, B., Thewissen, J.G.M. (Eds.), *Encyclopedia of Marine Mammals*. Academic Press, San Diego, U.S.A., pp. 164–169.
- Website 1. (accessed 23-7-2013) shutterbug.ucsc.edu/view_album.php?set_albumName=album81.html.
- Wild, R., Pedley, T.J., Riley, D.S., 1977. Viscous flow in collapsible tubes of slowly varying elliptical cross-section. *J. Fluid Mech.* 81, 273–294.
- Wood, R.C., Johnson-Gove, J., Gaffney, E.S., Maley, K.F., 1996. Evolution and phylogeny of the leatherback turtles (Dermochelyidae), with descriptions of new fossil taxa. *Chelonian Conserv. Biol.* 2, 266–286.
- Work, T.M., Balazs, G.H., 2002. Necropsy findings in sea turtles taken as by-catch in the North Pacific longline fishery. *Fish. Bull.* 100, 876–880.
- Work, T.M., Balazs, G.H., 2010. Pathology and distribution of sea turtles landed as bycatch in the Hawaii-based North Pacific pelagic longline fishery. *J. Wildl. Dis.* 46, 422–432.
- Wynneken, J., 2001. *The Anatomy of Sea Turtles*. NOAA Technical Memorandum NMFS-SEFSC-470. <http://courses.science.fau.edu/~jwynneken/sta/>.
- Zeek, P.M., 1951. Double trachea in penguins and sea lions. *Anat. Rec.* 111, 327–343.