Ontogenetic changes in tracheal structure facilitate deep dives and cold water foraging in adult leatherback sea turtles

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SUMMARY

Adult leatherbacks are large animals (300–500 kg), overlapping in size with marine pinniped and cetacean species. Unlike 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. Hatchlings are limited to the tropics and near-surface water. Adults, obligate predators on gelatinous plankton, encounter cold water at depth (<1280 m) or high latitude and are gigantotherms that maintain elevated core body temperatures in cold water. This study shows that there are great ontogenetic changes in tracheal structure related to diving and exposure to cold. Hatchling leatherbacks have a conventional reptilian tracheal structure with circular cartilaginous rings interspersed with extensive connective tissue. The adult trachea is an almost continuous ellipsoidal cartilaginous tube composed of interlocking plates, and will collapse easily in the upper part of the water column during dives, thus avoiding pressure-related structural and physiological problems. It is lined with an extensive, dense erectile vascular plexus that will warm and humidify cold inspired air and possibly retain heat on expiration. A sub-luminal lymphatic plexus is also present. Mammals and birds have independently evolved nasal turbinates to fulfil such a respiratory thermocontrol function; for them, turbinates are regarded as diagnostic of endothermy. This is the first demonstration of a turbinate equivalent in a living reptile.

Key words: Leatherback turtle, *Dermochelys*, tracheal structure, vascular plexus, diving, gigantothermy.

INTRODUCTION

The leatherback sea turtle, *Dermochelys coriacea* (Vandelli 1761), the sole living species of the Family Dermochelyidae, is a most unusual chelonian anatomically, with a highly modified and reduced shell structure that, with the thick leathery skin, allows the animals to assume a barrel shape when in water. Dermochelyidae diverged from other chelonians 100–150 m.y.a. (Wood et al., 1996). Other living sea turtles (Family Cheloniidae) are not closely related to them, having separately evolved in the middle Tertiary some 35–50 m.y.a. (Zangerl, 1980). This remote relationship has been confirmed by molecular studies (Bowen et al., 1993), while the limb ossification system of leatherbacks is completely different from that of other sea turtles (Rhodin, 1985; Snover and Rhodin, 2008). Adult leatherbacks are large animals [typically 300–500 kg, but <916 kg (Eckert and Luginbuhl, 1988)], 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.

The leatherback is also highly unusual in terms of its feeding ecology, biogeography and physiology. *Dermochelys coriacea* is an obligate feeder on gelatinous organisms, predominantly medusae, pyrosomas and siphonophores throughout its life (den Hartog and van Nierop, 1984; Davenport and Balazs, 1991). Its diet is therefore of poor quality for a carnivore (see Doyle et al., 2007), especially as the leatherback is probably the fastest-growing living reptile species. This means that it has to eat large quantities of food (Duron,

1978), from more than 100% body mass per day in hatchlings (Lutcavage and Lutz, 1986) to at least 50% body mass per day in adults (Davenport, 1998).

The prey densities of adult leatherbacks are geographically very patchy and leatherbacks migrate over long distances (thousands of kilometres) 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 over ambient (to around 25°C) when in cool water (e.g. off Newfoundland) by virtue of their large size ('gigantothermy'), exercise, thick subcutaneous blubber (unique in living reptiles), plus vascular countercurrent heat exchangers in the flippers (Frair et al., 1972; Greer at al., 1973; Paladino et al., 1990; Davenport et al., 1990; James and Mrosovsky, 2004; Bostrom and Jones, 2007). They even have extensive intracranial insulation to keep brain and salt glands warm (Davenport et al., 2009). A recently published review (Wallace and Jones, 2008) considers the gigantothermic/endothermic status of the species in the context of metabolic rate. There is good palaeoecological evidence that the link between the distribution of Dermochelyidae into cool waters (and hence of gigantothermy) is of great antiquity [>40 million years (Albright et al., 2003)].

Adult leatherbacks are also accomplished divers. Dives beyond 1000 m were first inferred (Eckert et al., 1986; Eckert et al., 1989) and subsequently confirmed by satellite tags (Houghton et al., 2008). Maximum recorded dive depths and dive durations have now been extended to 1280 m (Doyle et al., 2008) and 86.5 min (López-

Mendilaharsu et al., 2008), respectively. However, it has recently been determined from satellite telemetry that very deep dives (>300 m) are rare (Houghton et al., 2008), making up only 0.4% of all dives, and exceed calculated aerobic scope for the species. Leatherbacks are mostly (99.6%) limited to aerobic dives of <300 m, but they conduct thousands of dives per year so there are appreciable total annual numbers of very deep dives as well. It is generally assumed that diving is associated with foraging upon gelatinous organisms during periods when these are abundant at depth rather than at the surface; recent satellite telemetry studies of the sunfish *Mola mola*, another large specialist feeder on gelatinous organisms, have revealed that they spend days or weeks at substantial depths (roughly in the range of 100–400 m), migrating nearer to the surface at night and descending during the day, presumably in pursuit of their vertically migrating prey (Sims et al., 2009). This provides additional support for the existence of plentiful supplies of medusae, pyrosomas and salps at depth. Overall, the dive performance capacity of leatherbacks is comparable with that of deep diving marine mammals such as the southern elephant seal *Mirounga leonine* (Boyd and Arnbom, 1991) and sperm whale *Physeter macrocephalus* (Watkins et al., 1993).

While knowledge of adult leatherback biology is increasingly plentiful, data concerning early life history stages (beyond on-shore egg incubation and hatching) remain limited. Hatchlings swim straight out to sea for 24 h ['swimming frenzy' (Wyneken and Salmon, 1992)] and larger juvenile and subadult leatherbacks have rarely been seen in the field, although there are reports that juveniles <100 cm carapace length are restricted to waters of 26°C and above (Eckert, 2002). Until recently, leatherback turtles were found to be difficult to rear in captivity, most succumbing to dietary problems or infections within a few weeks. A few specimens were reared to a carapace length of 25–30 cm (e.g. Bels, 1988) and used in studies of feeding biomechanics (e.g. Bels et al., 1998). However, Jones (Jones, 2009) has reared young leatherbacks for as much as 815 days (the turtle concerned having a body mass of 42.65 kg and a straight carapace length of 72.0 cm), but the emphasis of his study was on growth and energetics, not on respiratory physiology or respiratory tract anatomy. Hatchling leatherbacks are not able to dive more than a short distance from the sea surface, and dive for <3 min (Wyneken and Salmon, 1992).

Skeletochronological evidence suggests that leatherbacks grow quickly by comparison with other sea turtles, reaching sexual maturity in 9–15 years (Zug and Parham, 1996), a finding supported by the observed rapidity of recovery of nesting beaches following effective protection (e.g. Dutton et al., 2005), and by the recent laboratory growth studies of Jones (Jones, 2009). However, at present we do not know when leatherbacks switch from being surface-dwelling animals to becoming deep divers.

When an adult leatherback dives from the surface to 300 m, the volume of air in the respiratory tract will decline to about 3.2% of the initial value. At 1200 m the corresponding value will be only 0.8%. Clearly, if extreme pressure differences between the inside of the respiratory tract and the surrounding tissues are not to develop, progressive collapse of the tract must occur. *Dermochelys* has a highly reduced plastron that consists of a thin, peripheral, elliptical cartilaginous ring (Wood et al., 1996; Wyneken, 2001). Functionally the plastron is therefore absent (uniquely in chelonians) and there is no ventral bony protection for the viscera. Spotila (Spotila, 2004) reported that the ventral surface becomes visibly concave when leatherbacks start dives, which is consistent with respiratory tract collapse, though compression of gas in the gut might also be occurring.

No detailed study of the anatomy of the leatherback upper respiratory tract appears to have been made. A highly detailed sea turtle anatomical manual (Wyneken, 2001) presents images of the lung and lower bronchi (both similar to those of cheloniid sea turtles, but the lungs of leatherbacks are more complex), but the only image that shows a leatherback trachea is that of a hatchling, and the trachea is rather indistinct, but appears to consist of cartilage rings and connective tissue. However, the manual does contain a clear image of the trachea of an adult cheloniid, the hawksbill turtle *Eretmochelys imbricata*. In this case 'the large tracheal diameter is maintained by cartilaginous rings. The trachea is lined by smooth epithelium' (Wyneken, 2001). The hawksbill is a shallow diver that rarely descends beyond 15 m (e.g. van Dam and Diez, 1996; Storch et al., 2006).

In this study we present evidence derived from adult and hatchling *Dermochelys* that demonstrates great ontogenetic changes in upper tracheal structure which relate to the deep-diving and thermoregulatory abilities of the adults.

MATERIALS AND METHODS
Study specimens

Material was collected from two freshly stranded adult leatherback turtles. The first turtle (Specimen 1) was found close to the shore at Ballycotton, East Cork. A female, it was 168 cm in standard curved carapace length, so would have weighed around 450 kg (see Georges and Fossette, 2006). The head and neck were removed (amputated as close as possible to the body) and transferred to a freezer; the remainder of the carcass was disposed of at sea. The head and neck were later embalmed in the Department of Anatomy, University College Cork. The carotid arteries were isolated on the severed surface of the neck by minor dissection. A large straight artery tube (length 120 mm, diameter 6 mm) was inserted into each artery and clamped into place. A 2% aqueous solution of 38% formaldehyde was pumped into each artery using a Duotronic embalming pump (Modesto, CA, USA) and pumping continued until the solution was observed to be emerging from the cut surface of the muscle tissues (as well as the jugular veins). The carotid arteries were then clamped and the head/neck allowed to settle for 24 h before submerging it in a 2% aqueous solution of 38% formaldehyde, allowing for fixation of the external tissues on the cut surface. Material from this specimen was later prepared for histology.

The second adult turtle (Specimen 2) was observed live in Strangford Lough, Co. Down, Northern Ireland on 18 February 2008. It was found dead (apparently of infection following an earlier, partially healed, severe head wound) on 21 February 2008 and autopsied. During the autopsy the larynx and a short length of upper trachea were removed and fixed/preserved in formalin.

A single hatchling (Specimen 3: Museum code NHM 1969.2804) was dissected under permit at the Natural History Museum, London on 13 June 2008. It had originally been collected in French Guiana in 1969, so was also from the Atlantic population of leatherbacks. It had been stored in alcohol after initial fixing in formalin. Permit and CITES rules prevented removal of material from the Museum, so study was limited to display of the gross anatomy of the larynx, trachea and bronchi.

Computed tomography (CT) scanning

The head and neck of Specimen 1 were CT scanned (1 mm slice acquisition with a bony reconstruction algorithm) using a Siemens Somatom Plus 4 scanner (Erlangen, Germany). Analysis was conducted using Osirix v. 3.21 Software (Osirix Foundation, Geneval, Switzerland) on a Macbook Pro workstation (Cupertina, CA, USA).

Gross anatomy

Gross dissection was conducted on all three specimens to reveal features of the adult and hatchling tracheae, within the limitation that adult material for the lower trachea was not available. Sections of trachea were removed for photography and (in the case of Specimen 1) histological investigation.

The hatchling material was very friable because of the duration of its storage, and some tracheal tearing took place during removal of the thick ventral skin. Images of dissections were captured with a Casio 10.1 megapixel digital camera (Shibuyaku, Tokyo, Japan).

Histology

Specimens of the adult turtle trachea were taken for histology. These were sectioned longitudinally and transversely in order to show the microscopic features of the cartilages, their perichondria and the joints between them, of the mucosa and of the vascularization of all of these, in particular of the mucosa. Staining was with haematoxylin and eosin, and van Giesson and Mallory's trichrome methods. Sections were inspected and photographed with a Canon trinocular camera microscope (Ohta-Ku, Tokyo, Japan) connected to a Dell workstation (Round Rock, TX, USA).

RESULTS
CT scanning

Fig. 1 displays selected CT sections of the head and neck of Specimen 1. The sagittal and coronal sections (Fig. 1A,B) showed that the trachea was wide-bored, with a smooth lumen; the images showed no obvious ring structure and the walls of the trachea appeared continuous and showed no evidence of calcification. The sagittal section showed that the dorsal wall of the trachea in the midline was much thinner (*ca.* 2 mm thickness) than the ventral wall (*ca.* 4 mm). From both of these sections it is evident that the cartilage around the anterior, laryngeal portion of the trachea was much thicker.

The rather rectangular transverse section in Fig. 1C is at the larynx; it showed a small lumen and thick cartilage walls. Posteriorly (Fig. 1D,E) the trachea was elliptical in cross-section. Again, the thicker ventral wall was evident.

Adult dissection

The cut surface of the neck of Specimen 1 (Fig. 2A) shows that the cartilaginous layer of the trachea, at that level, had an elliptical section (shorter diameter dorso-ventral) with the following measurements; external diameter 42×29 mm; inner diameter 33×23 mm; maximum dorsal thickness 2.1 mm, maximum ventral thickness 3.6 mm. From Fig. 2B it may be seen that the trachea was lined by a continuous layer of vascular tissue of varying thickness. Dorsally, in the midline, the vascular tissue was 0.5 mm thick, ventrally 2.9 mm.

Fig. 3 shows details of a short length (38 mm) of the trachea dissected from the cut surface of the neck. It can be seen that the tracheal wall in this region included a near-continuous tube of cartilage plates that form a mosaic, with only small amounts of intervening connective tissue. The rings were more regularly circular
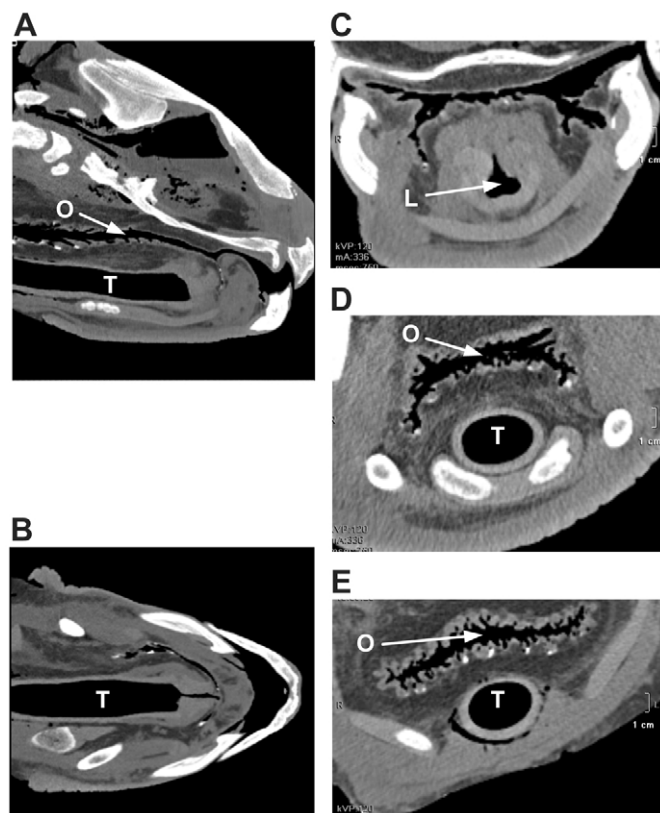

Fig. 1. Selected CT images of the head and neck of adult *Dermochelys coriacea*. (A) Sagittal section, (B) coronal section and (C) transverse section of buccal cavity posterior to tongue. (D) Transverse section in upper oesophageal region. (E) Transverse section in neck. T, trachea; O, oesophagus; L, larynx.
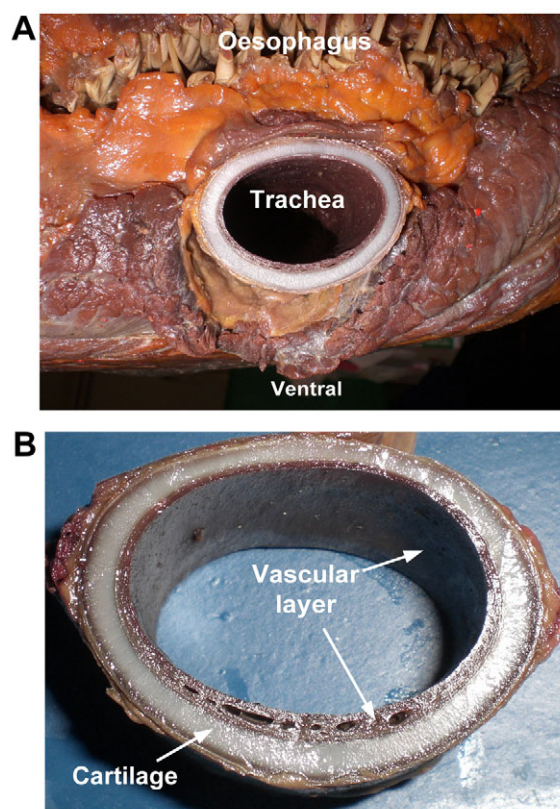

Fig. 2. Gross dissection of adult *Dermochelys coriacea*. (A) Cut surface of the neck of adult *D. coriacea*. Note the thick, yellow adipose layer between the trachea and oesophagus. (B) Close-up of a portion of the trachea removed from the cut surface. Note the thick vascular layer lining the trachea, with large venous vessels visible ventrally.
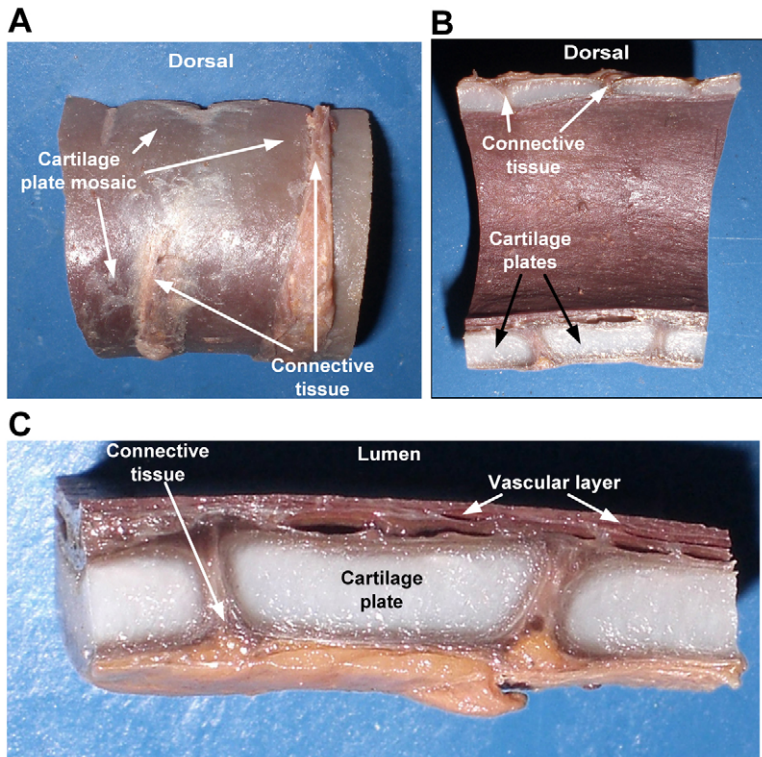
Fig. 3. Gross structure of adult trachea in the neck region (38 mm long portion). (A) External surface with connective tissue removed. In this region of the trachea there are no complete tracheal rings, but a mosaic of cartilage plates with narrow segments of connective tissue (fibrous joints). (B) Luminal surface of trachea lined with vascular tissue. (C) Longitudinal section of a portion of trachea. Note chamfered edges of interlocking cartilage plates.

in the most rostral parts of the trachea. Before the section was dissected further, it was found that the trachea could easily be completely compressed between the fingers, with the thinner dorsal surface bending far more readily than the thicker ventral surface. The luminal surface of this section was lined throughout by vascular tissue. The longitudinal section (Fig. 3C) demonstrates that neighbouring cartilage plates interlocked as they had chamfered edges, lending strength to the tracheal tube.

Fig. 4 shows the larynx taken from Specimen 2. This section of the trachea was also lined with vascular tissue, in which a longitudinal arrangement of blood vessels can be discerned (Fig. 4C). The ventral surface was composed of fused cartilage, with no interruptions save for paired vascular foramina; dorsally the plate/ring structure with intervening connective tissue was more

evident. Even this thicker-walled part of the trachea could be compressed to some extent by finger pressure alone.

Hatchling dissection

The hatchling trachea of Specimen 3 (Fig. 5A,B) consisted of cartilaginous rings separated by near-transparent connective tissue. For most of the tracheal length, the cartilaginous rings were much narrower than the connective tissue areas between them. In the region (coded 2 in Fig. 5A) of the trachea corresponding to the adult neck portions described above, cartilaginous rings made up 32% of the tracheal length, connective tissue 68%. The hatchling thus differed markedly from the adult in terms of the contribution of cartilage to the tracheal wall. Inspection (by binocular microscope) of cut parts of the trachea revealed that there was no sign of a vascular tissue lining to the lumen, while the cross-sectional shape was consistently
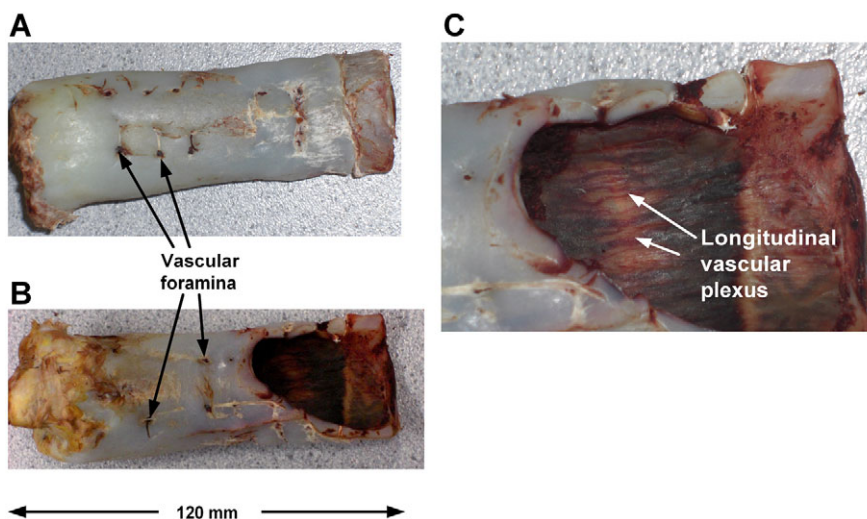

Fig. 4. Upper portion of adult trachea (including larynx). Anterior is to the left of the figure and ends at the opening of the trachea. (A) Dorsal surface of trachea after removal of connective tissue. Note paired vascular foramina. (B) Ventral surface cut obliquely to reveal vascular layer of lumen. (C) Close-up of vascular layer, showing longitudinal pattern of blood vessels within vascular plexus.
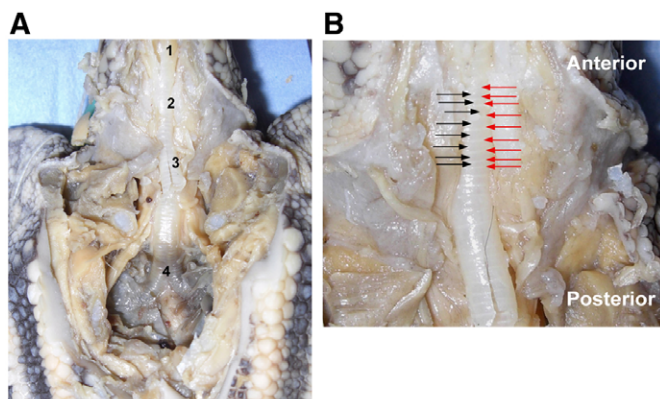
Fig. 5. Dissection of hatchling *Dermochelys coriacea* (Specimen 3: NHM 1969.2804). (A) Demonstration of trachea from larynx (1, ventral surface of larynx removed) to 4 (level of bifurcation into two bronchii); 2 is the region of the trachea corresponding to the trachea of adult Specimen 1; 3 is the region that was inspected for tracheal section shape. (B) Close-up of region 2. Red arrows indicate the position of cartilaginous tracheal rings; black arrows indicate intervening bands of translucent connective tissue.

circular. The ring/connective tissue structure continued anteriorly into the head region; only the larynx immediately posterior to the mandible did not have a ring structure (Fig. 5A).

Dissection continued posteriorly to the level where the trachea bifurcated to give two bronchi. A ringed structure was evident throughout, with cartilaginous rings becoming more numerous posteriorly, with narrower intervening sections of connective tissue. Immediately anterior to the bifurcation, the cartilaginous tracheal rings made up about 70% of the tracheal length, connective tissue 30%.

Histology

The adult cartilages were avascular (Fig. 6A) and were covered by perichondrium (Fig. 6B) which was thickest externally where it consisted of alternating layers, in which the fibrous tissue bundles were arranged at approximately right angles to one another. It contained a few blood vessels. The internal perichondrium (Fig. 6C) was thinner than the external one and lacked the layering of the latter; it was largely avascular. The chondrocytes were relatively densely packed and uniformly distributed (Fig. 6A). Most were in single lacunae.

In the intercartilaginous joints the fibrous tissue was densely packed (Fig. 6D). That in the centre of the joint was finer than elsewhere. Joints contained nerves and also blood vessels which passed between the external and internal surfaces.

The mucosa comprised a background of fibrous tissue in which large blood vessels predominated (Fig. 6C). These varied in diameter, many being about 20 μm wide. They tended to lie in all but the most superficial layers of the mucosa. Most ran longitudinally and there were prominent cross-connections between them. They contained clotted blood, in which the nucleated cells and the serum had
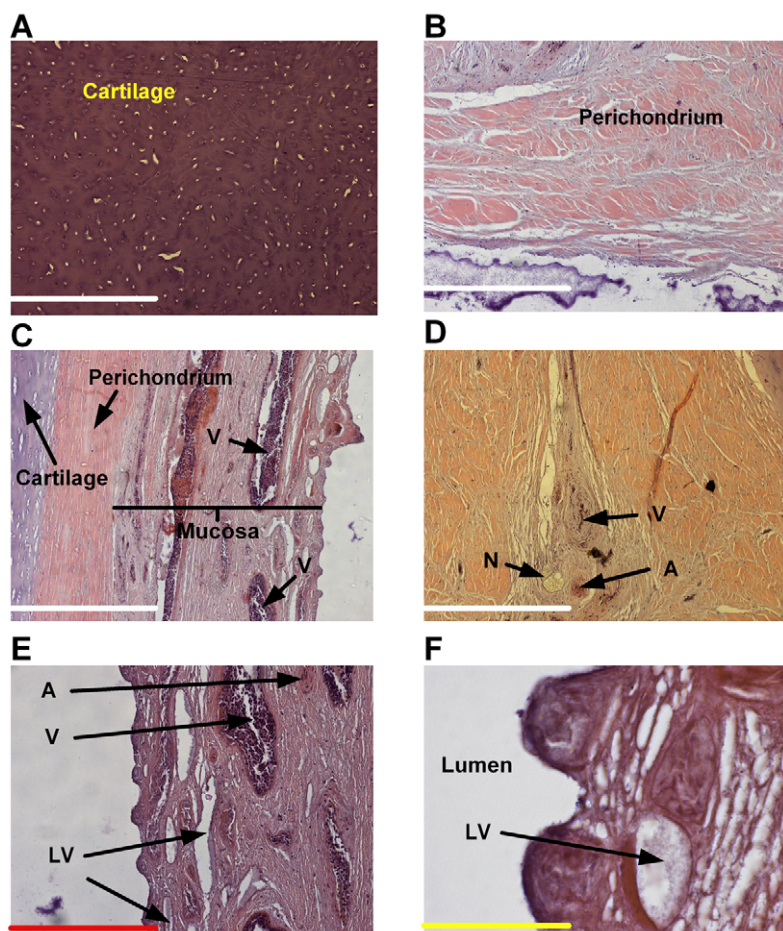

Fig. 6. Histology of adult leatherback trachea. (A) Cartilage. (B) External perichondrium. (C) Section through cartilage, internal perichondrium and mucosa. (D) Section of fibrous joint between cartilaginous plates (not visible) of trachea. (E) Mucosa containing prominent (longitudinally arranged) vascular and lymphatic tissue. (F) Close-up of mucosal surface showing lymphatic vessels and nodules. White scale bars, 50 μm; red scale bar, 25 μm; yellow scale bar, 5 μm; A, artery; V, vein; LV, lymphatic vessel; N, nerve.

undergone post-mortem separation. Vessels were composed of both arteries and veins, distinguishable by their differing wall thicknesses (Fig. 6E). However, in some cases it appeared that the vessel wall was thicker on its mucosal than on its cartilaginous side. Capillaries were not prominent.

Lymphatics were evident towards the mucosal surface, where they formed a dense plexus in places (Fig. 6E,F). Their walls consisted of highly attenuated squamous epithelial cells. Occasional valve-like elements were seen. Their contents were clear or granular; cells were absent. Some were embedded in a reticulum, the interstices of which were flattened and parallel to one another and to the overlying mucosal surface in some cases.

The mucosal surface was generally flat. The appearance of the covering epithelium was unclear, because of post-mortem changes. However, the mucosa overlying the lymphatics was irregular in places and had a prominent nodular appearance (Fig. 6E,F). Some nodules contained a central space surrounded by apparently concentric elements. The exact nature of these features is unclear, but the nodular form and association with lymphatic vessels suggests that they are lymphoid.

DISCUSSION

Air-breathing deep divers face a diverse suite of physiological challenges. As well as requiring adequate myoglobin and haemoglobin oxygen stores to support prolonged dives, they need to cope with the interaction of gas volume in the respiratory tract with great changes in ambient pressure. Compression of the respiratory air has the potential to cause collapse of the lungs, trachea and bronchial tree as the animal dives. Indeed, it is desirable that the lungs should collapse (progressively from the periphery to the central parts of the lungs to prevent trapping of air) to minimize exchange of gas between the respiratory tract and the blood. Any gas exchange of the compressed air with the blood can theoretically cause problems of nitrogen narcosis at depth, or decompression sickness ('bends') on a return to the surface. A consequent decrease in body volume could impose secondary problems of structural integrity in the thoracic region ('squeeze'). Deep-diving marine mammals have been extensively studied and the means by which they avoid or minimize these problems is now well established. Some allow the lungs and thorax to collapse during the first 25–50 m of dives [e.g. in phocid seals (Falke et al., 1985)], thus avoiding the 'squeeze', and minimizing significant interaction of pulmonary gas with the circulatory system. However, full alveolar collapse does not occur until much greater depths in otariid seals (Kooyman and Sinnett, 1982), while chronic bone damage caused by nitrogen emboli ('bends') is characteristic of some fossil and extant diving reptiles and mammals (e.g. Moore and Early, 2004; Rothschild and Martin, 2005), so a certain amount of interaction still takes place. Reinflation of the lungs on ascent in marine mammals depends upon the presence of surfactants (Spragg et al., 2004). Because the respiratory tract has graded rigidity, with the upper portions of the respiratory tract being more rigid than the lower portions, compressed air moves from the highly flexible lungs into the bronchial tree and trachea as the animals dive. The tracheae of terrestrial mammals, like those of most air-breathing vertebrates, consist of relatively narrow cartilaginous rings (incomplete dorsally in most mammals, but complete in birds and many reptiles) interspersed with wide sections of relatively thin connective tissue. This arrangement allows great flexibility, especially in the neck region, but is a poor structural design for resisting mechanical compression. While shallow diving marine mammals (e.g. sea otters, *Enhydra lutris*) tend to have calcified tracheal rings and greatly

reduced amounts of connective tissue, deep divers such as the harp seal *Pagophilus groenlandicus*, which dives beyond 200 m, have cartilaginous rings, although much expanded and overlapping, with some ring fusion (Tarasoff and Kooyman, 1973). Functionally it appears that the deep diving mammals have a cartilaginous tracheal design that will facilitate progressive collapse with increasing depth, whereas shallow divers have rigid upper portions of the tracheae that remain patent during dives.

Three novel findings for *Dermochelys* were revealed by this study. First, it is evident that great changes in tracheal structure occur between the hatchling and adult stages. Second, the adult leatherback trachea has a structure markedly different from that of other living sea turtles. It consists of an elliptical tube of near-continuous uncalcified cartilage, rather than a sequence of circular, closely packed tracheal rings. The tube is easily compressible, with the thinner dorsal sector of the tube being much easier to compress than the thicker ventral sector. Third, the trachea is lined throughout by a continuous vascular plexus. This contains a high proportion of longitudinally arranged, large-diameter blood vessels lying mainly in the deeper two-thirds of the mucosa, with prominent cross-connections between them. The arrangement is consistent with their functioning as a counter-current arrangement, retaining heat and maintaining body temperature. The predominant, densely packed, relatively large calibre veins in the mucosa resemble the arrangement found in, for example, human nasal mucosa. *Inter alia*, this arrangement is compatible with a role in warming the inspired air. The continuity between the mucosal blood vessels and those around the trachea through communications in the fibrous joints, facilitates outward flow of the blood when intratracheal pressure is relatively high, as in diving, when the warming function is unnecessary.

The compressible, elliptical cartilaginous tracheal tube is clearly an adaptation to the species' impressive adult diving abilities. As the turtle dives and its lungs collapse, air will move into the upper parts of the respiratory tract, minimizing problems of nitrogen exchange. Progressive collapse of the trachea itself will offset the development of pressure gradients, and it seems probable that only a small volume of air will remain in the laryngeal region at the greatest depths that this species reaches. This tracheal design is similar to that seen in delphinid cetaceans (Fanning and Harrison, 1974), though in cetaceans the trachea is much shorter, as the animals are effectively neck-less, while some of the tracheal rings are incomplete dorsally. Mechanically the leatherback design appears superior, but will inevitably have an effect on neck mobility. Because the adult leatherback has an elliptical pipe of near-continuous cartilage running from just behind the mandible through the neck into the body, the adult's ability to move the head laterally will be reduced in comparison with the hatchling (and probably in comparison with cheloniid sea turtles). However, the prey of adult leatherbacks is slow moving and lacks manoeuvrability, so this will not be a significant disadvantage.

A deficiency of the present study is that we did not have access to respiratory tract material caudal to the neck region (it was not logistically feasible to preserve whole turtles). We therefore do not know whether the observed chondrification of the trachea extends rearwards as far as the bifurcation into the bronchii (or even into the bronchii themselves). Further investigation will require access to new material.

The finding of a continuous mucosal lining of vascular tissue in the adult trachea of *Dermochelys* is unique amongst living sea turtles, indeed amongst reptiles. The adult leatherback can sustain elevated core body temperatures in cold temperate waters by a suite of

anatomical, metabolic and behavioural features (for review, see Wallace and Jones, 2008). Whether *Dermochelys* is gigantothermic or endothermic has provoked much discussion, but participants in the debate do not challenge the existence of a considerable thermal gradient between the core and the outside world (although this gradient is usually considered in the context of external water temperature, not air temperature). A feature of endothermic birds and mammals is that both groups have separately evolved nasal turbinate bones (complex thin-walled calcareous structures with erectile vascular tissue covering). Their function is to warm and humidify inspired air before delivery to the lungs; they also recover respiratory heat and water during expiration. The possession of turbinates has been regarded as diagnostic of endothermy (Hillenius, 1992) and living reptiles do not possess them. We believe that the vascular lining of the long adult leatherback trachea functions in analogous fashion to nasal turbinates. At high latitude a turtle taking a breath will inspire air of much lower temperature than the core temperature. As it travels along the trachea the air will be progressively warmed by the mucosal vascular plexus. The mucosa may have a thermal gradient along it if there is an opposed layout of longitudinal arterial and venous vessels, which would further enhance conservation of heat. Histologically this seems highly likely. If the air is less than saturated by water vapour, it will also be humidified. Expired air will be cooled and will lose water by condensation before release to the environment. Study of leatherback breathing behaviour and frequencies (Reina et al., 2005) revealed that *Dermochelys* exhales in about 1 s and immediately inhales, again in about 1 s. This breathing cycle occurs while the animal lifts its head out of the water, before it is lowered until the next breath. Leatherbacks breathe at about 4 breaths min⁻¹ when at the surface after dives. This implies long pauses (*ca.* 12–13 s) between breaths and contrasts strongly with the breathing behaviour of a marine pinniped such as the elephant seal *Mirounga angustirostris*, in which adults breath at about 15 breaths min⁻¹ in continuous fashion during short surface intervals after dives (Le Bouef et al., 2000). Because of the long pauses between breaths, it is feasible that the non-pulmonary fraction of the inspired air (i.e. that in the larynx, trachea and bronchii) will warm to central body temperature and that some of its heat will be lost on expiration, negating (at least to some extent) the heat conservation aspects of the counter-current system. Much depends on details of the control of blood flow through the tracheal mucosa. At present, experimental study to elucidate further details of the mechanism is not feasible. Only during nesting are live adult females readily accessible – and then on tropical beaches where temperature differences between air and turtle core are much smaller. Indeed, during heavy exercise when climbing tropical beaches and during the nesting process, heat loss *via* the respiratory system may be desirable, and the mucosal blood flow adjusted appropriately.

Intriguingly, delphinic cetaceans do not possess turbinates. In a recent paper (Cozzi et al., 2005) it was demonstrated that the trachea of the striped dolphin *Stenella coeruleoalba* (which dives to around 200 m) has a similar erectile vascular tracheal lining. They interpreted the existence of the mucosal layer as being an adaptation to reinflate the collapsed trachea after dives. We believe that the elasticity of tracheal cartilage (combined with expansion of the remaining tracheal air during ascents) will be sufficient to reinflate the trachea, and that the vascular lining of the dolphin trachea also helps maintenance of respiratory temperature and water balance.

The finding of substantial tracheal structural changes between the hatchling and adult stages is of great interest. In terms of morphological development, the increase in the proportion of cartilage

that takes place evidently results from marked chondrification of the tracheal wall. This process is regular in rostral parts of the trachea, since the adult rings are regular at that level. However, it is irregular caudally, since the adjacent edges of the rings come to be reciprocally curved and interdigitated. Nevertheless, the end result is similar in the two locations: by far the greater part of the tracheal wall is cartilaginous. This confers homogeneity and strength on it, and provides a consistent pattern of elasticity and resistance to compression around its circumference. This will facilitate the thousands of cycles of compression and reinflation that occur during the species' foraging dives. Eckert (Eckert, 2002) reported that juvenile leatherbacks (<100 cm carapace length) were not found in cool surface waters. However, so far there have been no opportunities to study diving behaviour in juvenile and subadult animals, so we do not know whether such juveniles can also not dive. *Dermochelys* is an endangered, protected species, so it will be necessary to wait for juvenile specimens to become available for dissection before the developmental changes in tracheal structure can be investigated further. The recent growth studies of Jones (Jones, 2009) yielded specimens up to 2 years old and more than 40 kg in body mass. Live animals reared in this fashion might be subject to CT scanning (as well as fitted with satellite tags and released to determine juvenile diving capabilities). They might also be subject (within ethical limitations) to investigations of respiratory physiology, while any that died of natural causes could profitably be dissected.

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