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Functional morphology of the nasal region of a hammerhead shark

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Abstract

We describe several novel morphological features in the nasal region of the hammerhead shark *Sphyrna tudes*. Unlike the open, rounded incurrent nostril of non-hammerhead shark species, the incurrent nostril of *Sphyrna tudes* is a thin keyhole-like aperture. We discovered a groove running anterior and parallel to the incurrent nostril. This groove, dubbed the minor nasal groove to distinguish it from the larger, previously described, (major) nasal groove, is common to all eight hammerhead species. Using life-size plastic models generated at 200 µm resolution from an X-ray scan, we also investigated flow in the nasal region. Even modest oncoming flow speeds stimulate extensive, but not complete, circulation within the model olfactory chamber, with flow passing through the two main olfactory channels. Flow crossed from one channel to another via a gap in the olfactory array, sometimes guided by the interlamellar channels. Major and minor nasal grooves, as well as directing flow into the olfactory chamber, can, in conjunction with the nasal bridge separating incurrent and excurrent nostrils, limit flow passing into the olfactory chamber, possibly to protect the delicate nasal structures. This is the first simulation of internal flow within the olfactory chamber of a shark.

Keywords: Flow visualisation, hammerhead shark, olfactory organ, X-ray micro-computed tomography.

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1. Introduction

The gross morphology of the nasal region of a fish¹ determines how flow behaves in this region, and therefore has an important influence on the fish's olfactory abilities (Cox 2008). In this report we describe the functional morphology of the nasal region of a hammerhead shark. To put the work in context, we first present what is known about the functional morphology of the nasal region of actively swimming sharks in general and then hammerheads in particular. We then briefly review previous hydrodynamics studies of the nasal regions of fish.

1.1 Functional morphology of the nasal region of sharks with an active habit

Typically sharks have two olfactory organs placed symmetrically towards the front and on either side of the head (Fig. 1A; Tester 1963; Herberhold 1969; Zeiske et al., 1986; Theisen et al., 1986; Zeiske et al., 1987; Bell 1993). Each organ has a nostril through which flow enters the olfactory chamber - the incurrent nostril - and a nostril through which flow leaves the chamber - the excurrent nostril (Figs. 1A and B). In sharks with an active habit, such as the spiny dogfish, *Squalus acanthias*, the nasal region is isolated from the mouth (Theisen et al., 1986, Fig. 1A). Both incurrent and excurrent nostrils lie close to each other (Figs. 1A and B; Zeiske et al., 1986, Fig. 1). They are separated from each other by a complex set of internal appendages (Zeiske et al., 1986, Figs. 1 and 3). In this report we refer to this collection of appendages as the nasal bridge². Generally, the incurrent nostril has a semicircular appearance, with a rounded rim (Theisen et al., 1986, Fig. 1B). A shallow depression may precede it (Zeiske et al., 1987, Fig. 2D). This depression, along with the incurrent nostril, is presumed to guide flow into the olfactory chamber (Zeiske et al., 1986, p. 389). The excurrent nostril is often characterised by the presence of a triangular fold (Theisen et al., 1986, Fig. 1B), thought to stop flow passing into the olfactory chamber via this nostril (Theisen et al., 1986, p. 81). The excurrent nostril may be linked to a shallow posterior depression (Zeiske et al., 1987, Fig. 2D).

Fig. 1

The ellipsoidal olfactory chamber of a shark (Figs. 1A and B) is almost completely filled with several tens of wing-shaped folds, or lamellae, arranged in pairs and stacked in parallel; a central ridge known as a raphe separates the lamellae into two separate rows (Fig. 1C; Zeiske et al., 1986, Fig. 2). The lamellae are subdivided into secondary folds (up to at least 30 - Zeiske et al., 1987, Fig.

¹ By which we mean both the internal and external features of the olfactory organ.

² This term should not, however, be confused with the nasal bridge of bony fish (Kleerekooper 1969, p. 51).

5A), giving their surfaces a fan-like appearance (Fig. 1C). In transverse section the lamellae appear pinnate (Fig. 1D; Fishelson and Baranes 1997, Fig. 3). Secondary folds on adjacent lamellae create a single, continuous, albeit convoluted, channel (Fig. 1D; Zeiske et al., 1986, Fig. 2B). This interlamellar channel is narrow, with a hydraulic diameter (White 2003, p. 376) of approximately 100 μm (Appendix A1).

The sensory epithelium is located on the ridges and in the troughs of the secondary folds of the olfactory lamellae; it consists of olfactory receptor cells, fluid-propelling multiciliated non-sensory cells and the occasional goblet cell (Zeiske et al., 1986, p. 385; Theisen et al., 1986, p. 77; Zeiske et al., 1987, p. 2409). Multiciliated non-sensory cells form the major component of the sensory epithelium, and indeed their cilia fully cover it (Schluessel et al., 2008, p. 1377).

The apical sections of lamellae in adjacent rows of the olfactory array of a shark physically contact each other, creating two separate channels, one directly above the raphe, the other above the lamellar array (Fig. 1C, inset; Zeiske et al., 1986, p. 384 and Fig. 2). The channel lying directly above the raphe is connected to the incurrent nostril, and the channel above the lamellar array is connected to the excurrent nostril (Zeiske et al., 1986, p. 384). Consequently these channels are referred to here as the incurrent and excurrent channels, respectively (Fig. 1C, inset). The excurrent channel appears considerably larger in cross-section than the incurrent channel (Zeiske et al., 1986, Fig. 2A; see also Tester 1963, Fig. 6).

Where the incurrent and excurrent nostrils meet, one or two additional extensions from the sides of the olfactory chamber ('valve flaps' in the terminology of Theisen et al., 1986, p. 74 – they are part of the nasal bridge mentioned above) insert between the free edges of the lamellae to create three separate channels (Zeiske et al., 1986, Fig. 3). One of these channels is a continuation of the incurrent channel. The other two channels are blind subsidiary excurrent channels which feed the main excurrent channel. Zeiske et al. (1986, p. 384) refer to these two subsidiary excurrent channels as galleries.

Passage of water through the olfactory organ is thought to occur as follows. Flow entering the incurrent nostril passes along the incurrent channel (Fig. 1E; Zeiske et al., 1986, Fig. 2A), where it is then drawn through the intricately branched interlamellar channels (Figs. 1D and E). Flow then passes out of the interlamellar channels into peripheral channels on the outer lamellar edges, and from these channels into the main excurrent channel or galleries (Fig. 1E; Zeiske et al., 1986, Fig.

2A). Flow in the galleries passes into the main excurrent channel. Flow in the main excurrent channel passes out of the olfactory chamber via the excurrent nostril.

In sharks with an active habit, flow through the olfactory chamber is probably generated by a pressure difference between the incurrent and excurrent nostrils arising largely from the forward motion of the fish (Zeiske et al., 1986, p. 389; Theisen et al., 1986, p. 81; Zeiske et al., 1987, p. 2410; Chi et al., 2004). The pressure difference itself arises because the forward-facing incurrent nostril experiences both the static and dynamic pressure of the flow and the rear-facing excurrent nostril experiences only the static pressure (Cox 2008, p. 583). In addition, flow over the excurrent nostril could draw flow from that aperture by viscous entrainment (Cox 2008, p. 583).

Whether the multiciliated non-sensory cells in the shark's olfactory epithelium are water-propelling or mucus-propelling is not known. The olfactory epithelium may even contain separate populations of both types of cell. Zeiske et al. (1986, p. 388; 1987, p. 2409) could not detect water flowing through the olfactory chamber of a stationary, inverted lemon shark, suggesting that if water-propelling multiciliated non-sensory cells are present in the olfactory chamber, the water currents they produce are weak. Consequently, flow through the interlamellar channels in this instance is probably generated by a pressure difference arising from the externally induced flow in the incurrent and excurrent channels. However, these are suppositions based on just one preliminary experiment.

Excessive flow through the olfactory chamber could potentially damage its lamellae. Zeiske et al. (1986, p. 389) proposed several mechanisms that might regulate flow through the chamber, thereby avoiding this damage. Externally, flow encountering the incurrent nostril could be directed away from it by the nasal bridge, with the diverted flow passing into the shallow depression posterior to the excurrent nostril. Internally, flow through the olfactory chamber could be 'short-circuited' by, for example, the physical contact between the apices of lamellae in adjacent rows of the olfactory array (Fig. 1C) being broken, creating a new channel between incurrent and excurrent channels.

1.2 Functional morphology of the nasal region of hammerhead sharks

Hammerhead sharks are of course distinguished from all other sharks by their bilaterally expanded and flattened heads. There are eight recognised species of hammerhead shark (Nelson 1994, p. 50; Compagno 1988, p. 367 and p. 370), each with a unique cranial morphology, ranging from the greatly extended wings of *Eusphyra blochii*, to the spade-shaped head of *Sphyrna tiburo* (Gilbert 1967, Fig. 22). The subject of this report, the smalleye or golden hammerhead (Castro 1989, p. 3),

Sphyrna tudes, has what one might consider to be a more classical head shape (Fig. 2), and one that is very similar to the scalloped hammerhead, *Sphyrna lewini*. This point is worth emphasising, since we used the swimming behaviour of *Sphyrna lewini* to inform the flow visualisation experiments reported here.

Fig. 2 (Colour in Web version; black and white in print version)

Incurrent and excurrent nostrils of the paired olfactory organs of a hammerhead shark (the following description relates mainly to the scalloped hammerhead, *Sphyrna lewini*, although reference is also made to the figures of *Sphyrna tudes* accompanying this report) are situated on the anterior edge of the head (Figs. 2 and 3A; Tester 1963, Fig. 2). The two nasal regions are widely spaced (Parker 1914, p. 68; Gilbert 1962, p. 62), suggesting an enhanced ability to locate an odour source by klinotaxis (Kajiura et al., 2005, pp. 253-254). The nasal regions are also remote from the mouth, indicating that 1) internal cranial movements are unlikely to assist water circulation within the olfactory chamber (E. Zeiske and J. Kux, personal communication) and 2) the pressure difference between incurrent and excurrent nostrils presumed to drive flow through the olfactory chamber must be largely induced by forward motion of the fish. In actual fact, hammerheads are obligate swimmers (Lowe 1996, p. 2605), relying on ram ventilation (Vogel 1994, pp. 68-69) to produce the respiratory current. The respiratory current cannot therefore contribute to flow through the olfactory chamber, as it does for example in the small-spotted catshark, *Scyliorhinus canicula* (Theisen et al., 1986, p. 81). The anterior location of the incurrent nostrils should generate maximum positive pressure at the incurrent nostrils (Vogel 1994, p. 68) - approximately 100 to 2,000 Pa (Appendix A2) - and also reduce or remove the delay in detecting potential odorants incurred by the boundary layer associated with the swimming fish (Denny 1993, pp. 138-140; Cox 2008, pp. 578-579). There is no information in the literature on the form of the incurrent nostril, apart from the fact that it is a 'relatively large opening' (Tester 1963, p. 256). However, it is known that in four species – *Eusphyra blochii*, *Sphyrna zygaena*, *Sphyrna lewini* and *Sphyrna tudes*, a 'deep, narrow groove' extends along the anterior edge of the head to link with the incurrent nostril (Fig. 3A; Tester 1963, Fig. 2 and p. 256; Gilbert 1967, Table 1). This is often referred to as the prenarial groove (Kajiura et al., 2005, p. 254), but in light of our discovery of another, smaller nasal groove, we now refer to it as the major nasal groove. Like other sharks, the excurrent nostril of the hammerhead nasal region is characterised by the presence of a triangular fold (Figs. 3 and 4; Tester 1963, p. 256; Kajiura et al., 2005, Fig. 3). Incurrent and excurrent nostrils are separated by a 'small, concealed, fleshy flap' (Tester 1963, p. 256) presumably equivalent to the nasal bridge of other sharks.

Fig. 3 (Colour in Web version; black and white in print version)

Fig. 4 (Colour in Web version; black and white in print version)

Each elongated olfactory chamber lies towards the front edge of the head (Meng and Yin 1981, pp. 16-17; Kajiura et al., 2005, Fig. 3) and may contain up to 150 or so pairs (i.e. rows) of olfactory lamellae (Kajiura et al., 2005, Table 2). Although this is a considerable number of lamellae, due to the bilaterally expanded and flattened head the lamellae are relatively small and the overall olfactory surface area is the same as or even smaller than that of other, non-hammerhead species of sharks (Kajiura et al., 2005, p. 261). The olfactory lamellae appear similar in shape and structure to those of other sharks (Tester 1963, Fig. 7; Schluessel et al., 2008, Fig. 6S): they have secondary folds, and when in an array the lamellae would give rise to channels corresponding to the incurrent and excurrent channels. Although there is no detailed information on the olfactory epithelium of hammerhead sharks, it is reasonable to suppose that, given the similarity of their olfactory lamellae to other sharks, their lamellar surfaces will possess a substantial population of multiciliated non-sensory cells.

The major nasal groove is thought to direct flow arising from the forward motion of the fish into the incurrent nostril (Tester 1963, p. 256). Thus it may be thought of as an exaggerated version of the anterior nasal depression of other sharks (Zeiske et al., 1987, Fig. 2D). Flow passing through the incurrent nostril presumably circulates within, and passes out of, the olfactory chamber in a manner similar to that proposed for other sharks with an active habit.

1.3 Previous hydrodynamics studies of the nasal regions of fish

Currently there is only one detailed hydrodynamics study relating to the olfactory organ of a fish (Kux et al., 1977, 1978, 1988). This study concerned two fish from the order Cyprinodontiformes (Nelson 1994, p. 269). Flow through the olfactory chambers of these fish is generated by the pumping action of a single accessory sac (Zeiske 1973, 1974; Zeiske et al., 1976). Reference is made in passing to another, unpublished, study relating to the hydrodynamics of the olfactory organ of the adult garpike, *Belone belone* (Zeiske and Hansen 2005, p. 22), which is exposed and relies on the motion of the fish for its ventilation (Theisen et al., 1980; Cox 2008, p. 587). Both ventilation mechanisms are quite different from the proposed ventilation mechanism discussed above for the shark olfactory organ.

There are two brief reports in the literature of hydrodynamics studies relating to the nasal region of sharks. The subject of the first (Metcalf 1973) is in fact the subject of this paper, *Sphyrna tudes*, but details in this report are entirely lacking. In the second report, Chi et al. (2004) outlined some results obtained from flow visualisation studies of the nasal region of *Squalus acanthias*. They confirmed that flow through the olfactory organ could be induced by forward movement of the fish.

The description of the gross morphology of the nasal region of *Sphyrna tudes* given here is based on visual inspection of a museum specimen together with X-ray micro-computed tomography (micro-CT; e.g. Mazik et al., 2008) of this specimen. The three-dimensional data set from the micro-CT was used to make life-size plastic models of the *Sphyrna tudes* head, which were then used for flow visualisation studies in a water tunnel.

2. Materials and Methods

2.1 Specimens

The specimen of *Sphyrna tudes* used to make the models described below resides in the Natural History Museum, London (Table 1). It was collected by trawl off the coast of Guyana in 1959 and has been preserved in 70 % industrial methylated spirits, 30 % distilled water ever since. As a result, it may have experienced some shrinkage (Nadeau et al., 2009, and references therein). The specimen consists of the head and part of the gill region (Fig. 2). The sex of the specimen is unknown. The total length of the specimen is estimated to be approximately 90 cm (Appendix A3). Since adult *Sphyrna tudes* vary in size from 1 to 1.5 m (Compagno 1984, p.552), this specimen was probably a juvenile approaching maturity.

The presence of a minor nasal groove (Section 3.1) in the other seven hammerhead species was investigated by inspecting museum specimens (*Eusphyra blochii*, *Sphyrna lewini*, *Sphyrna tiburo* and *Sphyrna zygaena*) or photographs of museum specimens (*Sphyrna corona*, *Sphyrna media* and *Sphyrna mokarran*) (Table1).

Table 1

2.2 Micro-CT

After removal of the specimen from the preservative it was mounted for X-ray scanning in a cling film-covered recess cut from a block of florist's foam. Each olfactory chamber was emptied of preservative prior to the scan. Scanning was performed using an HMXST 225 CT system (Metris

X-Tek, Tring, UK). The X-rays were generated from a tungsten target using a voltage and current of 180 kV and 105 μA , respectively. A total of 3,142 angular projections were collected at 0.1146° intervals in a single 360° rotation. The radial projections were reconstructed into a three-dimensional matrix of 1,897 x 1,830 x 630 (L x W x H) 124.5 μm cubic voxels using the software package CT-Pro (Version 2.0, Metris X-Tek, Tring, UK). To generate the stereolithography files described below, the matrix was exported as a set of 1,897 DICOM images (<http://medical.nema.org/>) in the sagittal plane (Evans 1993, pp. x-xi). The number of lamellae in the olfactory array of *Sphyrna tudes* was estimated from another set of DICOM images in the transverse plane.

2.3 Generation of stereolithography files and plastic models

The set of 1,897 DICOM images was converted into several stereolithography (STL) files using the image-processing software ScanIP (Simpleware Ltd, Exeter, UK). Four STL files were generated, corresponding to: 1) the entire head; 2) the entire head minus the right nasal region; 3) the right nasal region; 4) the olfactory channels of the right nasal region, including the incurrent and excurrent channels, the interlamellar channels and the two galleries. To create the STL file corresponding to the entire head, the DICOM images were imported into ScanIP and cropped in the *x*-direction to remove the gill region (a section approximately 3 cm thick), ultimately creating a flat surface at the back of the model. This was used to attach the aluminium mounting required for the flow visualisation experiments (Section 2.6). In order to generate an STL file of a manageable size, the data were resampled with a pixel spacing of 200 μm . The Floodfill segmentation tool was used to fill unwanted internal cavities in the data. Surface artifacts were removed using the Recursive Gaussian filter after application of a binarisation filter. Some features in the olfactory chamber were manually refined using the Paint segmentation tool. To generate the STL file, the Pre- and Post-smoothing options were selected; in addition, the number of triangles was reduced by 50% (Decimation). Pre-smoothing preserves the volume and topology of the original structure. STL files were generated in binary format. STL files for the other three models were generated from the same set of cropped images, essentially in the manner described. All STL files have a resolution of 200 μm x 200 μm x 200 μm .

STL files 1, 2 and 4 were converted into plastic models by Laser Lines Ltd (Banbury, UK) using fused deposition modelling (FDM). The models were built on a Dimension Elite 3D printer from a copolymer of acrylonitrile, butadiene and styrene (ABSplus; printer and material both Stratasys, Eden Prairie, USA). In FDM the three-dimensional part is created in layers. The Dimension Elite 3D printer produces parts with layers 178 μm thick, i.e. with a slightly greater accuracy than the

STL files. The layers in the ABSplus models of the complete and incomplete heads were perpendicular to the body axis (Fig. 2). The interior of the ABSplus models consisted of a three-dimensional lattice with hole sizes in the range 2.5 – 3 mm. The models were not waterproofed since it was found that the sealant partially blocked the incurrent nostril and altered the surface features. The ABSplus models were an off-white colour, providing good contrast with the red dye used for flow visualisation (Section 2.6). In addition, a transparent model of the right nasal region was made by Ogle Models and Prototypes Ltd (Letchworth, UK) using stereolithography. The model was built using the Viper SLA system from Accura 60 plastic (printer and material both 3D Systems, Rock Hill, South Carolina, USA). Like FDM, stereolithography produces layered parts; in this case the layers were 50 µm thick. Following stereolithography the part was polished. The transparent model was glued to the ABSplus model of the entire head lacking the right nasal region. In the ensuing text this hybrid model of the head is referred to as the transparent model and the ABSplus model of the complete head the opaque model. All models are actual size.

2.4 Accuracy of the models

In general, the external surfaces of the plastic models of the *Sphyrna tudes* head are faithful representations of the actual specimen. Details such as the openings of the ampullae of Lorenzini (Schmidt-Nielsen 1997, p. 558) on the dorsal and ventral surfaces of the head (Kajiura 2001, Fig. 2), the sharp edges of the excurrent nostrils and the slightly jagged nature of the posterior edge of the wings of the specimen have all been reproduced. However, the nasal grooves are not as clearly defined as in the specimen, and the detail of the small, slightly forward-pointing flap on the ventral edge X of the excurrent nostril (Figs. 3B and 4) has been lost.

Internally, although the incurrent and excurrent channels and two galleries are clearly defined, the olfactory lamellae and the interlamellar channels are not - only about 40 of the possible 180 or so interlamellar channels are discernible in the transparent model and the model of the olfactory channels (Fig. 5). The larger channels evident in these two models correspond to those between adjacent clumps of lamellae, which are larger than those between adhered lamellae (Fig. 6A and Fig. 6B, arrow). Details of the lamellar array that can be seen through the incurrent nostril of the specimen (Fig. 3B) have been lost in the models.

Fig. 5 (Colour in Web version; black and white in print version)

Fig. 6

2.5 Observations of swimming scalloped hammerhead sharks

Observations of swimming adult scalloped hammerhead sharks (*Sphyrna lewini*, three individuals) used to inform the flow visualisation experiments were made at the Outer Bay exhibit of the Monterey Bay Aquarium, California.

2.6 Flow visualisation

Flow visualisation experiments were conducted in closed-circuit free-surface water tunnels in the engineering departments of Cambridge and Bath. Details of the individual water tunnels are given in Appendix A4. Speed of flow in the water tunnels was altered between 20, 30 and 40 cm s⁻¹. Swimming speeds of unconstrained (juvenile) *Sphyrna lewini* range from 0.6 to 2 total lengths per second (Lowe 1996, p. 2607). Thus, assuming that the swimming behaviour of *Sphyrna tudes* is similar to that of *Sphyrna lewini*, with an estimated total length of 90 cm (Appendix A3) the minimal swimming speed of the *Sphyrna tudes* specimen described here would have been 54 cm s⁻¹. Therefore, at 0.4 - 0.7 of the estimated minimal swimming speed of our specimen, the speed of flow in the water tunnels was not ideal.

Experiments with the opaque model were performed in the Cambridge water tunnel. Most experiments with the transparent model were performed in the Bath water tunnel. For all experiments, an aluminium block was attached to the back of the model heads and the models held in the tunnels by three vertical stainless steel struts descending to the aluminium block from a support placed across the top of the tunnel. In this arrangement the models were approximately central (± 2 cm) width-wise to the working section of the tunnel. The models were positioned such that the plane of the wings was parallel either to the base of the water tunnel (opaque model) or to its sides (transparent model). The angle of attack (Vogel 1994, Fig. 11.1) was 0°. This is generally how *Sphyrna lewini* hold their heads when swimming (their heads are in fact capable of moving between -15° to +32° in the vertical plane; Nakaya 1995, p. 332). Although we noted that *Sphyrna lewini* can sweep their heads through an arc of up to 40° in the horizontal plane during their anguilliform/subcarangiform swimming movement (Vogel 1994, pp. 280-283; Lowe 1996, p. 2608), we only explored part of this arc. Thus the body axis (Fig. 2) of the transparent model was held in three positions relative to the oncoming flow: 1) parallel; 2) inclined approximately 10° downwards (i.e. to the left of the swimming shark); 2) inclined approximately 15° upwards (i.e. to the right of the swimming shark). In these three positions the incurrent nostril of the right nasal region of the model head was respectively 28, 33 or 27 cm from the base of the Bath water tunnel. Flow visualisation experiments focused on the right nasal region. The cross-sectional area of the model heads in frontal profile (equivalent to the view in Fig. 3A) does not exceed 57 cm²; the effect

on flow in the vicinity of the models from the walls of the tank is therefore negligible, based on standard corrections (Barlow et al., 1999, p. 361). The models were illuminated with halogen lamps from below (opaque) or simultaneously from the side and behind (transparent). Visualisation was aided by white sheet placed behind the models.

The dye used for visualising flow was red food colouring, diluted in a ratio of 4 parts water to one part dye to ensure that it was neutrally buoyant and therefore gave an accurate portrayal of flow (Lim 2000, p. 44). It was introduced via 1 or 2 mm external diameter horizontal stainless steel tubing. A smooth, well-defined dye filament emitted from the horizontal tubing indicated that dye and freestream velocities were matched (Lim 2000, Fig. 3.1). Dye was directed at the anterior edge of the head in five different locations (Fig. 3C): 1) the lateral region of the incurrent nostril; 2) the middle of the incurrent nostril; 3) the lateral region of the major nasal groove; 4) the middle of the major nasal groove; 5) towards the medial edge of the major nasal groove. The distance from the tip of the horizontal tubing to the anterior edge of the models varied between 3 and 15 cm, sufficient to avoid spurious effects. To examine the fate of the flow leaving the excurrent nostril, the model olfactory chamber was manually injected with dye via the incurrent nostril using a syringe equipped with a 1.5 mm external diameter needle. This experiment was performed at 20 cm s^{-1} .

Video footage of the flow visualisation experiments was taken with digital video camcorders, recording at 25 frames s^{-1} on to MiniDV videotape. Footage was then transferred to DVD for analysis. Typically the footage was of the ventral surface of the model head.

Results

3.1 Gross morphology of the nasal region

We found the incurrent nostril of *Sphyrna tudes* to be a narrow elongated aperture (Fig. 3A) with a slightly expanded medial portion (K in Fig. 4), giving the overall impression of a thin keyhole. The main axis of the incurrent nostril is inclined at an angle of approximately 75° to the body axis. In an anterior view of the head, the expanded medial portion of the incurrent nostril is obscured by the lateral edge of the major nasal groove (Fig. 3A). The raphe and incurrent aperture are broadly co-axial (Fig. 3B).

In transverse section the micro-CT data set shows the incurrent nostril to be a narrow channel (circle in Fig. 6C) linked to an incurrent channel typical of shark olfactory lamellar arrays (Section 1.1). The dorsal and ventral walls of this narrow channel are formed by slim infoldings of the dorsal

and ventral cranial surfaces (Fig. 6C). At the medial edge of the incurrent nostril, these infoldings appear to fuse, creating a forked structure that physically separates the incurrent and excurrent nostrils (NB in Fig. 6D); this structure is the ‘small, concealed fleshy flap’ mentioned by Tester (1963, p. 256), i.e. the nasal bridge (Fig. 7, NB). Externally, the nasal bridge is a wedge-like feature that originates from the dorsal surface of the incurrent nostril region and inserts into its ventral surface. This arrangement was clearer in specimens of *Sphyrna lewini* and *Sphyrna tiburo* that we examined. The infoldings described above are equivalent to the valve flaps of Theisen et al. (1986, p. 74; in particular, compare Fig. 6C with Fig. 7 of Zeiske et al., 1987).

Fig. 7 (Colour in Web version; black and white in print version)

Running parallel with and immediately anterior to the incurrent nostril on the dorsal surface of the head is a shallow but well-defined nasal groove (Figs. 3, 4, 6C and 7). This groove - referred to here as the minor nasal groove to differentiate it from the other, larger nasal groove, i.e. the major nasal groove - runs the length of the incurrent nostril, expanding and becoming more rounded towards its medial end, where it meets and crosses the major nasal groove (Fig. 4). At its medial end, the minor nasal groove merges with the lateral flank of the nasal bridge. We found that the minor nasal groove was also present in the other seven species of hammerhead shark.

The major nasal groove extends from the medial edge of the wing to the incurrent nostril along the anterior edge of the head, expanding as it does so (Fig. 3A).

The excurrent nostril, which unlike the incurrent nostril is a relatively open aperture, has four edges (labelled X, Y, Z and NB in Figs. 3, 4 and 7). The two anterior edges, X and Z, are fused by a thick section of tissue to give a triangular fold (Figs. 3 and 4). The rigidity of this triangular fold suggests that, unlike in other sharks (Zeiske et al., 1987, p. 2411), the excurrent nostril does not close when the fish is swimming rapidly. The triangular fold forms the lateral edge of the major nasal groove (Fig. 3A). Extending from the ventral edge (X) of the triangular fold is a small, slightly forward-pointing flap (Fig. 3B). The lateral edge (Z) of the triangular fold links with the anterior edge of the minor nasal groove and the nasal bridge (NB in Fig. 7). The nasal bridge and edge Y form the two posterior edges of the excurrent nostril (Fig. 7). The nasal bridge, together with edges X and Z, are relatively sharp, and should therefore reduce energy losses associated with exiting flow (Massey 1989, p. 92).

A broad shallow depression lies immediately behind the nostrils (Figs. 3, 4 and 7).

The overall shape of the olfactory chamber is that of an asymmetric ellipsoid (Fig. 6A). Each chamber lies approximately perpendicular to the body axis, and extends from the lateral edge of the incurrent nostril to the medial edge of the wing, with the bulk of the chamber medial to the nasal bridge. The olfactory lamellae occupy the full extent of the chamber; there are about 90 pairs in each chamber. Generally the lamellae lie perpendicular to the main axis of the chamber, but at the lateral and medial edges they fan outwards (Fig. 6A). At certain angles one can actually see the lamellar array extending across the incurrent nostril, with about 20 lamellae (and raphe) visible through the narrow aperture (Fig. 3B). The array is set back slightly in the incurrent nostril (Fig. 6C), which is why it may be easily overlooked.

Although individual olfactory lamellae could be identified and counted in the micro-CT data set, the resolution (124.5 μm) was not sufficient to identify any secondary folds present. Furthermore, the lamellae were clumped together (bracket in Fig. 6B), rather than being regularly spaced as presumably they would be in the marine environment. The sequence of patches (AP in Figs. 6C and D) along the lamellar edges that coincides with the location of the peripheral channels is likely to be air pockets trapped between the lamellae. In transverse section the lamellae are noticeably thinner where they contact the wall of the olfactory chamber (Fig. 6B).

An incurrent channel is formed by the apical sections of the lamellae in adjacent rows of the olfactory array, although in the olfactory chamber medial to the nasal bridge they do not quite make physical contact (Fig. 6E, arrow). In the region of the incurrent nostril, the incurrent channel is linked directly to the external environment by the narrow channel formed by the dorsal and ventral cranial infoldings (Fig. 6C, circle). The model of the olfactory channels shows that the incurrent channel is connected to the excurrent channel by a hairpin bend such that together incurrent and excurrent channels form a single, continuous channel linking incurrent and excurrent nostrils (Fig. 5; see also Fig. 1F).

The cranial infoldings in the incurrent nostril region, together with the lamellar tips, create two galleries (Section 1.1; Figs. 5, 6C and D) that join the excurrent channel just medial to the nasal bridge.

3.2 Flow in the nasal region

Dye directed at any position along the anterior edge of the nasal region of the model head, including the major nasal groove, nearly always resulted in circulation of dye within the olfactory chamber

(Video clip 1). Dye was observed travelling down this groove towards the incurrent nostril (Video clip 2).

Video clip 1

Video clip 2

The one instance where we could not detect dye entering the chamber occurred when the head was inclined to what would be the shark's left and the dye filament was directed at the medial edge of the major nasal groove. Indeed, although dye did circulate within the chamber when dye was directed at other positions along the anterior edge of the nasal region when the head was inclined to the left, the amount of dye entering the chamber in these instances was generally less than when the head was inclined to the right or held with its body axis (Fig. 2) parallel to the direction of the oncoming flow.

The point at which dye entered the keyhole aperture of the incurrent nostril depended upon where the filament struck the anterior edge of the model head (Fig. 8). Flow striking either the lateral edge or middle of the incurrent nostril passed into the nascent, scoop-shaped incurrent channel in those regions via the slit-like portion of the incurrent aperture (Fig. 8). Alternatively, flow could be deflected along the minor nasal groove and into the enlarged medial part of the keyhole aperture (K in Fig. 4; Video clip 3). A dye filament striking any part of the major nasal groove and channelled into the olfactory chamber only entered the enlarged medial part of the keyhole aperture, however (Video clip 2).

Fig. 8

Video clip 3

Having passed through the incurrent nostril, dye travelled along the incurrent channel, often as rapidly moving filaments. Passage of dye from the incurrent to the excurrent channel could occur anywhere along the internal section of the model array, from the nasal bridge almost up to the hairpin bend. Dye passed from the incurrent to the excurrent channel via the gap in the apical section of the lamellar array (Fig. 6E, arrow), often streaming through in distinct waves and often guided by the interlamellar channels (Video clip 4). We noticed that the point at which the dye filament struck the anterior edge of the nasal region influenced the point at which dye crossed from

the incurrent to the excurrent channel, although there was no obvious trend to this transfer. For example, dye striking the lateral edge of the major nasal groove with the model inclined to the (shark's) right mainly crossed just medial to the nasal bridge (arrow I in Fig. 8). However, dye striking the lateral edge of the incurrent nostril with the model again inclined to the right mainly crossed further into the chamber (arrow II in Fig. 8). Increasing the speed of the oncoming flow could result in greater penetration along the incurrent channel. The greatest penetration was observed with a speed of oncoming flow of 40 cm s^{-1} , when dye almost reached the hairpin bend (arrow III in Fig. 8). On no occasion did it reach this feature, however.

Video clip 4

Dye was noticeably more dispersed in the excurrent channel than in the incurrent channel (Video clips 1, 2 and 4), and generally appeared to flow through this channel at a slower speed. Towards the excurrent nostril, the dye stream appeared to flow through a constriction in the excurrent channel (Fig. 8, white circle).

The dye stream exiting the olfactory chamber generally extended from the lateral tip of the excurrent nostril to the medial edge of the nasal bridge (Fig. 8, lower inset), towards the medial edge of the broad shallow depression lying behind the nostril region (Fig. 7). The effluent stream therefore tended to avoid the posterior edge (Y) of the excurrent nostril (Fig. 7).

It was immediately apparent from the flow visualisation experiments that a significant portion of the dye striking the anterior edge of the model head was in fact deflected *over* the nasal region rather than into the incurrent nostril (Fig. 8, upper inset). The two nasal grooves, the nasal bridge and the triangular fold of the excurrent nostril were responsible for deflecting flow. Thus dye could pass along either groove and then be directed away from the nasal region and on to the ventral surface of the model head by the nasal bridge (Video clip 3). Dye that had passed over the nasal bridge merged with the dye stream exiting the excurrent nostril (Video clip 3). Alternatively dye could pass along the major nasal groove and then be guided on to the ventral surface of the head by the triangular fold of the excurrent nostril (Video clip 2). Dye was also observed 'leaking' from both grooves and passing directly over the head. The broad shallow depression posterior to the nostrils (Figs. 3, 4 and 7) appeared to facilitate passage of flow over the incurrent nostril region, as well as from the excurrent nostril.

We did not detect any flow in the galleries, probably due to residual air blocking these two channels.

Discussion

4.1 Gross morphology of the nasal region

Apart from two unusual features, the nasal region of the hammerhead shark *Sphyrna tudes* is architecturally similar to the nasal region of other sharks with an active habit (Section 1.1). These similarities include: 1) the incurrent and excurrent nostrils lying in close proximity, separated from each other by a nasal bridge (the ‘small, concealed fleshy flap’ of Tester 1963, p. 256); 2) a triangular fold forming part of the edge of the excurrent nostril; 3) a broad shallow depression lying behind the nostrils; 4) the olfactory chamber housing an extensive lamellar array with essentially the same system of olfactory channels, including galleries. The nasal region is also very well integrated into the head – only a small ventral portion of the triangular fold of the excurrent nostril protrudes (Fig. 3A).

As noted by Theisen et al. (1986, p.74), the olfactory lamellae are noticeably thinner where they contact the wall of the olfactory chamber (Fig. 6B), due to the absence of secondary folds (Fig. 1D). These thinner regions, matching the location of the peripheral channels (Fig. 1D), generate relatively deep interlamellar channels, and should therefore reduce resistance to flow (Vogel 1994, p. 294) and facilitate passage of water through these channels. This arrangement is similar to that in the olfactory chamber of the ribbon eel, *Rhinomuraena ambonensis* (Holl et al., 1970, Fig. 4).

An aspect of the olfactory lamellar array not previously commented on is a hairpin bend joining incurrent and excurrent channels such that they form one single, continuous channel between incurrent and excurrent nostrils (Fig. 5; see also Fig. 1F).

The olfactory lamellar array also had a relatively small gap in its apical section (Fig. 6E, arrow), linking incurrent and excurrent channels. This gap had a marked effect on flow through the chamber (Section 4.2). We do not know whether the gap is an artifact or not. It may have arisen postmortem (Nadeau et al., 2009, p. 434), or it may have been caused by shrinkage of the olfactory lamellae in the alcoholic preservative solution (Section 2.1). Quantifying the exact amount of shrinkage in alcohol-preserved specimens is difficult for two reasons (Hughes 1984, pp. 645-646). First, shrinkage is not necessarily isometric, due to varying tissue composition. Second, the pattern of shrinkage is not always the same between species, or even specimens from the same species. Based

on measurements of alcohol-preserved secondary lamellae from the gills of the nursehound, *Scyliorhinus stellaris*, shrinkage could be up to 30 % (Hughes et al., 1986, p. 39). Alternatively, the gap may have arisen when the X-ray scan was performed in air. We note that the gap is also present between pairs of olfactory lamellae from *Sphyrna lewini* (Tester 1963, Fig. 6B; Schluessel et al., 2008, Fig. 6S). The clumping of the olfactory lamellae observed in the X-ray scan (Figs. 6A and B) was certainly the result of the scan being performed in air, and arose from surface tension and a lack of support from the air; fish gills are affected in the same way (Hughes 1961, p. 346).

One unusual feature of the nasal region of *Sphyrna tudes* is its incurrent nostril which, in contrast to the more open, semicircular incurrent nostrils of other sharks, is slit-like (Fig. 3A). This difference must of course be dictated by the flattened head. The second unusual feature is a groove that runs parallel and anterior to the incurrent nostril (Fig. 7). We refer to this groove as the minor nasal groove, to distinguish it from the other, larger nasal groove running along the anterior edge of the head (Fig. 3A). The minor nasal groove has not been remarked upon before, although it does seem to be apparent in Fig. 2 of Tester (1963). It is present in all eight species of hammerhead shark. Its two functions are discussed in Section 4.2.

4.2 Flow in the nasal region

Using a life-sized model of a head of *Sphyrna tudes* placed in a water tunnel, we showed that oncoming flow is sufficient to produce circulation within the olfactory chamber, even at speeds slightly lower than the minimal speed of a freely swimming hammerhead shark. Although circulation within the olfactory chamber was extensive, it did not quite fully penetrate the chamber (Fig. 8). Circulation of flow within the chamber was probably assisted by flow passing over the triangular fold of the excurrent nostril and also by flow passing over the nasal bridge, entraining fluid (Cox 2008, p. 583) from the excurrent nostril. In both instances it was noticeable that flow over these features merged with flow leaving the model chamber via the excurrent nostril.

The route taken by flow through the olfactory chamber was that proposed by Zeiske et al. (1986, p. 388): incurrent nostril – incurrent channel – excurrent channel – excurrent nostril (Video clip 5). Zeiske et al. also proposed that flow passes from incurrent to excurrent channels via the interlamellar channels, either directly, or via the peripheral channels (Fig. 1E). We would suggest that flow can also pass from incurrent to excurrent channel via the hairpin bend that we identified (Fig. 5 and dashed line in Fig. 8; see also Fig. 1F and Video clip 5). We were, however, unable to confirm either of these two routes, for two reasons. Firstly, the lamellae and interlamellar channels were poorly defined in the transparent model because of the clumping of the lamellae in the X-ray

scan and because of the low resolution of the model (200 μm). Secondly, flow passing along the incurrent channel did not quite reach the hairpin bend. No doubt at higher speeds of oncoming flow than those used in this investigation, flow would have passed around it. However, we found that flow passed from incurrent to excurrent channels via the gap in the apical section of the model lamellar array (Fig. 6E, arrow), thereby short-circuiting the hairpin bend. Interestingly, this inadvertently provided a physical demonstration of one of the mechanisms proposed by Zeiske et al. (1986, p. 389) for regulating flow through the olfactory chamber of a shark (Section 1.1).

Video clip 5

Flow speeds in the incurrent channel appeared generally faster than those in the excurrent channel. Given the latter's larger transverse cross-sectional area (Fig. 6E), and given that the two are linked via the apical gap in the lamellar array, this result is, based on the principle of continuity (Vogel 1994, pp. 32-34), to be expected – in a continuous channel of varying cross-sectional area, the speed of flow in the smaller channel will be faster than that in the larger channel. Fast flow speeds in the incurrent channel would impart fluid with the momentum required for it to reach the medial end of the olfactory chamber, exposing it to a greater proportion of the lamellar array and its sensory surfaces. The apparent narrowing of the excurrent channel in the vicinity of the excurrent nostril (Fig. 8, white circle) is reminiscent of the constriction present on the outlet apertures of sponges, the nozzle-like effect of which is thought to prevent unnecessary refiltration of effluent (Vogel 1994, p. 39).

The galleries running along most of the length of the incurrent nostril (Fig. 5) presumably allow flow that has circulated through the interlamellar channels in this region to pass into the excurrent channel and out of the excurrent nostril (Section 1.1). That flow goes in the other direction in the galleries seems physically unreasonable. However, we were unable to investigate flow in the galleries, probably due to their blockage by residual air in the model.

Apparently the point at which flow strikes the anterior edge of the head in the nasal region influences the degree to which flow penetrates the olfactory chamber, and therefore also the principal point at which flow crosses from incurrent to excurrent channel. Chi et al. (2004) noticed a similar effect in the spiny dogfish, *Squalus acanthias*, and suggested that this may be important in extracting olfactory information from the surroundings. However, we could not establish any particular pattern in our results, and are therefore loathe to comment on this point further.

We verified Tester's assertion (1963, p. 256) that the major nasal groove directs flow into the incurrent nostril. However, we were also able to show precisely where flow guided by this groove entered the incurrent nostril, namely the enlarged medial part of the keyhole aperture (K in Fig. 4). Flow directed along the minor nasal groove could also pass into the olfactory chamber via this part of the keyhole aperture. Thus the minor nasal groove can, like its larger partner, act as an agent to channel flow into the olfactory chamber.

We were able to provide support for another type of flow regulatory mechanism proposed by Zeiske et al. (1986, p. 389; Section 1.1), and confirmed their prediction that the nasal bridge and shallow depression behind the incurrent and excurrent nostrils would be involved in this mechanism: flow striking the anterior edge of the head was deflected along the minor nasal groove, and then guided onto the ventral surface of the head by the nasal bridge. Thus the minor nasal groove can also act as a baffle, redirecting flow across the anterior face of the incurrent nostril, possibly to protect the olfactory lamellae that are exposed to the oncoming flow in this region (Fig. 3B). Similarly flow that had been channelled along the major nasal groove could be guided away from the incurrent nostril by the nasal bridge. The role of the broad shallow depression posterior to the nostrils seemed to be to assist flow passing over the incurrent nostril and out of the excurrent nostril.

Our study could be improved in several ways:

1) The clumping of the olfactory lamellae observed in the micro-CT data could be avoided by recording three-dimensional (3D) data with the specimen submerged in an aqueous medium rather than in air, using magnetic resonance imaging (MRI), for example (Craven et al., 2007). MRI may also be able to determine whether the gap in the apical section of the olfactory lamellar array was a consequence of the X-ray scan having been performed in air. In addition, MRI should be able to determine details which the micro-CT data could not, e.g. the exact structural composition of the nasal bridge. The resolution of any future 3D data should be such that the olfactory lamellae and interlamellar channels are resolved.

2) Flow visualisation could be performed at Reynolds numbers (Massey 1989, pp. 134-141; Vogel 1994, pp. 84-88) identical to those of freely swimming hammerhead sharks rather than slightly less. The Reynolds number could be maintained by using a larger model at slower speeds, or by using a life-sized model in conjunction with another visualisation technique such as particle image velocimetry (Gharib and Dabiri 2000).

3) Flow in the nasal region of the model head may have been affected by the absence of a body. However, since the streamlines at the truncation should not be radically altered by the missing body, the modification to upstream flow should be small and localised. But the only way of verifying this claim would be to perform the comparative experiment with a model body present.

4) Although the sweeping of the head associated with the hammerhead's swimming action (Gilbert 1962, p. 62) was partly taken into account by varying the angle at which flow struck the model head, the head was static rather than dynamic. A more accurate picture of flow in the nasal region could be achieved by using a motorised model head that sweeps through an arc and at a frequency similar to that of freely swimming hammerhead sharks (40° and 0.6 – 0.8 Hz respectively for adult *Sphyrna lewini*).

Needless to say, comparison with flow in the nasal regions of the other seven hammerhead species would also be of considerable interest.

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Appendix

A1. Hydraulic diameter of the interlamellar channels in the olfactory chambers of sharks

Due to the presence of the secondary folds on the olfactory lamellae of sharks, the interlamellar channels are highly convoluted (Fishelson and Baranes 1997, Fig. 3). The diameter of a convoluted duct may be represented by the hydraulic diameter, $D_h = 4A/P$, where A and P are the cross-sectional area and wetted perimeter of the duct, respectively (White 2003, p. 376). The diagrams used here to measure the hydraulic diameter of the interlamellar channels of a shark are Fig. 2B of Zeiske et al. (1986), Fig. 3 of Fishelson and Baranes (1997) and Fig. 11B of Schluessel et al. (2008), a drawing and two light micrographs respectively. The central and right interlamellar

channels were chosen for measurement in the case of Fig. 2B of Zeiske et al. (1986), the central channel from Fig. 3 of Fishelson and Baranes (1997) and both channels from Fig. 11B of Schluessel et al. (2008). The Fishelson and Baranes figure did not have a scale bar; the scale was estimated from the magnification given in the figure legend. The cross-sectional area and perimeter of the interlamellar channel in each case were calculated using the software package Rhinoceros (Version 3.0, Robert McNeel and Associates; see Cox 2008, Appendix A, p. 2 for general methodology). The upper and lower parts of the peripheral channels were not included in the measurements. The estimated hydraulic diameters are given in Table A1.

Table A1

Note that the hydraulic diameter of the interlamellar channel calculated from Fig. 3 of Fishelson and Baranes (1997) is an order of magnitude lower than that from the other two figures. The form of the lamellae in this figure is similar to Fig. 2B of Zeiske et al. (1986), which does have a scale bar. If the Fishelson and Baranes figure is scaled in accord with the Zeiske et al. figure, the hydraulic diameter becomes 80 μm rather than 14 μm , more in agreement with the hydraulic diameters calculated from the other two figures. Overall then, the hydraulic diameter of the interlamellar channels in the olfactory chamber of a shark is approximately 100 μm .

A2. Pressure difference between incurrent and excurrent nostrils of a hammerhead shark

The pressure difference between incurrent and excurrent nostrils of a hammerhead shark was estimated according to Cox (2008, Appendix A, p. 5) using the equation $\frac{1}{2}\rho U^2$ (Vogel 1994, p. 53), where in this instance ρ is the density of seawater at 10°C and U is the speed at which the fish swims. Based on a swimming speed of 0.6 to 2 total lengths per second (Section 2.6), if the total length of the fish is 90 cm (Appendix A3) and the density of seawater (salinity 35 ‰) at 10 °C is 1,027 kg m⁻³ (Denny 1993, p. 24), then the pressure difference across the nostrils falls in the range 100 - 2,000 Pa.

*A3. Estimate of total length of the *Sphyrna tudes* specimen*

The total length of a shark is the distance from the anterior tip of the head to the posterior tip of the tail fin (Gilbert 1967, Fig. 1a). From a drawing of a full length specimen of *Sphyrna tudes* (Gilbert 1967, Fig. 19), we estimated the ratio of the depth of the head (anterior tip to posterior tip of wing) to the total length of the fish as 1:9. The depth of the head of the *Sphyrna tudes* specimen described here is 10 cm. Therefore the total length of the specimen is estimated to be 90 cm.

Table A2

Fig. A1

Figure legends

Fig. 1. Schematics illustrating the functional morphology of the nasal region of sharks with an active habit. (A) Ventral surface of head of lemon shark, *Negaprion brevirostris* (based on Fig. 1A of Zeiske et al., 1987). Box: right nasal region. Dotted line: approximate location of olfactory chamber. (B) Boxed region in (A). Lines labelled 'C & E' and 'F' indicate positions of front face of sections in panels (C), (E) and (F), respectively. (C) Sagittal section through olfactory chamber (based on Fig. 2A of Zeiske et al., 1986). View towards medial end of chamber. The thinness of the lamellae where they are attached to the wall of the olfactory chamber is due to the absence of secondary lamellae (Theisen et al., 1986, p. 74). Secondary lamellae are shown on the left lamella only. Scale bar: 5 mm. Inset: outlines of incurrent and excurrent channels created by lamellae and the roof of the olfactory chamber. (D) Transverse section through two lamellae, towards the side wall of olfactory chamber, showing convoluted nature of interlamellar channel. (E) Flow through olfactory chamber. Same view as (C). (F) Cut-away view to one side of medial end of olfactory chamber, showing principal flow (arrowed line) through incurrent and excurrent channels. The hairpin bend in the path taken by the flow is an assumption based on the morphology of the olfactory chamber of the hammerhead shark *Sphyrna tudes* (Section 4.2). The interlamellar gaps and secondary lamellae present in (C) and (E) have been omitted for clarity. Similarly the detail of the peripheral channels is not shown. The thickness of the olfactory lamellae is exaggerated in (C), (E) and (F), again for clarity. In reality there would be many more lamellae in these sections. In (C) - (F) the lamellae and internal wall of the olfactory chamber are unshaded, the external wall of the olfactory chamber is light grey and the peripheral channels mid-grey. Mid-grey arrows: incurrent flow; clear arrows: excurrent flow; dark arrows: flow in interlamellar channels. EC: excurrent channel; EN: excurrent nostril; iC: interlamellar channel; IC: incurrent channel; IN: incurrent nostril; L: lamella; PC: peripheral channel; R: raphe; SF: secondary fold.

Fig. 2. Ventral view of the head of a specimen of the hammerhead shark *Sphyrna tudes*. Right nasal region outlined. a: Anterior; l: lateral; m: medial; p: posterior. Scale bar: 5 cm.

Fig. 3. (A) Anterior view of the right nasal region of *Sphyrna tudes*. (B) Anteroventral view of same region, with the lamellar array visible through the incurrent nostril. (C) Area between arrowheads in (A), indicating the points (1, 2, 4 and 5) and region (3) where the dye filament was directed in the flow visualisation experiments (Section 2.6). See also Fig. 8. Anatomical compass in (A) also applies to (B) and (C). d: Dorsal; l: lateral; m: medial; v: ventral. EN: excurrent nostril region; IN: incurrent nostril; R: raphe; X and Z: ventral and lateral edges of excurrent nostril, respectively. Scale bars: 1 cm.

Fig. 4. Anterolateral view of nasal region of *Sphyrna tudes*, highlighting the keyhole aperture of the incurrent nostril. a: Anterior; d: dorsal; p: posterior; v: ventral. EN: excurrent nostril region; K: keyhole aperture (the narrow lateral part of the aperture is mostly obscured by the eye region); X and Z: ventral and lateral edges of excurrent nostril, respectively. Note that the photograph shows the left nasal region, but it has been reversed in the horizontal plane to correlate with the anterior views of the head shown in Fig. 3. Scale bar: 1 cm.

Fig. 5. Ventral aspect of model of channels in the right olfactory chamber of *Sphyrna tudes*. The approximate extent of the two galleries is indicated with broken lines. a: Anterior; l: lateral; m: medial; p: posterior. DG: dorsal gallery; EC: excurrent channel; EN: excurrent nostril; HB: hairpin bend; iC: large interlamellar channel (see also Fig. 6B, arrow); IC: incurrent channel; IN: incurrent channel in incurrent nostril region; VG: ventral gallery. Scale bar: 1 cm.

Fig. 6. Micro-CT images of the head of *Sphyrna tudes*. (A) Dorsal plane. Right olfactory chamber outlined. (B) Transverse section through right olfactory chamber. Bracket: clumping of lamellae. Arrow: large interlamellar channel (see also Fig. 5). (C) - (E): Sagittal sections through left olfactory chamber in incurrent nostril region (C), nasal bridge region (D), and towards the centre of the lamellar array (E; see also Fig. 8 for approximate locations of these three sections). Circle in (C): narrow channel connecting external environment to incurrent channel. Square in (D): excurrent nostril. In (E) the excurrent channel has a cross-sectional area about five times that of the incurrent channel. Anatomical compass in (E) also applies to (C) and (D). Arrow in (E): gap between incurrent and excurrent channels. a: Anterior; d: dorsal; l: lateral; m: medial; p: posterior; v: ventral. AP: possible air pocket; EC: excurrent channel; G: gallery; IC: incurrent channel; L: lamella; NB:

nasal bridge; PC: peripheral channel. White: cartilage (or enameloid teeth); grey: soft tissue; dark grey: air. Scale bar in (A): 5 cm; scale bars in (B) - (E): 1 cm. Cut-away views of the model head of *Sphyrna tudes*, indicating the locations of these micro-CT images, are shown in Fig. A1, Appendix A5.

Fig. 7. Ventral aspect of right nasal region of *Sphyrna tudes*. a: Anterior; l: lateral; m: medial; p: posterior. EN: excurrent nostril; IN: incurrent nostril region; NB: nasal bridge; X and Y: ventral and posterior edges of excurrent nostril, respectively. Scale bar: 1 cm.

Fig. 8. Schematic showing flow in the model of the right nasal region of *Sphyrna tudes*. Ventral aspect. Numbers associated with black circles or squares indicate the points or region where the dye filament was directed at the anterior edge of the model head: 1) lateral region of incurrent nostril; 2) middle of incurrent nostril; 3) lateral region of major nasal groove (between two squares); 4) middle of major nasal groove; 5) towards medial edge of major nasal groove (see also Fig. 3C). References to Fig. 6 are the approximate locations of the micro-CT images shown in that figure. White section of olfactory chamber: incurrent channel; light grey section of chamber: excurrent channel.

Diamond: medial extent of major nasal groove. White circle: possible constriction in excurrent channel. Clear arrow: direction of oncoming flow; dark arrows: flow in the nasal region; arrows I - III: points at which flow may cross from incurrent to excurrent channels (Section 3.2). Dashed line: route assumed to be taken by principal flow *in vivo*. Scale bar: 1 cm. Lower inset: main route taken by flow leaving excurrent nostril. Upper inset: routes taken by flow deflected away from nasal region. a: Anterior; l: lateral; m: medial; p: posterior.

Fig. A1. Cut-away views of model head of *Sphyrna tudes*, indicating locations of micro-CT images shown in Fig. 6. Note that panel (A) shows a section through the right nasal region only, whereas Fig. 6A shows a section through the entire head.

Video clips

Video clips 1 - 4 were recorded from experiments conducted in the water tunnel in the Department of Mechanical Engineering at the University of Bath. The flow speed in the water tunnel was 20 cm s^{-1} . A Sony HDR-FX7E digital video camcorder was used to obtain these clips. The views in video clips 1 - 4 are of the ventral surface of the right nasal region of the transparent model head.

Video clip 1. Circulation of flow within model olfactory chamber. Body axis of head parallel to the direction of flow; dye filament directed at lateral region of major nasal groove.

Video clip 2. Channelling of flow towards incurrent nostril by major nasal groove. Head inclined to shark's right; dye filament directed at middle of major nasal groove.

Video clip 3. Flow in the region of the incurrent nostril. Body axis of head parallel to the direction of flow; dye filament directed at lateral region of minor nasal groove.

Video clip 4. Passage of flow from the incurrent to the excurrent channels. Head inclined to shark's right; dye filament directed at middle of major nasal groove.

Video clip 5. Simulation demonstrating probable route taken by the principal flow through the olfactory chamber of *Sphyrna tudes*. 26 - 29 s: passage through lateral part of incurrent nostril; 29 - 45 s: passage through incurrent channel (gap in apical section of lamellar array to right); 45 - 49 s: passage around hairpin bend; 49 - 57 s: passage through excurrent channel (gap in apical section of lamellar array to right); 57 - 58 s: passage past nasal bridge; 58 - 60 s: passage through excurrent nostril.

Table 1. Specimens of hammerhead shark examined in this study.

Species	Museum	Catalogue number
<i>Eusphyra blochii</i>	Natural History Museum, London, UK	BMNH 2009.6.3.1
<i>Sphyrna corona</i>	Los Angeles County Museum of Natural History, Los Angeles, USA	LACM 30728-3
<i>Sphyrna lewini</i>	Natural History Museum, London, UK	BMNH 2009.6.3.2
<i>Sphyrna media</i>	Smithsonian Institution, Suitland, USA	USNM 196140
<i>Sphyrna mokarran</i>	Smithsonian Institution, Suitland, USA	USNM 394494
<i>Sphyrna tiburo</i>	Natural History Museum, London, UK	BMNH 1903.5.15.346-349
<i>Sphyrna tudes</i>	Natural History Museum, London, UK	BMNH 1984.8.8:19
<i>Sphyrna zygaena</i>	Natural History Museum, London, UK	BMNH 1867.10.4.4

Table A1. Hydraulic diameters (D_h) of the interlamellar channels in the olfactory chambers of sharks.

Species	D_h (μm)	Interlamellar channel	Reference
Lemon shark, <i>Negaprion brevirostris</i>	147	Central	Fig. 2B, Zeiske et al., 1986
Lemon shark, <i>Negaprion brevirostris</i>	141	Right	Fig. 2B, Zeiske et al., 1986
Bigeye houndshark, <i>Iago omanensis</i>	13	Central	Fig. 3, Fishelson and Baranes 1997
Brownbanded bamboo shark, <i>Chiloscyllium punctatum</i>	143	Left	Fig. 11B, Schluessel et al., 2008
Brownbanded bamboo shark, <i>Chiloscyllium punctatum</i>	102	Right	Fig. 11B, Schluessel et al., 2008

Table A2. Details of water tunnels used in the engineering departments of Cambridge and Bath.

Feature	Cambridge	Bath
Working section (L x W x H, cm)	800 x 90 x 40	152 x 38 x 51
Temperature (°C)	20	18 - 19
Distance downstream from start of working section (cm)	300	50
Make of tunnel	Purpose-built	Eidetics 1520
Flow speed (cm s ⁻¹)	20	20, 30, 40
Regulation of dye flow	Gravimetric	Needle valve from reservoir under a pressure of 10 ⁵ Pa