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Flow through the nasal cavity of the spiny dogfish, *Squalus acanthias*

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Abstract. The nasal cavity of spiny dogfish is a blind capsule with no internal connection to the oral cavity. Water is envisioned to flow through the cavity in a smooth, continuous flow pattern; however, this assumption is based on previous descriptions of the morphology of the olfactory cavity. No experimentation on the flow through the internal nasal cavity has been reported. Morphology of the head of the spiny dogfish (*Squalus acanthias*) does not suggest a close external connection between the oral and nasal systems. However, dye visualization showed that there was flow through the nasal apparatus and from the excurrent nostril to the mouth when respiratory flows were simulated. The hydrodynamic flow through the nasal cavity was observed from flow tank experiments. The dorsum of the nasal cavity of shark heads from dead animals was exposed by dissection and a glass plate was glued over of the exposed cavity. When the head was placed in a flow, dye was observed to be drawn passively into the cavity showing a complex, three-dimensional hydrodynamic flow. Dye entered the incurrent nostril, flowed through the nasal lamellae, crossed over and under the nasal valve, and circulated around the nasal valve before exiting the excurrent nostril. When the nasal valve was removed, the dye became stagnant and back flowed out through the incurrent nostril. The single nasal valve has a hydrodynamic function that organizes a coherent flow of water through the cavity without disruption. The results suggest that the morphology of the nasal apparatus in concert with respiratory flow and ambient flows from active swimming can be used to draw water through the olfactory cavity of the shark.

1 Introduction

Chemoreception (taste and smell) in fishes relies upon hydrodynamic flow to aid in localization of a food odor source [1]. The hydrodynamic flow through the nasal apparatus in fishes is important in transporting water and dissolved odorous chemicals over the olfactory organs for initial sensory processing [2]. The nasal apparatus of most

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fishes, with the exception of hagfishes, lungfishes, and certain specialized species, are not internally connected to the pharynx [2]. The typical olfactory organs of sharks (Chondrichthyes, Neoselachii) are blind capsules that are usually located ventrally on the rostrum and anterior to the mouth [3–9]. The opening of the nasal cavity of sharks is partially divided into anterior and posterior nostrils by nasal valves and flaps in which water enters and exits the nasal cavity, respectively [9]. The nasal valve(s), or protrusions of the posterior/anterior nasal flaps, were postulated to provide a hydrodynamic function affecting the flow of water through the nasal apparatus [10,11].

The morphology of the olfactory cavities of sharks has been studied previously [4,8–13], but visualization of the flow has only been performed on plastic and computational models of the nasal cavity of the highly derived hammerhead shark (*Sphyrna tudes*) [8,14]. The water flow through the plastic model of the hammerhead shark was induced by an ambient flow based on swimming by the shark. It was proposed that respiration might also induce water flow through the olfactory cavities of sharks [1,3,4,12,16–19]. The position of the mouth relative to nasal openings in most sharks would allow flow through the nasal apparatus that is induced by suction through the mouth. Respiratory-induced flow was demonstrated in sharks, where the nostrils were in close proximity to the mouth [3,10,18,20]. However, it had only been presumed that ventilation of the nasal apparatus was primarily accomplished by a pressure difference generated by active swimming [21]. For either swimming-induced or ventilatory-induced flow, there have been no empirical observations of flow through the actual nasal apparatus of a shark.

A study was undertaken to determine the pattern of flow through the nasal cavity of a spiny dogfish (*Squalus acanthias*). This species has excurrent nostrils located on the anterior of the head and remote from the mouth. In addition, the nasal apparatus has a single nasal valve, which could influence water flow. The primary objectives of this study were to experimentally observe the hydrodynamic flow through the blind nasal cavity of the spiny dogfish, determine the function of a single nasal valve, and determine the feasibility of swimming-induced and ventilatory-induced flow through the nasal apparatus. Flow experiments were conducted by following the movement of dye through the exposed nasal cavity of dissected heads of the spiny dogfish. In addition, it was expected that the flow through the nasal apparatus should be smooth without observable turbulence and that modification of the nasal apparatus should induce an unsteady flow.

2 Materials and methods

2.1 Preparation of shark heads

Heads of mature spiny dogfish (*Squalus acanthias*) were purchased from a commercial vender (Carolina Biological Supply Company). The heads were well preserved initially in a formalin solution, washed in water, and held in Carosafe® (2-Amino-2-Ethyl-1,3-Propanediol, 2-Phenoxyethanol, and Propylene Glycol). Dissections of thirteen specimens were made in preparation for experimentation. The olfactory organ was dissected to expose the internal nasal cavity, including the lumen of the nasal cavity and lamellae. Incisions with a scalpel were made on the left side of the head from the tip of the rostrum to anterior of the eye in the frontal plane above the nostrils and along the mid-sagittal plane. The dorsal portion of flesh was removed, exposing the ventral part of the olfactory organ, including the single nasal valve.

The left olfactory cavity of each specimen was prepared in one of three ways (Fig. 1): Preparation 1- no modifications of the nasal cavity were made so that the nasal valve (i.e., projection from the posterior nasal flap) and lamellae (i.e., sensory

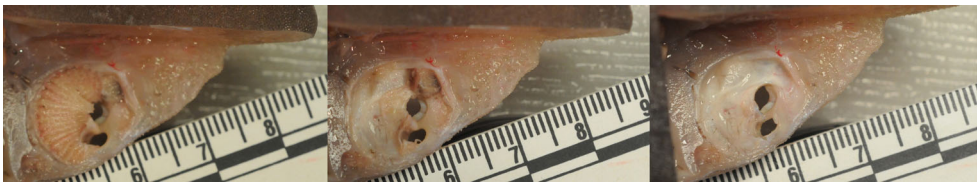

Fig. 1. Example of olfactory cavity preparation (dorsal view with anterior direction to the right). Preparation 1 (left) with no modification of nasal cavity, Preparation 2 (center) with lamellae removed, and Preparation 3 (right) nasal valve and lamellae removed.

Fig. 2. Head of the spiny dogfish (*Squalus acanthias*) prepared with coverslip glued on top of the exposed olfactory cavity and small cannula positioned anterior of the incurrent nostril for dye injection.

epithelium arranged as multiple plates) [8,10,11,13] were kept intact; Preparation 2-the nasal valve was kept intact and lamellae were removed; Preparation 3-the lamellae and nasal valve were both removed.

After the initial preparation of the nasal cavity, a glass coverslip (25×25 mm) was glued to the margins of the exposed cavity with silicone sealant. Cutting into the nasal cavity and the application of the coverslip would change the geometry of the nasal cavity. Although the full three-dimension flow in the nasal cavity may be affected, the basic flow pattern observed in the ventral portion of the cavity may not be subsequently changed from the unmodified condition. A small cannula was then attached anterior of the incurrent nostril; with the opposing end left open for later attachment of the dye injection apparatus (Fig. 2).

2.2 Flow tank experimentation

Hydrodynamic experimentation was conducted in a recirculating flow tank, based on the flow tank design of Vogel and LaBarbera [22]. The channels of the flow tank were constructed of PVC pipe (106 mm I.D.). The working section of the flow tank had walls constructed of 12.7 mm thick, clear Lucite. The dimensions of working section were $0.7 \times 0.17 \times 0.13$ m. The working section was bounded upstream and downstream by a plastic grid (commercially termed "egg crate") that removed turbulence from the flow. Water speed through the working section was controlled with a 1/8 HP variable speed electric motor (GKH Type HST20N) connected to a Series H motor controller (G. K. Heller Corp., Floral Park, NY), which drove a propeller situated downstream

of the working section. Water speed was determined from the time a drop of ink traversed a measured distance through the working section. The flow tank contained freshwater that was maintained at between 22 and 23°C.

Each head preparation was mounted on a tri-prong spearhead, which was suspended in the flow tank on a metal stand. The head was placed on its right side, with the dorsal side facing the camera and anterior end facing forward toward the direction of the water flow. The projected frontal area of the shark accounted for 12% of the cross-sectional area of the working section of the flow tank.

A blue dye was used to visualize the flow of water through the nasal apparatus. The dye was composed of a water-soluble blue ink mixed with whole milk in a one to four ratio. The mixture was slightly negatively buoyant. Such dyes are used for flow visualization, where the milk retards diffusion of the dye into the surrounding water [23,24]. The blue dye was introduced from a 3 mm (E.D.) cannula that was positioned anterior of the incurrent nostril. Dye flowed to the cannula through tubing by a gravity feed from a glass funnel held above the level of the nostril and controlled with a stopcock for a consistent flow. The water velocity in the flow tank was maintained at 70 mm/s. This velocity is slower than the reported 123 mm/s swimming speed for the spiny dogfish [25]. However, using higher flow speeds in our experiment made it difficult to document the flow pattern and follow the dye through the olfactory cavity.

Video recordings of the water flow through the nasal cavity were made with a Sony Color Video Camera 3CCD (model DXC-390) system with a Zoom 6000 II lens and Navitar. 67X adapter tube. The video camera had a resolution of 800 television lines. The camera was positioned approximately 30 mm from the side of the working section of the flow tank on a tripod. The camera was positioned at the level of the exposed nasal cavity. Video from the camera was recorded at 30 frames/s onto Hi8 videotape using a Sony GV-A500 video recorder. The recordings were viewed on a Panasonic CT 2600M video monitor.

2.3 Respiratory flow simulation

To simulate the influence of respiratory-induced water flow through the olfactory cavities by flow into the mouth, heads of spiny dogfish were first prepared by sealing the spiracles and gills with duct tape. A thin (2.2 mm) wooden strut was used to prop open the center of the mouth. Dimensions of the mouth opening were determined from digital photographs of the frontal view of the head using ImageJ (NIH, ver. 1.47). The width and height of the open mouth were 45 mm and 10 mm, respectively, with a projected frontal area of 67.5 mm^2.

Heads were submerged in a glass aquarium (1.6 × 1.2 × 1.4 m), with no water flow. The heads were oriented ventral side up on the bottom of the tank and a 5 kg mass was placed on top of the head for stabilization. A Tygon tube (6 mm E.D.) was passed anteriorly through the esophagus into the oral cavity. The opposite end of the tube was connected to a suction pump (Proquatics Canister Filter 2400). The suction pump was used to pull water through the mouth at low (0.06 L/s) and high (0.13 L/s) mass flow rates, simulating respiratory flow. The water pulled through the mouth by the pump was not returned to the tank so that no secondary flows were produced around the head. The flow velocity through the mouth was calculated as mass flow divided by the area of the open mouth and equaled 880 mm/s and 1926 mm/s for low and high flow rates, respectively. Blue dye was introduced approximately 5–10 mm anterior to the incurrent nostril from a 3 mm (E.D.) tubing connected to a 10 cc syringe. The same dye as used in the flow tank experiment was introduced slowly in a coherent stream from the ending of the tubing. The flow of the dye was recorded at

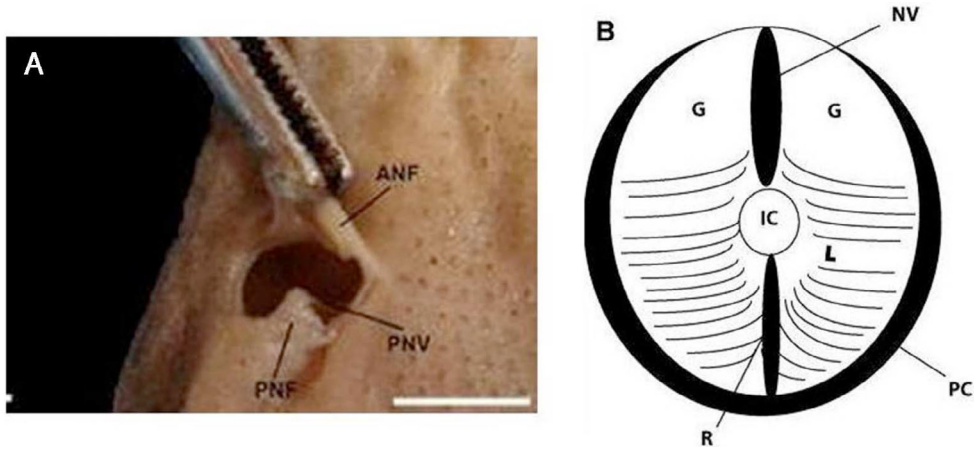

Fig. 3. Structure of nasal apparatus of the spiny dogfish (*Squalus acanthias*). A) Image of external nasal openings showing anterior nasal flap (ANF), posterior nasal valve (PNV), and posterior nasal flap (PNF). B) Diagram of internal olfactory cavity showing gallery (G), nasal valve (NV), inlet chamber (IC), lamellae (L), raphe (R), and peripheral canal (PC). Outlet chamber is not visible in this view. The white scale bar represents 40 mm.

30 frames/s with a Sony Hi8 video camera (CCD-V701) positioned above the water surface and directly over the submerged shark head. Surface distortions were removed by recording through a piece of clear acrylic floating on the water surface.

3 Results

3.1 Structure of the nasal apparatus in spiny dogfish

Spiny dogfish possess a conical shaped head and pointed rostrum. An anterior incurrent nostril and posterior excurrent nostril were formed from a thin anterior nasal flap that separates the two nostrils (Fig. 3A). The incurrent and excurrent nostrils were wide and circular in shape. A single nasal valve (PNV) protruded from the posterior nasal flap (PNF). The posterior nasal valve separated the incurrent and excurrent cavities. The nostrils were located anteriorly on the rostrum separate from the mouth. No external grooves connected the excurrent nostrils and the mouth. The shark possessed a single nasal valve protruding from the posterior nasal flap. The posterior nasal valve separates the incurrent and excurrent cavities.

The olfactory cavity of the shark was functionally divided with nasal valves, flaps and lamellae into inlet and outlet chambers, two galleries, and a peripheral canal (Fig. 3B). The incurrent nostril leads into the inlet chamber and the outlet chamber leads to the external nostril. The peripheral canal was on the perimeter of the cavity, with olfactory lamellae in the posterior of the cavity. The lamellae had secondary folding to increase surface area.

3.2 Flow through the nasal apparatus

With the lamellae and nasal valve intact (*Preparation 1*), water flow was complex. Dye entered the incurrent nostril in a laminar manner and moved into the inlet chamber, where water took one of several pathways (Fig. 4). Dye either passed directly over

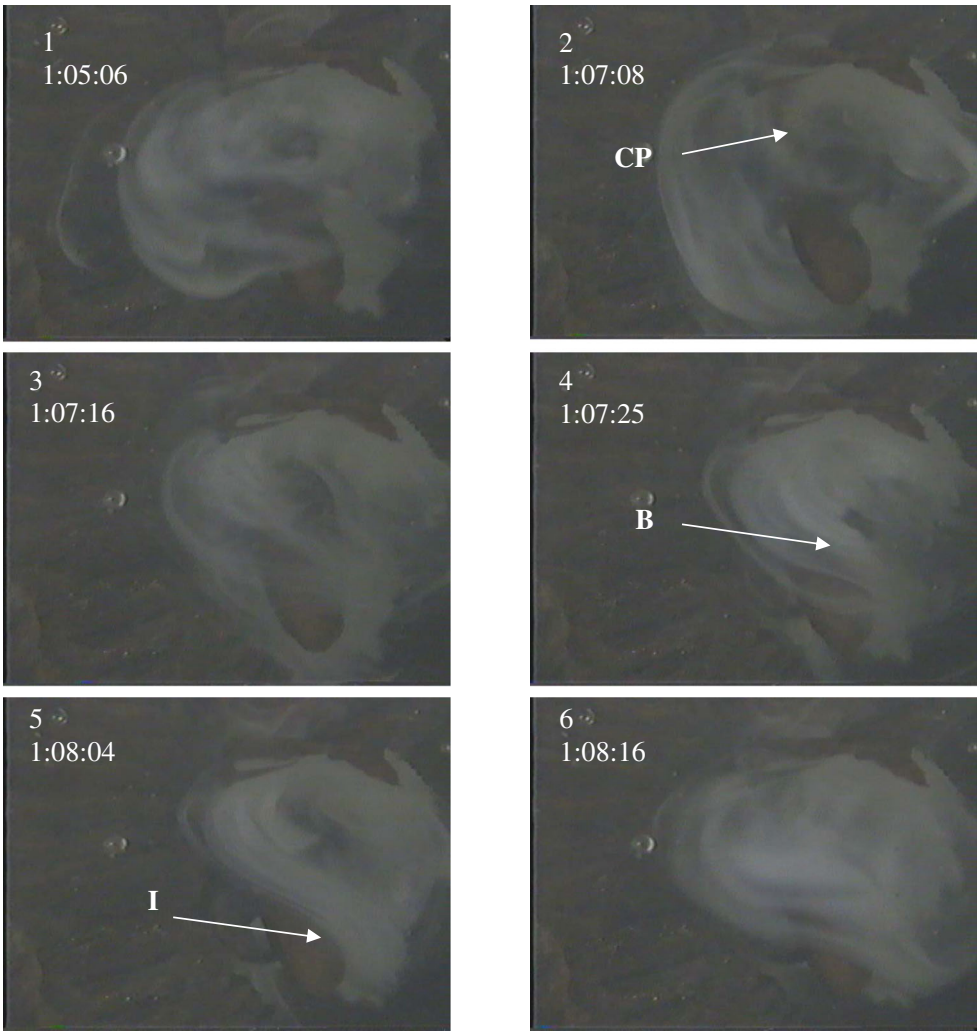

Fig. 4. Video sequence of water flow through nasal cavity of the spiny dogfish (*Squalus acanthias*). The nasal cavity is approximately 12 mm in diameter. Dye first circulated at the apex curvature (CP) on the nasal valve upon entering the incurrent nostril. Circulation was then observed at the bulbous position (B) and indentation (I) on the nasal valve. Time indicates when the dye was flowing around the nasal valve upon dye injection and is not indicative of when the dye was introduced. Orientation of all the images has anterior of the nasal cavity directed to the right and lateral directed to the top.

the lamellae, or passed above and/or below the nasal valve before entering the outlet chamber and exiting the excurrent nostril. Dye was observed to circulate around three specific points on the nasal valve: the curvature at the apex of the valve immediately at the entrance of the incurrent nostril, a bulbous point located at the midpoint of the nasal valve, and the indentation on the tip of the valve (Fig. 5). The movement of the dye appeared to be faster at these three locations on the nasal valve, in comparison to dye flowing directly posterior to the lamellae, before exiting the excurrent nostril.

For specimen with the lamellae removed but nasal valve intact (*Preparation 2*), the flow appeared to be similar to the flow observed for Preparation 1.

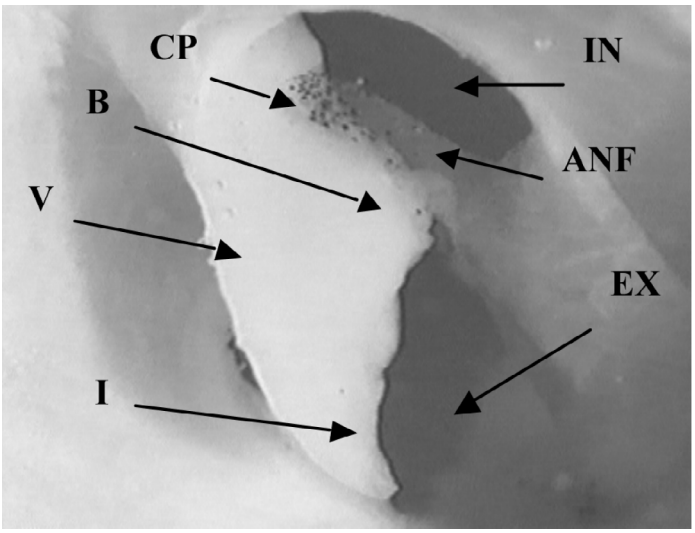

Fig. 5. Illustration of the olfactory cavity of the spiny dogfish where the nasal valve (V), incurrent and excurrent nostril (IN and EX, respectively), and anterior nasal flap are visible. The bulbous point (B) and indentation (I) are where water circulates around before exiting the excurrent nostril (EX). Incurrent nostril is on lateral side of shark and excurrent nostril is more medially positioned.

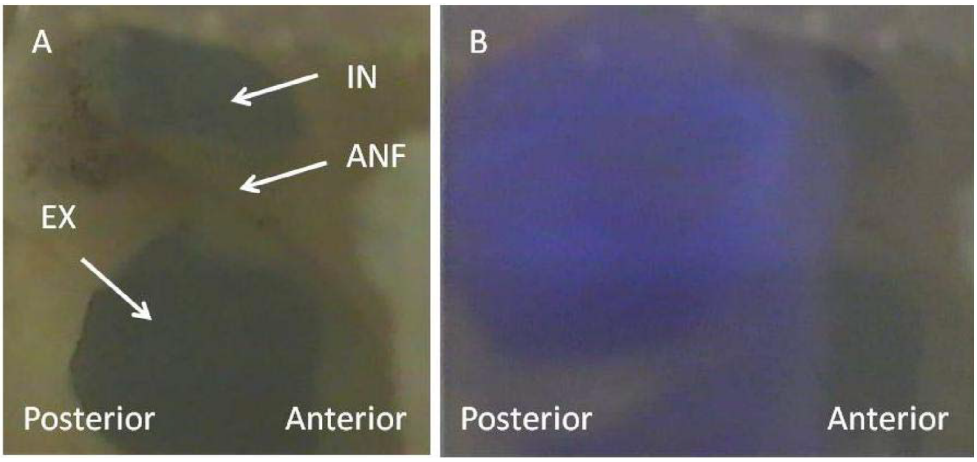

Fig. 6. Dorsal view of the olfactory cavity of the spiny dogfish with lamellae and valve removed (A). When the posterior nasal valve was removed, introduced dye became stagnant within the nasal cavity (B). IN, incurrent nostril on lateral side; EX, excurrent nostril on medial side, ANF, anterior nasal flap.

With both the lamellae and nasal valve removed (*Preparation 3*), dye did not initially enter the incurrent nostril. As a result, dye stagnated within the nasal cavity. Most of the dye that entered the incurrent nostril did not exit the nasal cavity through the excurrent nostril. Small traces of dye exited the excurrent nostril but flowed back into the incurrent nostril (Fig. 6). Dye was observed to stagnate in the inlet chamber, and the dye progressively grew more abundant in the cavity. Mixing of inlet and outlet dye streams was observed.

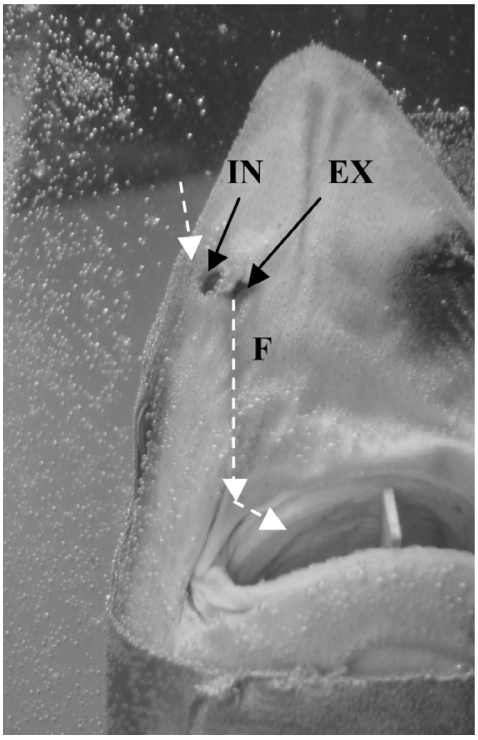

Fig. 7. Ventral view of spiny dogfish head. The olfactory cavity was filled with blue dye. The dye exited the excurrent nostril and flowed straight into the mouth of the shark. IN, incurrent nostril; EX, excurrent nostril; F, water flow.

3.3 Respiratory-induced nasal flow

Dye introduced anterior to the incurrent nostril for both high and low flow rates was drawn into the olfactory cavity through the incurrent nostril. The dye subsequently exited from the excurrent nostril. Upon exiting the excurrent nostril, the dye flowed posteriorly in a straight line along the ventral side of the rostrum toward the corner of the mouth. The dye subsequently angled inward toward the central part of the oral cavity and was drawn into the mouth (Fig. 7).

4 Discussion

The nasal valve of the spiny dogfish has a hydrodynamic function that serves to facilitate a smooth, unidirectional flow through the nasal cavity. Observations showed that in an ambient flow, dye entered the nasal cavity through the incurrent nostril and exited through the excurrent nostril. Removal of the nasal valve disrupted flow both into and through the nasal cavity. In the absence of the valve, there was no longer a morphological separation within the nasal cavity. The olfactory cavity was not functionally divided. Flow was not separated between the inlet and outlet chambers, and without the nasal valve, the dye tended to stagnate. As a result, the dye built up within the entire cavity, and did not exit the excurrent nostril, but rather back flowed out through the incurrent nostril with absence of the valve. The nasal valve therefore is necessary to maintain continuous flow through the nasal apparatus.

The position and number of nasal valves in sharks have been proposed to have a hydrodynamic function in regulating the flow of water for ventilation of the nasal apparatus [10,26]. The valve structure of teleost fishes, if present, enables unidirectional flow through the nasal cavity [2]. The milk shark, *Rhizoprionodon acutus*, also possesses a single nasal valve, which is assumed to function in the same manner as the spiny dogfish.

The spiny dogfish possesses anterior nasal flaps and a posterior nasal valve, which guides water into the nasal cavity. The anterior nasal flaps and nasal valve may temporarily reduce the speed of the water flow into the incurrent nostril, producing a pressure difference between incurrent and excurrent nostrils [2]. Dye appeared to flow into the inlet chamber at a slower velocity than in the outlet chamber upon exiting the olfactory cavity. The experimental modification of the nasal cavity may have affected the flow velocity due to the presence of the coverslip and reduction in nasal cavity volume. However, the general flow pattern as indicated by the movement of the dye appeared to be in agreement with the expectations of the experiment.

The flow pattern produced by the anterior nasal flaps is similar to the Pitot mechanism that has been proposed for teleost fishes with nasal flaps and valves that direct water into the incurrent nostril [2]. In sharks, the incurrent and excurrent nares are oriented perpendicular to each other. In the Pitot- mechanism, one opening (incurrent naris) is positioned into an oncoming flow or current [27]. The incurrent naris would experience both static and dynamic pressures, whereas the perpendicularly oriented excurrent naris would experience only static pressure [2]. Therefore, a pressure difference can be created and a secondary flow would be generated through the blind olfactory cavity of a shark. Such a Pitot-like mechanism can regulate pressure within the nasal cavity for a smooth, continuous flow [10,28]. Most sharks show a positive rheotaxis and orient themselves into a current [29]. This orientation enables a shark to harness the pressure difference that would be generated by a Pitot mechanism [2]. Computational fluid dynamic simulations of the hammerhead shark nasal apparatus resulted in high and low pressures at the incurrent and excurrent nostrils, respectively [14]. The pressure difference would induce a flow through the olfactory chamber.

Cox [2] argued that water must have been actively circulating within the olfactory cavity of sharks. Although active mechanisms may play a role in ventilation of the nasal cavity, observations of flow in the present study show that passive mechanisms can facilitate circulation through the nasal cavity. The constant swimming of the spiny dogfish would produce a steady current of water to ventilate the nasal cavity [10]. A transparent plastic model of the hammerhead shark cephalofoil placed in a water tunnel showed extensive, but incomplete ventilation of the nasal apparatus [8]. Pelagic sharks appear to use active swimming to maintain a constant flow through the olfactory cavities in concert with ram ventilation and respiratory ventilation (i.e., buccopharyngeal pump) [3,10,15,17,30].

The spiny dogfish may use buccopharyngeal pumping to actively ventilate the nasal apparatus. A steady flow drawn through the mouth of the spiny dogfish was found to induce a flow through the nasal apparatus. Dye entered the incurrent nostril and exited the excurrent nostril even in stagnant conditions, where flow from the oral cavity was the only flow present to pull water through the olfactory cavity. This induced flow occurred despite a head and nasal morphology that does not suggest a close association of the olfactory and respiratory systems, such as external grooves between the excurrent nostrils and the mouth [9]. Although the flow through the mouth was not cyclically pulsed as is typical of ventilatory flow in fish [31], the use of steady flow indicated that respiratory flows could be coupled with flow through the nasal apparatus. In addition, various fishes that utilize suspension-feeding and ram ventilation have flow velocities through the mouth and buccal cavity that are

within the range of the low flow condition in this study and approach the high flow condition [32,33]. Even cyclic ventilatory flows in smaller fish have flow velocities that approach the low flow condition [34,35]. Flow through the nasal apparatus and into the mouth was consistent with inferences made in previous reports [10,11].

Previous dye-injection studies of respiratory-induced nasal flow found that water could move through the nasal apparatus to the mouth and through the gills for the catshark (*Scyliorhinus canicula*), nursehound (*Scyliorhinus stellaris*), and dusky smooth-hound (*Mustelus canis*) [3,18]. The nasal morphology of these species was closely associated with the mouth and the flow of water through the nostrils was likely driven by respiratory pumping [20]. Based on the head and nasal morphology, the spiny dogfish was designated to "functional group" A, which was comprised of neoselachians that have excurrent nostrils situated on the anterior or lateral edge of the head and remote from the mouth [20]. This functional group consists of many shark species that are noted to be fast and continuous swimmers. These sharks were considered to have flow through the nasal apparatus driven primarily by the forward movement of the shark with respiratory actions being unlikely to greatly influence the flow [20]. However, results from the present study indicate that respiratory-induced flow could provide a mechanism to augment passive nasal ventilation.

The ability to detect prey over hundreds of meters by smell is important in sharks, which are considered to have an acute sense of smell [36]. Blood concentrations can be detected that are less than one part blood to one million parts of water. The passive movement of water through the nasal apparatus helps maintain a continuous sensing of the environment to find potential prey. The flow of water induced by the swimming motions of the shark or gill ventilation effectively couples olfaction with other mechanical systems. Furthermore, the continuous flow through the nasal apparatus on each side of the head is used to localize prey by differential olfactory stimulation as the shark swims along a sinusoidal path [37].

In summary, the hydrodynamic flow through the blind nasal cavities of sharks is a complex three-dimensional flow. The single nasal valve of the spiny dogfish facilitates a smooth, unidirectional flow through the nasal cavity. For the olfactory system of a shark to function effectively, the water surrounding the animal's head must be sampled continuously [12]. Flows induced passively by the forward movement of sharks and actively by respiration are mechanisms that act in concert with the head shape to ventilate the nasal apparatus. In addition, this study demonstrated that respiratory flow of the spiny dogfish could induce water to move through the olfactory cavity and into the mouth in a smooth, continuous flow [4,5,16].

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