Sensory Systems in Sawfishes.

2. The Lateral Line

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Key Words

Mechanoreception • Pristidae • Sawfish • Rostrum • Pit organs • Lateral line

Abstract

The lateral line system allows elasmobranchs to detect hydrodynamic movements in their close surroundings. We examined the distribution of pit organs and lateral line canals in 4 species of sawfish (*Anoxypristis cuspidata*, *Pristis microdon*, *P. clavata* and *P. zijsron*). Pit organs could only be located in *A. cuspidata*, which possesses elongated pits that are lined by dermal denticles. In all 4 pristid species, the lateral line canals are well developed and were separated into regions of pored and non-pored canals. In all species the tubules that extend from pored canals form extensive networks. In *A. cuspidata*, *P. microdon* and *P. clavata*, the lateral line canals on both the dorsal and ventral surfaces of the rostrum possess extensively branched and pored tubules. Based on this morphological observation, we hypothesized that these 3 species do not use their rostrum to search in the substrate for prey as previously assumed. Other batoids that possess lateral line canals adapted to perceive stimuli produced by in-faunal prey possess non-pored lateral line canals, which also prevent the intrusion of substrate particles. However, this hy-

pothesis remains to be tested behaviourally in pristids. Lateral line canals located between the mouth and the nostrils are non-pored in all 4 species of sawfish. Thus this region is hypothesized to perceive stimuli caused by direct contact with prey before ingestion. Lateral line canals that contain neuromasts are longest in *P. microdon*, but canals containing neuromasts along the rostrum are longest in *A. cuspidata*.

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Introduction

Mechanoreception, which is also referred to as distant touch, is used by aquatic vertebrates to detect the presence, absence and direction of water currents and shift waves caused by other animals (e.g. predators, prey and conspecifics) moving through the water [Dijkgraaf and Kalmijn, 1963]. It also allows fish to orient themselves in their environment and detect obstacles, but the detection range is limited to within a few body lengths of the receiver [Kalmijn, 1989]. Receptors of the lateral line system detect local accelerations of the surrounding water relative to the animal [Kalmijn, 1989].

The peripheral end organs of the lateral line system are distributed bilaterally and symmetrically over the head,

trunk and tail of the fish. Their sensory unit, the neuromast, is categorized depending on its position: free (or superficial) neuromasts are located on the epidermal surface with their cupulae extending into the surrounding water. In batoids, they are located in pits, which are surrounded by non-specialized denticles and are called pit organs [Maruska, 2001; Peach and Marshall, 2009]. Canal neuromasts are enclosed in lateral line canals, which can be embedded within skin, bone or cartilage. Canals are fluid-filled and connected to the environment via pores, which can be covered by a membrane. The vesicles of Savi described in elasmobranchs are another example of enclosed neuromasts.

To describe the functional properties of the lateral line, the detection characteristics have to be understood at different levels. When stimulated sinusoidally, hair cells are displacement detectors, as their receptor potentials are proportional to the displacement of the ciliary bundles relative to the cell body [Kalmijn, 1989]. Under constant displacement, hair cells adapt rapidly and cease to respond to the stimulus [Kalmijn, 1989]. At the organ level, most lateral line neuromasts are velocity detectors when stimulated sinusoidally, as the cupula overlying the cilia is displaced by viscous drag forces proportional to the water velocity [Kalmijn, 1989]. At the system level, arrays of free neuromasts act as velocity detectors, whereas the lateral line canal system detects acceleration [Kalmijn, 1989]. It is the spatial array of the lateral line canals over the head and body which enables the detection of localized acceleration differences distributed over this surface [Kalmijn, 1989].

The lateral line is a low-frequency system that detects hydrodynamic oscillations between 1 and 200 Hz [Kasumyan, 2003], but differences in the morphology of the peripheral structures can alter its functional characteristics. For example, the areas of maximum sensitivity of canal and free neuromasts differ [Kasumyan, 2003]. In teleosts, a membrane sealing the pores of a canal aids in noise reduction and amplifies oscillations between 5 and 10 Hz [Kasumyan, 2003]. Similarly, the non-pored canals of batoids display increased sensitivity towards low frequencies and function as touch receptors [Maruska and Tricas, 2004].

As structures of the lateral line system are stable morphological characters, they are useful for species identification and the assessment of interspecific relationships [Kasumyan, 2003]. We describe here the distribution of mechanosensory lateral line canals in 4 species of pristid sawfish, namely *Anoxypristis cuspidata* (Latham 1794), *Pristis microdon* (Latham 1794), *P. clavata* (Garman

1906) and *P. zijsron* (Bleeker 1851). We discuss its role in the detection of water movements and the use of the rostrum in the detection of prey. The distribution of pit organs is not known in any species of sawfish, but Hoffmann [1912] and Chu and Wen [1979] provided detailed but incomplete descriptions of the lateral line canals of *P. pectinata* and *A. cuspidata*.

Materials and Methods

Sawfish were obtained through the Queensland Department of Primary Industries observer programme or donated by other researchers (DPI&F Permit 87591 to B.E.W. and S.P.C.). As all species of sawfish are listed in CITES Appendix 1, even incomplete samples were dissected (table 1). Samples were obtained frozen, and thawed in 10% formalin in 0.1 M phosphate buffer for 24 h. They were transferred into another change of 10% formalin in phosphate buffer in which they were left for up to 2 weeks before being transferred into 70% ethanol or phosphate buffer (pH = 7.4, 0.1 M). All procedures were approved by the University of Queensland Animal Ethics Committee (Approval No. VTHRC/717/06, VTHRC/835/07 and VTHRC/974/08 (NF)).

To visualize the sub-dermal canal system of the lateral line, specimens were stained with methylene blue solution (approx. 1% in distilled water) applied to the skin under slight pressure, following the method of Wueringer and Tibbetts [2008]. If a lateral line canal was wide enough, methylene blue was injected directly into the canal with a syringe. Specimens were viewed using a Heerbrugg Wild M650 or Olympus SZ40 stereomicroscope and images were taken using a Canon Powershot A620 camera. Canals were dissected and traced along their whole extent. Several types of canals were distinguished: pored canals, non-pored canals, canals with branched or straight tubules, canals situated in the deep layer of the dermis or canals loosely associated with the connective tissue underneath the dermis. Maps of the lateral line system were redrawn in Adobe Illustrator CS3 (www.adobe.com).

We assessed if the neuromast receptor organs of sawfish were present in lateral line canals but absent in tubules, as reported for other elasmobranch species [Maruska, 2001]. The method of Marzullo et al. [2011] was used. The stain Janus green (0.1% in distilled water) was injected into lateral line canals and tubules with a syringe, and the canal/tubule was cut open and viewed. The presence of neuromasts was defined according to the following criteria. Firstly, canals that contained neuromasts were filled with a jelly which was interpreted as the dissolved upper layers of the cupula. Secondly, even though Janus green is a known vital stain for neuromasts in teleosts [Rouse and Pickles, 1991], in the fixed tissue it stained the walls of lateral line canals/tubules dark blue, whereas the cupula and neuromasts stained green. Thirdly, by removing neuromasts from the canal and viewing them with a Leitz Laborlux S light microscope, nerves extending from the neuromasts became visible. Fourthly, canals, from which neuromasts had been removed, contained regular perforations on the bottom of the canal, through which nerves reached the neuromasts. If a canal contained neuromasts, this was marked in schematic drawings. However, the positioning of neuromasts in relation to the canal axis, and also the distance between neuromasts,

could not be quantified due to the limited quality of frozen and fixed samples.

Lengths of lateral line canals that contained sensory structures were measured in ImageJ software (<http://rsb.info.nih.gov/ij/>) from the schematic drawing that was created for each species. The drawing was standardized to represent an animal with a body length (BL) of 100 cm. BL is defined as the length from the tip of the tail to the anterior fontanelle, which eliminates the differences in rostrum length (RL) across species. RL is measured from the tip of the rostrum to the anterior border of the anterior fontanelle. In order to graphically estimate the RL for a specimen of 100 cm BL, all BL measurements of 1 species were plotted in a graph against the total length.

The general arrangement of the lateral line of *P. microdon* (sp. 10) was analysed using standard histological methods. Tissue samples were kept in neutrally buffered formalin for several weeks. Samples containing denticles were decalcified in 15% EDTA for up to 4 days, washed in water, dehydrated in an ascending alcohol series (70, 90, 100, 100% EtOH for 45 min each), transferred into 100% xylene and embedded in paraffin wax at 60°C in a vacuum oven. Sections (6 µm) were cut with a Spencer 820 microtome and stained with Mayer's haematoxylin-eosin.

Identification of cartilages followed Wilga and Motta [1998], Wueringer and Tibbetts [2008] and Compagno [1999]. The terminology of the lateral line canals follows McEachran et al. [1996] and Chu and Wen [1979]. The fine structure of pit organs (of *P. pectinata*) is known from Budker [1938].

Results

All specimens of sawfish were juveniles (table 1). Results on *P. zijsron* were preliminary, as only one specimen was dissected, but no more samples were available. All specimens possessed dermal denticles distributed over the disc. In smaller specimens of *A. cuspidata*, dermal denticles are denser on the ventral side of the rostrum, but they are also present around sensory pits. In *Pristis* spp., dermal denticles are well developed in neonates and cover the whole skin surface.

Pit Organs

In *A. cuspidata*, as in other batoids, the sensory structures of pit organs are located in an elongated groove in the skin, which is oriented perpendicular to the body axis (fig. 1). In some neonates and small juveniles (n = 5, sp. 3–7), the elongated groove was bordered by a row of 2–3 denticles along the posterior and anterior sides. One sawfish (sp. 5) possessed 5 denticles on each side of the groove. Another neonate (sp. 8) did not possess any denticles and pit organs were not visible. Dorsal pit organs are distributed medially along the body axis on the dorsal surface of the skin (fig. 2b). They commence posterior to the spiracle and extend from the medial axis to lateral of the pos-

Table 1. General measurements of sawfish used in the study of dissections of the lateral line

No.	Parts	TL	BL	RL	Sex	Collection site
<i>A. cuspidata</i>						
1	H, P, S	166.5	96.1	70.4	M	Ducie River
2	H, S	146.5	109.0	37.5	F	Halifax Bay, Qld.
3	H	143.3			M	Halifax Bay, Qld.
4	H	140.8			M	Halifax Bay, Qld.
5	H, P, S	131.2	94.2	37.0	–	Taylors Point, Qld.
6	H, S	124.3	90.8	33.5	M	Bowling Green Bay, Qld.
7	H, P	121.3			F	–
8	H, S	74.6	52.0	22.6	F	–
9	H, P, S	67.0	44.6	22.4	F	–
<i>P. microdon</i>						
10	H, P, S	99.0	72.8	26.2	M	Norman River
11	H, P, S	142.0	106.6	35.4	F	Norman River
12	H, P, S	146.5	109.0	37.5	M	Norman River
13	H, P, S	149.5	112.1	37.4	F	Norman River
14	H, P, S	155.3	115.4	39.9	F	Leichhardt River
15	H, P, S	170.8	129.3	41.5	M	Leichhardt River
<i>P. clavata</i>						
16	H, P, S	–	–	33.7	–	Ducie River
17	H, P, S	77.0	60.1	16.9	F	–
18	H, P, S	71.6	54.2	17.4	F	–
19	H, P, S	150.0	119.1	30.9	M	Ducie River
20	H, P	212.0	172.2	39.8	M	Ducie River
<i>P. zijsron</i>						
21	H, P	287.0	213.9	73.1	F	Ducie River

TL = Total length. All specimens are juveniles. 'Parts' indicates if the specimen is complete with head (H), pectorals (P) and saw (S). Measurements of the total length of *A. cuspidata* in italics were calculated (see Wueringer et al., 2011). All measurements are in centimetres.

Fig. 1. Schematic representation of the mechanosensory lateral line canals and free neuromasts (pit organs) on the dorsal and ventral surfaces of pristids. Main canals, tubules and secondary tubules are indicated in lines of varying thickness. Only in *A. cuspidata* are secondary tubules drawn; in all species of *Pristis* spp. the extent of their networks and pore fields is indicated in light purple. In *A. cuspidata*, the area that can contain free neuromasts is marked with a green line, and schematic neuromasts are drawn in green. **Inset** Magnification of rostral lateral line canals. DILC = Dorsal infraorbital lateral line canal; DHLCL = dorsal hyomandibular lateral line canal; DSLC = dorsal supraorbital lateral line canal; MALC = mandibular lateral line canal; MLC = medial lateral line canal; NLC = nasal lateral line canal; PLC = posterior lateral line canal; PNLC = prenasal lateral line canal; SCLC = scapular lateral line canal; VHLC = ventral hyomandibular lateral line canal; VSLC = ventral supraorbital lateral line canal.

terior lateral line canal. The dorsal pit organs were counted in each body half of three specimens that were donated with pectorals and gill area attached ($n = 6$, 17.5 ± 7.4 dorsal pit organs). No spiracular pit organs or pit organs on the ventral surface were found in *A. cuspidata*.

Pit organs could not be quantified in *Pristis* spp., as they could not be located in the skin with the methods used. The dermal denticles of *Pristis* spp. are quite dense, and successful location of pit organs may require the use of electron microscopy, or vital staining.

Lateral Line Canals

The mechanosensory lateral line system forms a subepidermal bilaterally symmetrical net on the dorsal and ventral surface in all pristids (fig. 1). Most canals are embedded in the fibrous layer of the dermis and contain sensory neuromasts along their length. Canals embedded in the fibrous layer can penetrate into the deep fascia of the dermis and become only loosely associated with the dermis. Canals are either non-pored or pored. Pored canals possess either only a single terminal pore or additional pored tubules along their length (fig. 2e). Tubules can be branched or straight.

Tubules of *Pristis* spp. were divided into primary and secondary tubules, as they can be quite extensive, both in number and branching. Primary tubules branch off the main lateral line canals, whereas secondary tubules branch off primary tubules. Furthermore, canal walls of secondary tubules are not reinforced by dense connective tissue like the canal walls of main lateral line canals or primary tubules (fig. 2e–h). Secondary tubules often form extensive networks in the dermis. They do not contain sensory neuromasts, whereas primary tubules contain sensory neuromasts in certain locations (fig. 3). In general, tubules are more extensively branched in *P. microdon* than in *P. clavata* or *P. zijsron*. Interestingly, the skin of *P. clavata* was about twice as thick as that of a similar-sized specimen of *P. microdon*.

Dorsal Lateral Line Canals

In all pristids, the dorsal infraorbital lateral line canal (DILC) connects bilaterally over the neurocranium anterior to the pores of the endolymphatic duct. Up to there, the DILC is non-pored and embedded within the deep fascia, which is closely associated with the neurocranium. Lateral to the endolymphatic ducts, it connects the posterior lateral line canal (PLC) to the dorsal supraorbital lateral line canals (DSLCL). In *Pristis* spp. this junction is separated into two junctions, a posterior one between the PLC and the DILC, and an anterior one between the

DILC and the DSLCL. These two junctions are connected by a stretch of the DILC where it runs from anterior to posterior. From its connection point to the two other canals, the DILC continues laterally between the spiracle and the eye, forming a thick non-pored tube that is loosely associated with the deep fascia. Antero-lateral to the spiracle, the DILC bifurcates into 1 anterior and 2 posterior branches in *A. cuspidata*, *P. clavata* and *P. zijsron* (2 anterior and 2 posterior branches in *P. microdon*).

In all pristids the 2 posterior branches run parallel to each other in the caudal direction. They become more closely associated with the deep fascia and terminate in branched tubules lateral of the caudal margin of the spiracle. In all pristids, the anterior branch or branches of the DILC run lateral to the lower margin of the orbita, extending anteriorly to the eye onto the nasal capsule. In *A. cuspidata* it ascends into the fibrous layer of the dermis and terminates in branched tubules on both sides. When passing laterally along the eye, the anterior branch of the DILC is non-pored and is loosely associated with the deep fascia. In *P. microdon* the more lateral branch terminates lateral to the anterior end of the eye, but the medial branch extends further anterior and onto the nasal capsule, where it ascends into the fibrous layer of the dermis and connects to the ventral infraorbital lateral line canal (VILC). In *P. clavata* and *P. zijsron*, the 1 anterior branch connects to the VILC slightly anterior of the orbits. In *Pristis* spp. the area between and around all 3 or 4 branches contains a network of tubules that connects all of them with each other, and also with the anterior part of the dorsal hyomandibular lateral line canal (DHLC).

Lateral and slightly anterior of the endolymphatic pores, the PLC extends from a junction with the DILC in all pristids. From there, the PLC continues in a posterior direction lateral to the dorsal midline. Numerous tubules branch off medially and laterally in *A. cuspidata*, *P. microdon* and *P. clavata* (in *P. zijsron* only medially), and possess even smaller, secondary tubules branching off along their length (fig. 1). The secondary tubules form a network in the dermis of *Pristis* spp.

In all pristids the scapular lateral line canal (SCLC) branches off the PLC above the pectoral girdle. In *Pristis* spp. the PLC bends laterally at this junction. In *P. microdon*, long tubules extend from this area anteriorly. In *A. cuspidata* the PLC does not possess secondary tubules caudal of this junction. Situated in the fibrous layer of the dermis, the SCLC follows along the pectoral girdle. The SCLC forms 3 branches in *P. microdon* and *P. zijsron*, but only 1 in *P. clavata* and *A. cuspidata*, which run parallel

Fig. 2. Structures of the mechanosensory lateral line of pristids. **a** On the ventral surface of the rostrum of *A. cuspidata*, branched tubule (LL) of the DSLC are intermixed with pores of the ampullae of Lorenzini (AOL). **b** Pit organs (P) of *A. cuspidata* are surrounded by 2–5 scales on each side. **c** Near the cranial foramen (CF) of *A. cuspidata*, ampullary pores (AOL) intermix with tubules of the DSLC (LL). **d** A neuromast (Ne) was removed from the rostral DSLC of *P. microdon*, after staining with Janus green. The nerve (N) is attached. **e** Cross section of the epidermis and

dermis of the DHLC lateral and anterior of the eye of *P. microdon*. The lumen (Lu) of the primary lateral line canal contains a neuromast (Ne). Primary (T1) and secondary (T2) tubules are closer to the skin surface and do not contain neuromasts. **f** The network formed by secondary tubules (T2) extending from **(g)** a pored (Po) primary lateral line canal (Ca) of the DHLC of *P. microdon*. **h** The network formed by secondary tubules (T2) of the rostral DSLC of *P. microdon* as seen under a light microscope.

Fig. 3. Distribution of the neuromast epithelium in the lateral line canals of 3 species of pristids. Canals of the lateral line system that do not contain neuromasts are drawn in grey.

over the metapterygial cartilage towards the posterior base of the pectoral fin. In *P. microdon* additional tubules branch off these three canals, forming a network of pored tubules. Above the mesopterygial cartilage, the scapular canal connects to the DHLC. From this junction, the SCLC continues along the metapterygial cartilage to the posterior base of the pectoral fin, where it ends. In *A. cuspidata* the SCLC possesses numerous tubules, some of which are branched.

In all pristids the DHLC loosely follows the propterygial cartilage from its junction with the SCLC, while remaining embedded in the fibrous layer of the dermis. In *Pristis* spp. primary tubules extend laterally from the DHLC, connecting to a network formed by secondary tubules which ends in minute pores (fig. 1). Before the DHLC extends to the ventral side, lateral to the posterior border of the eye, it is integrated into a network of primary and secondary tubules that connect it to the DILC. In *A. cuspidata* tubules extend laterally from the DHLC, connecting to a smaller lateral line canal that runs parallel to and lateral of the DHLC. From this smaller canal, more straight tubules extend laterally, carrying second-

ary pored tubules along their length. Lateral to the midline of the eye, the tubules of the DHLC cease and the smaller parallel canal fuses with the DHLC. Anterior to the eye, the DHLC extends to the ventral side.

In all pristids the DSLC originates from the junction of the PLC and DILC, which occurs lateral to the endolymphatic pores. From there, it continues in the anterior direction lateral to the dorsal midline. Above the neurocranium the DSLC is embedded in the deep fascia, which is closely associated with the neurocranium and forms an extensive pore field between the endolymphatic pores and the anterior fontanelle. Anterior to the anterior fontanelle, the DSLC then extends onto the rostrum running parallel to the edge of the rostrum at a distance of 1/5 of the rostrum width (1/3 in *Pristis* spp.) from the edge. The DSLC forms a pore field with highly branched tubules along the dorsal side of the rostrum (fig. 2). Along the rostrum of *P. microdon* and *P. clavata* primary tubules extend both medially and laterally from the DSLC. They are connected to the surface via secondary tubules. The secondary tubules form an intricate pattern: right above the DSLC they form a canal network, from which parallel tubules extend only laterally. Near

the rostral appendix (in *P. microdon* halfway between the first and second rostral teeth), the DSLC breaks through to the ventral side and connects to the ventral supraorbital lateral line canal (VSLC). The rostrum of *P. zijssron* was missing.

Ventral Lateral Line Canals

On the ventral surface, an abdominal lateral line canal (ALC) was present in *P. microdon* and *P. clavata*, but absent in *A. cuspidata*. The ALC is located in the skin above the ventral pectoral girdle and possesses primary tubules branching off the main canal to both sides. In *P. zijssron* the canal may have been present in the abdominal skin not donated with the sample.

In all pristids the mandibular lateral line canal (MALC) is situated on the mandible. In *A. cuspidata*, *P. zijssron* and *P. clavata*, the MALC is pored with tubules along its length. In *A. cuspidata* and *P. clavata*, the MALC does not connect across the midline. In *P. microdon* and *P. zijssron*, it runs parallel to the mouth at half the width of the mandible and the two bilateral canals connect across the body midline. In *P. microdon*, the MALC is connected to the surface via terminal pores but possesses no tubules.

In all pristids the ventral hyomandibular lateral line canal (VHLC) forms a loop projecting posteriorly between the lateral edge of the nasal capsule and the first gill opening. In *P. microdon* straight primary tubules radiate in both directions from the medial part of the loop, but radiate only laterally from the lateral part of the loop, whereas in *A. cuspidata*, *P. clavata* and *P. zijssron* straight primary tubules radiate in only one direction. These primary tubules are connected to the surface via highly branched secondary tubules. In *A. cuspidata* the outer branch of the VHLC connects to the DHLC, but also sends a branch forward, which connects to the inner branch of the VHLC. In *Pristis* spp. the outer branch of the VHLC connects only to the DHLC.

Posterior to the nostril of *A. cuspidata*, *P. clavata* and *P. zijssron*, the nasal lateral line canal (NLC) draws from the midline towards the edge of the disc. It is positioned closer to the mouth in *P. microdon*. Lateral of the nostril, the NLC forms a junction with the VSLC, from which the VSLC extends anteriorly, and the NLC extends posteriorly. Along its path the NLC is non-pored. More laterally, the NLC ends in a junction, from which the VILC extends anteriorly, while the VHLC extends posteriorly.

In *A. cuspidata* the VILC does not exist. In *Pristis* sp. the VILC runs lateral of the VSLC, and connects to the DILC anterior of the nostril. In *P. microdon*, but not in *P. clavata* or *P. zijssron*, the VILC possesses primary and sec-

Table 2. Length of the sensory epithelium in the lateral line canals of a hypothetical specimen of each species of sawfish, with a standardized BL of 100 cm

	AC	PM	PC
TL	142.2	134.2	120.0
BL	100	100	100.0
RL	42.2	34.2	20.0
SCLC	10.54	13.20	7.71
DHLC	16.77	21.88	9.69
PLC	13.42	18.82	9.17
DILC	9.19	10.49	7.61
DSLC	46.64	38.45	23.08
ALC	0	7.47	4.21
MALC	1.28	3.30	1.11
VHLC, NLC, MLC	26.44	31.46	19.15
VSLC	45.04	46.25	32.56
PNLC	43.04	35.78	21.33
Total dorsal	96.56	102.84	57.25
Total ventral	115.80	124.26	69.36
Total	212.36	227.10	126.61

All measurements in centimetres. AC = *A. cuspidata*; PM = *P. microdon*; PC = *P. clavata*; TL = total length; MLC = medial lateral line canal; see text for other lateral line canal abbreviations.

ondary tubules along its path, which also connect it to the VSLC.

In all species of sawfish, the VSLC forms a non-pored loop anterior to the nostril, which is embedded in the fibrous layer of the dermis. From this loop, the VSLC extends along the rostrum, at 1/10 of the rostrum width in *A. cuspidata* (1/5 in *Pristis* spp.). On the rostral appendix (between the first and second rostral tooth in *P. microdon*) the VSLC penetrates the rostrum and connects to the DSLC. The VSLC possesses pored primary and secondary tubules along the rostrum, which draw only laterally. In *P. zijssron*, the VSLC could only be traced to slightly anterior of the nostril, where the rostrum was cut. On the dried rostrum only the general direction of the canals could be seen, which followed that described in the 2 other species of sawfish, but the extent of tubules could not be evaluated. This area is therefore represented with '?' in the schematic drawing (fig. 1).

The pre-nasal lateral line canal (PNLC) extends medial to the VSLC along the rostrum, at about 1/5 of the rostrum width in *A. cuspidata* (about 1/3 in *Pristis* spp.). Its two bilaterally symmetrical canals fuse across the midline on the rostral appendix. Along the rostrum, the PNLC possesses primary and secondary tubules that

form a fine network and project towards the midline in all species except *P. zijsron*, in which this canal could not be examined. Medial to the nostril, the PNLC curves towards the midline, where its 2 bilaterally symmetrical branches fuse and connect to the NLC via a short median canal.

Distribution of the Sensory Epithelium in the Lateral Line Canals of Pristids

Janus green was injected into the lateral line canals to locate sensory neuromasts in *A. cuspidata*, *P. microdon* and *P. clavata*. All species possessed neuromasts contained within an epithelium on the bottom of the canals (fig. 2d, e). Thus, measurement of the length of canals that contained neuromasts provided an approximation of the length of neuromast epithelia. However, the positioning of neuromasts in relation to the canal axis was not quantified. Removal of neuromasts exposed perforations in the canal wall, which allow innervation. These perforations were indicative of the presence of neuromasts in a canal even if the sensory structures did not clearly absorb the stain.

The length of the sensory epithelium was measured and compared to the hypothetical specimens with a standardized BL of 100 cm (fig. 3). Comparison of the BL and RL between *P. microdon* and *A. cuspidata* (table 1) shows that the percentage of RL of *P. microdon* (34.2 cm) is smaller than in *A. cuspidata* (42.2 cm). Thus, the rostral neuromast epithelium is shorter in *P. microdon* than in *A. cuspidata* (table 2). However, the total neuromast epithelium is longer in *P. microdon* both ventrally and dorsally (227.1 cm), as the canals on the head are longer than in *A. cuspidata* (212.4 cm). *P. clavata* had both the shortest rostrum (20.0 cm) and also the shortest lateral line epithelium (126.6 cm) in this comparison.

Discussion

The lateral line system is a purely aquatic sensory system that is present in all fish and some amphibians [Coombs and Braun, 2003; Montgomery et al., 1995]. The morphology of the sensory unit – the neuromast – is quite stable in an evolutionary sense, while the morphology of the accessory structures – the lateral line canals and structures surrounding free neuromasts – is quite variable in relation to the ecology of a species [Coombs and Braun, 2003]. As the morphological characteristics of the accessory structures influence and guide the detection capabilities of the neuromasts, their morphological variations are functionally important and provide insights

into biologically significant stimuli for a given species [Coombs and Braun, 2003].

Pit Organs

To date, only one study has examined superficial neuromasts (pit organs) in pristids [Budker, 1938]. In adult *P. pectinata* they are difficult to locate, as pit organs are small fissures between tightly packed denticles that are not indicated by any particular morphology of these denticles [Budker, 1938]. Similarly, in our study we failed to locate pit organs in any species of *Pristis*. *A. cuspidata* is the only species of sawfish that does not possess any denticles at birth [Deynat, 2005; Taniuchi et al., 1991; Wueringer et al., 2009]. When the first denticles develop, those that line the elongated pits develop first, presumably to protect these minute sensory structures from abrasion. Initially, 3 denticles line each side of the pit organ, but this number seems to increase with age. Before covering the whole skin surface, denticles form along scars. Later, more denticles develop which eventually cover the skin completely. In the largest specimen the head and saw were completely covered with denticles, but more irregularly than in other rays (e.g. *P. microdon* or rhinobatids).

A. cuspidata possesses open slit-type pit organs bordered by accessory denticles. This condition is identified as plesiomorphic, contrasting the apomorphic conditions of having either overlapping denticles that cover the sensory structures in galean sharks or no accessory denticles as in ‘recent batoids’ (e.g. mylobatiformes) [Peach and Rouse, 2004]. Both the mylobatiforms and lamnids have independently lost their spiracular pit organs, which may be due to their pelagic lifestyle [Peach and Rouse, 2004]. Whether the absence of spiracular pit organs in *A. cuspidata* is related to a pelagic lifestyle is unclear; a comparison with other pristids is needed first. Nevertheless, fisheries data from the Australian Gulf of Carpentaria indicates that *A. cuspidata* regularly ventures further offshore than any other pristid species [Peverell, 2006].

Mandibular pit organs are absent in *A. cuspidata*. This group of sensory receptors appears to be present only in sharks and rhinobatid rays [Peach, 2003]. Its absence in all other batoids is regarded as an apomorphic condition, and in mylobatid rays, the reduction of ventral pit organs may be related to the reduction of accessory denticles, and therefore the loss of protection against abrasion [Peach and Rouse, 2004].

Lateral Line Canals

The results of this study on the morphology of the lateral line system of pristids are in accordance with, but also

expand the previous description of *A. cuspidata* [Chu and Wen, 1979; Hoffmann, 1912]. All 4 species of sawfish described here possess pored lateral line canals dorsally and ventrally along the rostrum. This finding is of particular interest, as sawfish are commonly described as using their rostrum to rake through the substrate in search of cryptic prey [Breder, 1952]. However, this behaviour would predict the presence of non-pored lateral line canals, at least along the ventral side of the rostrum, which would allow the location of infaunal prey to be detected via tactile stimuli, which would not be detected by pored canals [Maruska and Tricas, 2004]. Moreover, non-pored canals are superior to pored canals for an animal that searches through the substrate, as the arrangement prevents contamination by sand. The rostral cartilage, which underlies all rostral lateral line canals, may also restrict the depression of lateral line canals and would therefore limit the detection of mechanotactile stimuli along the rostrum. The length of the rostral teeth of freshwater sawfish *P. microdon* is known to vary according to the location of capture and the predominant substrate type [S.C.P., pers. commun. to B.E.W.], indicating that these sawfish sharpen their teeth on the substrate. This does not contradict our hypothesis that these animals do not rake through the substrate in search for infaunal organisms, as during tooth-sharpening behaviour the ventral side of the rostrum is not necessarily buried in the substrate.

The distribution of pored and non-pored lateral line canals is comparable in the 4 pristid species, and canals located ventrally between the nostrils and the mouth and between the nostrils and the base of the rostrum are non-pored. During the final stages of prey manipulation, when a sawfish repositions its mouth on top of the prey to ingest it [B.E.W. pers. obs.], the area of the disc around the mouth and the nostrils is most likely to come into close contact with the substrate and the prey. Thus non-pored canals are more functional, as pored canals could be easily blocked by sand grains [Maruska and Tricas, 2004], and mechanotactile stimuli created by the prey depressing the skin around the sawfish's mouth may overstimulate pored canals.

Interestingly, while *A. cuspidata* does not possess an ALC, it is present in 2 *Pristis* species and all rhinobatids and rhynchobatids studied to date [Chu and Wen, 1979; Wueringer and Tibbetts, 2008], but absent in all other batoids studied to date [Chu and Wen, 1979]. As sawfish evolved from a rhinobatid-like ancestor [Cappetta, 1974; Schaeffer, 1963; Wueringer et al., 2009], the absence of the ALC in *A. cuspidata* is considered an apomorphy. It is unclear, however, if the similarity of the branched ALC

in *Aptychotrema rostrata* and *P. microdon* is caused by ecological or phylogenetic constraints and which factors lead to *Glaucostegus typus* possessing an open-groove ALC [Wueringer et al., 2009].

In *A. cuspidata*, the canal that branches off the VHLC and connects to the NLC could represent a remnant of the VILC, but as it morphologically resembles a tubule, it has not been named. The VILC of *Pristis* sp. runs lateral to the nostrils, which do not reach the lateral margin of the head. In *A. cuspidata*, however, the nostrils form a groove in the lateral margin of the head that channels the hydrodynamic flow into the nostril. Thus the reduction of the VILC may be caused by the need to increase the olfactory sampling space and acuity in *A. cuspidata*, which of all the pristids ventures furthest offshore [Peverell, 2006].

The lateral line system of sawfish possesses highly branched tubules along most of its extent. The branched lateral line canals of *A. cuspidata* form a network of tubules that is comparable to that formed by the secondary tubules of *P. microdon* anterior of the endolymphatic pores in only the DSLC. Otherwise, the branched canals of *A. cuspidata* do not form extensive networks, and secondary tubules are straight and short. In *P. microdon*, however, branched lateral line canals generally possess a network of primary and secondary tubules that form the most intricate pattern. Primary tubules branch off the main canals, either to one or both sides, and form a network above the main lateral line canals. Secondary tubules are located between the primary tubules and the epidermis, and form an even more intricate network that connects the canals to the outer medium via pores.

Two factors may explain the intricate networks. In bony fishes, the network of lateral line canals on the head is often more complexly branched than on the body [Kasumyan, 2003]. Usually, more intricate tubules are found in species that grow large, to provide connectivity between the neuromasts ontogenetically with increasing canal length while the numbers of neuromasts remain the same [Kasumyan, 2003]. In the European sole *Solea vulgaris* the number of pores increases soon after metamorphosis, but pores remain small to prevent particle intrusion while maintaining good resolution [Kasumyan, 2003]. As *P. microdon* can attain total lengths of around 5 m [L.S., pers. obs.] and juveniles occur in murky waters with large amounts of particulate matter suspended in the water column, both arguments may explain its extensively branched tubules. *A. cuspidata*, on the other hand, attains a maximum total length of 350 cm [Last and Stevens, 2009], and ventures offshore into clearer waters than *P. microdon* [Peverell, 2006].

A direct comparison between *P. clavata* and *P. zijsron* is difficult. Historically, *P. clavata* was thought to remain relatively small, hence the common name 'dwarf sawfish', but recent evidence indicates that specimens up to 230 cm total length are still immature [Thorburn et al., 2008]. The lateral line of this species forms a network that is as intricate as that of *A. cuspidata*, but not as intricate as that of *P. microdon*. *P. zijsron* – as far as could be assessed from only one specimen – potentially possesses the least intricate lateral line system of all the 4 species studied, although this species attains recorded total lengths of over 500 cm [Peverell, 2006].

The morphology of the lateral line of *P. clavata* has another unique feature: its canals are the straightest of all sawfish species studied. This may be related to the thickness of its skin, which can be up to 2 mm thick even in small specimens – which makes it at least 4 times thicker than in the other species of sawfish examined. Not enough is known of the biology of this species of sawfish to hypothesize on ecological factors that may have contributed to such a thick epidermis.

Comparison of the Sensory Epithelium

Interspecific comparison between the lengths of the sensory epithelium inside the lateral line canals can shed light on the relative importance of this sensory system in sawfish. The BL of 100 cm was chosen as this falls within the length range of samples used for this study and should therefore produce a smaller error than extrapolating from the sample and choosing a body size outside of the

sample range. However, data from other researchers indicates that the RL calculated for *P. clavata* may be too small [J. Whitty, pers. commun.]. The use of a standardized BL shows that a longer rostrum in *A. cuspidata* simultaneously increases the length of its sensory epithelium along the rostrum. The specialization differences between *A. cuspidata* and *P. microdon* are clear, as the latter possesses a longer lateral line epithelium along the head, suggesting this region is specialized for the detection of hydrodynamic disturbances.

In all 4 species of sawfish studied, the lateral line is a well-developed sensory system widely distributed over the cephalic disc including the rostrum. Its occurrence on both sides of the rostrum indicates a potential role in prey manipulation. Additionally, the presence of pored canals along both sides of the rostrum indicates that sawfish may not use their rostrum to rake through the substrate for prey, but this needs confirmation via behavioural experiments.

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