Morphology of the Mechanosensory Lateral Line System in the Atlantic Stingray, *Dasyatis sabina*: The Mechanotactile Hypothesis

KAREN P. MARUSKA* AND TIMOTHY C. TRICAS
Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida

ABSTRACT   The biological function of anatomical specializations in the mechanosensory lateral line of elasmobranch fishes is essentially unknown. The gross and histological features of the lateral line in the Atlantic stingray, *Dasyatis sabina*, were examined with special reference to its role in the localization and capture of natural invertebrate prey. Superficial neuromasts are arranged in bilateral rows near the dorsal midline from the spiracle to the posterior body disk and in a lateral position along the entire length of the tail. All dorsal lateral line canals are pored, contain sensory neuromasts, and have accessory lateral tubules that most likely function to increase their receptive field. The pored ventral canal system consists of the lateral hyomandibular canal along the disk margin and the short, separate mandibular canal on the lower jaw. The extensive nonpored and relatively compliant ventral infraorbital, supraorbital, and medial hyomandibular canals form a continuous complex on the snout, around the mouth, and along the abdomen. Vesicles of Savi are small mechanosensory subdermal pouches that occur in bilateral rows only along the ventral midline of the rostrum. Superficial neuromasts are best positioned to detect water movements along the transverse body axis such as those produced by tidal currents, conspecifics, or predators. The pored dorsal canal system is positioned to detect water movements created by conspecifics, predators, or possibly distortions in the flow field during swimming. Based upon the stingray lateral line morphology and feeding behavior, we propose the Mechanotactile Hypothesis, which states that the ventral nonpored canals and vesicles of Savi function as specialized tactile mechanoreceptors that facilitate the detection and capture of small benthic invertebrate prey. J. Morphol. *238*:1–22, 1998.   © 1998 Wiley-Liss, Inc.

KEY WORDS: elasmobranch; lateral line; mechanosensory; neuromast; mechanotactile

The mechanosensory lateral line occurs in all fishes and aquatic amphibians (Dijkgraff, '63; Lannoo, '87; Webb, '89; Northcutt, '92) and detects near field water movements relative to the skin surface (Harris and Van Bergeijk, '62; Kalmijn, '89; Coombs, '94; Coombs et al., '96). The functional unit of the lateral line system is the neuromast, composed of mechanosensory hair cells and support cells covered by a gelatinous cupula. Displacement of this cupula by viscous drag modulates the hair cell receptor potential and associated primary afferent neural discharges (Hoagland, '33). Morphological features of the peripheral lateral line system determine the characteristics of information sent to processing centers in the brain. For example, the spatial distribution of neuromasts defines the receptive field on the body (Denton and Gray, '83), innervation patterns influence system sensitivity, and provide information on peripheral processing mechanisms (Munz, '89), and neuromast morphology determines which component of water motion (e.g., acceleration or velocity) is encoded (Kalmijn, '89; Kroese and Schellart,

*Correspondence to: Karen P. Maruska, Dept. of Biological Sciences, Florida Institute of Technology, 150 W. University Blvd., Melbourne, FL 32901–6988. E-mail:maruska@fit.edu or E-mail: tricas@fit.edu

'92). Assessment of characteristics such as neuromast morphology, distribution, and patterns of innervation are essential to evaluate the functional organization of the lateral line system.

With the exception of the review by Boord and Campbell ('77) on shark lateral line systems, studies on the organization of the lateral line periphery in elasmobranchs are generally incomplete and make little reference to function. Three types of neuromasts occur in most elasmobranchs. Superficial neuromasts (SN), or pit organs, are located on the skin surface with the cupula directly exposed to the water. Canal neuromasts (CN) are situated in dermal or subdermal canals that are in contact with the water via pores on the skin. Vesicles of Savi (VS) are isolated mechanoreceptors situated in subdermal pouches primarily on the ventral surface of some torpediniform and dasyatid batoids. Lateral line canals are generally described separately (Garman, 1888; Tester and Kendall, '69; Chu and Wen, '79; Puzdrowski and Leonard, '93) from superficial neuromasts (pit organs) in elasmobranchs (Tester and Nelson, '69) and are considered together only in the skate, *Raja batis* (Ewart and Mitchell, 1892). Thus complete descriptions of the anatomy of the peripheral mechanosensory system within a single elasmobranch species are extremely limited.

The lateral line system in teleosts functions in the detection of water flow across the skin, and facilitates schooling behavior (Partridge and Pitcher, '80), localization of stationary objects (Campenhausen et al., '81; Hassan, '89), social communication (Satou et al., '91, '94), and prey detection (Hoekstra and Janssen, '85, '86; Montgomery and Saunders, '85; Montgomery et al., '88; Montgomery, '89; Montgomery and Milton, '93; Janssen et al., '95). However, use of lateral line-mediated mechanoreception for these functions in elasmobranch fishes is presently uninvestigated. The organization of the lateral line system in elasmobranchs differs from that of all teleosts primarily in the location of multiple neuromasts between adjacent pores and the existence of extensive nonpored canals on the head (Chu and Wen, '79; Webb and Northcutt, '97; Webb, '89). These morphological differences likely represent unique transduction mechanisms between teleost and elasmobranch lateral line systems that may be related to function.

The Atlantic stingray, *Dasyatis sabina*, is the most abundant and widely distributed batoid elasmobranch in estuarine waters of the southeastern United States. It feeds almost exclusively on small benthic infaunal invertebrates (e.g., amphipods, mysids, isopods, ophiuroids, polychaetes, and bivalves) that it excavates from the sand substrate (Turner et al., '82; Cook, '94) and feeds almost continuously throughout the day and night (Bradley, '96). We used this species to examine the gross anatomy, morphology, spatial organization, and innervation patterns of lateral line mechanoreceptors to interpret the function of these different receptor types with special emphasis on their role in predation. As a result of this analysis, we propose the Mechanotactile Hypothesis to explain at least one function for the unique, nonpored lateral line canals found in elasmobranch fishes.

MATERIALS AND METHODS

General morphology, distribution, and innervation patterns of the mechanosensory lateral line system were examined in the Atlantic stingray, *Dasyatis sabina*. Canal topography and neuromast distribution, innervation and axon characteristics, and distribution of cutaneous nerve endings were examined by gross dissection and standard histological techniques. Observations on stingray feeding behavior were conducted in the field and used in conjunction with morphological data to formulate hypotheses on the possible function of lateral line mechanoreceptors in the detection and capture of benthic invertebrate prey.

Canal topography and neuromast distribution

Mature adult Atlantic stingrays, *Dasyatis sabina*, of both sexes were collected with a 1" mesh seine net from the Banana River (FL). Rays were anesthetized (MS222) and pithed, fixed in 10% formalin, and stored in 50% isopropyl alcohol. Superficial neuromasts were stained in six live rays by immersion in a 0.05% methylene blue-seawater solution for 4–6 h. Superficial neuromasts are located on raised areas of epidermis (termed papillae) that contain a central groove with a sensory epithelium that sits at the groove base. Immersion of the ray in methylene blue solution stains the edges of the papillae grooves light blue. Each superficial neuromast papilla was marked with colored latex

paint and counted under a dissecting microscope. Orientation of the superficial neuromast papilla groove was recorded as the angular deviation from the rostrocaudal body axis and midsagittal plane.

Locations of the dorsal and ventral hyomandibular, supraorbital, infraorbital, posterior lateral line, and mandibular canals were mapped after pressure injection of a 0.5% methylene blue solution into the canals. Vesicles of Savi and canal neuromasts were located by injection of 0.5% toluidine blue stain into the lateral branches of each canal and visualized under a dissecting microscope. Classification of the canals as hyomandibular, supraorbital, infraorbital, and mandibular follows that of Ewart and Mitchell (1892) and Tester and Kendall ('69). However, we adopt the term "posterior lateral line canal" (PLL) used by Puzdrowski and Leonard ('93), which is based on innervation of this canal by the posterior lateral line nerve, to replace the term "lateral canal" used by others (Ewart and Mitchell, 1892; Tester and Kendall, '69; Chu and Wen, '79).

Neuromasts and surrounding tissue dissected from preserved stingrays were dehydrated in a graded alcohol series, cleared in toluene, and infiltrated with and embedded in paraffin (paraplast ®). Tissue was sectioned at 8 μm on a microtome, mounted on chrom-alum coated slides, and stained with Mayer's hematoxylin and eosin or Gomori's trichrome stain (Humason, '79). The anatomy of mechanoreceptors (canal neuromasts, superficial neuromasts, and vesicles of Savi) was then examined under a compound microscope, but the polarity and density of hair cells were not examined in this study. General neuromast dimensions (length and width) were measured with an ocular micrometer. The thickness of the epidermis and dermis (stratum compactum and stratum spongiosum) overlying the dorsal and ventral hyomandibular lateral line canals (n = 6 individuals) was also measured as an indicator of distance between the skin surface and canal roof. Further, the number of fibroblast cells (identified as nucleated cellular components within the connective tissue canal wall) within the dorsal and ventral hyomandibular canal walls (n = 6 individuals) was counted and compared by a student's t – test to estimate structural differences and relative canal wall rigidity.

Innervation and axon characteristics

Innervation patterns of neuromasts were determined by dissection and application of 99% isopropyl alcohol saturated Oil red O stain to nerve fibers and surrounding tissue. Connective and muscle tissue was then destained with 70% alcohol and rinsed with tap water. Nerves stained a darker red than the surrounding connective and muscle tissues. Nerve fibers were traced distally to the canal or neuromast base by dissecting away connective and muscle tissue.

Nerves that innervate individual canal neuromasts, superficial neuromasts, or vesicles of Savi were removed at the mechanoreceptor base from formalin-fixed stingrays, embedded in Tissue Tek OCT compound, cut in cross section at 2–10 μm on a cryostat, mounted on chrom-alum-coated slides, and stained with sudan black B in propylene glycol. The numbers of total fibers, afferent, and efferent axons were counted for each nerve. Efferents were defined as axons 3–5 μm smaller in diameter than the surrounding afferent axons, but identification was based solely on relative size (Roberts and Ryan, '71). Differences in the number of total afferent and efferent axons associated with neuromasts in ten different canal locations (n = 8 nerves per location in a single ray) were tested by oneway analysis of variance (ANOVA) with replication and a subsequent T-method test for unplanned comparisons (Sokal and Rohlf, '81).

Distribution of cutaneous nerve endings

The distribution of putative tactile nerve endings in the skin was examined in five female rays that ranged in disk width (DW) from 26.5–28.0 cm to determine relative tactile sensitivity in areas around the mouth and on the rostrum relative to other regions of the body. This analysis was important because of the possibility that enhanced somatosensory sensitivity rather than the ventral nonpored canals and vesicles of Savi could mediate localization of prey near the mouth. Skin samples (1 cm²) of epidermis, dermis, and underlying muscle tissue that included a section of the hyomandibular lateral line canal (dorsal disk) were removed from the dorsal middisk area caudal to the branchial chamber. Tissue samples from the ventral surface were dissected from: (1) the middisk area lateral to the gill slits and not associated with any lateral line canals (ventral disk), (2) an area lateral to the mouth

that included sections of the supraorbital or infraorbital lateral line canals (jaw), and (3) an area on the rostrum that included a section of the infraorbital lateral line canal (rostrum). Tissue was fixed in 10% formalin, cut in cross section (to the rostrocaudal body axis) at 32 μm on a cryostat, and mounted on chrom-alum-coated slides. Slides were dried and then stained with the Winkelmann and Schmit ('57) silver method. The number of nerve fibers observed within the layers superficial to the muscle (stratum compactum, stratum spongiosum, and epidermis) was counted under a compound microscope on six randomly chosen 32-μm-thick sections (192 μm total tissue analyzed per location per animal) from each of the four locations in five female rays and analyzed by oneway analysis of variance (ANOVA) with a subsequent T-method test for unplanned comparisons (Sokal and Rohlf, '81). Classification of the dermis into the superficial stratum spongiosum (SS) and deeper stratum compactum (SC) layers follows the terminology of Murray ('61) and Hawkes ('74) for elasmobranch and teleost skin, respectively. These layers (stratum spongiosum and stratum compactum) also correspond to the superficial dermal region and stratum compactum, respectively, used by Motta ('77) to describe the dermis of several shark species. The elasmobranch general cutaneous system consists of free nerve endings and specialized nerve terminals that terminate within the dermal layers of the skin (see review by Roberts, '69a). All cutaneous nerves not associated with lateral line mechanoreceptors were counted in this study and no attempt was made to distinguish between or quantify morphologically different nerve types.

Behavioral observations

Dasyatis sabina feeds almost exclusively on small benthic invertebrates (amphipods, isopods, ophiuroids, bivalves, mysids, polychaetes) that they mechanically excavate from the sand with characteristic motions of the pectoral fins and mouth to displace sand beneath the body and expose buried prey (Cook, '94; Bradley, '96). The foraging and prey capture behaviors of *D. sabina* were recorded in the field to assess any potential role for the ventral lateral line system in feeding. Stingrays were observed on the sand flats and seagrass beds of the Banana River (FL) at the study site of Cook ('94) over a period of several months during daylight hours in 1–2 h sessions for a total of 200 h.

Feeding stingrays were followed from a distance of 2–4 m and behaviors recorded from the time they presumably detected prey (time when animal stopped and began mechanical excavation to expose buried prey) until the animal swam away from the feeding depression. Body, head, hyoid, and jaw movements were qualitatively recorded before, during, and after prey consumption. Specific attention was given to relationships between prey localization and capture behaviors and the associated lateral line system.

RESULTS
Canal topography and neuromast distribution

Neuromasts are composed of sensory hair cells and support cells that are covered by a gelatinous cupula and are innervated by branches of the posterior (PLLN) and anterior lateral line (ALLN) nerves. *Dasyatis sabina* has superficial neuromasts on the skin surface, canal neuromasts in subdermal pored and nonpored canals that include the hyomandibular, infraorbital, supraorbital, posterior lateral line and mandibular canals, and vesicles of Savi in isolated subdermal pouches on the ventral rostrum (Fig. 1A-D).

The posterior lateral line nerve innervates both canal (posterior lateral line canal) and superficial neuromasts on the trunk and tail. Superficial neuromasts occur on papillae in bilateral rows near the midline that extend from the spiracle to the tip of the tail only on the dorsal body surface ($\bar{x}$ = 101.8 ± 10.5 SD) (Fig. 2). Sensory epithelia are positioned at the base of the papilla groove with cupulae directly exposed to the surrounding water through the groove (Fig. 1A). The raised papillae are ~0.5–0.75 mm wide and 0.3–0.5 mm high above the surrounding skin, whereas the sensory epithelia of the neuromasts are 50–100 μm wide (perpendicular to the groove axis) (Fig. 3A). The main axis of superficial neuromast grooves near the spiracle lie at 100–160° to the rostrocaudal body axis, whereas grooves from the suprabranchial region to the tail base lie at ~90° to the dorsal midline. Groove axes of superficial neuromasts on the tail are positioned laterally and lie in the dorsoventral axis (Fig. 2B).

Posterior lateral line canal (PLL) distribution overlaps that of the superficial neuromasts on the dorsal surface of the disk and tail (Fig. 4). The posterior lateral line canal begins near the suprabranchial midline, but
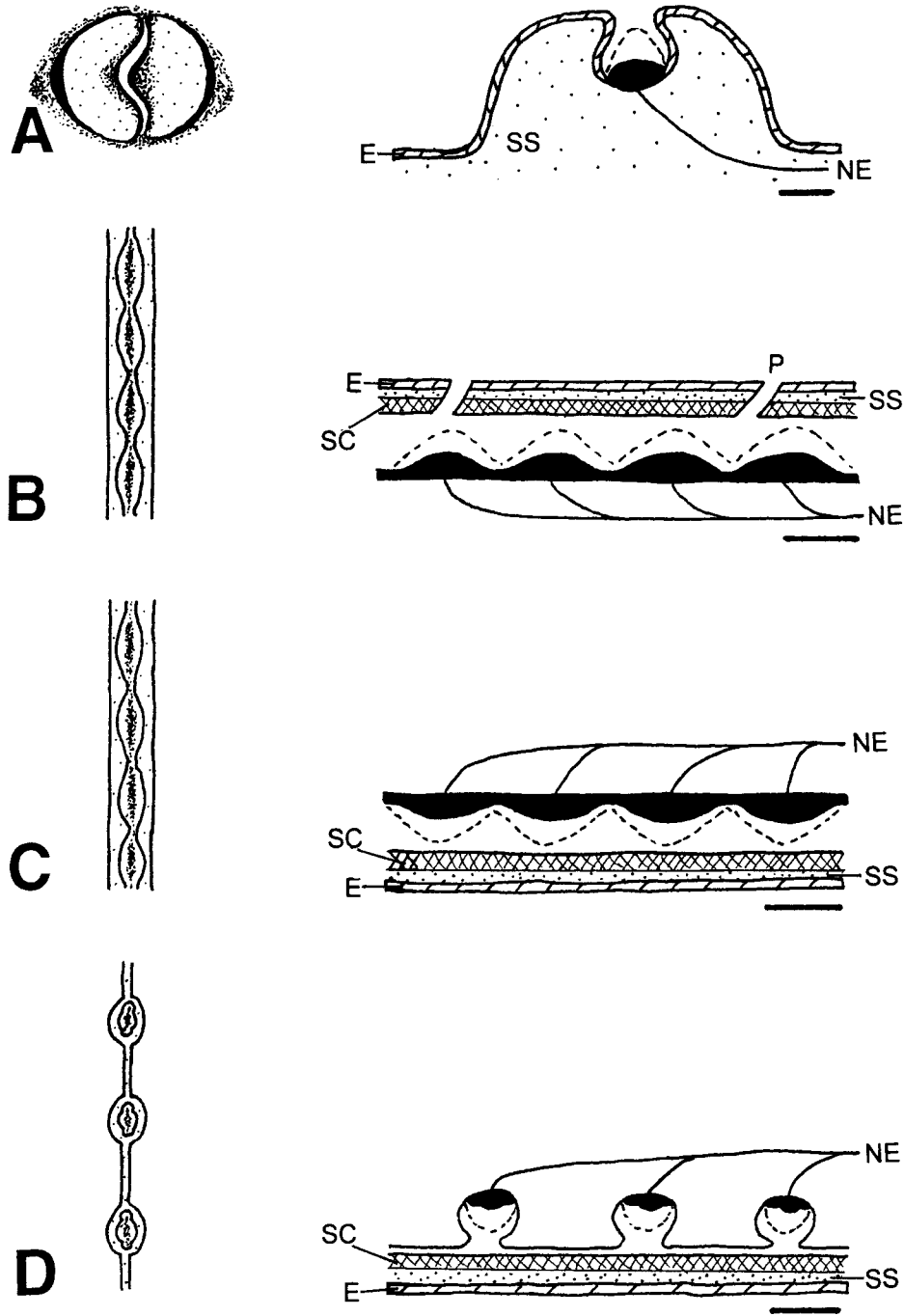
Fig. 1. Schematic diagram of mechanoreceptor types found in the Atlantic stingray, *Dasyatis sabina*. **A:** Superficial neuromasts are located at the base of a central groove through a papillae on the skin surface. The sensory epithelium is presumably covered by a gelatinous cupula and is directly exposed to the water via the groove. **B:** Pored canal neuromasts are enclosed in subdermal canals with pores (P) that open to the water. **C:** Nonpored canals have no connection to the surrounding water. **D:** Vesicles of Savi are located in isolated subdermal pouches on the ventral rostrum midline. Schematic dorsal view of mechanoreceptors shown at left (not to scale) and a lateral view shown at right. Ventral is down on C and D. Bar = 100 μm for A and 1 mm for B-D. Neuromasts are indicated in solid black, and dashed lines over each neuromast represent the predicted, but undetermined cupula location. Single hatches represent the epidermis (E), dots indicate the stratum spongiosum (SS), and cross hatching indicates the stratum compactum (SC) layers of the dermis. NE, nerve.
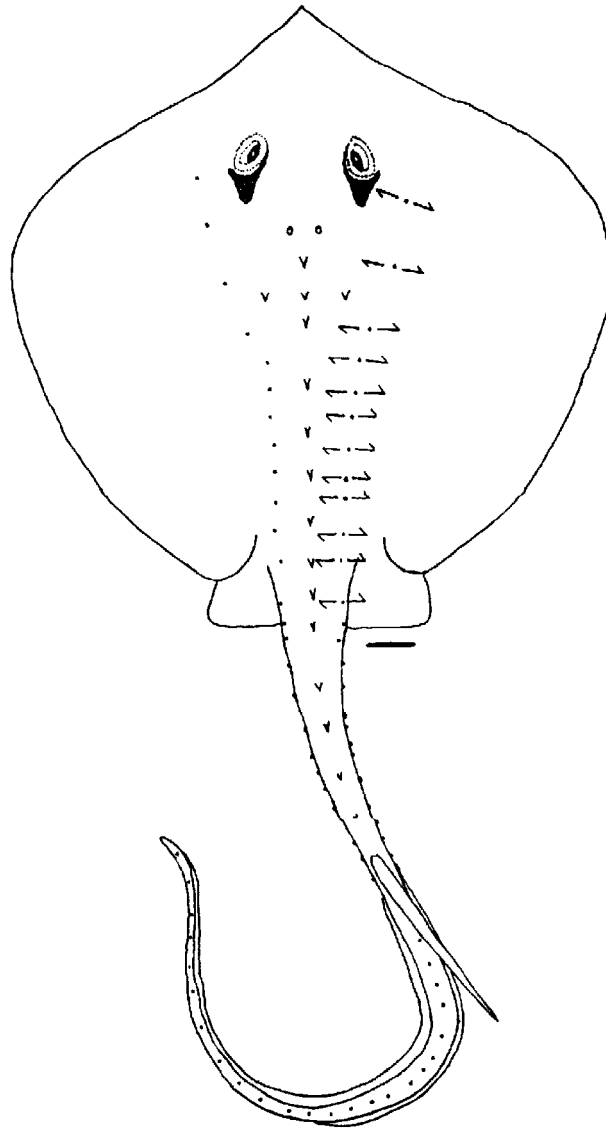
A

B

Fig. 2.   Distribution and groove orientation of superficial neuromasts on the dorsal surface of the Atlantic stingray, *Dasyatis sabina*. Each dot represents a single superficial neuromast papilla and arrows (right side) show the groove orientation. There are approximately 100 superficial neuromasts situated at the base of papillae grooves on the skin along the dorsal midline and tail.

A: Neuromast grooves near the spiracle are positioned 100–160° and grooves from the suprabranchial region to the base of the tail are near 90° to the rostrocaudal body axis. Bar = 0.5 cm. **B:** Neuromasts assume a lateral position on the tail and all grooves are positioned in the dorsoventral axis (arrow). Bar = 1.0 cm.

like the superficial neuromasts is positioned laterally along the tail (Fig. 4). The posterior lateral line canal is continuous with the dorsal supraorbital canal on the head and has lateral tubules that lack neuromasts and terminate in pores in the suprabranchial region as well as above and below the canal along the tail (Fig. 4).

The mechanosensory lateral line system innervated by branches of the anterior lateral line nerve is more complex and consists of three dorsal and four ventral canals. The head canals include the bilateral and interconnected hyomandibular (HYO), infraorbital (IO), and supraorbital (SO) canals and the nonconnected mandibular (MAN) canal on the lower jaw. Neuromasts form a nearly continuous sensory epithelium within each of these canals (Fig. 1B,C). Each canal neuromast is oval and ≤ 1 mm long and ≤ 0.5 mm wide. Adjacent neuromasts are either continuous or separated by <100 μm (Fig. 1B,C). Histological techniques often cause destruction of the cupula, so it is unknown whether this structure is also continuous or what proportion of the canal opening it occupies. There are no neuromasts in the lateral tubules that terminate in pores in any of the canals.

The thickness of the epidermis overlying the canals does not differ between canals on the ventral ($\bar{x} = 76.3 \pm 11.6$ μm SD) and dorsal ($\bar{x} = 81.2 \pm 12.9$ μm SD) surfaces (t-test, $df = 18$, $P = 0.38$). However, the dermal layers between the epidermis and canal roof are characterized by a different organization (Fig. 3C,E). The thickness of the stratum spongiosum does not differ between dorsal and ventral surfaces (t-test, $df = 18$, $P = 0.25$), but the stratum compactum, which sits directly over the canal, is thicker on the dorsal surface than on the ventral (t-test, $df = 18$, $P = 0.005$) (Fig. 3C,E). Further, dorsal hyomandibular canal walls contain more fibroblast cells ($\bar{x}$ number $= 309.3 \pm 40.8$ SD) (measured as the mean number of fibroblast cells per 8 μm canal wall cross section from six individuals) than the ventral hyomandibular canal ($\bar{x}$ number $= 151.6 \pm 54.8$ SD) (t-test, $df = 20$, $P = 0.0003$). However, there is no difference in the number of fibroblast cells between the pored and nonpored sections of the ventral hyomandibular canal (t-test, $df = 10$, $P = 0.37$). Thus the thicker stratum compactum and greater number of fibroblast cells indicate that dorsal canal walls are more rigid in structure compared to their ventral pored and nonpored counterparts.

The canals of the dorsal surface are interconnected (HYO, IO, SO) but independently penetrate the disk to join their ventral counterparts. The dorsal hyomandibular canal extends from the caudal suprabranchial region anteriorly around the branchial chamber and rostral to the eye where it penetrates the disk to join the ventral hyomandibular canal (Fig. 4). Tubules that lack neuromasts extend from the dorsal hyomandibular canal and furcate to terminate in between one and six pores near the disk margin. The dorsal supraorbital canal is partially embedded in the cartilage of the chondrocranium and extends from caudal to the eye, anteriorly to the tip of the rostrum. A short canal section posterior to the endolymphatic pores (surface openings that connect the inner ear to the water) joins the right and left supraorbital canals across the midline. Short lateral tubules extend from the supraorbital canal and terminate in pores on the head, but there are no tubules or pores associated with the supraorbital canal on the rostrum (Fig. 4). The dorsal infraorbital canal is continuous with the dorsal supraorbital canal on the chondrocranium caudal to the eye. The infraorbital canal is located between the eye and spiracle and extends to the disk where it bifurcates to terminate in pores both rostral and caudal to the eye and spiracle. The infraorbital canal penetrates the disk adjacent to the dorsal hyomandibular canal on the rostrum to join the ventral infraorbital canal (Fig. 4).

The ventral hyomandibular, infraorbital, and supraorbital canals are also interconnected, and they independently penetrate the disk to join their dorsal counterparts. In contrast, the mandibular canal is short and isolated from other canals and extends across the midline with a single pore at the lateral edge of the lower jaw (Fig. 5). The ventral hyomandibular canal is located along the anterior disk margin but continues medially and away from the margin caudally. This portion of the hyomandibular canal near the anterior disk margin contains short (< 2 cm) tubules that lack neuromasts and terminate in pores within 1 cm of the rostral disk edge. However, the portion of the hyomandibular canal that extends from the caudal pectoral fin edge anteriorly along the midline and around the gill slits lacks pores (Fig. 5). The ventral infraorbital canal forms an undulat-
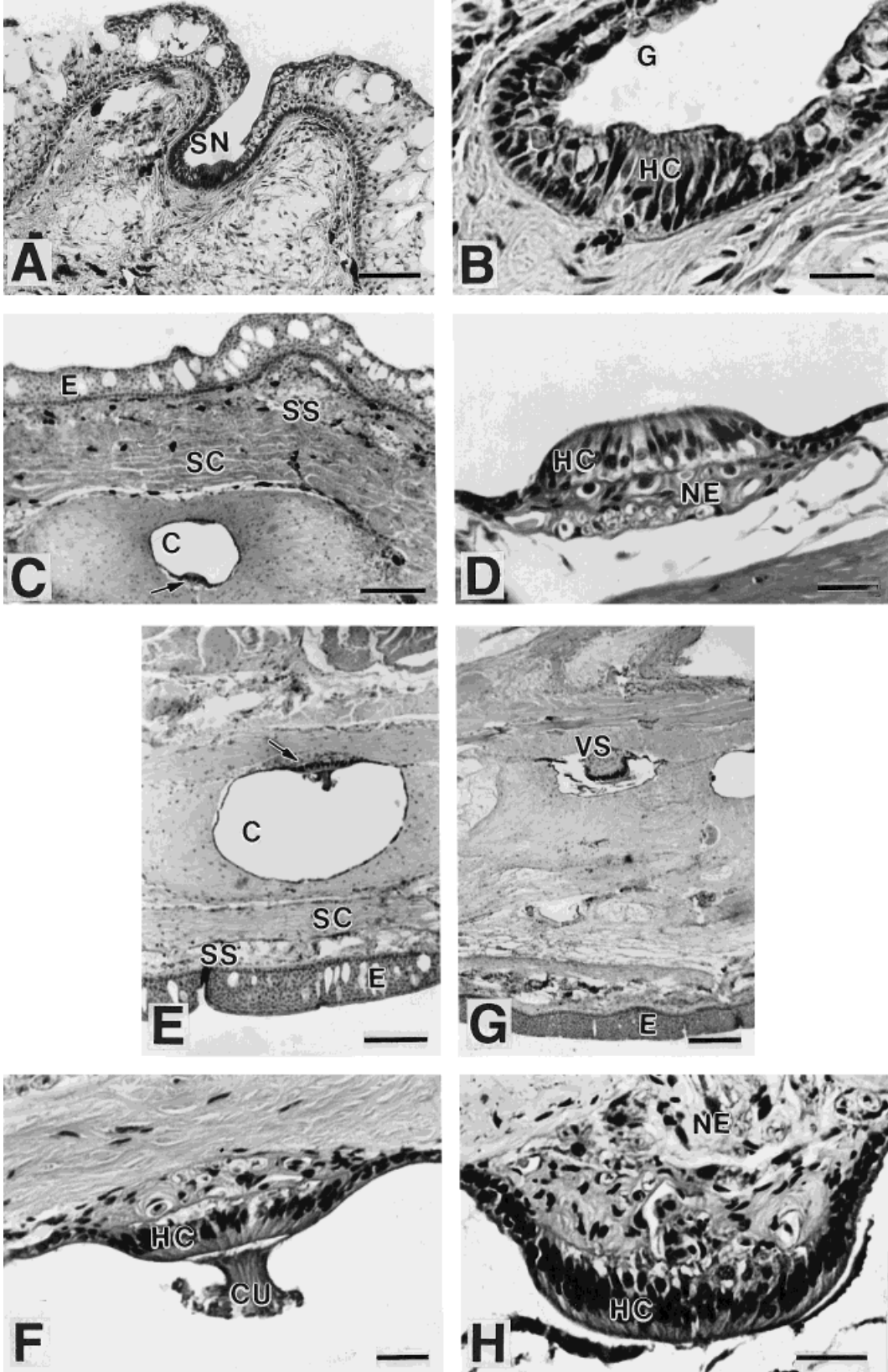
Figure 3

ing loop from the hyomandibular canal near the first gill slit to the rostrum where it penetrates to join the dorsal infraorbital canal. The infraorbital canal also extends medially from the hyomandibular canal to the posterior edge of the naris and along the rostrum midline. The ventral infraorbital canal also lacks pores and the two sides are joined across the midline by a deep commissural canal rostral to the upper jaw (Fig. 5). The ventral supraorbital canal composes the medial portion of the infraorbital canal loop lateral to the naris and mouth and extends around the rostral edge of the naris where it is continuous with the row of vesicles of Savi on the rostrum.

Vesicles of Savi occur in bilateral rows that are continuous with the anterior end of the ventral supraorbital canal and extend to the rostrum tip ($\bar{x}$ number = 8.4 ± 1.8 SD), but the location and number of vesicles varies bilaterally (Fig. 6). Each vesicle is composed of a single neuromast in a thin-walled pouch along the ventral midline of the rostrum (Fig. 1D). Each pouch is ~0.5–1.0 mm long (along the rostrocaudal axis), 200–500 μm wide at the center, and situated ~1–3 mm below loose collagenous tissue layers (Fig. 3G). The neuromast is ~100 μm wide (at the center) and 150–200 μm long (along the rostrocaudal axis) with tapered ends (Fig. 1D). The lumina of adjacent vesicles
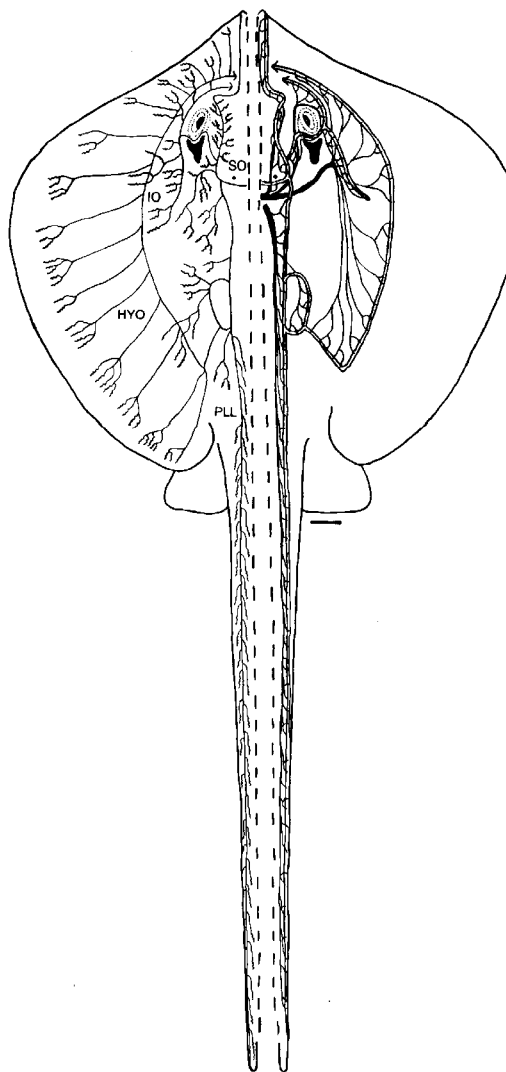

Fig. 4. Distribution and innervation of the lateral line canal system on the dorsal surface of the Atlantic stingray, *Dasyatis sabina*. Left side illustrates location of all canals and pores. Right side shows lateral line nerves that innervate the canal neuromasts. Canals are bilateral, subdermal, and interconnected both between sides and within sides. Neuromasts form a near continuous sensory epithelium in the canals close to the midline. HYO, hyomandibular canal; IO, infraorbital canal; PLL, posterior lateral line canal; SO, supraorbital canal. Bar = 0.5 cm.

Fig. 3. Histological cross sections of mechanoreceptors in the Atlantic stingray, *Dasyatis sabina*. **A:** Superficial neuromasts (SN) are located in raised papillae on the dorsal skin surface. Bar = 100 μm. **B:** Each superficial neuromast is composed of sensory hair cells (HC) positioned at the base of a groove (G) with cupulae (not shown) exposed to the water. Bar = 25 μm. **C:** Dorsal canal neuromasts (arrow) are located in canals (C) beneath the epidermal (E), upper stratum spongiosum (SS), and dense stratum compactum (SC) dermal skin layers. Bar = 500 μm. **D:** Canal neuromasts are composed of sensory hair cells (HC) and support cells normally covered by a gelatinous cupula (not shown) and are innervated by afferent and efferent nerve fibers (NE, seen here in cross section). Bar = 50 μm. **E:** Ventral canal neuromasts (arrow) are located in canals (C) above epidermal (E) and loose, compliant stratum spongiosum (SS) and stratum compactum (SC) dermal skin layers. Bar = 500 μm. **F:** Ventral canal neuromasts also consist of sensory hair cells (HC) and support cells covered by a gelatinous cupula (CU). Bar = 50 μm. **G:** Vesicles of Savi (VS) are located in subdermal pouches above epidermal (E) and loose, compliant layers on the ventral surface. Bar = 250 μm. **H:** These neuromasts also consist of sensory hair cells (HC) covered by a gelatinous cupula (remnants visible above neuromast) and are innervated by afferent and efferent nerve fibers (NE, seen here in cross section). Bar = 25 μm.

are joined together by a thin-walled tubule ~20–50 μm in diameter that lacks both neuromasts and pores. The distance between adjacent vesicles ranges from 2–20 mm ($\bar{x}$ = 8.1 ± 5.5 mm SD) with vesicles closest to the
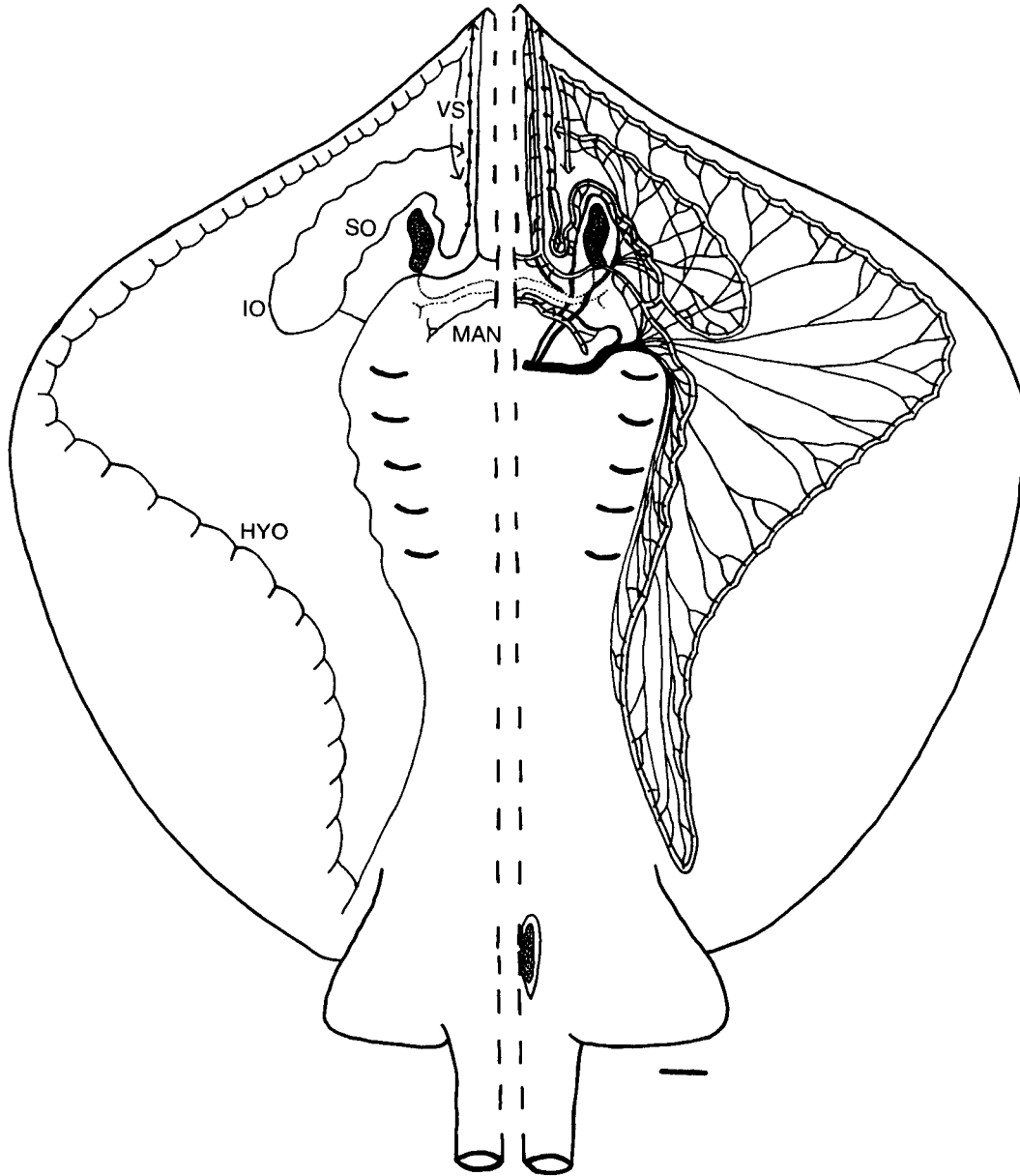
Fig. 5. Distribution and innervation of the lateral line canal system on the ventral surface of the Atlantic stingray, *Dasyatis sabina*. Left side illustrates location of all canals and pores for the hyomandibular and mandibular canals. Right side shows lateral line nerves that innervate canal neuromasts. Sections of the hyomandibu-lar, infraorbital, and supraorbital canals along the mid-line, on the snout and around the mouth lack pores, but do contain innervated neuromasts. HYO, hyomandibu-lar canal; IO, infraorbital canal; MAN, mandibular ca-nal; SO, supraorbital canal; VS, vesicles of Savi. Bar = 0.5 cm.

supraorbital canal positioned closer together than those near the rostrum tip.

Innervation and axon characteristics

The dorsal and ventral canals (HYO, IO, SO, MAN) and vesicles of Savi are inner-vated by separate branches of the anterior lateral line nerve complex, whereas the pos-terior lateral line canal and superficial neu-romasts are innervated by the posterior lat-eral line nerve. The external mandibular branch of the anterior lateral line nerve
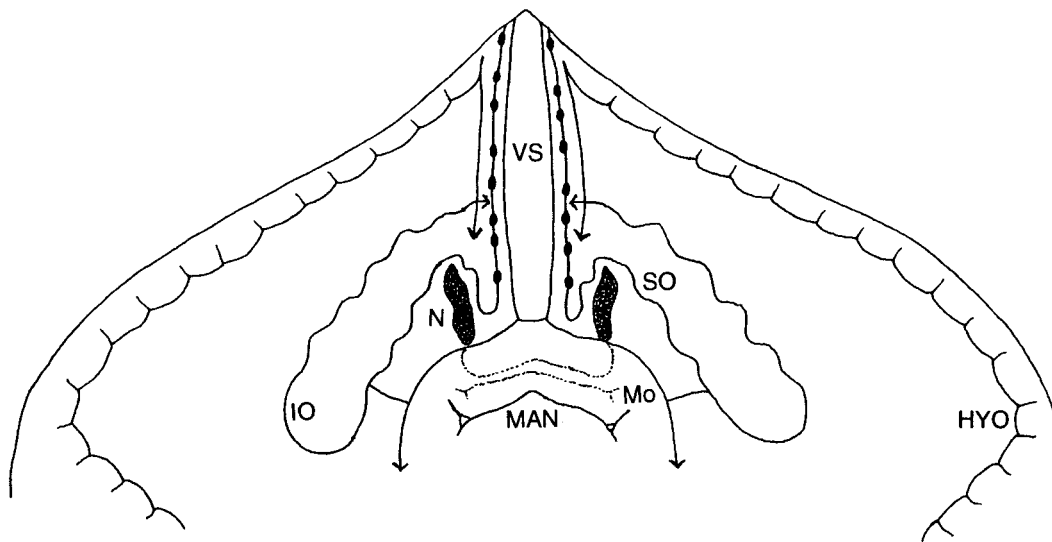
Fig. 6. Distribution of the ventral canals on the rostrum and vesicles of Savi in the Atlantic stingray, *Dasyatis sabina*. Vesicles of Savi (large dots) occur in bilateral rows immediately lateral to the medial infraorbital canals on the rostrum. The vesicles are interconnected via a thin-walled neuromast-free tubule and are not open to the surrounding water. HYO, hyomandibular canal; IO, infraorbital canal; MAN, mandibular canal; Mo, mouth; N, nare; SO, supraorbital canal; VS, vesicles of Savi.

innervates the dorsal and ventral hyomandibular and the mandibular canals. The dorsal and ventral supraorbital canals and vesicles of Savi are innervated by the superficial ophthalmic branch and the dorsal and ventral infraorbital canals are innervated by the buccal branch of the anterior lateral line nerve. The posterior lateral line nerve innervates the canal and superficial neuromasts behind the branchial chamber and on the tail. Innervation of superficial neuromasts anterior to the branchial chamber was not determined due to the complex network of fibers in this area and the inability to trace the small nerves back to the main branch using Oil red stain. Nerves show extensive peripheral branching as they divide multiple times prior to innervating a neuromast (Figs. 4, 5).

The number of primary afferent fibers per neuromast varies among mechanoreceptor type (canal neuromast, superficial neuromast and vesicles of Savi) and canal location (Table 1). There are ~five total axons for each superficial neuromast ($\bar{x}$ = 5.3 ± 1.5 SD), 16 for each canal neuromast ($\bar{x}$ = 15.8 ± 2.1 SD), and 19 for each vesicle of Savi ($\bar{x}$ = 19.4 ± 4.4 SD). The total number of axons and afferent axons per canal neuromast differs only between the dorsal infraorbital and dorsal supraorbital canals, which represent the lowest and highest means, respectively (ANOVA, *df* = 9, 70, F = 2.96, *P* = 0.04, T-method test) (Fig. 7). There are ~12 afferent ($\bar{x}$ = 12.4 ± 1.9 SD) and three efferent ($\bar{x}$ = 3.4 ± 0.6 SD) axons in each nerve branch with a mean afferent:efferent axon ratio of 4.5 ± 0.7 SD for a single canal neuromast. There was no difference in the number of efferent axons associated with each neuromast among canal locations (ANOVA, *df* = 9, 70, F = 1.04, *P* = 0.17). In summary, the dorsal supraorbital canal neuromasts have a higher number of primary afferent fibers than the dorsal infraorbital canal neuromasts, but the efferent innervation is similar among all canal locations.

Distribution of cutaneous nerve endings

Cutaneous nerves not associated with lateral line mechanoreceptors were characterized as single fibers, groups of axons, or coiled endings in layers superficial to the muscles in accordance with descriptions and photographs from other elasmobranch species (Murray, '61; Roberts, '69a,b). However, distribution of morphologically different cutaneous nerves was not quantified in this study. All nerves were observed within or beneath the dermal layers (stratum spongiosum and compactum) and no fibers penetrated the layer of cuboidal cells that forms

TABLE 1. *Number of axons (mean ± SD, n = 8 nerves per location in a single individual) that innervate individual canal neuromasts, superficial neuromasts, and vesicles of Savi in the Atlantic stingray, Dasyatis sabina*

Location[1]	Total axons	Afferent axons	Efferent axons	Afferent/efferent ratio
Ventral HYO (P)	13.5 ± 5.5	10.6 ± 3.7	2.9 ± 2.2	5.3 ± 3.1
Ventral HYO (NP)	16.3 ± 4.3	12.5 ± 3.0	3.8 ± 1.6	4.2 ± 3.2
Dorsal HYO	13.5 ± 2.8	10.5 ± 2.1	3.0 ± 1.1	4.1 ± 2.1
Ventral IO	18.0 ± 2.3	14.5 ± 2.8	3.5 ± 1.6	5.5 ± 3.8
Dorsal IO	12.5 ± 3.0	10.1 ± 2.6	2.4 ± 0.9	4.8 ± 1.9
Ventral SO	15.5 ± 4.6	12.1 ± 3.9	3.4 ± 1.3	3.9 ± 1.6
Dorsal SO	19.9 ± 4.1	15.3 ± 3.3	4.6 ± 1.7	3.5 ± 1.0
MAN	14.1 ± 2.9	11.1 ± 1.8	3.0 ± 1.5	4.6 ± 2.5
PLL − trunk	15.6 ± 2.7	12.5 ± 2.3	3.1 ± 1.0	4.3 ± 1.4
PLL − suprabranchial	18.6 ± 5.8	14.8 ± 4.8	3.9 ± 1.6	4.5 ± 2.6
Grand mean	15.8 ± 2.1	12.4 ± 1.9	3.4 ± 0.6	4.5 ± 0.6
SN	5.3 ± 1.5			
VS	19.4 ± 4.4	15.3 ± 2.4	4.1 ± 0.9	4.2 ± 1.3

[1]HYO, hyomandibular; P, pored; NP, nonpored; IO, infraorbital; SO, supraorbital; MAN, mandibular; PLL, posterior lateral line; SN, superficial neuromast; VS, vesicles of Savi.

the basal epidermal layer. Larger nerves furcated just beneath the stratum compactum and sent axons toward the skin surface where they often branched again within the stratum compactum or spongiosum and terminated just below the epidermis.
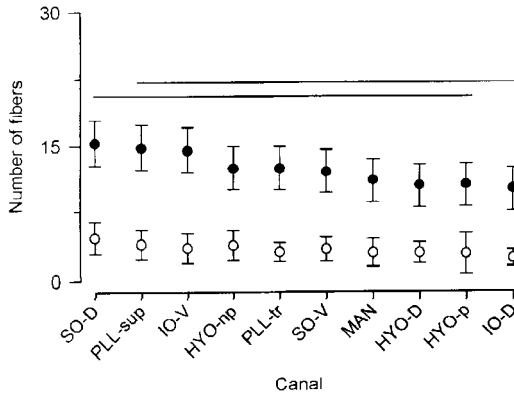

Fig. 7. Total number of afferent (closed circles) and efferent (open circles) axons that innervate a single canal neuromast from ten different locations on the Atlantic stingray, *Dasyatis sabina*. Each point represents the mean ± 95% confidence limits (T-method test for unplanned comparisons) for afferents and the mean ± SD for efferents. Horizontal lines lie above afferent fiber values that do not differ. There is no difference in the number of efferent fibers among canals and a difference in the number of afferent fibers occurs only between the dorsal supraorbital and the dorsal infraorbital canals, which represent the highest and lowest means respectively. HYO-D, dorsal hyomandibular; HYO-p, ventral pored hyomandibular; HYO-np, ventral nonpored hyomandibular; IO-D, dorsal infraorbital; IO-V, ventral infraorbital; MAN, mandibular; SO-D, dorsal supraorbital; SO-V, ventral supraorbital; PLL-sup, posterior lateral line (suprabranchial); PLL-tr, posterior lateral line (trunk).

More than three times as many cutaneous fibers occur on the ventral disk as on the dorsal disk of the ray (ANOVA, $df = 3,20$, $P = 0.00003$, T-method test) (Table 2). However, there was no difference in the number of cutaneous fibers between the ventral disk, jaw, and rostrum locations (ANOVA, $df = 2$, 15, $P = 0.15$). These data indicate that there is no specialized distribution of cutaneous or putative tactile nerve endings around the mouth and on the rostrum that would serve to increase tactile sensitivity in these areas.

Feeding behavior

Atlantic stingrays feed almost exclusively on small benthic invertebrates (e.g., amphipods, mysids, polychaetes, ophiuroids, bivalves, isopods) that they excavate from the sand substrate (Cook, '94). Observations on feeding behavior in the field indicate that the detection of prey likely involves multiple sensory systems that possibly include electroreception and olfaction. Search behavior involves slow swimming at ∼ 0.1–0.25 m/sec and 1–5 cm above the sand substrate followed by an abrupt stop to examine an area for prey (Fig. 8A, B). During inspection behavior, the ray briefly lies motionless on the

TABLE 2. *Number of cutaneous fibers not associated with lateral line mechanoreceptors (mean ± SD, n = 5 individuals) within the dermis of the Atlantic stingray, Dasyatis sabina*

Location	No. of cutaneous fibers
Dorsal disk	7.9 ± 1.8
Ventral disk	29.9 ± 9.2
Jaw	42.7 ± 10.6
Rostrum	37.8 ± 8.8

bottom presumably to detect prey beneath the body by cutaneous contact, stimulation of the lateral line, or electroreception. This period of quiescence may be followed by periodic movements to reposition the body. When prey are detected within the substrate, the ray begins to displace the sand beneath the body by swift undulations of the tips of its pectoral fins (Fig. 8C). This excavation exposes prey within a feeding depression that is about the diameter of the disk. Excavated and often motile prey are retained in the feeding depression beneath the disk and the body is moved to position the mouth over the prey (Fig. 8D-F). In some cases, the ray will brace the pectoral fins against the substrate, raise its body above the feeding depression, and pause for several seconds before it makes subsequent strikes at prey. Prey located deeper in the substrate, such as tube dwelling polychaetes, are further excavated within the original feeding depression by a strong suction action of the mouth that creates a small deep pit slightly larger than the diameter of the mouth. Prey are consumed by rapid biting motions of the jaws and associated rapid spiracle and gill movements, displacement of sand to the side of the pectoral fins, or exhalation of sediments through the spiracles.

DISCUSSION

This study provides the first description of the canal topography, neuromast distribution, and innervation patterns of the entire peripheral lateral line system in an elasmobranch species. Further, it is the only study to show the distribution of superficial neuromasts in a batoid since the pioneer study by Ewart and Mitchell (1892) of the skate, *Raja batis*. We formulate the Mechanotactile Hypothesis of lateral line function that proposes that the nonpored canals and vesicles of Savi function as touch receptors that encode displacement of the underlying skin caused by prey. Finally, we propose a model for the role of the ventral lateral line system in predation in *Dasyatis sabina* based on its natural food habits, feeding behavior, and organization of the peripheral lateral line system.

Canal topography and neuromast distribution

Superficial neuromast distribution in the stingray, *Dasyatis sabina*, is similar to that of both shark and skate species only with regard to the line along the dorsal midline and tail (dorsal-lateral line) (Ewart and Mitchell, 1892; Tester and Nelson, '69). Budker ('58) suggested that superficial neuromasts in sharks follow the generalized plan of a line of superficial neuromasts posterior to the mouth, a line between the pectoral fins, lines on the dorsal-lateral portion of the body and caudal fin, and a pair of neuromasts anterior to the endolymphatic pores. Superficial neuromasts in the skate, *Raja batis*, are located in bilateral rows on the dorsal midline with a single pair anterior to the endolymphatic pores (Ewart and Mitchell, 1892). The stingray, *D. sabina*, differs from all shark species examined thus far (Tester and Nelson, '69) and *Raja* by its lack of a pair of superficial neuromasts anterior to the endolymphatic pores. *Dasyatis sabina* further differs from all shark species examined because it lacks the lines of superficial neuromasts posterior to the mouth and between the pectoral fins. The ~100 superficial neuromasts in *D. sabina* most closely resemble the 77 superficial neuromasts seen in the dogfish, *Squalus acanthias*, but this number (77) is lower than that observed for other shark species examined (e.g., > 600 SN for *Sphyrna lewini*, Tester and Nelson, '69). The arrangement and number of superficial neuromasts on the stingray may have some functional significance, but evolutionary trends cannot be determined until a larger number of taxa are examined.

All superficial neuromasts observed in *Dasyatis sabina* occur on the dorsal body surface with their grooves oriented perpendicular (90°) to the rostrocaudal body axis. Thus, the best response of superficial neuromasts on the dorsal disk should be to water movements that are along the transverse body axis. However, superficial neuromasts along the entire tail are in a lateral position with a dorsoventral orientation of the grooves. This rotation of groove orientation indicates that superficial neuromasts on the tail will have a best response to water movements that are parallel to the dorsoventral axis and a weak response in the direction parallel to the anteroposterior body axis. However, these proposed axes of best and least sensitivities are based solely on gross morphology and assume the neuromast hair cells have a best sensitivity parallel to the groove axis.

The superficial neuromast sensory epithelium is located inside a well-developed groove
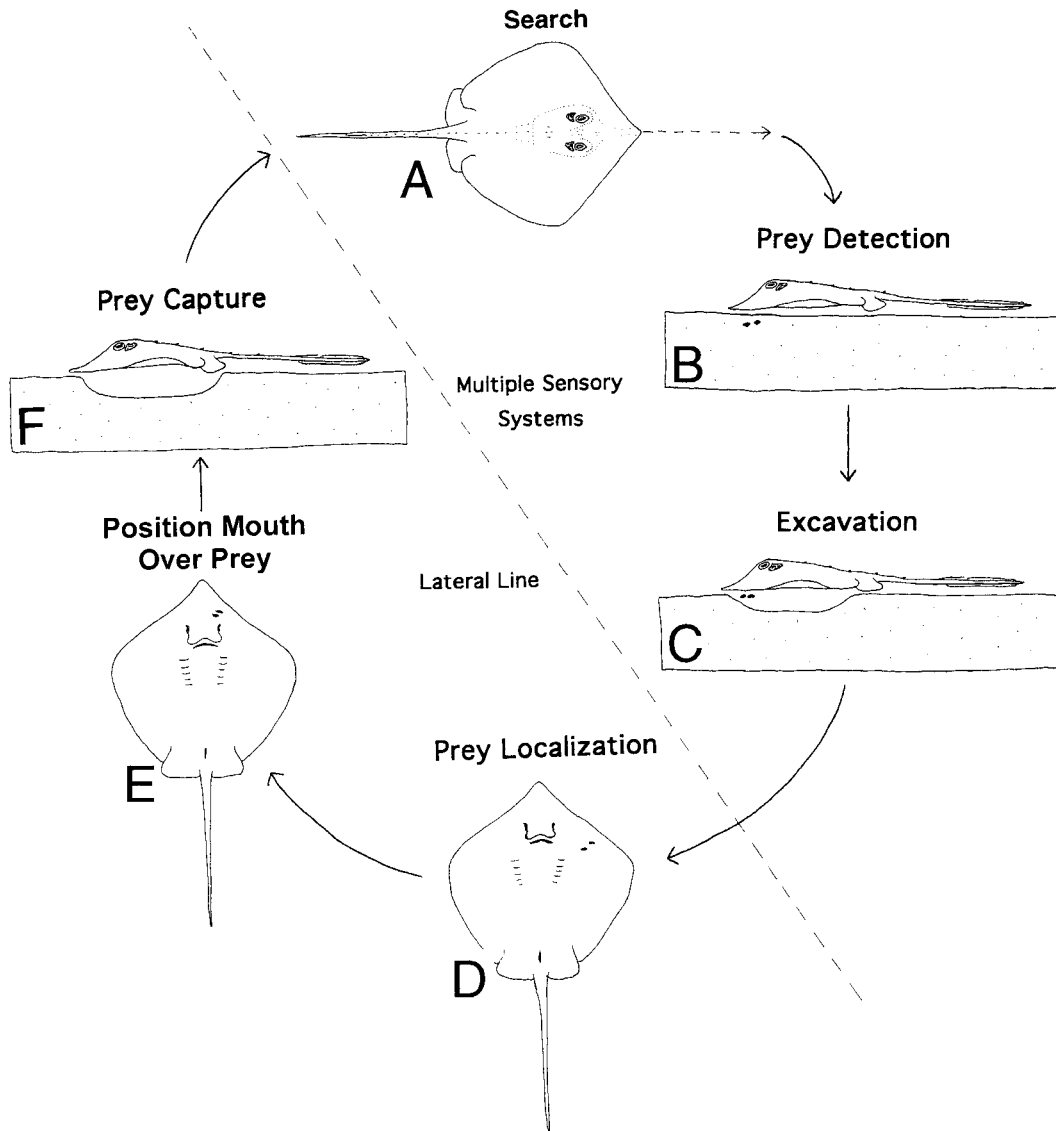
Search

Fig. 8. Diagrammatic summary of the feeding behavior of the Atlantic stingray, *Dasyatis sabina*. Rays search for and detect concealed invertebrate prey (small dots beneath ray in **B,C,D,E**) with multiple sensory systems such as electroreception, olfaction, or lateral line (**A**, B) (see text). Mechanical motion of the mouth and pectoral fins is used by the ray to excavate and expose the buried prey (C). The lateral line is hypothesized to help localize prey (D), then position (E) and guide (**F**) the mouth over the prey for capture. Other senses such as electroreception are also hypothesized to function in conjunction with mechanoreception during the final stages of prey capture (see text).

above the skin surface that would promote water flow parallel to the cupula, enhance directional sensitivity, and protect the cupula from abrasion or damage. This differs considerably from the pit organs of sharks in which superficial neuromasts lie between the bases of modified scales and are recessed

below the skin surface (Tester and Nelson, '69). Lateral placement of superficial neuromasts on the tail of *Dasyatis sabina* and other batoids may reflect their benthic and often sedentary behavior where they rest motionless on the bottom. This system would function to detect low frequency water move-

ment across the dorsal surface generated by tidal currents, conspecifics or predatory bull sharks, *Carcharhinus leucas*, which are the major predators of *D. sabina* in the Indian River Lagoon (Snelson et al., '84). In addition, superficial neuromasts may mediate rheotaxis behavior (orientation to water currents) in the stingray as recently demonstrated for several teleost species (Montgomery et al., '97).

Canal topography in *Dasyatis sabina* is similar to that described for other batoid species (Garman, 1888; Chu and Wen, '79). Canal organization was described for *D. sabina* by Puzdrowski and Leonard ('93), but the distribution of neuromasts within canals was not assessed. Canal neuromasts in the stingray form a nearly continuous sensory epithelium in the canals (PLL, HYO, IO, SO, MAN) close to the midline. This neuromast configuration was first described in elasmobranch canals by Johnson ('17) and differs from teleost lateral line systems that typically contain a single discrete neuromast between two adjacent pores (Coombs et al., '88). Placement of neuromasts close to the midline in canals on the dorsal surface may be an adaptation to reduce the self-generated noise caused by pectoral fin undulations during swimming.

Nonpored canals in *Dasyatis sabina* contain hundreds of neuromasts that form a nearly continuous epithelium within a single canal. The absence of pores in these canals suggests a unique mechanical transduction mechanism since these receptors would not respond to water movements parallel to the skin surface. However, since cupular dimensions and associated hair cell mechanics are also important parameters that influence neuromast transduction properties (van Netten and Kroese, '89a,b; van Netten and Khanna, '94), it would be necessary to determine the cupular morphology and organization among adjacent neuromasts before the physics of these unique mechanoreceptors can be modeled.

The numerous branched tubules that lack neuromasts and terminate in pores on the dorsal surface of *Dasyatis sabina* likely function to increase sampling area, displace the receptive field to the disk periphery, and improve signal-to-noise ratio. The canals on the dorsal surface of *D. sabina* are in the best position to detect water movements generated by conspecifics or predators, but not by benthic prey. Branched tubules are char-

acteristic of most elasmobranchs (Garman, 1888; Chu and Wen, '79) and some teleosts (Stephens, '85), and the number and complexity of branches usually increase with body size. A similar phenomenon occurs in *D. sabina* where the number of branches per hyomandibular tubule on the dorsal surface increases from one to two in embryos to as many as six in adults (pers. obs.). Bleckmann and Munz ('90) showed that the highly branched lateral line of the black prickleback, *Xiphister atropurpureus*, increases the receptive field only if nearby objects create water displacements just above threshold levels or if the stimulus is masked by background noise. Branched tubules in *Dasyatis sabina* may also increase the receptive field if the stimulus is masked by the background noise of wave action, currents, or swimming. The pored canals on the dorsal surface may also function to detect underwater objects by perceiving distortions in the flow field generated around the body while swimming, but this remains to be tested.

The ventral canal system of *Dasyatis sabina* differs from the dorsal canal system in that it contains long segments that entirely lack surface pores. Nonpored head canals occur in all shark, skate, and ray species (Garman, 1888; Chu and Wen, '79). This lack of connection between canal and surrounding water indicates they will not respond to water motion across the skin as for pored canals and superficial neuromasts. The mechanical properties of excitation in canals without pores should be considerably different from pored canal systems, but knowledge of canal, neuromast, and cupular dimensions is necessary to predict canal flow and transduction mechanisms (Denton and Gray, '88). However, preliminary neurophysiological recordings have verified that primary afferents from nonpored canal neuromasts are not sensitive to water motion, but do respond to tactile stimulation of the underlying skin (pers. obs.). Ventral canals typically have a less dense dermal layer over the canal relative to their dorsal counterparts and therefore should be more compliant and sensitive to displacements of the underlying skin. Although nonpored canals are described for a number of elasmobranch species, an ecological function for this system has previously not been proposed. One advantage of a nonpored canal system in benthic feeding batoids would be to eliminate or reduce lateral line stimulation by extrane-

ous water currents produced during prey excavation or burying behavior. Water movement specifically created by these behaviors would stimulate pored canals, especially on the ventral surface. Thus such self-generated noise would be greatly reduced in the nonpored canal system but still permit detection of prey that displace the skin surface.

Vesicles of Savi also appear to be specialized mechanoreceptors primarily found on the ventral surface of many torpedo rays (Torpedinidae), electric rays (Narcinidae), and stingrays (Dasyatidae) (Norris, '32; Szabo, '68; Nickel and Fuchs, '74; Chu and Wen, '79; Barry and Bennett, '89). In *Dasyatis sabina*, vesicles of Savi contain individual mechanoreceptors within subdermal pouches that form a single row on each side of the ventral rostrum midline. Each vesicle of Savi in the torpediniform rays (Narcinidae and Torpedinidae) contains one large central neuromast between two smaller peripheral neuromasts (Norris, '32; Szabo, '68; Nickel and Fuchs, '74; Chu and Wen, '79; Barry and Bennett, '89). At the light microscopy level, this appears to differ in *D. sabina* by the presence of only a single neuromast, but this needs to be verified by scanning electron microscopy. Further, the ventral lateral line canal system in torpediniform rays is often reduced or absent, but the number and surface area covered by the vesicles of Savi are increased. This differs from many of the dasyatid rays that have both an elaborate canal system and a reduced vesicle system on the ventral surface. The functional and evolutionary significance of these differences among torpediniform and myliobatiform rays remains to be determined.

The lack of a direct connection to the surrounding water in the vesicles of Savi and close association with cartilaginous skeletal elements also indicates a function different from pored lateral line canals. Like the nonpored lateral line canals, the vesicles of Savi would not respond to pressure differences across the skin surface caused by water movements. However, they should also be sensitive to direct displacement of the underlying skin, which would cause bi-directional flow of fluid from the overlying vesicle and excite hair cells of opposite polarity in adjoining rostral and caudal vesicles. Thus vesicles of Savi could also function as touch receptors. Vesicles in the lesser electric ray, *Narcine brasiliensis*, are sensitive to vibrations of 150–200 Hz and were suggested possibly to function to detect substrate borne vibrations (Szabo, '68; Barry and Bennett, '89), but this needs to be tested directly. The spatial separation of vesicles in the stingray (2–20 mm) would function as an excellent point source detector when prey are immediately rostral to the mouth. We propose that these vesicles in *Dasyatis sabina* are used to guide the mouth (via body movement) toward invertebrate prey immediately before capture.

Innervation and axon characteristics

Neuromast innervation patterns are an important determinant of the spatial resolution and sensitivity of the lateral line system. Canal neuromasts usually exceed superficial neuromasts both in size and in associated fiber innervation, or number of fibers per neuromast (Tester and Kendall, '67; Munz, '89). Canal neuromasts in *Dasyatis sabina* are larger than superficial neuromasts and contain about three times more fibers. Furthermore, the number of fibers that innervate an individual canal neuromast does not differ among locations except between the dorsal infraorbital and dorsal supraorbital canals. The dorsal infraorbital canal contains fewer afferent fibers and may reflect increased sensitivity in canals around the eyes provided that hair cell numbers in these two canals are the same. One advantage of increased sensitivity to water movements around the eyes would be to facilitate detection of objects, predators or prey at night or in turbid water when visual cues are reduced. A second advantage of increased lateral line sensitivity within the visual field would be the multimodal integration of visual and mechanosensory information in higher processing centers of the brain (e.g., the tectum). The coincident detection of visual and lateral line stimuli may be necessary to elicit a behavioral response (e.g., escape response) similar to that seen with the overlapping visual and electrosensory fields mapped in the tectum of the skate (Bodznick, '91). Therefore, it would be advantageous to have increased mechanosensory sensitivity within the visual field if these stimuli are integrated in tectal or other processing regions of the brain.

Canal neuromasts are innervated by both afferent and efferent axons with a ratio of ~5:1. Efferent fibers are several microns smaller in diameter than afferent fibers in the same nerve (Roberts and Ryan, '71) and although distinct axon diameter size classes

were observed in fibers that innervate canal neuromasts, none were found in superficial neuromast nerves. Efferent neurons can be activated by other sensory stimuli (e.g., visual, tactile, and vestibular) and function in the peripheral processing of mechanoreceptive information (Roberts and Russell, '72; Highstein and Baker, '85; Tricas and Highstein, '91). The apparent lack of efferent innervation in the superficial neuromasts of *Dasyatis sabina* (based solely on differential axon diameter data) would indicate that mechanosensory stimuli at the periphery are not modified by efferent activity as they are in canal neuromasts. Another hypothesized function of efferent fibers is to reduce the firing rate of primary afferents and prevent transmitter depletion in response to swimming activity (Roberts and Russell, '72; Russell and Roberts, '72). However, since superficial neuromast morphology in *D. sabina* indicates an axis of best response perpendicular to the rostrocaudal body axis, stimulation of these mechanoreceptors would be minimal with forward swimming motion, possibly reducing the need for efferent innervation. Studies of immunocytochemical labeling for acetylcholinesterase will be necessary to confirm the absence of superficial neuromast efferent axons in the stingray.

Each vesicle of Savi is innervated by ~20 axons, which is four times that of superficial neuromasts and 1.3 greater than the overall mean for canal neuromasts in *Dasyatis sabina*. Vesicles also have an afferent:efferent fiber ratio of 4.2 $\pm$ 1.3 SD, which is similar to that observed in the canal neuromast locations. The greater number of afferent fibers that innervate each vesicle of Savi may be associated with increased hair cell numbers, or possibly represent a higher hair cell to axon ratio that would increase the sensitivity of these mechanoreceptors to tactile stimuli. However, unknown parameters such as hair cell density, neuromast size, and cupula dimensions must be determined before relative sensitivity and transfer functions can be adequately determined or modeled.

The Mechanotactile Hypothesis: Role of the lateral line in predation

One important function of the teleost mechanosensory system is to detect and localize prey (Hoekstra and Janssen, '85, '86; Montgomery and Saunders, '85; Montgomery et al., '88; Montgomery, '89; Montgomery and Milton, '93; Janssen et al., '95). How-
ever, whereas the use of olfaction, electroreception, and vision for the detection and localization of prey is established in many elasmobranch species (Tester, '63; Gilbert, '63; Kalmijn, '71), use of mechanoreception for feeding remains uninvestigated. *Dasyatis sabina* feeds almost exclusively on small benthic invertebrates (e.g., amphipods, isopods, mysids, polychaetes, bivalves and ophiuroids) that it excavates from the sand substrate (Cook, '94) and feeds throughout most of the day and night (Bradley, '96). The majority of these prey items are found beneath the substrate surface and are not in the visual field. The electrosensory system of the stingray, which consists of the ampullae of Lorenzini, is used to detect weak bioelectric fields emitted by concealed natural prey. Use of this sensory system for prey detection and localization is well documented in elasmobranch species (Kalmijn, '71; Blonder and Alevizon, '88). Initial detection of prey by *D. sabina* likely involves multiple sensory systems such as electroreception (Blonder and Alevizon, '88), olfaction (Silver, '79), and possibly mechanoreception (proposed below) followed by mechanical excavation to expose these invertebrate prey. Therefore, prey detection and capture are likely mediated by several sensory systems that work in concert to enhance the feeding efficiency of the ray. We suggest that the lateral line system is also an important sense used in the feeding behavior of this species.

We propose the Mechanotactile Hypothesis to explain a potential functional role for the ventral nonpored lateral line canals and the vesicles of Savi in the stingray, *Dasyatis sabina*. Water movements produced by motile or sessile prey may be detected by the ventral pored lateral line canals along the rostral disk margin and posterior middisk area (Fig. 9:1). If prey are buried beneath the sand substrate, the ray will excavate a feeding pit below the body to expose and contain the prey. Once detected, the ray moves its body to position the prey beneath the nonpored canals on the rostrum and around the mouth. These canals do not respond to water movements, but rather function as touch receptors when stimulated by direct contact of prey with the skin surface (the subject of the deformation of the skin is ripe for a biomechanical analysis). The neuromast hair cells are stimulated by movement of canal fluid and subsequent cupula displacement (Fig. 9:2). The ray may then

move its body again to position the rostrum and vesicles of Savi over the prey. Similar to nonpored canals, vesicles of Savi do not respond to local water motion but may function as point-source touch receptors (Fig. 9:3). The narrow diameter of the interconnecting tubules indicates that prey contact with the skin surface between two adjacent vesicles should only stimulate those two vesicles compared to stimulation of multiple neuromasts in the nonpored canal system. The relatively wide spatial separation between vesicles (2–20 mm) includes the size range of amphipod and isopod prey most common in the natural diet. These features of the vesicle of Savi system may facilitate a more accurate source location than the nearly continuous neuromasts of the nonpored canals. The ray can then move its mouth toward the prey as the tactile stimulus is passed along a sequence of vesicles on the rostrum. Final prey guidance to the mouth for capture is facilitated by the short mandibular canal on the lower jaw and the nonpored infraorbital and supraorbital canals on the rostrum (Fig. 9:4). When prey are positioned beneath the mouth, the jaw can protrude to capture and consume the prey.

This mechanotactile mechanism suggests a novel function not previously described for the lateral line system. Mechanoreceptive detection of benthic prey is likely also used by other batoid species that possess nonpored canal systems on the ventral surface. For example, *Myliobatis* and *Rhinoptera* contain extensive convoluted nonpored canals on the ventral surface of the rostrum (Chu and Wen, '79) and feed on benthic invertebrate prey (e.g., bivalves, polychaetes, crustaceans) that require excavation from the substrate (Ridge, '63; Smith and Merriner, '85). Also, Montgomery and Skipworth ('97) recently demonstrated the importance of the ventral canal system of the short-tailed stingray, *Dasyatis brevicaudata*, in the detection of water currents produced by the respiration and filter feeding of bivalves. The ventral lateral line system of this species contains a nonpored canal system similar to *Dasyatis sabina*. Whereas the pored canals along the anterior disk margin may detect these water movements generated by bivalve prey, the nonpored canals may be directly stimulated by skin displacements as described above. Thus the proposed function of the ventral lateral line system in feeding

is supported by the fact that most batoids possess either nonpored canals or vesicles of Savi on the ventral surface that likely function as mechanotactile receptors and that the majority of species feed on benthic organisms.

Nonpored canals on the head are not restricted to batoids, but are characteristic of all elasmobranch species studied thus far (Garman, 1888; Chu and Wen, '79). The presence of nonpored canals on the ventral surface of sharks may also serve a mechanotactile function in the localization and capture of prey as described above for the stingray. For example, in the wild the great hammerhead shark, *Sphyrna mokarran*, pins stingrays to the bottom with its laterally expanded head and pivots to bring the ray's wing under the head (Strong et al., '90). Most hammerhead species have nonpored canals on the dorsal laterally expanded head as well as on the ventral rostrum and around the mouth (Chu and Wen, '79). These canals would be stimulated by this direct contact and provide the shark with positional information used to orient the mouth over the stingray wing to bite. Although the nonpored canal system might serve a mechanotactile function in feeding in some species, what function might these nonpored canals serve on a pelagic shark that does not feed on the bottom? Many pelagic species such as the mako (*Isurus glaucus*) and blacktip (*Carcharhinus limbatus*) sharks have nonpored canals only on the dorsal snout in front of the eyes and on the ventral rostrum anterior to the mouth (Garman, 1888; Chu and Wen, '79). One possible function of these canals would be to reduce stimulation of the entire canal system caused by water movements generated during forward swimming motion. Forward swimming behavior of pelagic sharks generally includes movement of the head from side to side that would stimulate pored canals on the snout while this self-generated noise would be reduced with a nonpored canal system. A second possibility is that these canals function as mechanotactile receptors during the final stages of prey capture, prey handling, or to localize a prey item (e.g., a fish) and guide it to the mouth after an initial unsuccessful strike. A third possible function for these nonpored canals in any elasmobranch would be as mechanotactile receptors used to facilitate body position during copulatory behavior. Elasmobranchs have internal fertilization that
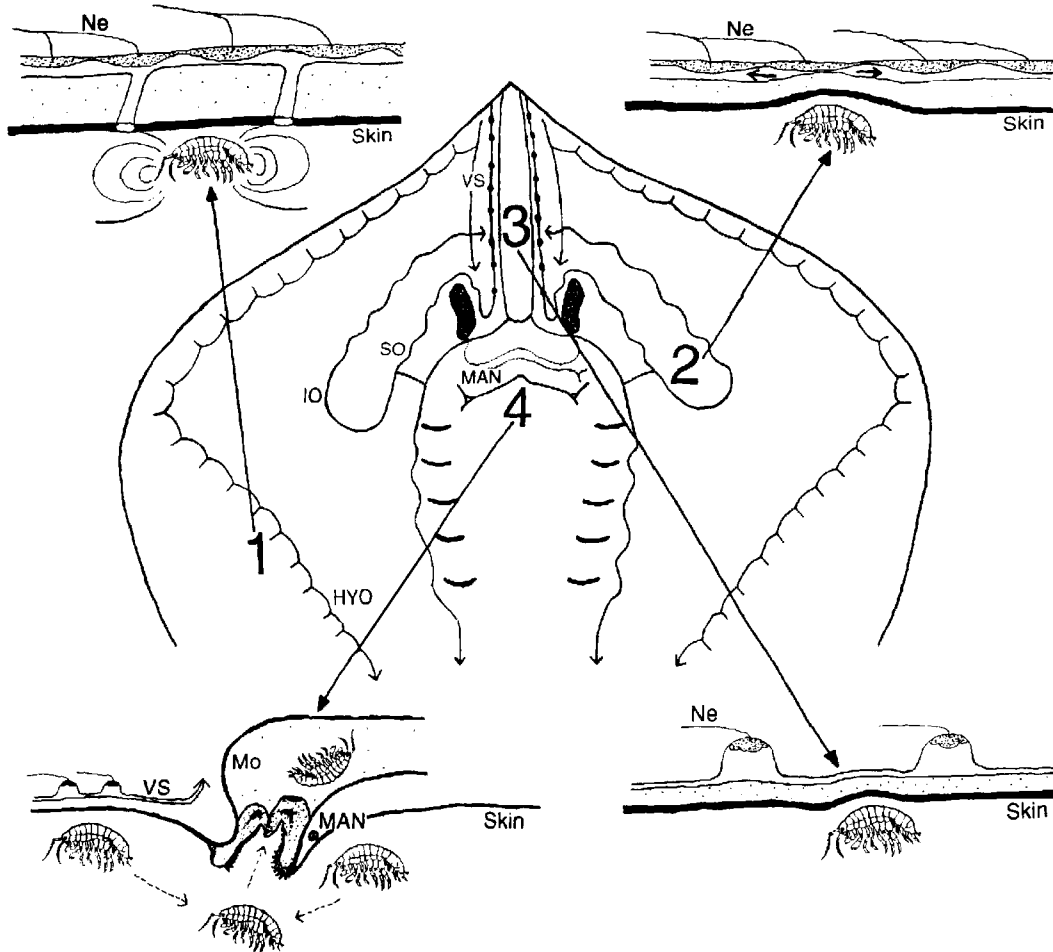
Fig. 9.   Diagrammatic representation of the Mechano-tactile Hypothesis for the function of the ventral lateral line system of the Atlantic stingray, *Dasyatis sabina*, in predation on small benthic infaunal prey. Exposed invertebrate prey (e.g., an amphipod or patch of amphipods) are contained by the pectoral fins in an excavated feeding depression beneath the ray. 1. The pored hyomandibular canal detects water movement caused by motile or sessile prey at the peripheral ventral disk surface. 2. Nonpored canals function as mechanotactile receptors to position and guide the mouth or vesicles of Savi toward the prey. 3. Vesicles of Savi also function as mechanotactile receptors to detect the exact location of prey and position the rostrum and mouth above it. 4. The ray repositions its body to place the prey beneath the mouth for capture. This final guidance probably involves both the vesicles of Savi and the short mandibular canal on the lower jaw. HYO, hyomandibular canal; IO, infraorbital canal; MAN, mandibular canal; Mo, mouth; Ne, nerve; SO, supraorbital canal; VS, vesicles of Savi.

requires the male to bite the pectoral fin of the female allowing the pair to position their bodies ventral-to-ventral for the male to insert the clasper (intromittent organ) into the female's cloaca. Thus the nonpored canals around the mouth and on the rostrum may also function to facilitate body positioning for copulation. However, these speculative functions of nonpored canals and vesicles of Savi in elasmobranchs remain to be experimentally tested.

Elasmobranchs also possess a general cutaneous system of free nerve endings and specialized nerve terminals (e.g., corpuscles of Wunderer and endings of Poloumord-winoff) that provide proprioceptive, tactile, and possibly temperature sensation (Roberts, '69a; Bone and Chubb, '75,'76). Murray

('61) recorded discharges from cutaneous sensory endings to displacement stimuli of the skin surface as low as 20 μm. A 20-μm displacement of the skin surface over a ventral nonpored canal would also cause strong movement of canal fluid at a velocity amplitude proportional to displacement and cupular displacement proportional to canal fluid movement (van Netten and Kroese, '89b; Kroese and van Netten, '89). The most sensitive canal lateral line afferents in teleosts generally respond to water motion in the nanometer range, which is often also the threshold for a behavioral response (Munz, '85; Coombs and Janssen, '89). Therefore, a skin displacement far less than 20 μm should stimulate the lateral line system, but not the tactile receptors. In addition, the distribution of cutaneous nerve endings in *Dasyatis sabina* does not differ between different locations on the ventral surface, which indicates these putative tactile receptors have neither the specialized distribution nor the increased sensitivity needed for the specific function of prey localization. This anatomical evidence supports the hypothesis that ventral nonpored canals and vesicles of Savi function as relatively hypersensitive, localized touch receptors. Further physiological studies on the frequency tuning, sensitivity, and projections of these nonpored canal neuromasts and the vesicles of Savi are needed to model their response properties and central processing. In addition, more studies of feeding behaviors on natural prey are required to determine the biological relevance of the lateral line system in other elasmobranch fishes.

ACKNOWLEDGMENTS

LITERATURE CITED

Barry, M.A., and M.V.L. Bennett (1989) Specialized lateral line receptor systems in elasmobranchs: the spiracular organs and vesicles of Savi. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory Lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 591–606.

Bleckmann, H., and H. Munz (1990) Physiology of lateral-line mechanoreceptors in a teleost with highly branched, multiple lateral lines. Brain Behav. Evol. *35*:240–250.

Blonder, B.I., and W.S. Alevizon (1988) Prey discrimination and electroreception in the stingray, *Dasyatis sabina*. Copeia. *1*:33–36.

Bodznick, D. (1991) Elasmobranch vision: multimodal integration in the brain. J. Exp. Zool. Sup. 5:108–116.

Bone, Q., and A.D. Chubb (1975) The structure of stretch receptor endings in the fin muscles of rays. J. Mar. Biol. Ass. U.K. 55:939–943.

Bone, Q., and A.D. Chubb (1976) On the structure of corpuscular proprioceptive endings in sharks. J. Mar. Biol. Ass. U.K. *56*:925–928.

Boord, R.L., and C.B.G. Campbell (1977) Structural and functional organization of the lateral line system of sharks. Am. Zool. *17*:431–441.

Bradley, J.L. (1996) Prey energy content and selection, habitat use and daily ration of the Atlantic stingray, *Dasyatis sabina*. Florida Institute of Technology thesis, 49 pp.

Budker, P. (1958) Les organes sensoriels cutanes des selaciens. In: Traite de zoologie. Library de l'Academie de Medicine. Paris: Masson, pp. 1033–1062.

Campenhausen, C. von, I. Riess, and R. Weissert (1981) Detection of stationary objects by the blind cave fish *Anoptichthys jordani* (Characidae). J. Comp. Physiol. *143*:369–374.

Chu, Y.T., and M.C. Wen (1979) A study of the lateral-line canal system and that of Lorenzini ampullae and tubules of elasmobranchiate fishes of China. In Anonymous (eds.): Monograph of Fishes of China. Shanghai, China: Academic Press, pp. 1–132.

Cook, D.A. (1994) Temporal patterns of food habits of the Atlantic stingray, *Dasyatis sabina* (LeSeur, 1824), from the Banana River Lagoon, Florida. Florida Institute of Technology thesis, 45 pp.

Coombs, S. (1994) Nearfield detection of dipole sources by the goldfish (*Carassius auratus*) and the mottled sculpin (*Cottus bairdi*). J. Exp. Biol. *190*:109–129.

Coombs, S., M. Hastings, and J. Finneran (1996) Modeling and measuring lateral line excitation patterns to changing dipole source locations. J. Comp. Physiol. A *178*:359–371.

Coombs, S., and J. Janssen (1989) Peripheral processing by the lateral line system of the mottled sculpin (*Cottus bairdi*). In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 29–319.

Coombs, S., J. Janssen, and J.F. Webb (1988) Diversity of lateral line systems: Evolutionary and functional considerations. In J. Atema, R.R. Fay, A.N. Popper, and W.N. Tavolga (eds): Sensory Biology of Aquatic Animals. Heidelberg: Springer-Verlag, pp. 553–593.

Denton, E.J., and J.A.B. Gray (1983) Mechanical factors in the excitation of clupeid lateral lines. Proc. R. Soc. Lond. *B218*:1–26.

Denton, E.J., and J.A.B. Gray (1988) Mechanical factors in the excitation of the lateral line of fishes. In J. Atema, R.R. Fay, A.N. Popper, and W.N. Tavolga (eds): Sensory Biology of Aquatic Animals. Heidelberg: Springer-Verlag, pp. 595–593.

Dijkgraaf, S. (1963) The functioning and significance of the lateral line organs. Biol. Rev. *38*:51–106.

Ewart, J.C., and H.C. Mitchell (1892) On the lateral sense organs of elasmobranchs. II. The sensory canals of the common skate (*Raja batis*). Trans. R. Soc. Edinb. *37*:87–105.

Garman, S. (1888) On the lateral canal system of Selachia and Holocephala. Bull. Mus. Comp. Zool. *17*:57–119.

Gilbert, P.W. (1963) The visual apparatus of sharks. In P.W. Gilbert (ed): Sharks and Survival. Boston: Heath, pp. 283–326.

Harris, G.G., and W.A. Van Bergeijk (1962) Evidence that the lateral-line organ responds to near-field displacements of sound sources in water. J. Acoust. Soc. Am. *34*:1831–1841.

Hassan, E.S. (1989) Hydrodynamic imaging of the surroundings by the lateral line of the blind cave fish, *Anoptichthys jordani*. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory Lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 217–227.

Hawkes, J.W. (1974) The structure of fish skin. I. General organization. Cell Tissue Res. *149*:147–158.

Highstein, S.M., and R. Baker (1985) Action of the efferent vestibular system on primary afferents in the toadfish, *Opsanus tau*. J. Neurophysiol. *54*:370–384.

Hoagland, H. (1933) Quantitative analysis of responses from lateral-line nerves of fishes. II. J. Gen. Physiol. *16*:715–732.

Hoekstra, D., and J.Janssen (1985) Nonvisual feeding behavior of the mottled sculpin, *Cottus bairdi,* in Lake Michigan. Environ. Biol. Fish. *12*:111–117.

Hoekstra, D., and J. Janssen (1986) Lateral line receptivity in the mottled sculpin (*Cottus bairdi*). Copeia *1*: 91–96.

Humason, G.L. (1979) Animal Tissue Techniques, 4th ed. San Francisco: W.H. Freeman, 661 pp.

Janssen, J., W.R. Jones, A. Whang, and P.E. Oshel (1995) Use of the lateral line in particulate feeding in the dark by juvenile alewife (*Alosa pseudoharengus*). Can. J. Fish. Aquat. Sci. *52*:358–363.

Johnson, S.E. (1917) Structure and development of the sense organs of the lateral canal system of selachians (*Mustelus canis* and *Squalus acanthias*). J. Comp. Neurol. *28*:1–74.

Kalmijn, A.J. (1971) The electric sense of sharks and rays. J. Exp. Biol. *55*:371–383.

Kalmijn, A.J. (1989) Functional evolution of lateral line and inner ear sensory systems. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory Lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 187–215.

Kroese, A.B., and N.A.M. Schellart (1989) Sensory transduction in lateral line hair cells. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 265–284.

Kroese, A.B., and N.A.M. Schellart (1992) Velocity- and acceleration-sensitive units in the trunk lateral line of the trout. J. Neurophys. *68*:2212–2221.

Lannoo, M.J. (1987) Neuromast topography in urodele amphibians. J. Morphol. *191*:247–263.

Montgomery, J. C. (1989) Lateral line detection of planktonic prey. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensroy Lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 561–574.

Montgomery, J.C., and A.J. Saunders (1985) Functional morphology of the piper *Hyporhamphus ihi* with reference to the role of the lateral line in feeding. Proc. R.Soc. Lond. B. *224*:197–208.

Montgomery, J.C., and R.C. Milton (1993) Use of the lateral line for feedingin the torrentfish (*Cheimarrichthys osteri*). N Zealand J. Zool. *20*:121–125.

Montgomery, J.C., and E. Skipworth (1997) Detection of weak water jets by the short-tailed stingray *Dasyatis brevicaudata* (Pisces: Dasyatidae). Copeia 4:441–883.

Montgomery, J.C., C.F. Baker, and A.G. Carton (1997) The lateral line can mediate rheotaxis in fish. Nature *389*:960–963.

Montgomery, J.C., J.A. Macdonald, and G.D. Housley (1988) Lateral line function in an Antarctic fish related to the signals produced by planktonic prey. J. Comp. Physiol. A *163*:827–833.

Motta, P.J. (1977) Anatomy and functional morphology of dermal collagen fibers in sharks. Copeia. *3*:454–464.

Munz, H. (1985) Single unit activity in the peripheral lateral line system of the cichlid fish *Sarotherodon niloticus* L. J. Comp. Physiol. A *157*:555–568.

Munz, H. (1989) Functional organization of the lateral line periphery. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory Lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 285–297.

Murray, R.W. (1961) The initiation of cutaneous nerve impulses in elasmobranch fishes. J. Physiol. *159*: 546–570.

Nickel, E., and S. Fuchs (1974) Organization and ultrastructure of mechanoreceptors (Savi vesicles) in the elasmobranch Torpedo. J. Neurocytol. *3*:161–177.

Norris, H.W. (1932) The laterosensory system of *Torpedo marmorata*, innervation and morphology. J. Comp. Neurol. *56*:169–178.

Northcutt, R.G. (1992) The phylogeny of octavolateralis ontogenies: a reaffirmation of Garstang's phylogenetic hypothesis. In D.B. Webster, R.R. Fay, and A.N. Popper (eds): The Evolutionary Biology of Hearing. New York: Springer-Verlag, pp. 21–47.

Partridge, B.L., and T.J. Pitcher (1980) The sensory basis of fish schools: relative roles of lateral line and vision. J. Comp. Physiol. *135*:315–325.

Puzdrowski, R.L., and R.B. Leonard (1993) The octavolateral systems in the stingray, *Dasyatis sabina*. I. primary projections of the octaval and lateral line nerves. J. Comp. Neurol. *332*:21–37.

Ridge, R.M. (1963) Food habits of the bat ray, *Myliobatis californica*, from Tomales Bay, California. Berkeley, University of California thesis, 56 pp. .

Roberts, B.L. (1969a) Mechanoreceptors and the behavior of elasmobranch fishes with special reference to the acoustico-lateralis system. In E.S. Hodgeson and R.F. Mathewson (eds): Sensory Biology of Sharks, Skates and Rays. Arlington, VA: Office of Naval Research, Department of the Navy, pp. 331–390.

Roberts, B.L. (1969b) The spinal nerves of the dogfish (*Scyliorhinus*). J. Mar. Biol. Ass. U.K. *49*:51–75.

Roberts, B.L., and I.J. Russell (1972) The activity of lateral line efferent neurones in stationary and swimming dogfish. J. Exp. Biol. *57*:435–448.

Roberts, B.L., and K.P. Ryan (1971) The fine structure of the lateral-line sense organs of dogfish. Proc. R. Soc. Lond. B *179*:157–169.

Russell, I.J., and B.L. Roberts (1972) Inhibition of spontaneous lateral line activity by efferent nerve stimulation. J. Exp. Biol. *57*:77–82.

Satou, M., H.A. Takeuchi, S., Kitamura, Y., Kudo, J., Nishii, and M. Tanabe (1991) Involvement of lateral line sense in intersexual vibrational communication during spawning behaviour in the hime salmon (landlocked red salmon, *Oncorhynchus nerka*). Neurosci. Res. Suppl. *14*:15.

Satou, M., H.A. Takeuchi, J. Nishii, M. Tanabe, S. Kitamura, N. Okumoto, and M. Iwata (1994) Behavioral and electrophysiological evidences that the lateral line is involved in the inter-sexual vibrational communication of the hime salmon (landlocked red salmon, *Oncorhynchus nerka*). J. Comp. Physiol. A *174*:539–549.

Satou, M., H.A. Takeuchi, S. Kitamura, Y. Kudo, J. Nishii, and M. Tanabe (1991) Involvement of lateral line sense in intersexual vibrational communication during spawning behaviour in the hime salmon (landlocked red salmon, *Oncorhynchus nerka*). Neurosci. Res. Suppl. *14*:15.

Silver, W.L. (1979) Olfactory responses from a marine elasmobranch, the Atlantic stingray, *Dasyatis* sabina. Mar. Behav. Physiol. *6*:297–305.

Smith, J.W., and J.V. Merriner (1985) Food habits and feeding behavior of the cownose ray, *Rhinoptera bonasus*, in lower Chesapeake Bay. Estuaries *8*:305–310.

Sokal, R.R., and F.J. Rohlf (1981) Biometry, 2nd ed. New York: W.H. Freeman., 859 pp.

Snelson, F.F., T.J. Mulligan, and S.E. Williams (1984) Food habits, occurrence, and population structure of the Bull shark, *Carcharhinus leucas*, in Florida coastal lagoons. Bull. Mar. Sci. *34*:71–80.

Stephens, R.R. (1985) The lateral line system of the gizzard shad *Dorosoma cepedianum* LeSeur (Pisces: Clupidae). Copeia *3*:540–555.

Strong, W.R., F.F. Snelson, and S.H. Gruber (1990) Hammerhead shark predation on stingrays: An observation of prey handling by *Sphyrna mokarran*. Copeia 3:836–840.

Szabo, T. (1968) Analyse morphologigicque et fonctionelle de l'epithelium sensoriel d'un mechanorecepteur. Actual. Neurol. *6*:131–147.

Tester, A.L. (1963) The role of olfaction in shark predation. Pac. Sci. *17*:145–170.

Tester, A.L. and J.I. Kendall (1967) Innervation of free and canal neuromasts in the sharks *Carcharhinus menisorrah* and *Sphyrna lewini*. In P.H. Cahn (ed): Lateral Line Detectors. Bloomington: Indiana University Press, pp. 53–69.

Tester, A.L., and J.I. Kendall (1969) Morphology of the lateralis canal system in the shark genus *Carcharinus*. Pac. Sci. *23*:1–16.

Tester, A.L., and G.J. Nelson (1969) Free neuromasts (pit organs) in sharks. In P.W. Gilbert, R.F. Mathew-son, and D.P. Rall (eds): Sharks, Skates and Rays. Baltimore: Johns Hopkins Univ. Press, pp. 503–531.

Tricas, T.C., and S.M. Highstein (1991) Action of the octavolateralis efferent system upon lateral line of free-swimming toadfish, Opsanus *tau*. J. Comp. Physiol. *169*:25–37.

Turner, R.L., Heatwole, D.W., and S.E. Stancyk (1982) Ophiuroid discs in stingray stomachs: Evasive autotomy or partial consumption of prey? In J.M. Lawrence (ed): Echinoderms: Proceedings of the International Conference, Tampa Bay. Rotterdam: A.A. Balkema, pp. 331–335.

van Netten, S.M., and S.M. Khanna (1994) Stiffness changes of the cupula associated with the mechanics of hair cells in the fish lateral line. Proc. Natl. Acad. Sci. *91*:1549–1553.

van Netten, S.M., and A.B.A. Kroese (1989a) Hair cell mechanics controls the dynamic behavior of the lateral line cupula. In J.P. Wilson, and D.T. Kemp (eds): Cochlear Mechanisms: Structure, Function and Models. New York: Plenum, pp. 47–55.

van Netten, S.M., and A.B.A. Kroese (1989b) Dynamic behavior and micromechanical properties of the cupula. In S. Coombs, P. Gorner, and H. Munz (eds): The Mechanosensory Lateral Line–Neurobiology and Evolution. New York: Springer-Verlag, pp. 247–263.

Webb, J.F. (1989) Gross morphology and evolutoin of the mechanoreceptive lateral line system in teleost fishes. Brain Behav. Evol. *33*:34–53.

Webb, J.F., and R.G. Northcutt (1997) Morphology and distribution of pit organs and canal neuromasts in non-teleost bony fishes. Brain Behav. Evol. *50*:139–151.

Webb, J.F. (1989) Gross morphology and evolution of the mechanoreceptive lateral line system in teleost fishes. Brain Behav. Evol. *33*:34–53.

Winkelmann, R.K., and R.W. Schmit (1957) A simple silver method for nerve axoplasm. Staff meetings of the Mayo Clinic. *32*:217–222.