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A Hydrodynamic Sensory Antenna Used By Killifish for Nocturnal Hunting

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A HYDRODYNAMIC SENSORY ANTENNA USED BY KILLIFISH FOR NOCTURNAL HUNTING

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The perception of sensory stimuli by an animal requires several steps, commencing with the capture of stimulus energy by an antenna. As the interface between the physical world and sensory transduction, the antenna modifies the stimulus in ways that enhance the animal's perception. For example, the mammalian external ear collects sound and spectrally alters it to increase sensitivity and improve the detection of directionality. The surface-feeding killifish *Aplocheilus lineatus* is able to hunt in darkness by detecting surface capillary waves with the lateral-line system atop its head. This cephalic lateral line consists of 18 stereotyped mechanosensitive neuromasts, each bordered by fleshy ridges. Each neuromast has a single axis of sensitivity along its longitudinal axis. The ridges bordering each neuromast form a channel parallel to the neuromast's axis of sensitivity. The ridges direct water oscillations along the length of the channel and are able to block the transmission of surface waves. The neuromasts respond to stimulation ranging from tens of hertz to hundreds of hertz at amplitudes from tens of nanometers to a few micrometers peak-to-peak. Each neuromast has a unique receptive field defined by its directional sensitivity to stimulation of the water surface. The receptive field is sharply modulated in angle, which cannot be explained either by changes in response to angle of the waves on the surface of the water or by the angular sensitivity of the hair cells within the neuromast. Instead, the receptive field is determined by the ridges altering the flow of water over and around each neuromast. Modification of the hydrodynamic environment by the

addition of a supplemental ridge perturbs the receptive fields of adjacent neuromasts and affects the transmission of waves over the head. We found that the addition of a supplemental ridge inhibits the fish's behavioral performance by significantly increasing both the fish's reaction time to a stimulus and the probability that it fails to respond to a stimulus. We propose that the ridges constitute a hydrodynamic antenna for the cephalic lateral line of *Aplocheilus lineatus*.

Dedicated to the memory of F. David Schnebly,

who introduced rigor to my enthusiasm for the natural world.

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1. Introduction

The evolution of a nervous system had myriad fitness advantages to early metazoans. It allowed colonial organisms to grow to large sizes yet still precisely coordinate activity across their bodies. Along with the development of muscles, the nervous system enabled colonial organisms to actively pursue prey and flee predators, forever changing the scale of interaction between organisms. At its simplest, the advantage of a nervous system is two-fold: it allows precise detection of physical stimuli and drives coordinated responses across tissues to those stimuli. However, even the simplest synapses have some capacity for plasticity and adaptation [Eccles 1951, Spencer 1989], implying that the advantage of a nervous system also includes some capacity for learning.

At the length and time scales relevant for unicellular organisms, sensory perception is statistical [Berg 1975]. For example, in bacterial chemotaxis the presence of a chemical on a chemoreceptor may slightly reduce the probability that the flagellum reverses direction. Averaged over the many chemoreceptors of the cell, the animal will tend to move toward an attractant or away from a repellant. However, given the very small surface area of a bacterium it is susceptible to pockets of low or high concentration and to statistical fluctuations in sampling. Animals, however, have the benefit of averaging responses across many cells covering large areas, allowing for the development of concrete objectification of the external world. This process is clearest in large and complicated animals, such as the mollusks, arthropods, and vertebrates, all of which display intricate structures specialized for sensory detection. The initial step of sensory perception is often described as sensory transduction: the process by which a physical stimulus is transduced into a neural signal. Effective perception, however, is dependent upon the structure of the sensory organ. This structure acts as the interface between the environment and the

transducing cells, functioning as a sensory antenna that often modifies or focuses a physical stimulus in order to improve transduction or to enable higher-order processing.

1.1. Sensory antennae

A general definition of an antenna is that it is a structure that converts freely propagating phenomenon into a localized phenomenon. Examples would be radio antennae, which collect radio-frequency electromagnetic waves, or a telescope, which collects visible-frequency electromagnetic waves. Antenna structures are implicit in sensory organs, from lenses for photodetection to vibrissae for mechanosensation. An antenna processes the stimulus prior to transduction, a step essential to perception. For example, myopia is not a defect in the ability of a photoreceptor to detect photons of light or send signals to the central nervous system. Instead, myopia results from convergence of light before hitting the retina, rather than at the retina [Goss 1997]. This is a defect of a sensory antenna: the eye is too long in one dimension or there is a refractive defect in the cornea or lens.

Sensory organs are often manifolds of many sensory antennae. For example, the mammalian ear is a complicated structure, often divided into the outer, middle, and inner components. The outer ear collects sound [Walsh 2008] and spectrally modifies it [Middlebrooks 1991]. The spectral modification proves necessary for determining the elevation of the sound source. The mammalian middle ear acts as a simple machine, coupling airborne compressive waves from the ear canal to the fluid-filled cochlea of the inner ear [Waterman 1971]. The mammalian cochlea spectrally decomposes sound on the basis of frequency [von Békésy 1956 and 1970, Waterman 1971] and amplifies the magnitude of vibrations [Hudspeth 2008]. The decomposition is essential for processing of

sound by higher brain areas, and the amplification provides far greater sensitivity to low-amplitude sounds and leads to finer frequency resolution [Hudspeth 2008].

Sensory organs are structured to act as antennae to improve the acuity of sensation. The elaborate antennae seen in male moths allow them to precisely localize a female moth by tracking the concentration of pheromones she releases [Vogt 1981]. The antennae of the moth also have proteins that inactivate the pheromone molecules upon binding, improving the sensitivity of the system by reducing the incidence of sensing the same molecule twice [Vogt 1981]. The pit organ, found in pit vipers (Crotalinae), pythons (Pythonidae), and boas (Boidae) is an infrared detector used to track infrared prey and predators [Gracheva 2010]. It consists of a thin, heavily vascularized membrane suspended across a pit on the snout of the animal. The membrane divides the pit into inner and outer chambers. The aperture of the pit is somewhat narrowed, which acts as a pinhole giving the animal directional sensitivity [Gracheva 2010]. The membrane is innervated by the trigeminal nerve, which in these snakes has a TRPA1 channel that is thermosensitive [Gracheva 2010]. The membrane acts as an antenna by catching infrared radiation entering the pit. The thinness of the membrane gives it very little thermal inertia, allowing it to respond with a temperature change to small amounts of incident infrared radiation. Its heavy vascularization ensures that it is maintained close to the ambient temperature of the environment [Gracheva 2010].

Cutaneous mechanosensitivity is mediated by a variety of touch receptors. One, the Pacinian corpuscle, detects high-frequency vibration [Mendelson 1964]. The corpuscle consists of a nerve surrounded by 20 to 60 lamellae of connective tissue. The nerve fires in response to a steady-state pressure but adapts within milliseconds. It was found that the lamellae act as

high-pass filters. They absorb steady-state deflections, attenuating the response of the nerve, but allow high-frequency stimuli to pass. Removal of the lamellae dramatically slowed the adaptation of the nerve, changing it from a fiber that fires only transiently in response to a steady-state deflection to one that fires an extended burst [Mendelson 1964]. All of these structures, the moth's antennae, the snake's pit-organ membrane, and the Pacinian corpuscle's lamellae, act as sensory antennae to enhance the response of the corresponding systems to relevant stimuli.

Morphological changes in sensory antennae drive much of the evolutionary history and diversity of visual systems. The simplest eyes are eyespots: planar conglomerations of photosensitive excitable cells. Eyespots are capable of detecting moving shadows, but little else [Land 1992]. The evolution of an eyespot in a pit allows for limited directional sensitivity and produces a common form of eye, referred to as a pit eye, occurring in 85% of animal phyla and evolving independently at least 40 times [Land 1992]. In six phyla, representing 96% of known animal species, a second stage of morphological adaptation has improved the optical characteristics of the eye. If the pit deepens, narrowing at its orifice, it can form a pinhole, which allows for improved directional sensitivity and for the formation of simple images. Such a structure is found in *Nautilus* and *Haliotis* (abalone). A lens develops from transparent tissue, followed perhaps by an iris and a cornea, leading to a simple eye, a structure that in various forms has evolved independently many times in animals [Land 1992]. In all, there are ten forms of eyes found in animals utilizing nearly every known optical technology including the pinhole eye, the homogeneous lenses, the inhomogeneous lens, arrangements of multiple lenses, arrangements of a refractive cornea and a lens, and the mirror lens (notable exceptions being zoom lenses and Fresnel lenses). The transition from

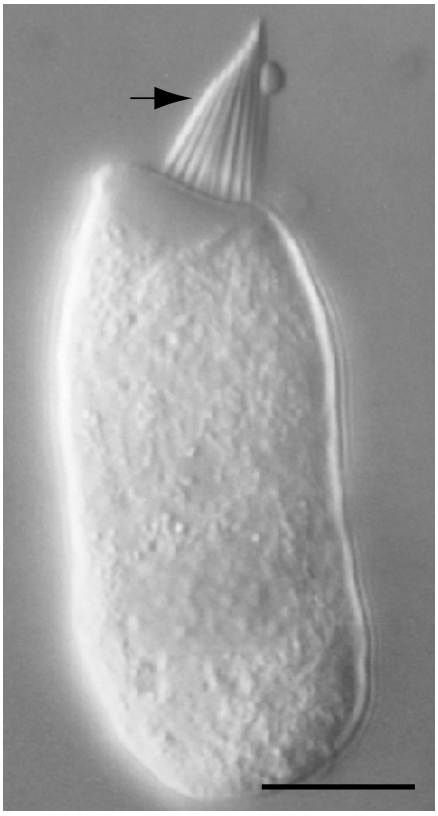


Figure 1.1 - Hair cell
A hair cell is a polarized neural cell type characterized by a bundle of stereocilia on its apical surface (indicated by the arrow). The stereocilia are arranged in a staggered array, from the shortest at one end to the tallest at the opposite. Adjacent to the tallest stereocilia is the kinocilium, with a ball at its tip. The hair cell is depolarized by deflections toward the kinocilium (to the right) and hyperpolarized by deflections away from it (to the left). At the base of the cell there are synapses contacted by afferent and efferent neurites. The scale bar represents approximately 5 μm.

an eyespot to a simple eye does not involve any fundamental changes to the photoreceptors; rather, the transformation is formed by changes to the structures surrounding the photoreceptors. Selection has repeatedly driven the development of sophisticated sensory antennae that allow for improved perception of the environment.

1.2. Vertebrate hair-cell mechanosensation

A similar process can be observed in vertebrate hearing, which is dependent on the acoustolateralis system. Basal aquatic vertebrates have, in addition to the ear, patches of mechanosensory hair cells on the exterior of their bodies called neuromasts. Tetrapods, particularly the most derived groups, the mammals and birds, have increasingly intricate organization of hair cells and surrounding structures, giving them sensitivity to an incredibly broad spectral and amplitude range of sound stimuli.

Hair cells are polarized neuroepithelial cells in which the apical surface is crowned by a hair bundle, an array of actin-filled hairs referred to as stereocilia (Figure 1.1). At one end of the stereociliary bundle stands a

cilium, the kinocilium. The stereocilia are staggered in height, with the shortest being on the side of the bundle opposite the kinocilium and the tallest being adjacent to the kinocilium. Neighboring stereocilia are tethered to each other by a number of linkages along their length [Assad 1991], the most critical being the tip links that run from the tip of each stereocilium to the lateral surface of the taller adjoining stereocilia. In series with the tip link is a transduction channel whose open probability is a function of the tension in the tip link such that deflections of the hair bundle toward the kinocilium depolarize the cell [Assad 1991]. Deflections of the hair bundle away from the kinocilium hyperpolarize the cell. The hair bundle has an axis of sensitivity running through the kinocilium and the midline of the bundle. The magnitude of the response to a particular deflection correlates with the cosine of the angle between the deflection and the axis of sensitivity of the hair bundle [Shotwell 1981]. Hair cells are the most sensitive mechanoreceptors found among vertebrates, capable of detecting deflections as small as a few nanometers at frequencies ranging from a few hertz to hundreds of kilohertz.

Hair cells are innervated by afferent and efferent neurites. The synapses apposed to the afferent neurites contain specialized presynaptic structures called synaptic ribbons [Bodian 1978]. The ribbons are thought to be important for maintaining the extremely high rate of sustained synaptic vesicular release with high temporal precision necessary for efficient signaling of auditory information [Moser 2006]. Hair cells are among the few neural cell types that exhibit graded vesicular release. Unlike neurons, which release a burst of vesicles when an action potential reaches the synaptic bouton, hair cells release vesicles continually [Moser 2006]. The rate of release is dependent on the polarization of the cell. Depolarization of the hair cell increases the rate of release, whereas hyperpolarization reduces it.

The simplest vertebrate acoustolateralis organs are the neuromasts of the lateral-line system found in fish and aquatic amphibians. Neuromasts are patches of hair cells in a planar array on the exterior of the animal (Figure 1.2A). Animals utilize them to detect low-frequency vibrations and water flows [Bleckmann 1993]. The lateral-line system consists of hundreds of neuromasts distributed across the body of the animal. Each neuromast contains from tens to hundreds of hair cells, depending on the species. The hair cells are covered by a gelatinous cupula that protects the hair cells and transfers water movements to their mechanosensitive hair bundles [McHenry 2007].

There are two classes of lateral-line systems: the superficial lateral line and the canal lateral line. The neuromasts of the superficial lateral line are directly situated on the exterior of the body, sometimes in a trough or pit [Bleckmann 1993]. Neuromasts of the superficial lateral line are sensitive to water velocity, low-frequency vibrations, and near-field disturbances such as

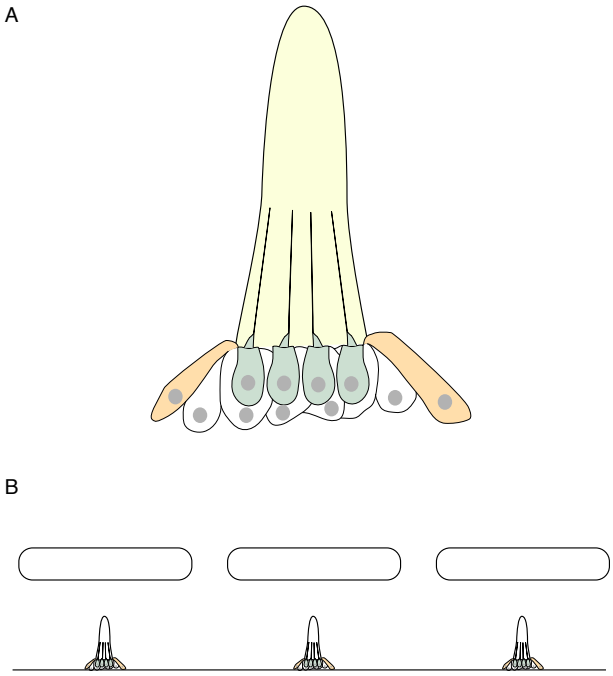


Figure 1.2 - Neuromast
A. A superficial neuromast consists of a patch of hair cells protruding from an epithelium into the water. There are two populations of hair cells with opposite polarities. The hair cells (green) are surrounded by supporting cells (white) and the entire neuromast is radially girdled by mantle cells (orange). The kinocilia of the hair cells are extended and project into the gelatinous cupula overlying the hair cells (yellow).
B. Canal neuromasts are similar in structure but lie in canals enclosed in the skin or bone. The canals are connected to the external water by pores.

turbulence produced by a swimming fish [Bleckmann 1993]. The neuromasts of the canal lateral line are enclosed within a channel recessed in the skin or underlying tissue (Figure 1.2B). Pores along its length connect the channel with the environment. Neuromasts of the canal lateral line are specialized to detect pressure differentials between the pores of the channel, allowing them to detect water accelerations, including any accelerative component of near-field disturbances. They can also detect low-frequency vibrations. Most species of fish (Chondrichthyes and Osteichthyes) have both superficial and canal lateral lines. However, freshwater fish from fast-flowing environments, such as trout (freshwater Salmoninae) and marine fish tend to have complicated canal lateral-line systems, whereas freshwater lake fish often have a reduced canal system and presumably depend more on their superficial lateral line [Bleckmann 1993].

The primary structure that acts as a sensory antenna for superficial neuromasts is the cupula, which can project into the water column hundreds of microns [Mukai 1994 and personal observation]. Canal neuromast responses are modulated by a more complex antennal structures: the canal. The canal diameter, pore diameter, and pore spacing, in addition to the cupula structure, define the sensitivity of the canal lateral line [Bleckmann 1993].

In many species of fish, modified hair cells in organs called ampullary organs, act as electroreceptors [Bullock 1982]. Ampullary organs are thought to have evolved independently at least three times in fishes: prior to the radiation of the Neopterygii, in two families of Osteoglossiformes, the Notopteridae and the Mormyridae, and in two orders of the Ostariophysi, the Gymnotiformes and the Siluriformes. Nearly all non-teleost fish have electroreceptors, but they are found in only these two groups of teleost fish [Bullock 1982]. The Mormyridiformes and Gymnotiformes also evolved tuberous electroreceptor

organs independently of each other. Unlike the ampullary organs, which are sensitive to modulations of an electric field at frequencies of less than 50 Hz, the tuberous receptors are sensitive to high-frequency modulations. The ampullary organs consist of hair cell-like sensory cells at the base of either a pit or jelly-filled canal [Bleckmann 1993]. The tuberous organs consist of 1 - 30 sensory cells whose apical surfaces are packed with microvilli. Both classes of receptors are innervated by the lateral-line nerve. Animals use these electrosensory systems for prey detection. It has been suggested that some animals may also use them to determine their orientation relative to the earth's magnetic field. Species from Mormyridiformes and Gymnotiformes also use electric field discharges from a specialized electric organ to actively probe their environment, giving them an ability to identify objects and to communicate with conspecifics [Bullock 1982, Hopkins 1988, Bleckmann 1993].

Among these species, the overall distribution of electroreceptors acts as a sensory antenna that allows the fish to measure a change in the shape of the electric field produced by their electric organ caused by the presence of a resistive or capacitative object in the field [Bullock 1982]. The canal and jelly overlying the ampullae presumably also function as antennae to improve the sensitivity in high-conductance environments such as sea water [Bullock 1982]. The electric organ, which produces a periodic electrical discharge that the animal uses both for object detection and for intraspecific communication, can also be thought of as an active sensory antenna. The structure of the organ affects the waveform and presumably also the electric-field shape of the discharge, which the fish uses to interact with its social and asocial environments [Hopkins 1995].

Early in craniate evolution, the sensory placodes that gave rise to the hair cells of the neuromast began invaginating to form otic vesicles bilaterally to the

hindbrain, forming the auditory labyrinth, or inner ear [Waterman 1971]. The inner ear is commonly divided into two components: the vestibular system and the auditory system. The vestibular system is used to detect accelerations of the body, including those owing to gravity, whereas the auditory system is involved in the detection of sound. This distinction is less clear in basal aquatic vertebrates, such as fish, which utilize vestibular structures for both the detection of accelerations and of sounds [Webb 2000].

The hair cells of the sensory epithelia in the vertebrate vestibular system are similar in their responses to mechanical deflections. However, complicated structures have evolved that act as antennae to define unique stimuli for each sensory epithelium of the vestibular system, producing for the animal a cohesive perception of acceleration and orientation.

The vestibular system consists of a complex of membranous tubules forming the semicircular canals and the vestibule (Figure 1.3). In all vertebrates except the live-bearing mammals (Theria), the vestibule contains the utricle, the saccule, and the lagena [Waterman 1971]. Therians do not have a lagena. Within the vestibular system there are two classes of hair-cell-containing sensory epithelia: cristae and maculae. The hair cells of the cristae have cilia that extend into a gelatinous cupula, much like the hair cells of a neuromast in the lateral-line system. The structure of the macula is similar, except that the gelatinous structure, referred to as the otolithic membrane, is shorter and is impregnated with small calcium carbonate crystals called otoconia (usually aragonite, but occasionally vaterite) [Hillman 1971]. The otoconia add mass to the membrane, increasing its inertia. The semicircular canals are generally three looping structures oriented approximately orthogonally to each other. Each canal contains a bulge called an ampulla, from one surface of which protrudes a crista ampullaris. The hair cells of each crista are sensitive to rotations of the

head around an axis defined by the semicircular canal. Both the semicircular canal and the cupula act as sensory antennae. The utricle, saccule, and lagena each contains a macula. In mammals, the saccule is oriented in a parasagittal plane so as to be sensitive to anteroposterior and vertical accelerations, such as those felt in an elevator. The utricle lies in the horizontal plane and is sensitive to head tilt. The otolithic membrane and hair-cell epithelium act as antennae to define the class of sensitivities of the utricle and saccule. The various sensory structures of the vestibular system of vertebrates demonstrate complex morphologies and act as antennae to tune the underlying hair cell epithelia to particular types of orientation or movement.

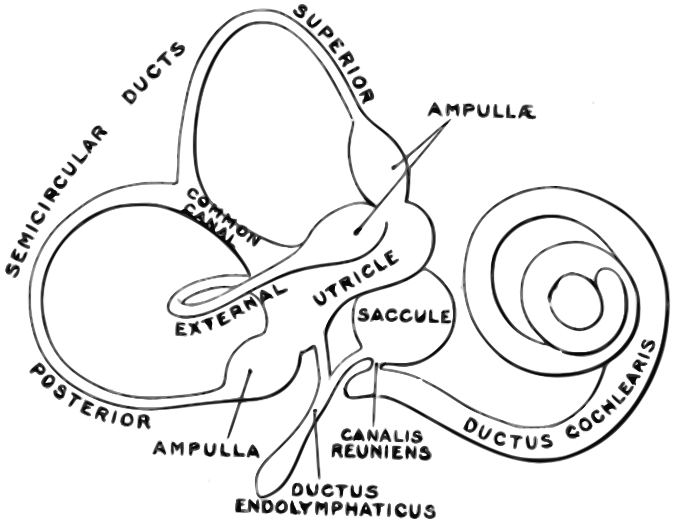


Figure 1.3 - Mammalian auditory labyrinth
The structures of the mammalian auditory labyrinth are portrayed in this lithograph from *Gray's Anatomy* [Gray 1918]. The semicircular canals and associated ampullae are clearly visible, as are the relative locations of the saccule, utricle, and cochlea.

The auditory system in most vertebrates consists of another class of hair cell-containing sensory epithelium: a papilla. In a papilla, only the tips of the hair cells are embedded in a gelatinous layer, usually referred to as a tectorial membrane [Waterman 1971]. The papilla is the basis of the specialized hearing structures in higher vertebrates. Fish lack papillae; they are thought to use the macula lagenae and macula sacculi for hearing [Webb 2000] and may also use the crista neglecta, which in most vertebrates is thought to act as an additional

semicircular canal crista [Webb 2000]. Amphibians use the saccule for low-frequency sound detection in addition to substrate-borne vibration. They have two papillae as well, the basilar papilla and the amphibian papilla. The basilar papilla is sensitive to high-frequency sound (over 1 kHz) whereas the amphibian papilla is sensitive to low-frequency sound (from 0.1 - 1 kHz) [Schoffelen 2008].

The basilar papilla is the primary hearing organ in amniotic vertebrates. Squamata reptiles, such as lizards, have an elongated basilar papilla that serves as their auditory organ. Crocodilians and birds have a further elongated basilar papilla with a vestibular macula lagenae situated at the apical end. Mammals have the most elongated basilar papilla, also called the organ of Corti, which is coiled into a spiral within the temporal bone. The structure is referred to as the cochlea.

In mammals, birds, and some other reptiles the frequency sensitivity of hair cells varies along the papilla from high frequency at the base of the papilla to low frequency at the apex, functionally a spectral decomposition of sound along the length of the papilla [Waterman 1971, Manley 1990]. The frequency sensitivity of a hair cell is dependent on both the cell's characteristics and the properties of the tympanic membrane overlying and the basilar membrane underlying the hair cells [Waterman 1971]. The bundles of hair cells sensitive to low frequency have relatively few, long, large-diameter stereocilia, whereas those sensitive to high frequency have numerous, short, small-diameter stereocilia [Tilney 1983]. Hair cells also display electrical oscillation in response to depolarizing current steps, indicating an electrical resonance [Crawford 1981, Hudspeth 1988, Miranda-Rottmann 2010]. The frequency of resonance varies along the length of the basilar papilla in birds with the overall frequency sensitivity of the hair cells. In mammals, the mechanical properties of the

basilar membrane also vary systemically along the length of the basilar papilla, being lighter and stiffer at the base and heavier and more pliable at the apex. The gradient in the mechanical properties of the basilar membrane changes the resonant frequency of the membrane along its length, further increasing the frequency selectivity of hair cells. In the mammalian cochlea, this gradient leads to a spectral decomposition by frequency of sound along its length. A related phenomenon is the traveling wave, which is related to the gradient of properties of the basilar membrane [von Békésy 1970] as well as other factors, such as mechanical amplification by hair cells [Hudspeth 2008[. The traveling wave is defined by the fact that for any given stimulation frequency there will be slow-moving waves along the basilar membrane that will peak in amplitude at a single point along the membrane where the resonant frequency accords with the frequency of stimulation. This gradient in mechanical properties of the basilar membrane acts as a sensory antenna to spectrally decompose sounds in the cochlea.

Vertebrates have evolved complicated mechanisms to transfer sounds from the environment to the ear. Many species of fish use the air-filled swim bladder as a vibratory resonator. In herring (Clupeidae) the swim bladder contacts the saccule directly [Waterman 1971, Hawkins 1993]. Another group of fish, the Ostariophysi, have developed a series of four bones, the Weberian ossicles, that couple the swim bladder to the membranous labyrinth of the ear. Ostariophysan fish have excellent hearing and dominate freshwater environments [Mayden 2009], where the threat of predation from terrestrial animals is likely to increase the importance of sound detection.

Terrestrial vertebrates face the problem of impedance matching when detecting sound: sound must be transferred from the compressible air in the environment, which has a low impedance, to the incompressible liquid of the

ear, which has a high impedance. Incoming sound vibrates the tympanum (eardrum), which in turn vibrates the ossicle or ossicles of the middle ear, which then vibrate the oval window of the labyrinth (Figure 1.4). The ossicle is referred to as the columella in the reptiles. Mammals have a series of three ossicles, the malleus, incus, and stapes, which act as levers, amplifying the force applied to the oval window [Waterman 1971]. In all mammals and reptiles, the large area of the tympanum acting on the small area of the oval window functions as an hydraulic lever. Muscles on the mammalian ossicles are able to vary their overall stiffness, functioning to reduce the impact of loud noises on the cochlea [Waterman 1971]. The middle ears acts as an antenna, transferring sound from the environment to the phonoceptive inner ear.

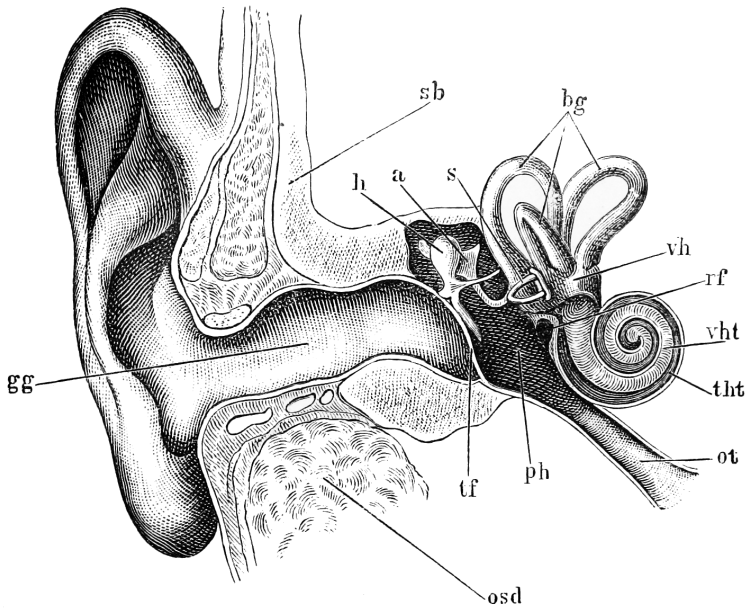


Figure 1.4 - Mammalian outer and middle ear
The external ear in mammals consists of a fleshy structure called a pinna and the ear canal or auditory meatus. The pinna collects sound in a directional manner. It also introduces a spectral modification into incoming sound that is processed for sound-localization information. The middle ear consists of the tympanum (tf) and three ossicles, the malleus (h), incus (a), and stapes (s). It functions to transfer air-borne sound waves into the cochlea. From *Gray's Anatomy* [Gray 1918].

The external environment of the ear canal can have significant effects on the perception of hearing. Mammals in particular have developed a large external ear called a pinna (Figure 1.4). The pinna channels sound from a large area into the ear canal, increasing the amount of sound collected and thereby enhancing the animal's sensitivity [Walsh 2008]. The pinna also improves the directional sensitivity of hearing by allowing mammals to aim their pinna in different bearings, focusing their sound sensitivity to particular areas of space, much as many animals can rotate the eye in its socket to track objects.

The pinna serves another function as well: it spectrally modifies sound. The ridges of the pinna generate destructive interference at a frequency that is dependent on the the elevation at which the sound originates relative to the animal. This is called the pinna notch. Because the frequency of the notch depends on the elevation of the source, the brain can use the spectral characteristics of the notch to localize the source of the sound. The general term for phenomena such as the pinna notch is head-related transfer function (HRTF). Non-mammalian tetrapods, most notably the owls (Strigiformes), also use HRTFs for sound-source localization. Owls utilize both the shape of the facial feathers [Hausmann 2009] and an asymmetry in the vertical position of the ear canal, which together dominate the HRTF, to improve their auditory object-localization ability [Witten 2006]. It is likely that other birds also utilize the HRTF for auditory object localization, albeit with less acuity [Köppl 2009]. The structures surrounding the ear canal that function as an external ear act as antennae essential for the higher-order processing of sound.

The diversity of mechanosensation abilities among vertebrates is extensive and is underlaid by the remarkable characteristics of the hair cells. Vertebrates use hair cells to detect external flows and turbulence, their own rotation and orientation, and compressive vibrations ranging from a few cycles

per second to hundreds of thousands of cycles per second. Although there is significant diversity in the hair cells themselves, much of this impressive breadth of detection ability can be explained by differences in the structures the lie between the hair cells and the environment, which I term sensory antennae. The canal of the lateral line changes hair cells' responses from velocities to accelerations, acting as a differentiator. The semicircular canals restrict the sensitivity of the hair cells in the cristae ampullaris to rotational accelerations around a particular axis, whereas the otolithic membranes of the various maculae allow for sensitivity to both linear accelerations and gravitational acceleration. The organ of Corti spectrally decomposes sound along its length, which is crucial for higher-order processing of tonality. Selection has acted to drive morphological variability in the sensory antennae that form the acoustolateralis organs, allowing vertebrates to sample a broad array of mechanical stimuli.

1.3. Lateral line-based behavior

The lateral line offers fish a unique ability that is difficult for us as terrestrial vertebrates to imagine: the sensation of near-field disturbances, also referred to as a sense of distant touch [Bleckmann 1993]. Given the evolutionary conservation of such a sensory system, one would expect to observe it functioning in a behavioral role. The lateral line is involved in a multitude of behaviors. Many species of fish have been shown to use the lateral-line system for rheotaxis, the act of orienting into a water current [Montgomery 1997, Kanter 2003]. Blinded fish are able to school, or swim in a coordinated manner with conspecifics. However, they lose the ability to do so if the lateral line is destroyed, suggesting that they are capable of normal schooling behavior using the lateral line [Pitcher 1976]. Blind cavefish (*Astyanax mexicanus fasciatus*) are

able to navigate in their environment and discriminate spaces between objects, both of which behaviors are presumably driven by the lateral-line system [Hassan 1986, Sharma 2009]. Numerous species utilize the lateral line for hunting prey. The efficiency of prey capture in the muskellunge (*Esox masquinongy*) is inhibited by either blinding or lateral-line inactivation [New 2001]. The two manipulations seem to affect different components of the behavior. Blinded fish do not stalk their prey, but still strike prey at short distances. Fish whose lateral lines have been inactivated stalk their prey, but are far more likely to miss when striking. The nocturnal piscivorous European catfish (*Siluris glanis*) actively tracks prey in three dimensions in darkness [Pohlmann 2004]. Inactivation of the lateral line eliminates this behavior, but elimination of the animal's external taste does not. Other fish, such as the willow shiner (*Gnathopogon elongatus caerulescens*) and the mottled sculpin (*Cottus bairdi*) are also able to hunt prey in darkness [Kanter 2003]. Removal of the lateral-line cupulae blocks this ability in the willow shiner [Mukai 1994]. Many species of surface-feeding fish can also hunt in darkness using their lateral-line system, including freshwater hatchetfish (Gasteropelecidae), freshwater butterflyfish (Pantodontidae), halfbeaks (Hemiramphidae) and many species of aplocheilid killifish (Aplocheilidae) [Schwartz 1971]. The striped panchax (*Aplocheilus lineatus*) performs admirably at this behavior and we chose to use it as a model to investigate the role of sensory antennae in the lateral-line system.

1.4. Introduction to *Aplocheilus lineatus*

Aplocheilus lineatus, a killifish in the family Aplocheilidae, is native to southern India and Sri Lanka. Found in a wide array of ecosystems, from jungle streams to brackish estuaries, they are voracious surface-feeding fish,

presumably targeting both surface-dwelling and surface-trapped insects. They display a remarkable ability to capture prey in the dark or when blinded [Schwartz 1965]. The behavior utilizes the cephalic lateral line on the dorsal surface of their head to detect capillary waves on the water surface.

On the basis of a single click stimulus, blinded *Aplocheilus* can accurately determine the direction and distance of a surface target to within about five degrees in angle and to within about ten percent of distance at a range up to eighteen centimeters [Schwartz 1965]. Pharmacological or mechanical ablation of all of the neuromasts eliminates the blinded animal's ability to localize [Müller 1982, Kaus 1987]. Ablation of individual neuromasts affects the fish's ability to estimate the direction of a target, but has very little effect on its ability to determine the distance to the target [Schwartz 1965, Müller 1982]. This suggests that the fish compares activity between neuromasts to estimate the target direction, but analyzes the waveform itself to determine the target distance. If all neuromasts but one are removed, the fish turns to the same angle independently of the correct target angle, suggesting that individual neuromasts have unique receptive fields that the fish uses to determine a target's direction [Müller 1982].

Aplocheilus appears to utilize the frequency-dependent dispersion of surface capillary waves to determine the distance to a target [Käse 1987]. Fish are unable to determine the target distance to a pure sinusoidal stimulus, but when stimulated with a click are still able to estimate the target distance when all neuromasts but one have been removed [Bleckmann 1982, Müller 1982]. Together these results suggest that the fish analyzes either the spectrum or the temporal structure of the waves to determine the target distance. The travel speed of a capillary wave depends on its frequency. Higher-frequency waves travel more quickly. A 100 Hz wave travels at approximately 36 $cm \cdot s^{-1}$ whereas a

250 Hz wave travels at approximately 48 $cm \cdot s^{-1}$. Either by measuring the rate of change of the instantaneous frequency of a wave packet or by determining the arrival interval between waves of specific frequencies, the fish could ascertain how far the wave has traveled from its source. Stimulation with artificially modulated waves that appear to have traveled further than they actually have consistently tricked the fish into overestimating the target distance [Bleckmann 1982]. The fish are able to distinguish between two frequencies if they differ by more than ten to fifteen percent [Bleckmann 1981, Vogel 1997], which should be sufficient frequency resolution for the fish to measure the wave dispersion.

The prey-localization behavior of *Aplocheilus* seemed to us to be a rich area to study the role of antenna structure of the lateral-line system in a complex behavior. We approached the system by first characterizing the anatomy of the head. We then investigated the neuromasts electrophysiologically. In parallel, we studied the water movements over the head of the fish in response to surface waves. Finally, we analyzed the relationship between the lateral-line sensory antenna and the fish's prey-localization ability.

2. Anatomy

I began in the Hudspeth lab with the project of describing lateral-line based behaviors in the zebrafish (*Danio rerio*). Zebrafish are tropical minnows native to slow-moving streams, river flood plains, lakes, and rice paddies of the southern Himalayan region including Nepal, India, Myanmar, and Bangladesh [Spence 2008]. Given their niche as a prey species, our prediction was that the lateral line would be used in predator avoidance, presumably through the Mauthner cell-mediated escape response [Korn 2005]. I also investigated the role of the lateral line in rheotaxis. Ablating the lateral line caused very minimal effect in either case, and the expected outcome was was a binary response: with an intact lateral line the fish succeeded in a test; without the lateral line it failed. It seems that zebrafish rely upon other senses for predator avoidance or have lost some acuity in their many generations of domestication.

From the literature I was aware of complex lateral-line-driven behaviors such as prey capture [Coombs 1997]. I conducted an anatomical survey of different fish species looking for unique lateral-line structure, focusing on nocturnal species and those with unique body shapes. Although several species had interesting phenotypes, one that I happened upon had a truly dramatic lateral line: *Aplocheilus lineatus* (Aplocheilidae; Day, 1889). A literature search made the potential of the species clear. *Aplocheilus* was documented to exhibit striking lateral line-based prey-capturing behavior. It seemed as if the characterization of the lateral line of *Aplocheilus* would make an interesting thesis project, so I began working on the fish.

The first step was to characterize the anatomy of the lateral-line system of *Aplocheilus*, a surface-dwelling and surface-feeding Cyprinodontiform fish. The dorsal surface of its head is flattened and displays a prominent premaxillary upper lip (Figure 2.1). The unique shape of its head attracted me to the fish

Figure 2.1 - *Aplocheilus* hunting posture The killifish hunts with its head immediately below and parallel with the surface of the water, maximizing sensitivity to capillary waves. Note the large lip. The fish has the classic long cigar-shaped body, rounded caudal fin, and very posterior dorsal and anal fins of a piscine ambush predators. Its common name in German, Streifenhechtling, translates to 'striped little pike.' This is a medium-sized adult of the 'Golden Wonder' color morph, the most common found in captivity. The scale bar represents 10 mm.

originally, and it proved to be a specialization that allows the unique cephalic lateral line to be in close apposition to the water surface. The physical phenomena driving the *Aplocheilus* prey-capturing behavior are the waves at the air-water interface, specifically the high-frequency surface waves called capillary waves. An insect striking or struggling on the surface of the water produces a packet of capillary waves. Given the shallow penetration of these waves [Hogan 1984], to detect them the fish must minimize the distance between the neuromasts on its head and the water surface. The flatness of the dorsum of the head allows the fish to optimally position its neuromasts so as to maximize sensitivity to capillary waves. The head anatomy is specialized at three levels: the flat head and lip, the arrangement of the neuromasts, and the presence of fine structure surrounding the neuromasts. We hypothesize that these structures act as sensory antennae enhancing the fish's ability to detect surface prey. To investigate this hypothesis we began by precisely characterizing the fine structure of the system.

2.1 Materials and methods

2.1.A. *Animal care*

Fish for the anatomical survey of the lateral line system were acquired from either a Petco retail store, fish2u.com, or Aquatics and Pets Inc. and maintained in filtered water at room temperature at pH 7 and a conductivity of 300-400 μS. *Aplocheilus* were captive bred individuals of the 'Golden Wonder' color morph. Water quality was monitored and the water was regularly refreshed. The experiments were approved by The Rockefeller University IACUC.

2.1.B. *Visualization of the lateral line*

For visualization of neuromasts, we exposed the fish to the fluorophore 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (4-Di-2-ASP; Sigma Aldrich), which is accumulated by hair cells. Fish were incubated in 200 μM 4-Di-2-ASP for five minutes followed by a five minute wash in fresh water. We then anesthetized the fish by placing them in an anesthetic, 670 μM ethyl 3-aminobenzoate methanesulfonate (tricaine, MS-222). We visualized the lateral line under a compound microscope (Olympus) using filtered light from a mercury lamp and acquired images using a color CCD camera (Olympus). We also imaged *Aplocheilus* under a dissection microscope (Leica) with filtered light from a mercury lamp, acquiring images with a CCD camera (Olympus).

2.1.C. *Actin staining*

To determine the orientation of hair cells within the neuromasts of *Aplocheilus*, we stained the actin of the hair cells using phalloidin conjugated to an Alexa Fluor dye (Invitrogen). Fish were euthanized by placing them in 670 μM MS-222, chilling them to 4° C, and transecting their brains at the base

of the hindbrain. The upper cranium was removed and fixed overnight in a solution of 4% formaldehyde in physiological saline (1X phosphate-buffered saline, PBS) at 4° C. The tissue was washed in PBS and blocked in PBS containing 10% bovine serum albumin and 0.2% Tween-20 for 1 hour at room temperature. The tissue was washed again with PBS, incubated in PBS containing 6.6 μM Alexa-conjugated phalloidin for either 4-6 hours at room temperature or overnight at 4° C, and washed in PBS with 0.2% Tween-20 for 2 hours. The tissue was washed and placed dorsum down in a 35 mm glass-bottom dish #1.5 (MatTek Corp.) and embedded in Vectashield anti-fade agent (Vector Labs). We visualized the staining using a laser confocal microscope with a 40X water-immersion objective lens (Olympus).

2.1.D. *Osmium staining*

We used osmium tetroxide to stain the nerve innervating the cephalic lateral line of *Aplocheilus*. Skull tissue was acquired as described above and fixed in 4% formaldehyde in PBS for 2 hours at room temperature. The tissue was washed in PBS and placed in a petri dish. A drop of 157 mM osmium tetroxide was placed a few centimeters from the tissue and the petri dish was closed. The tissue was checked every 5 minutes to monitor the staining of the nerve. When sufficient staining was acquired, the specimen was dehydrated in a series of increasing concentrations of ethanol and placed in methyl salicylate to clear. After clearing it was imaged under a dissecting microscope.

2.1.E. *Cupula staining*

The cupulae were marked using dyed 10 μm latex microspheres (Polysciences Inc.). An anesthetized fish was removed from the water and the

microspheres were dropped onto its head. The head was then gently washed and imaged under a dissecting microscope.

2.1.F. *Scanning electron microscopy*

Fish were placed in standard no-added-calcium anuran saline solution with 80 $\mu g \cdot ml^{-1}$ subtilopeptidase A (Sigma protease XXIV) for 30 minutes to loosen cupulae. Fish were anesthetized until inert and then immersed for 1 hour in a primary fixation solution containing 200 mM gluteraldehyde, 400 mM formaldehyde, 80 mM sodium cacodylate, 1 mM $CaCl_2$, 800 µM MS-222, and 200 µM tannic acid. The fish were washed for 5 minutes at 4° C twice and the upper crania were removed. The wash solution was water containing 80 mM sodium cacodylate and 4 mM $CaCl_2$. The specimens were then immersed in water containing 50 mM osmium tetroxide, 80 mM sodium cacodylate, and 4 mM $CaCl_2$ for 1.5 hours at 4° C and washed twice in distilled water for 5 minutes each time. The specimens were dehydrated by immersion in graded ethanol concentrations from 50% to 70% for 10 minutes at room temperature at each concentration. The specimens were then stored for 18 hours at room temperature in 70% ethanol followed by immersion in graded ethanol concentrations between 80 to 95% for 10 minutes each at room temperature. The specimens were placed in 100% ethanol for two periods of 45 minute each and then dried through liquid CO_2 without transition solvent in the Tousimis Autosamdri-815A apparatus; valve settings: cool, 0.55; fill, 0.35; purge/vent, 0.23; bleed, 0.27; purge time, 10 min (setting "2"). The specimens were mounted with colloidal-silver glue on aluminum stubs and maintained in vacuum for 20 min thereafter to allow removal of solvent without exposure to ambient moisture. They were then coated with gold-palladium in a Desk IV sputter-coater (Denton Vacuum) with continuous coating for 200 s at a 9 kV accelerating

potential (60% "sputter setpoint" setting), producing a 26-mA current and 50 mTorr vacuum after four argon flushes and with argon bleeding the specimen was rotated (10% setting). Specimens were examined in LEO 1550 SEM with 30-µm aperture at an accelerating voltage of 5 kV.

2.1.G. *Transmission electron microscopy*

Euthanasia and primary fixation was performed as for scanning electron microscopy with the addition of 20 mM sucrose to the fixation solution. After 1.5 hours in primary fixation, the fish were washed with 80 mM sodium cacodylate, 20 mM sucrose, and 1 mM $CaCl_2$ for 30 minutes. The crania were removed from the fish and the bone and skin were removed from the area surrounding the left group II of the cephalic neuromasts. The specimens were then stained in water containing 50 mM OsO_4, 80 mM sodium cacodylate, 20 mM sucrose, 5 mM K^+ ferrocyanide, and 1 mM $CaCl_2$ for 1.5 hours and then washed twice in distilled water for 5 minutes. The specimens were further trimmed and then decalcified in 100 mM EGTA and 10 mM sodium cacodylate for 18 hours at 4° C. The specimens were dehydrated in graded ethanol concentrations of 30%, 50%, 70%, 80%, and 95% at 4° C for 10 minutes at each concentration. The specimens were stained with uracyl acetate by immersing them in 95% ethanol containing 0.4% (weight/volume) uranyl acetate for 1 hour. The specimens were then dehydrated by twice immersing them for 1 hour in 100% ethanol. The specimens were immersed in propylene oxide twice for 1 hour each, embedded in a 50% “hard Epon” plastic mixture in propylene oxide for 24 hours, and then placed in the plastic mixture for 24 hours with rotary stirring. The plastic was outgassed at 50° C for 30 minutes under vacuum and then positioned on plastic coverslips and cured for 66 hours at 50° C under vacuum. After curing, the specimens were semi-thin sectioned with a “Histo”

diamond knife at 0.7 µm and stained with toluidine blue O basic fuchsin. Thin sections of 70 nm were then cut and stained for 2 minutes with 50% saturated aqueous uranyl acetate/ 50% acetone and then for 1.5 minutes with Sato's lead stain. The sections were examined using a transmission electron microscope.

2.2 Results

2.2.A. *Anatomical survey*

The standard lateral line system consisted of a prominent canal running along the lateral surface of the trunk, with a few canals on the head, and a distribution of stitches of superficial neuromasts across the head, trunk, and tail (Figure 2.2). Some freshwater fishes, such as zebrafish, do not develop the canal on their trunk.

In the anatomical survey we observed three different lateral-line phenotypes: standard, robust, and structured. The standard phenotype had a basic lateral-line anatomy, as previously described. Examples of fish with the standard phenotype are the zebrafish (*Danio rerio*) and the Siamese fighting fish (*Betta splendens*, Figure 2.3A). The robust phenotype had a

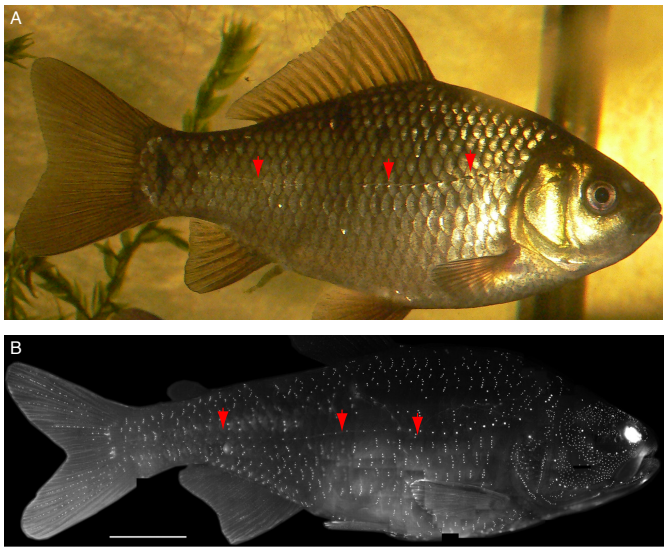


Figure 2.2 - Lateral line
A. The canal lateral line of *Carassius carassius* (the Crucian carp) is indicated by red arrowheads. From Wikimedia Commons.
B. Both the superficial and canal neuromasts are visible in this fluorescence image of a stained Blind cave tetra (*Astyanax mexicanus fasciatus*). The arrowheads indicate the canal. Adapted from Sapède 2002.

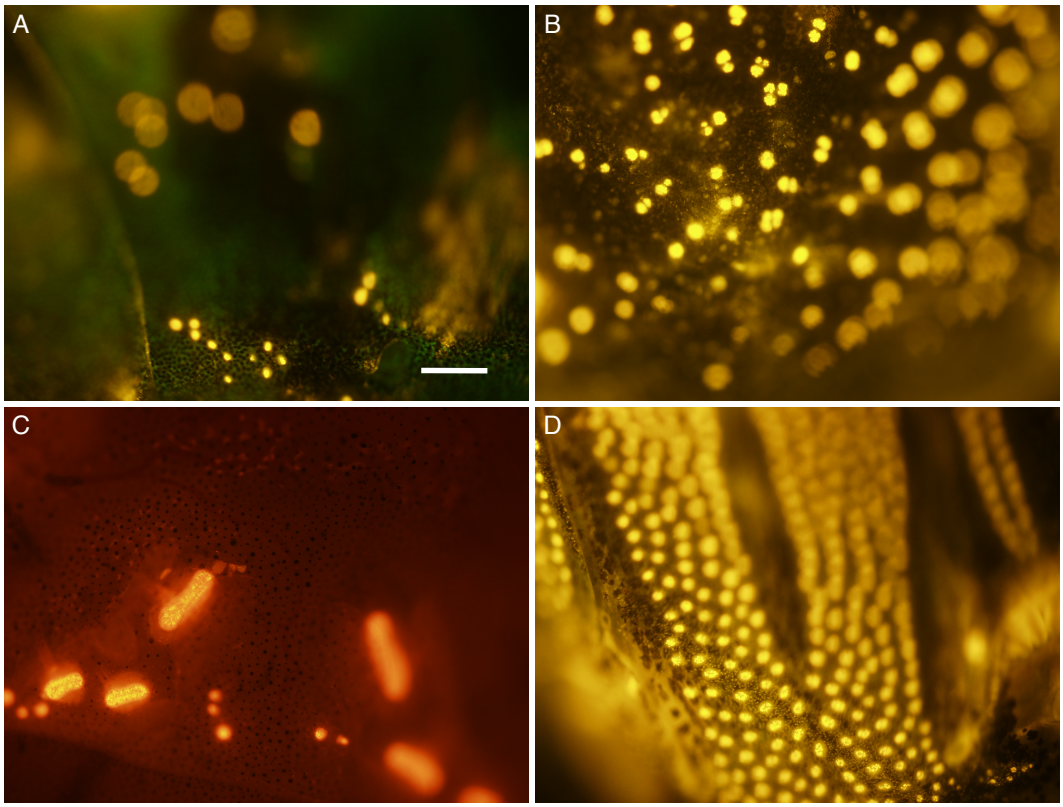


Figure 2.3 - Lateral-line diversity

Four fish snouts stained to show the neuromasts were imaged at 5X magnification. The same region, the middle of the snout immediately behind the upper lip was imaged. Rostral is down.

A. A Siamese fighting fish (*Betta splendens*). This is a standard cephalic lateral line on the snout. The scale bar represents 200 µm.

B. The iridescent shark (*Pangasius hypophthalmus*) is a nocturnal catfish. Its cephalic lateral line displays a robust phenotype, with a high concentration of neuromasts. A similar concentration of neuromasts is visible over much of its body.

C. The striped panchax (*Aplocheilus lineatus*) has a highly structured lateral line with enormous neuromasts. The rest of its body has a normal concentration of much smaller neuromasts.

D. The common hatchetfish (*Gasteropelecus sternicla*) has unique ridge on its crown covered with a packed array of many hundreds of neuromasts. The rest of its body has a normal concentration of neuromasts.

lateral-line anatomy similar to the standard, but with a far greater concentration of neuromasts. We observed this in nocturnal fish, such as the San Raphael catfish (*Platydoras armatulus,* Figure 2.3B) and the Mexican blind cavefish (*Astyanax mexicanus fasciatus,* Figure 2.2B), although the sighted morph of the

species (*Astyanax mexicanus*) had a standard lateral line. The third class of fish were those that had unique structures in their lateral line. Dramatic examples include *Aplocheilus lineatus* (the striped panchax, Figure 2.3C), the common hatchetfish (*Gastropelecus sternicla*, Figure 2.3D), the freshwater butterflyfish (*Pantodon buchholzi*), the blue panchax (*Aplocheilus normani*), and to a lesser degree the medaka (*Oryzia latipes*) and the platy (*Xiphophorus spp.*). All of those species have unique cephalic lateral lines. *Gastropelecus* has a densely packed ridge on its crown, containing many hundreds of neuromasts, and blinded animals are able to hunt prey on the water surface. *Pantodon* has a few large patches of neuromasts in pouches on its head. It also exhibits a robust blind surface-hunting behavior. The four other fish have cephalic neuromasts similar in structure to those of *Aplocheilus lineatus* with a stereotyped array of extremely large neuromasts. Only *Aplocheilus normani* has a comparably sophisticated array of neuromasts to *A lineatus*. *Xiphophorus* is in

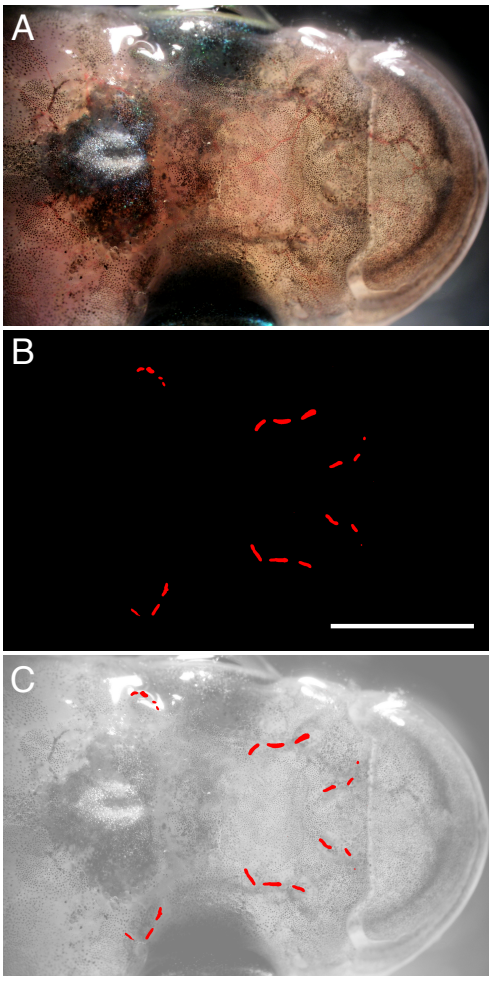


Figure 2.4 - *Aplocheilus* cephalic lateral line

A. The array of specialized neuromasts occurs on the animal's flattened head between the eyes and behind the prominent, semicircular lip.

B. Labeling with a red fluorophore concentrated by hair cells reveals the positions of the largest 16 of the 18 neuromasts. The scale bar represents 5 mm.

C. Superposition of the previous two images reveals the position of the neuromasts of the dorsal surface of the head.

the same order as *Aplocheilus* (Cyprinodontiformes) and *Oryzia* is in a sister order (Beloniformes), suggesting that the large neuromasts seen in *Aplocheilus* may be a synapomorphic trait shared between Cyprinodontiformes and Beloniformes.

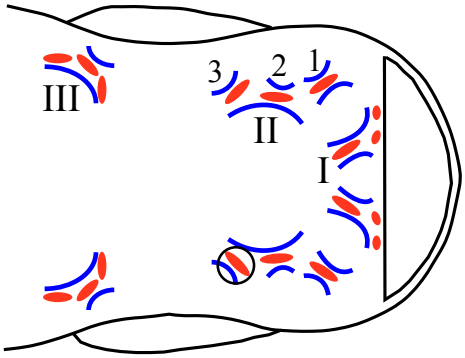


Figure 2.5 - Lateral-line schematic
A schematic diagram portrays the stereotyped placement of the neuromasts (red) and ridges (blue) in *Aplocheilus*. Each neuromast is identified from rostral to caudal by a group number, given in Roman numerals from I to III, and by an Arabic numeral from 1 to 3. The circled neuromast, for example, is the third in group II on the right side, or

Of these species, *Aplocheilus lineatus* seemed the most suited to further study. All of the fish with the robust lateral-line phenotype, or a closely related species, have been shown to utilize their lateral lines for hunting or navigation [Schwartz 1965, Hassan 1986, Pohlmann 2004, Sharma 2009], but the logistics of analyzing a sensory system used for detecting and analyzing three-dimensional waveforms in the water seemed overwhelming when compared to a system adapted to two-dimensional waveforms. We opted, therefore, to study a surface-hunting fish. Of the surface-feeding fish *Gasteropelecus*, *Pantodon,* and *Aplocheilus lineatus*, only *Aplocheilus* readily breeds in captivity. Moreover, its neuromasts are easily accessible on the surface of its head, unlike those of *Pantodon*. The blind prey-localization behavior of *Gasteropelecus* is more complicated than the behavior of *Aplocheilus*. Whereas *Aplocheilus* orients toward and then swims to a target stimulus, *Gasteropelecus* tends to spiral in toward a target stimulus (personal observation). We also discovered that there is a substantial body of work on the blind prey-localization ability of *Aplocheilus* on which to build.

2.2.B *Aplocheilus cephalic lateral line*

Aplocheilus lineatus hunts immediately below the surface of the water (Figure 2.1). Its cephalic lateral line consists of 18 large superficial neuromasts arranged on the head in a precise array (Figure 2.4). On each side of the head, the neuromasts are symmetrically arranged in three groups each containing three neuromasts. Individual neuromasts are designated by the side of the fish

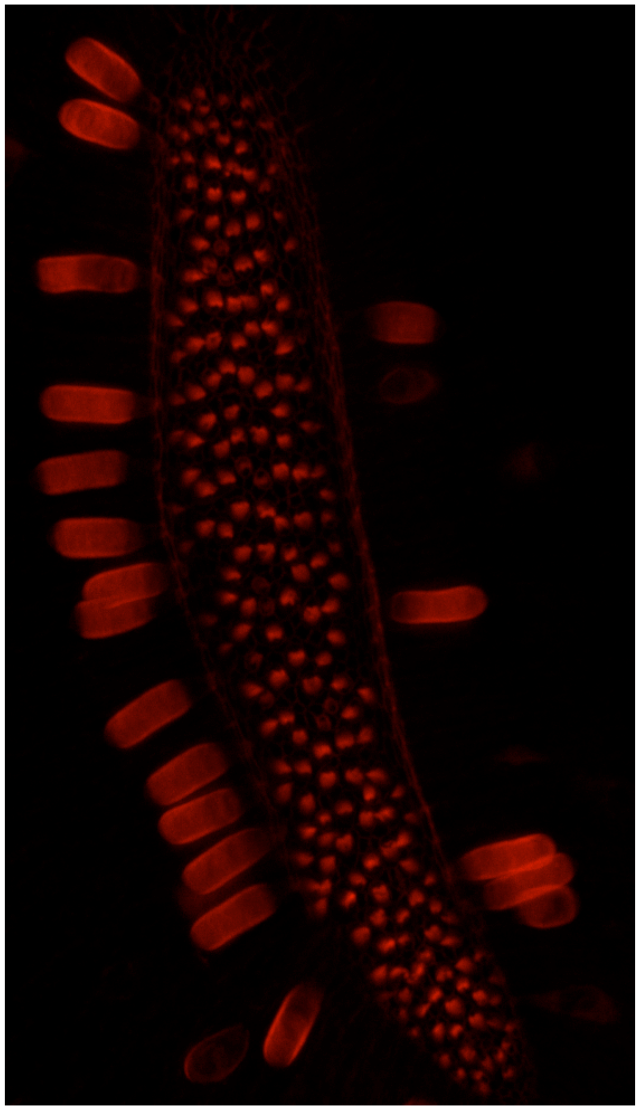


Figure 2.6 - Actin staining of hair bundles
The stereocilia are actin-filled protrusions whereas the kinocilium has a microtubule-based structure. As a result, the actin-binding compound phalloidin conjugated to a fluorescent dye marks the extent of the stereocilia while leaving the kinocilium unstained, allowing us to see the orientation of the bundles. The kinocilium marks the tall end of the bundle and denotes the side of the bundle toward which deflections depolarize the hair cell. This is neuromast LI3, which contains about 200 hair cells. The hair bundles are the polygonal blotches; the dark spot in each bundle indicates the position of the kinocilium. The axes of sensitivity of all hair cells lie along the long axis of the neuromast. The large, barrel-shaped objects are strange cells of unknown function or identity.

(L or R), their group (I to III), and by their identity within that group (1-3), (Figure 2.5).

Whereas the superficial neuromasts of most fish are circular and contain approximately 20 to 30 hair cells [Blaxter 1983], the cephalic neuromasts of *Aplocheilus* are ellipsoidal and contain from about 70 to over 200 hair cells (Figure 2.6). The actin stain also indicates the orientation of the hair cells: the unstained spot of each hair cell represents the kinocilium, which lies at the tall end of the bundle and indicates the direction of deflections that depolarize the hair cell. The hair cells of each neuromast are polarized along the long axis of the neuromast. There are two populations of hair cells with opposite polarities, giving the neuromast maximal sensitivity to deflections in either direction along its long axis. Scanning electron micrographs corroborate the actin stain, with all of the hair bundles clearly oriented along the long axis of the neuromast (Figure 2.7).

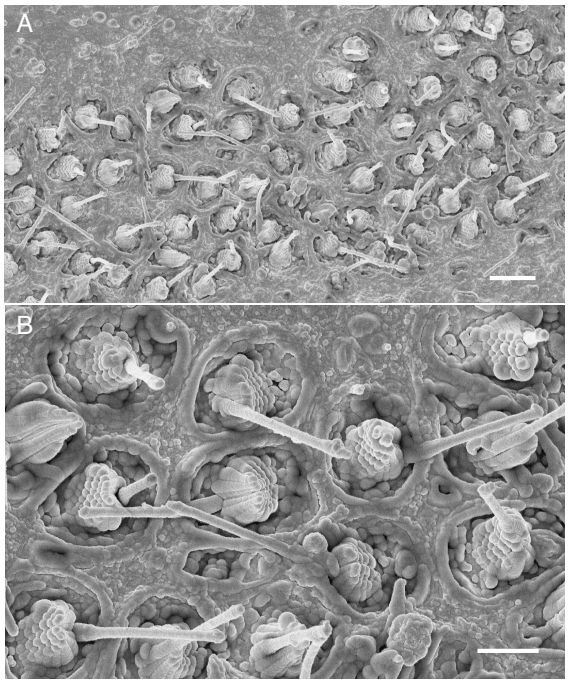


Figure 2.7 - Scanning electron micrograph of neuromast LII1

A. A low-magnification scanning electron micrograph shows part of a single cephalic neuromast of *Aplocheilus*. The cupula has been removed to expose the hair bundles, whose axes of sensitivity correspond to the long axis of the neuromast. The scale bar represents 4 μm.

B. A higher-magnification view resolves the orientations of individual hair bundles. Movement of a bundle toward its kinocilium, the single long process that ordinarily extends into the cupula, depolarizes the hair cell. The scale bar represents 2 μm.

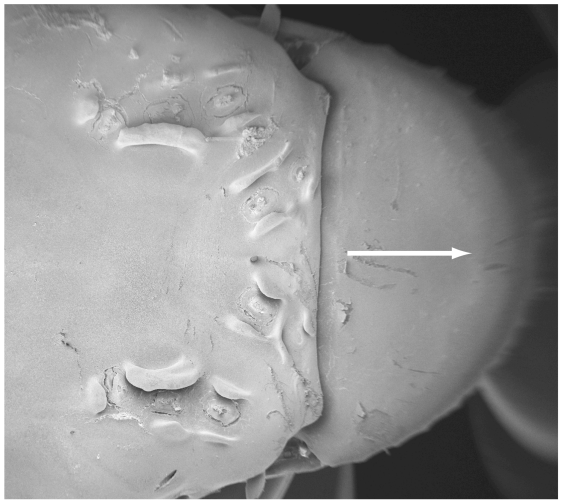


Figure 2.8 - Low-magnification scanning electron micrograph of dorsum
A scanning electron micrograph shows that each neuromast is bordered by fleshy ridges. A neuromast is an ellipsoidal structure with an outer ellipse indicating the area covered by the cupula and a narrow inner ellipse comprising the hair cells. The arrow indicates the anterior direction.

A low-magnification scanning electron micrographs illustrates the fine structure of the head. Each neuromast is bordered by a set of ridges (Figure 2.8). The ridge structure is very clear in a light micrograph of a thin section (Figure 2.9). The ridges are fleshy and packed with capillaries. We did not observe any innervation within the ridges, but we did see myelinated fibers innervating the neuromast itself. Using osmium tetroxide we were able to label the anterior lateral-line nerve running from the back of the cranium forward to each group of neuromast (Figure 2.10).

In a light micrograph, the cupula looks extremely reduced due to dehydration during the fixation process (Figure 2.9). Labeling the cupulae *in vivo* demonstrates, however, that they normally fill most of the channel produced by the ridges (Figure 2.11).

In both scanning and transmission electron micrographs the hair cells are of conventional structure (Figures 2.7 and 2.12). We observe strange, actin-filled cells adjacent to the hair cells. They are apparent with a phalloidin stain and are also clearly evident in electron micrographs (Figures 2.6, 2.9 and

2.12). Neither their identity nor their function is known.

2.3 Significance

The anatomy of the head of *Aplocheilus* is striking. It has a number of adaptations that seem likely to be related to its mechanosensory prey-localization ability. The head is severely flattened and has a large, protruding lip. The flattening of the head is ideally suited to

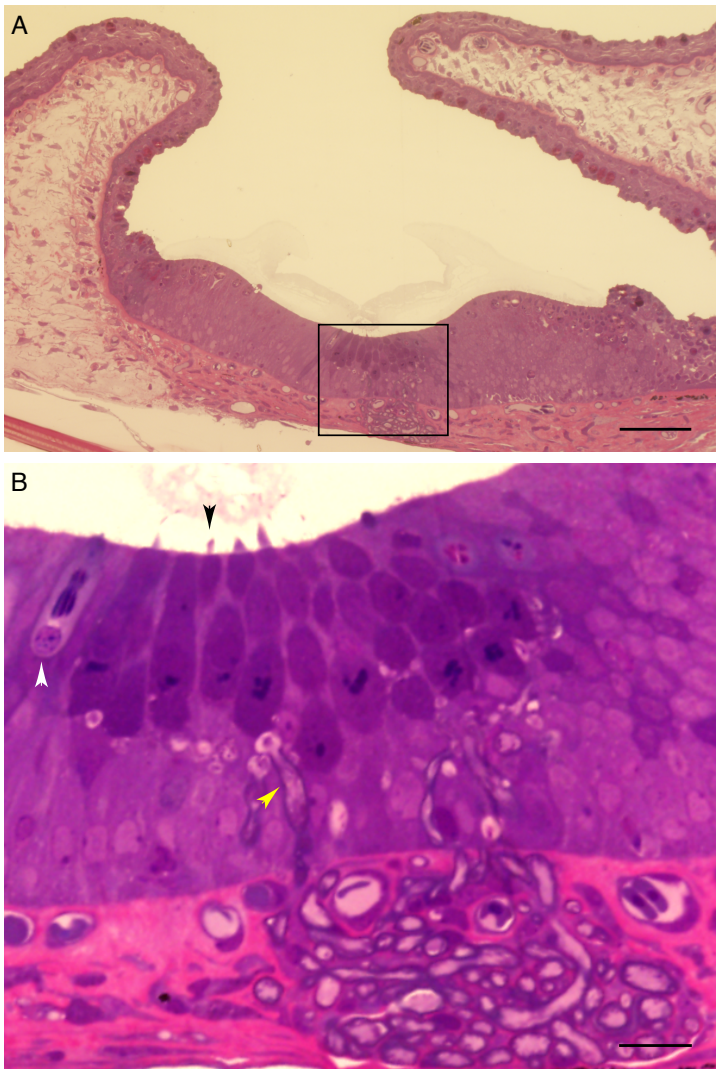


Figure 2.9 - Section of neuromast II2 and associated ridges
A. The fleshy ridges are very apparent in this image. The hair cells with their bundles can be seen in the middle of the epithelium below the ridges. The faint structure above the hair bundles is the cupula, which would fill much of the channel produced by the ridges *in vivo*, but here has collapsed during the fixation process. Note the many capillaries filling the ridges and the bundle of myelinated fibers below the hair cells. The scale bar represents 50 µm.
B. A higher-magnification image of the hair cells and nerves. A hair bundle is indicated by the black arrowhead. A myelinated nerve fiber that has passed through the basement membrane of the neuromast epithelium is indicated by the yellow arrowhead. Notice the bundle of myelinated fibers below the basement membrane. The unknown cell seen with the actin stain is also apparent here, indicated by the white arrowhead. Scale bar represent 10 µm.

placing the cephalic neuromasts in proximity to the surface of the water, where they will be maximally sensitive to capillary waves. The role of the lip is less clear. Capillary waves do not seem to translate over it. It could be acting as a refractive lens, forcing waves around it and amplifying any difference in wave-arrival times between the left and right side neuromasts. This could be important for detection of stimuli toward the front of the fish.

The neuromasts themselves are extremely large and have an unambiguous axis of sensitivity along their longitudinal dimension. The complexity and stereotypy of the neuromast array suggests that the individual neuromasts have unique receptive fields. This implication is strengthened by the behavioral effect of neuromast ablations. When all neuromasts but one are

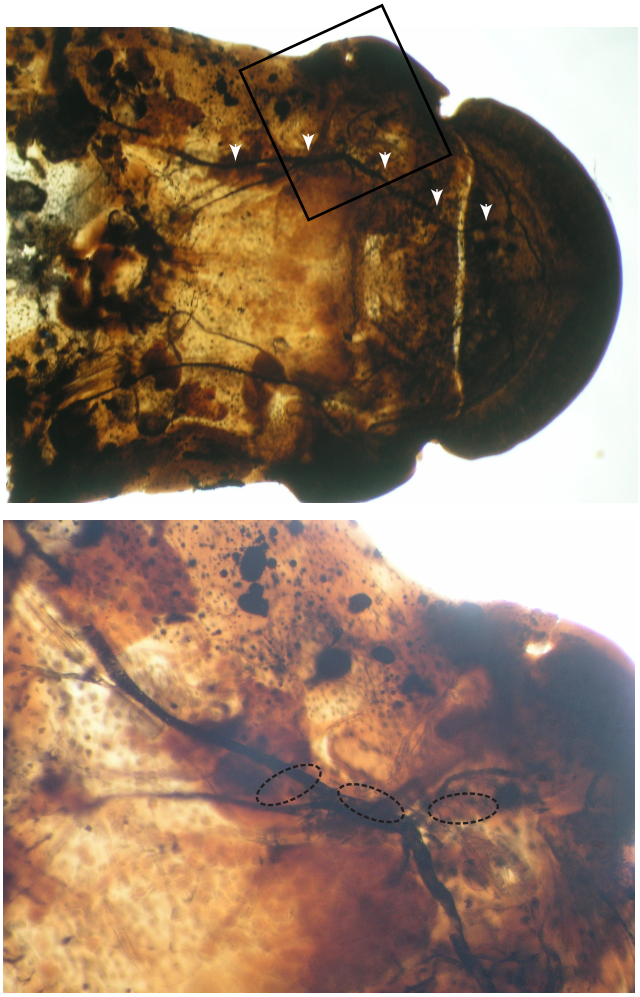


Figure 2.10 - Osmium stain of anterior lateral-line nerve

A. The anterior lateral-line nerve runs from the back of the cranium forward along the top of the orbit, under the three groups of neuromasts before branching into the lip. The left nerve is indicated by white arrowheads. The box indicates the region shown below at higher magnification.

B. A higher-magnification image of area around the left group II shows the approximate locations of neuromasts II3, II2, and II1 (from left to right).

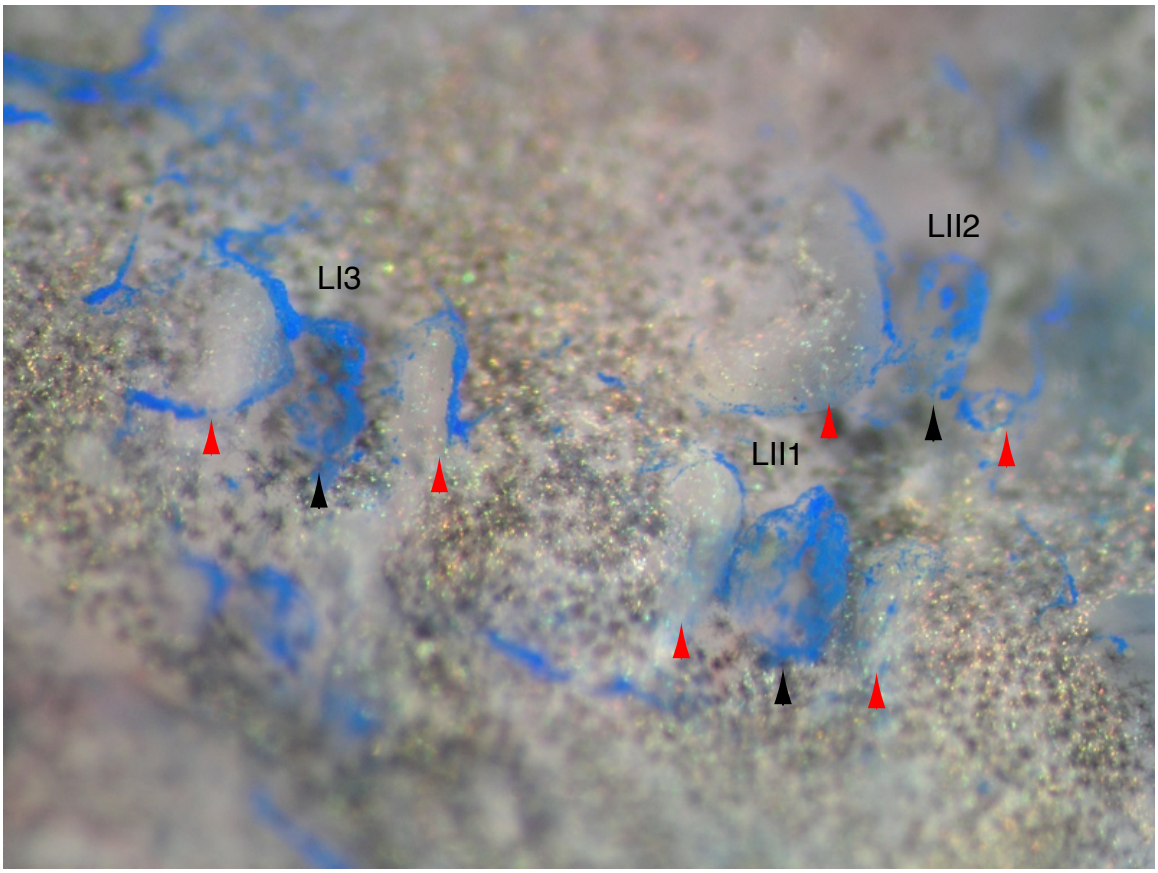


Figure 2.11 - Bead labeling of cupulae
The cupulae become apparent if dyed latex microspheres are applied to the head of the fish. Neuromasts LI3, LII1, and LII2 are apparent. The view is looking down on the front of the fish at approximately at 45° angle. The cupulae are indicated by black arrowheads. The ridges are indicated by red arrowheads. The cupulae appear to fill much of the channel produced by each ridge.

removed, the fish reacts identically to a wave stimulus sent from any position on the surface of the water, always turning in the same direction and to the same angle [Müller 1982]. The direction and angle depend on which neuromast remains.

If the neuromasts do have unique receptive fields, it seems likely that the ridges bordering each neuromast have a role in structuring them. The ridges lie in proximity to the neuromast, producing a channel that is largely filled by the

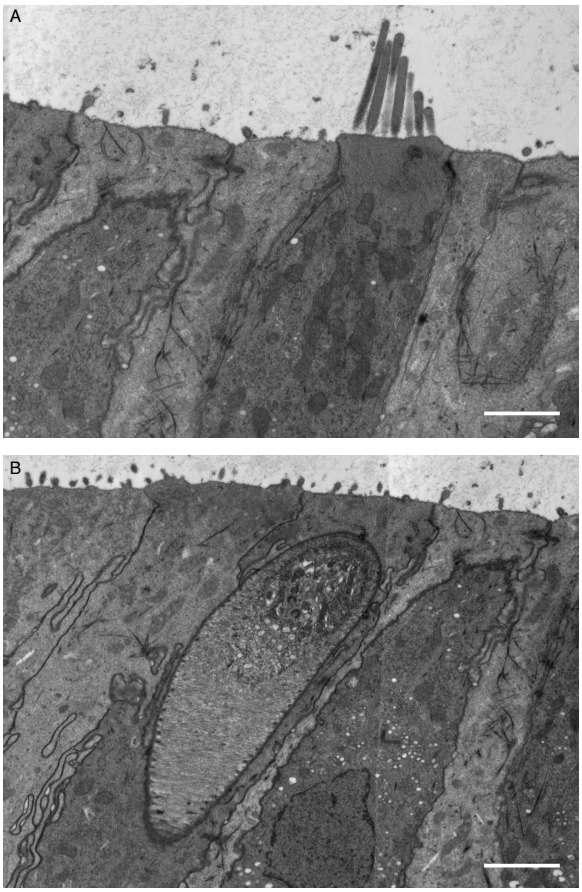


Figure 2.12 - Transmission electron micrograph of neuromast hair cell
A. TEM of a hair cell. It appears to be a conventional hair cell. Scale bar represents 2 μm.
B. TEM of the unknown cell. The white striated material consists of actin filaments. Scale bar represents 2 μm.

cupula. The ridges could restrict access to the neuromast to all waves but those coming from particular directions. They could also be limiting the movement of water in the channel that they form over the neuromast.

There are a large number of myelinated fibers running to each neuromast. The fibers maintain myelination after passing through the basement membrane of the neuromast epithelium, which is uncommon. Given that myelination increases the speed of nerve transduction, perhaps there has been strong selection for rapid transmission of signals from the neuromast to the central nervous system.

The anatomy of the cephalic lateral-line system is unique. There are a number of structures, such as the lip, ridges, and cupulae, that could be acting as sensory antennae. To further investigate these possibilities we decided to study the hydrodynamics of the system.

3. Hydrodynamics

Aplocheilus lineatus is able to detect capillary waves on the water surface and analyze those waves to determine the location of their source. It utilizes this ability to hunt prey in darkness. There are two primary restoring forces that act on water surface waves: gravity and cohesion. Gravity dominates low-frequency waves such as ocean waves, whereas cohesion (or capillary action) dominates high-frequency waves such as ripples. There is a transition between the two regimes at about 13 Hz [Käse 1987]. Waves with frequencies below 13 Hz are called gravity waves and and those with frequencies greater than 13 Hz

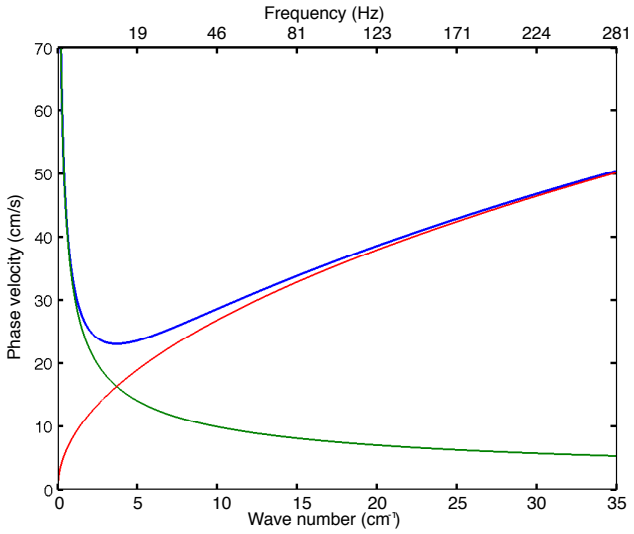


Figure 3.1 - Wave phase velocity as a function of wave number
Surface waves exist in two forms: gravity waves and capillary waves. The green trace represents the phase velocity as a function of wave number for gravity waves.

$$phase\ velocity_{\ gravity} = \sqrt{\frac{g}{K}}$$

The red trace represents the phase velocity as a function of wave number for capillary waves.

$$phase\ velocity_{capillary} = \frac{T \cdot K}{\rho}$$

The blue trace represents the overall equation.

$$phase\ velocity_{overall} = \sqrt{\left(g + \frac{T \cdot K}{\rho}\right) \cdot K^{-1}}$$

where g is the gravitational accelerations, K is the wave number ($2\pi \cdot \lambda^{-1}$), ρ is the density of water, and T is the coefficient of surface tension. Typical parameters were used (g = 981 cm·s^{-1} and $T \cdot \rho^{-1}$ = 72 cm^3·s^{-2}). The phase velocity reaches a minimum of 23 cm·s^{-1} at 13 Hz. Adapted from Käse 1982.

are called capillary waves (Figure 3.1). Gravity waves exhibit normal dispersion; that is, the higher a wave's frequency, the slower its phase velocity. Capillary waves exhibit anomalous dispersion such that the crests of higher-frequency waves move faster than those of lower-frequency waves. *Aplocheilus* appears to use this characteristic to determine the distance to a target stimulus [Bleckmann 1982]. There are other differences between gravity and capillary waves as well. Whereas translating gravity waves drive circular trajectories of water particles, with zero net flow, capillary waves drive spiraling trajectories of the water, resulting in a net flow parallel to the direction of the waves' translation (Figure 3.2, Hogan 1984). The amplitude of trajectories produced by a surface wave attenuate exponentially with depth. At a depth of one wavelength, the wave amplitude is negligible. For the behaviorally relevant frequencies of 100 - 250 Hz, the wavelengths are about 600 - 300 µm. This phenomenon likely underlies the fish's hunting posture with the dorsum of its head immediately below the water surface at a depth of less than 500 µm. The longitudinal component of the water movements may also increase, such as when ocean waves wash onto the beach.

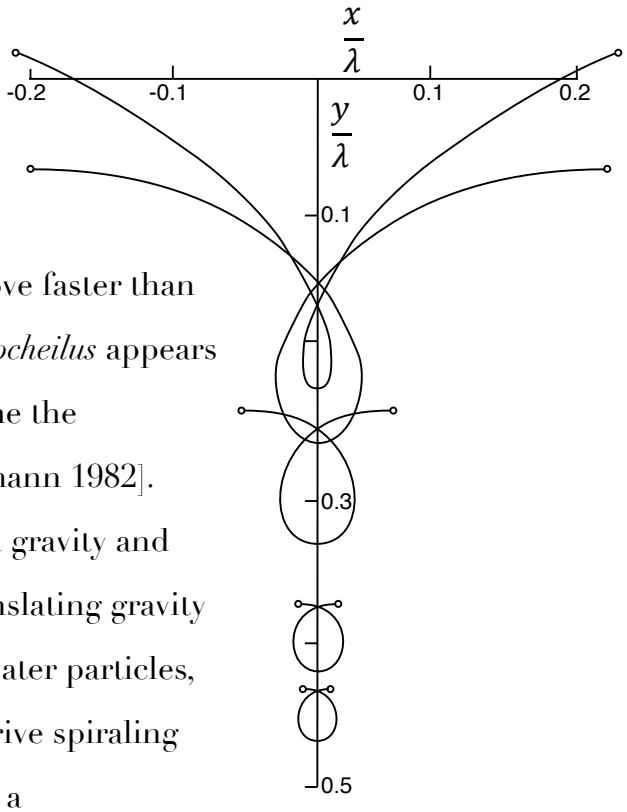


Figure 3.2 - Capillary-wave water movement
The translation of a capillary wave drives spiraling water particle trajectories. There is a net flow of water was a capillary wave passes. Capillary waves attenuate rapidly with depth. The units X and Y are both proportions of a wavelength. Adapted from Hanson 1984.

We hypothesized that the ridges on the top of the head act as sensory antennae by regulating water movement around the neuromasts. To test this hypothesis, and to observe the nature of hydrodynamics over the head in general, we developed a system to track the movement of beads on the water surface. We also investigated the effect that the addition of supplemental ridges had on hydrodynamics around the neuromasts.

3.1 Materials and methods

3.1.A. *Monitoring waveforms*

We were able to monitor the displacement of the surface of the water in response to different stimuli, a click stimulus, a struggling insect, and a sinusoidally-modulated air puff, using a heterodyne interferometer (OFV501 and OFV3001, Polytec GmbH) with its laser reflected from the water surface. The click stimulus is a broadband waveform produced by dropping a floating object, such as a food pellet, onto the water surface. The struggling insect was a small cricket dropped onto the water.

3.1.B. *Hydrodynamic experiments*

Each fish was anesthetized in 670 µM MS-222 in fish water at pH 7. The animal was secured with its head protruding from a flexible plastic tube and adjusted relative to surface of the water to approximate the fish's hunting position. It was important that the dorsum of the fish's head lie within about 200 µm of the surface in order to observe relevant water movements. Fine adjustments were made by adding or removing water from the chamber. Hollow phenolic beads 3 - 45 µm in diameter (Polysciences, Inc.) were scattered over the surface of the water. The beam of a helium-neon laser was passed through a beam expander and aimed at the surface of the water with a low angle of

incidence to illuminate the beads. A video camera (Motionscope PCI2000S, Redlake Instruments) recording at 500 frames per second was used to visualize bead movements over the head of the fish during sinusoidal stimulation of the water's surface at

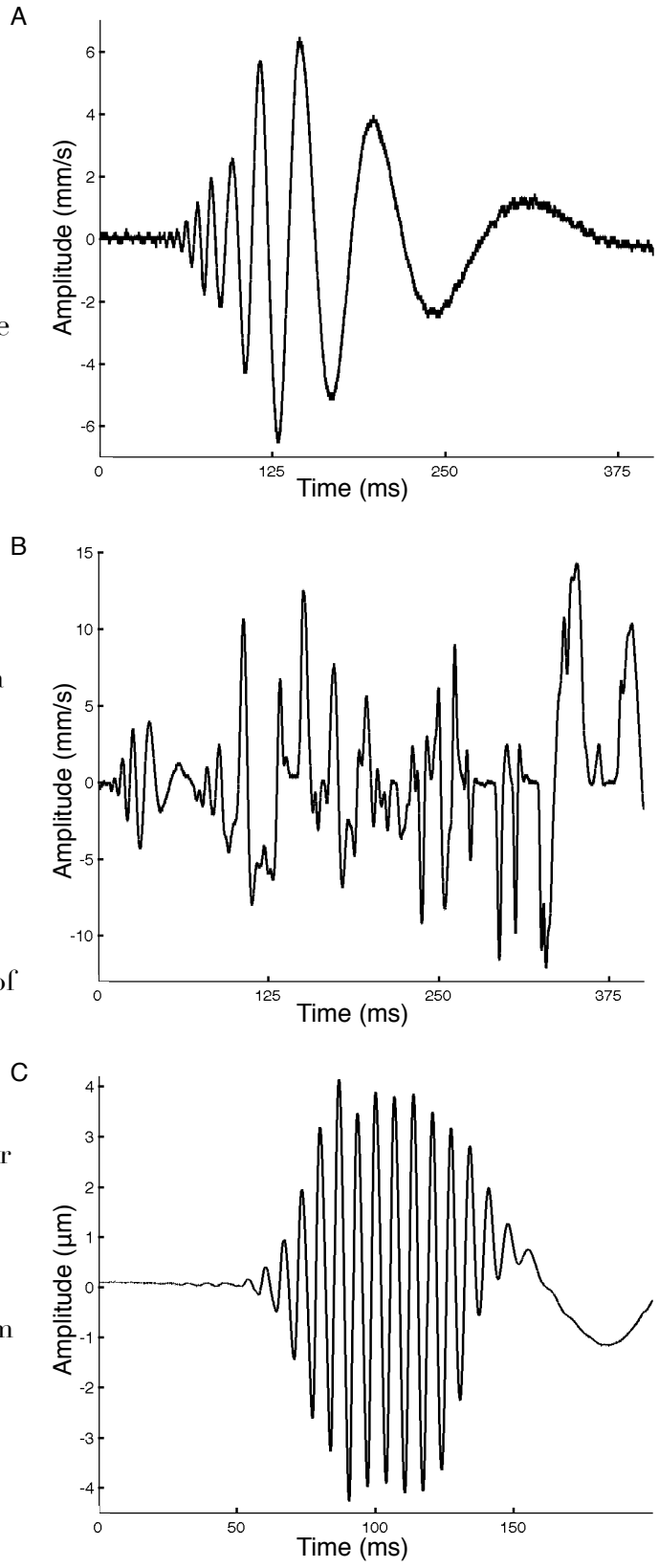


Figure 3.3 - Sample waves
A. The velocity waveform of a click stimulus on the surface of the water generated by dropping a food pellet onto the water. It has a broad spectrum and demonstrates dispersion with the high-frequency waves at the front of the wave packet and low-frequency waves at the back.
B. The velocity waveform of a cricket struggling on the water surface. It is erratic and displays energy at high frequencies.
C. The displacement waevform of a sinusoidal wave packet generated using our air-puff stimulation system. The waveform has a frequency of 150 Hz and its amplitude is sinusoidally modulated.

30 Hz. The stimuli were generated using an air-puffing system consisting of a shielded speaker driven by a stereo amplifier (SA1, Tucker Davis Technologies) that produced sinusoidally modulated wave stimuli on the water's surface. The speaker diaphragm was covered with a plastic petri dish to create a sealed chamber. A hole drilled through the center of the dish held a glass capillary

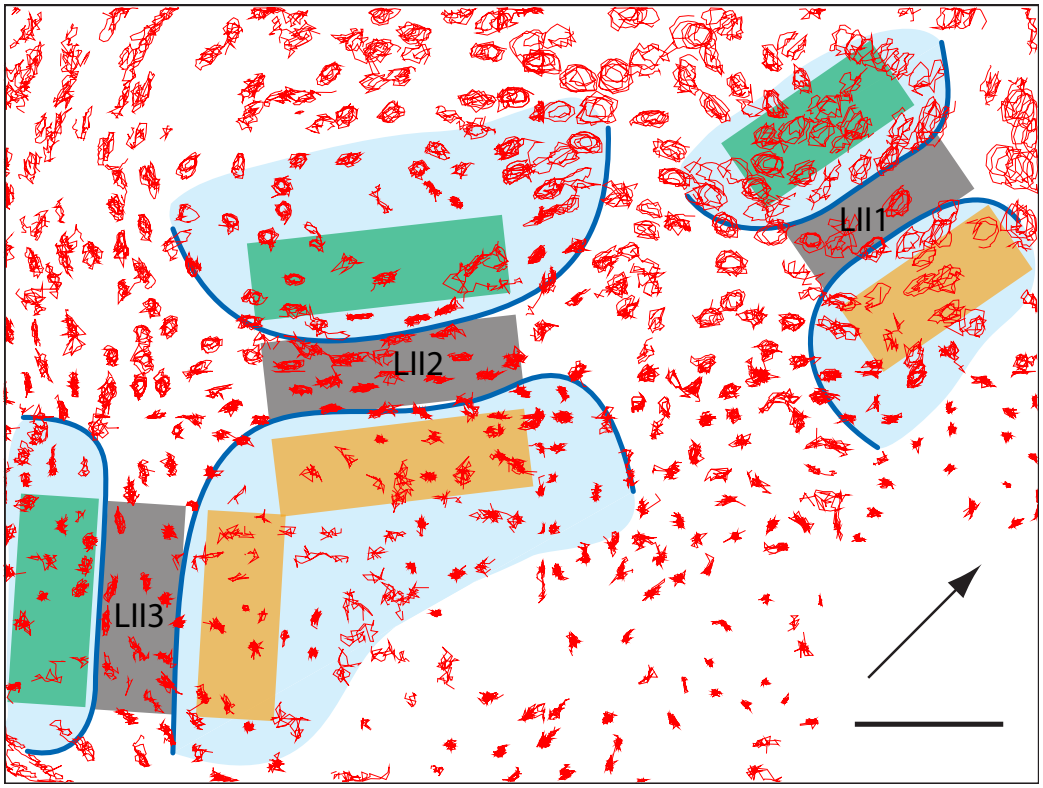


Figure 3.4 - Bead trajectories and regions of interest.
A sample of bead trajectories from a single experiment overlaid onto a schematic representing the anatomy and the regions of interest. The field of view is over the neuromasts of left group II. The anterior of the fish is to the right and lateral side is up. Each red line represents a different bead and its position over time. Notice that over the neuromasts the bead trajectories largely follow the longitudinal axis of the neuromast. Also note that those axes differ greatly between neuromasts. It seems that the ridges drive this transition. The regions of interest lie lateral to the neuromasts (green), directly above them (grey), or medial to them (orange). The longitudinal component of a measurement corresponds to the long axis of the rectangle and the transverse axis to its short axis. The arrow indicates the direction toward the wave source. The scale bar represents 200 μm.

tube 1.17 mm in internal diameter and 76 mm in length. Stimulation of the speaker sent pressure pulses down the capillary, whose orifice was positioned 1 - 2 mm above the water's surface, 70 mm from the fish and at an angle of 45° to the left of its longitudinal axis of the fish. Bead trajectories were detected with an ImageJ program for particle tracking [Sbalzarini 2005]. The 30 Hz Fourier components in the longitudinal and transverse direction of the neuromasts were determined with Mathematica7 (Wolfram Research). The corresponding averages and standard deviations were computed from approximately ten trajectories for each specific area.

3.1.C. *Ridge modifications*

The hydrodynamic environment of a fish's head was modified by adding supplemental ridges between the ridges lateral to neuromast LII2 and LII3. The supplement ridge was made from either cyanoacrylate glue or epoxy cement and attached to the head using the former adhesive. The ridges were removed with forceps. Successful experiments were performed on a total of two animals.

3.2 Results

3.2.A. *Waves produced by different stimuli*

The wave produced by a small object, such as an insect, dropping onto the water surface is referred to as a click stimulus. It generates a small wave packet with a broad frequency content (Figure 3.3A). Insects produce similar stimuli when dropping onto the surface. The struggling of an insect produces an erratic stimulation (Figure 3.3B). Using our stimulation system we were able to produce sinusoidal waves on the water surface (Figure 3.3C).

3.2.B. *Hydrodynamic effect of natural ridges*

Using a high-speed camera to track laser-illuminated floating beads as proxies for water movement, we were able to observe the hydrodynamic environment over the head of *Aplocheilus* in response to sinusoidal wave stimulation. We investigated the movement of individual beads at the stimulation frequency. Figure 3.4 displays an example of bead trajectories over the neuromasts and ridges of left group II.

Comparing water movement lateral to, medial to, and directly over the neuromasts of group II in response to sinusoidal stimulation allowed us to determine the effect of the ridges on water movements around the neuromasts (Figure 3.4). We quantified the water movements by decomposing the bead trajectories into two components: one parallel to the neuromast axis of sensitivity and the other orthogonal to that axis.

Lateral to the neuromasts, the water is minimally affected by the ridges and the water movements in response to the stimulation are large. In contrast, we observed a substantial reduction in water motion medial to the neuromasts, owing to partial blockage of the waves by the ridges (Figure 3.5). As expected if the ridges serve to channel waves onto the neuromasts, we measured an increase in the longitudinal water movement along a neuromast's axis of sensitivity relative to the transverse movement that occurs orthogonal to that axis when we observed the bead trajectories directly over the neuromast. The differences in water movement at different points on the head relative to the ridges is consistent with the hypothesis that these ridges modulate wave movements over the neuromasts.

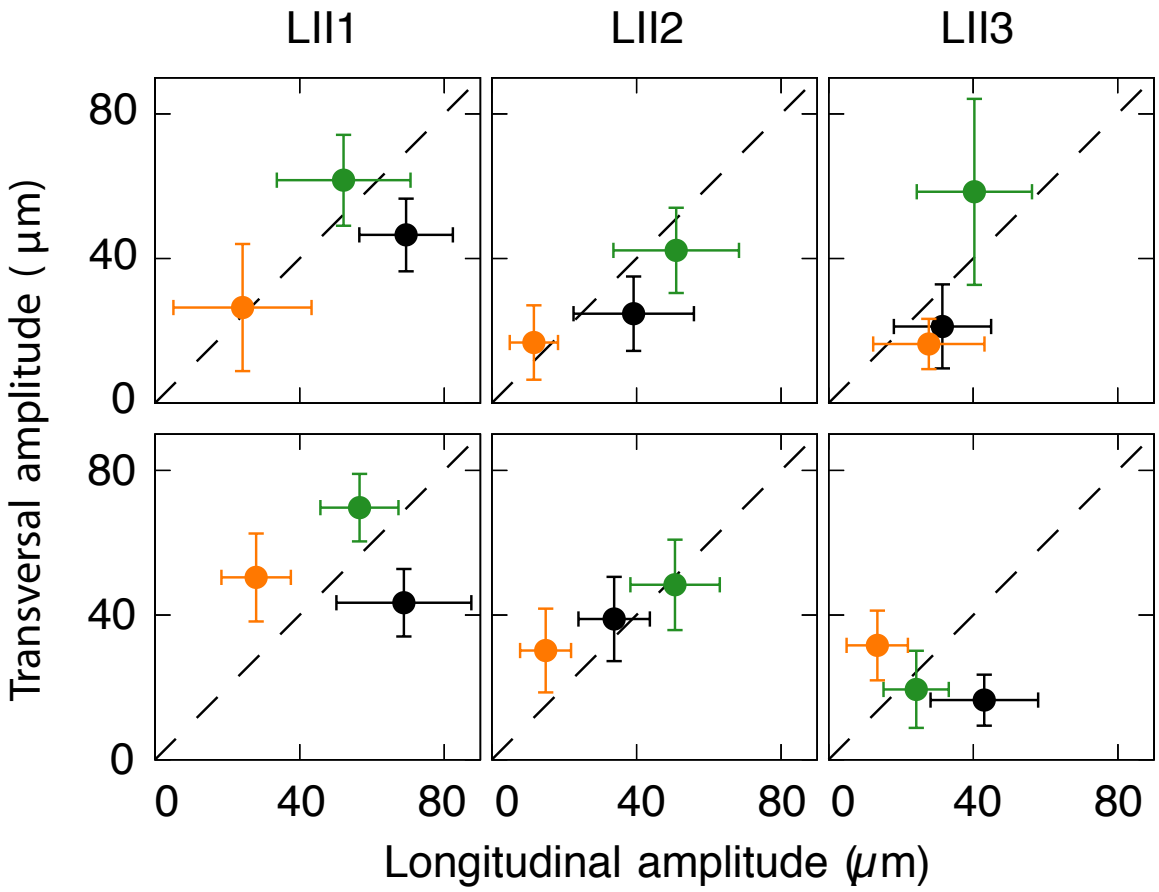


Figure 3.5 - Role of ridges in the hydrodynamics over the head
The natural ridges cause a difference in water movement at different points over a fish's head. The longitudinal amplitude is the vectorial component of the movement that lies along the axis of sensitivity of the neuromast; the transverse amplitude represents the component orthogonal to that axis. Water movements are analyzed lateral to the neuromast (green), over the neuromast (black), and medial to the neuromast (orange; as depicted in Figure 3.4). Water movements are largest lateral to and smallest medial to the neuromast. Directly above the neuromast the longitudinal amplitude exceeds the transverse amplitude, so the ridges direct water movement along the neuromast's inferred axis of sensitivity. The top panels are from fish 1 and the bottom panels from fish 2. The error bars in represent standard deviations.

3.2.C. *Hydrodynamic effect of a supplemental ridge*

We were able to affix to the head a supplemental plastic ridge of plastic similar in shape and dimension to the natural ridges. The supplemental ridge

was placed in the gap between the ridges lateral to neuromasts LII2 and LII3 (Figure 3.6).

The addition of a supplemental ridge had a noticeable hydrodynamic effect (Figure 3.7). The additional ridge consistently changed the water movement; removal of the ridge always produced at least a partial recovery. We observed that sometimes the ridge seemed to largely block water movements over the head in response to stimuli, as seen in fish 2 (Figure 3.7), but in other observations it seemed to change the nature of water movements, as seen in fish 1 (Figure 3.7).

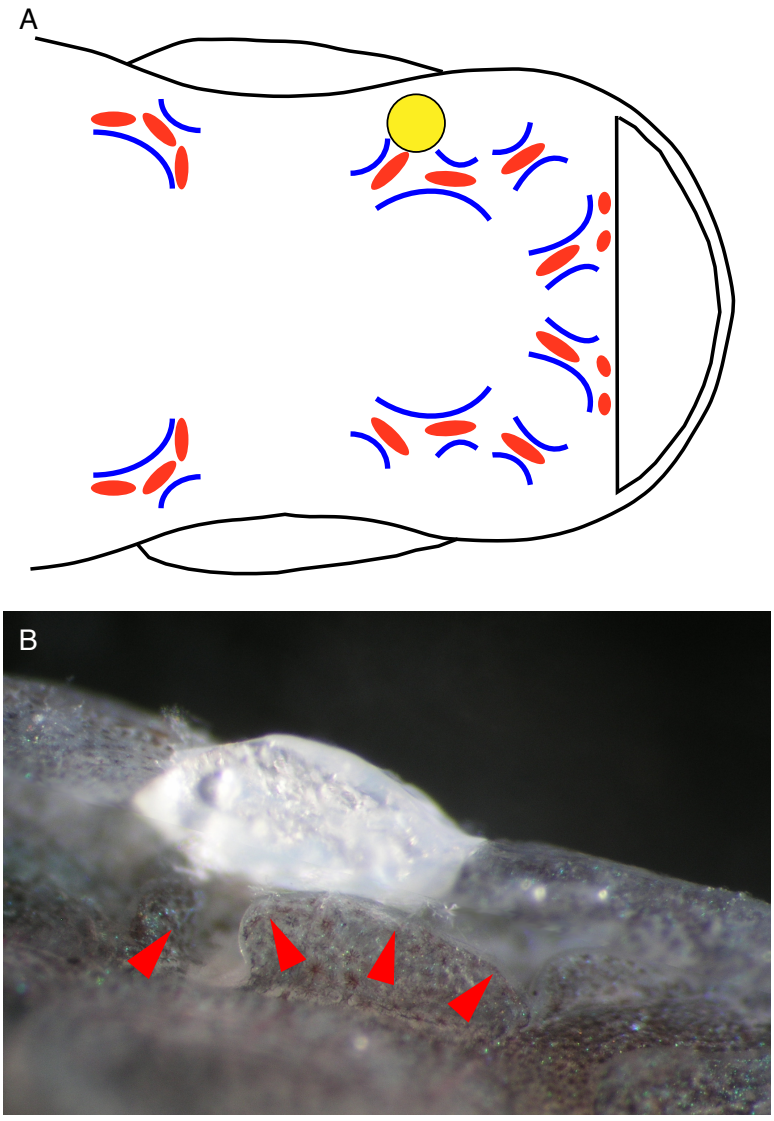


Figure 3.6 - Supplemental ridge
A. This schematic diagram illustrates the placement of a supplemental ridge (yellow circle) lateral to neuromast LII3.
B. A supplemental ridge is formed by a mass of acrylic cement of dimensions similar to those of natural ridges (arrowheads). The scale bar represents 200 μm.

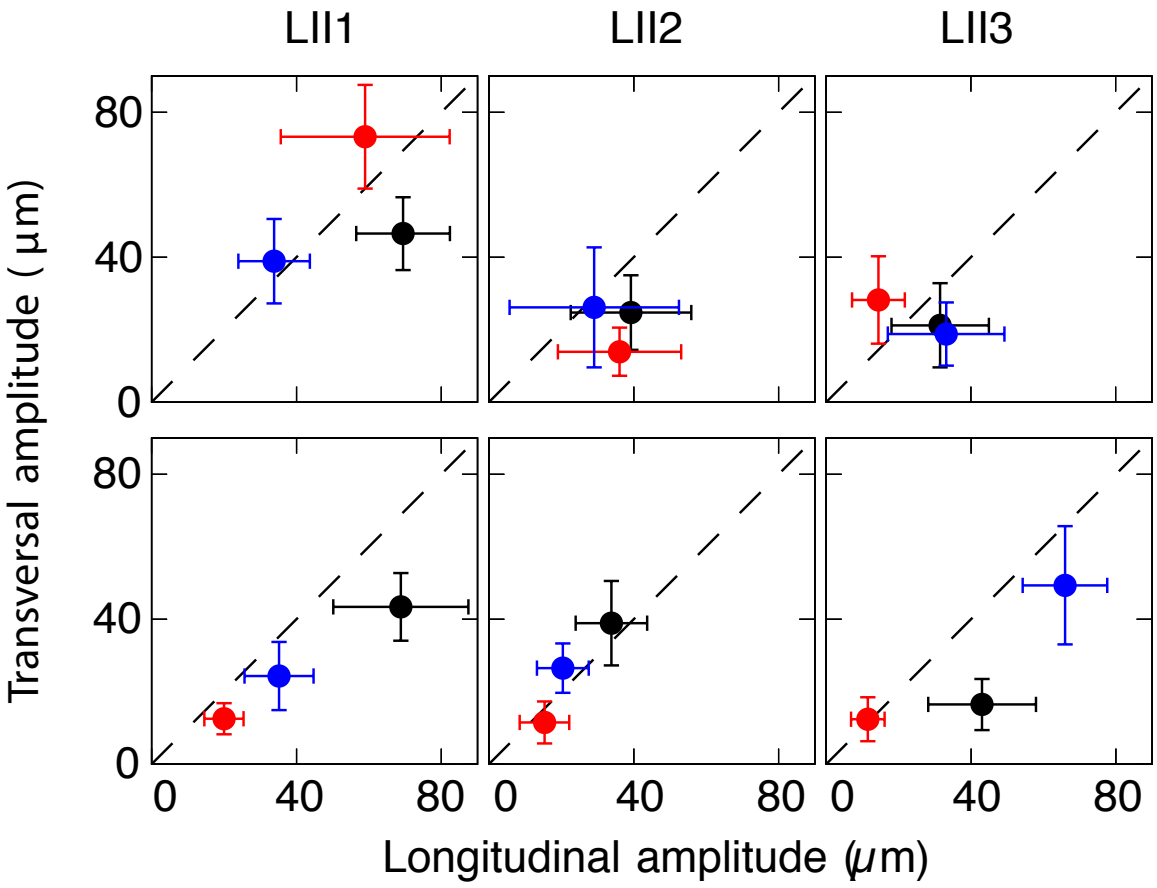


Figure 3.7 - Effect of a supplemental ridge on hydrodynamics over the head
Adding a supplemental ridge changes the water movements measured directly over the identical neuromasts. The control experiments are shown in black, the trials with the supplemental ridge in red, and the recovery data following removal of the ridge in blue. The top panels are from fish 1 and the bottom panels from fish 2. The error bars represent standard deviations.

3.3 Significance

We were able to observe the waveforms of the various stimuli we employed using a heterodyne interferometer (Figure 3.3). The click stimulus demonstrates the principle of dispersion, with high-frequency waves at the head of the wave packet and low-frequency waves at the tail. Dropping the cricket onto the water surface created a waveform very much like that of the click stimulus, although with a larger amplitude. The struggling of the cricket

produces an erratic waveform with substantial energy at high frequencies exceeding 50 Hz. Surface-feeding animals are thought to use high-frequency waves as indicators that the waves are of biologic origin (Lang 1980). We were also able to confirm the fidelity of the waveforms produced by our air-puff stimulation system, which produced a faithful sinusoidal waveform at the driving frequency.

By tracking particles over the surface of the head, we were able to estimate the vectors of water movement in response to sinusoidal surface wave stimulation. We were interested in any effect produced by the ridges bordering the neuromasts. The movement of the water was greatest lateral to the neuromasts, after the waves reached the fish's head but before reaching the ridges. Medial to the neuromasts, in an area largely blocked by ridges, we saw a dramatic attenuation of water motion. Over the neuromasts themselves, we saw an intermediate amplitude of water motion. However, the water motion was skewed in favor of displacements parallel to the neuromast axis of sensitivity rather than motions orthogonal to that axis. This suggests that the ridges act to channel water motion to maximize the neuromast sensitivity to incoming waves. The dramatic attenuation in the medial region demonstrates that the ridges are capable of blocking the translation of waves, further implying that they could have a role in limiting the sensitivity of a neuromast to waves from certain directions. Overall, the ridges seemed to have an important role in modulating the motion of water across the head of *Aplocheilus*.

The addition of a supplemental ridge had effects on the flow of water over the neuromast. For some neuromasts it seemed to mostly block the flow of water. For others it changed the ratio of transverse to longitudinal water movements. In all cases it had an effect on the water movements, corroborating the hydrodynamic role of the ridges in general.

We explored the function of the cephalic ridges surrounding the neuromasts of *Aplocheilus*. The ridges might offer protection to the neuromasts. They could also be used to detect the water depth, although we did not see any nerve fibers within them, casting doubt on this hypothesis as they are presumably unable to detect deformations in their shape (Figure 2.10.A). Both their structure, which creates channels around each neuromast, and our observations, which demonstrated that the ridges affect water flows over the head, make it seem probable that they are involved in modulating the water movement around the neuromasts. Perhaps they act as antennae to structure the receptive fields of the neuromasts. To investigate this possibility, we began electrophysiological experiments on the cephalic lateral line.

4. Electrophysiology

It is essential to understand the sensitivity of the sensory organs if one wishes to gain insight into how they signal the central nervous system and, through it, drive behavior. The striking appearance of the cephalic lateral line of *Aplocheilus*, with its stereotyped array of 18 large neuromasts each surrounded by ridges, suggests that the individual neuromasts sample different parts of the sensory field on water surface. The behavioral effect seen in experiments where all neuromasts but one are ablated also suggests that individual neuromasts have unique receptive fields [Müller 1982]. Rather than turning to the source, the fish always turns to a particular angle that depends on which neuromast remains. Both the anatomy and the behavior suggest that the neuromasts have unique angular sensitivities on the water surface. We sought to determine the receptive fields of each neuromast.

An advantage of working with the superficial lateral line is the accessibility of the hair cells, which are directly exposed to the environment. This allowed us to place a recording electrode adjacent to a neuromast and to record the extracellular microphonic potential produced by the hundreds of hair cells responding in synchrony. The size of the cupulae limits our proximity to the neuromast to about 100 μm, but fortunately the microphonic potential is large enough that we can still record microphonic potentials in the microvolt range. In each neuromast, the two populations of hair cells of opposite polarities generate microphonic responses at twice the stimulation frequency, since deflections in one direction depolarize about half of the hair cells and deflections in the opposite direction depolarize the other half. The two populations do not merely cancel each other because the voltage responses to depolarizing deflections exceed those to hyperpolarizing deflections.

Before investigating the receptive fields of the neuromasts, we first characterized their basic response characteristics. Past behavioral experiments have demonstrated that *Aplocheilus* is sensitive to stimulation from tens to a few hundreds of cycles per second [Bleckmann 1981A]. Its wave-amplitude threshold for behavior reaches a minimum at 100 Hz of wave amplitudes less than 10 nm [Bleckmann 1981B]. The activity threshold of the anterior lateral-line nerve indicates a slightly lower sensitivity, around 50 nm at 100 Hz, but a similar spectral sensitivity, with a sharp ramp in sensitivity from 10 Hz to 100 Hz, followed by a very gradual drop in sensitivity as the frequency of stimulation increases above 100 Hz. To test this we stimulated the cupula directly using a piezoelectric transducer while electrophysiologically recording from the neuromast.

In addition to directly manipulating the cupula, we also stimulated the the neuromast with sinusoidal surface waves produced using an air-puffing stimulator. This allowed us to observe the neuromast's response to more natural stimuli and to investigate the effect of water depth on microphonic responses. It also enabled us to stimulate from different positions around the head of the fish to investigate the receptive fields of the neuromasts. By manipulating the topography of the surface of the head through the addition of a supplemental ridge, we were able to test the hypothesis that the bordering ridges are acting as sensory antennae by modulating the responses of the neuromasts.

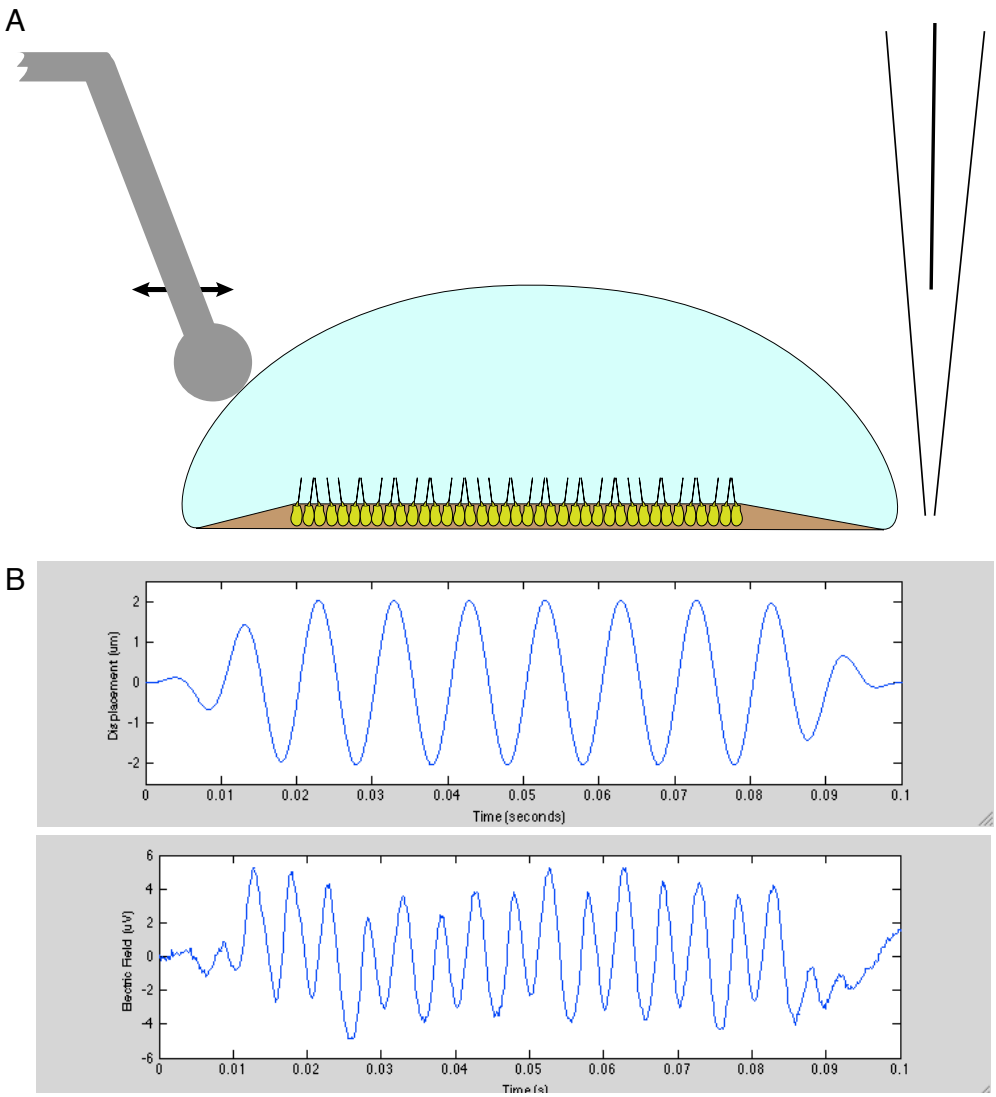


Figure 4.1 - Direct stimulation of the neuromast

A. A schematic representing the apparatus for stimulating the neuromast while recording microphonic potentials. The hair cells are in yellow and the cupula in blue. The longitudinal axis of the neuromast from left to right. The glass fiber on the left of the cupula is connected to a piezoelectric actuator, allowing us to mechanically deflect the cupula a few microns at frequencies up to 300 Hz. The recording pipette containing a silver/silver chloride electrode is at the right side of the cupula. Using it we record the microphonic potentials of the hair cells in response to deflections of the cupula.
B. Representative example of a direct stimulation microphonic recording. The upper trace is the command sent to the stimulator. The lower trace is the microphonic potential. Notice that the response is at twice the frequency of the stimulation due to the two populations of hair cells with opposite polarities.

4.1 Materials and methods

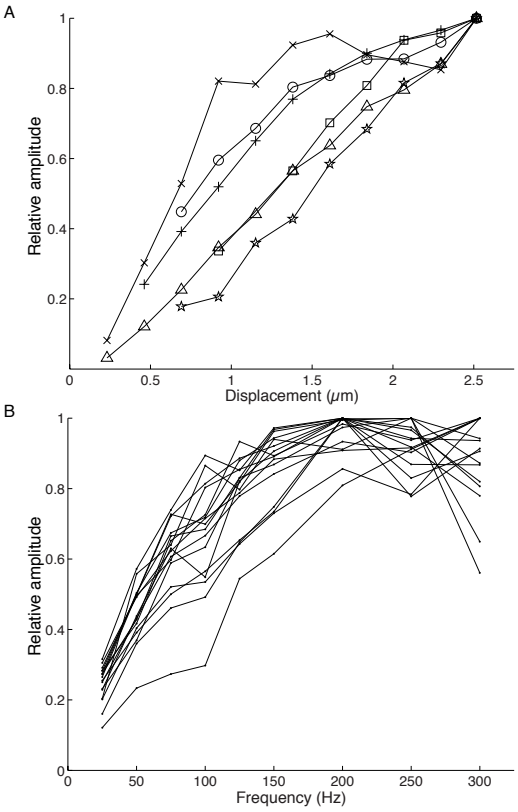
4.1.A. *Electrophysiological measurements*

Each fish was anesthetized in either 66 mM ethyl carbamate (urethane) or 670 μM tricaine (MS-222) in fish saline solution consisting of 116 mM NaCl, 2.9 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES at pH 7. The animal was secured as before (§3.1B), a glass micropipette of resistance 0.5 - 7 MΩ was placed about 00 μm lateral to a neuromast, and a ground electrode was situated in the bath. Using the recording pipette, the microphonic signal was detected (Axoclamp 2A, Axon Instruments), amplified (AM502, Tektronix, Inc.), and bandpass filtered between 10 Hz and 3 kHz. To block the transduction channels of the hair cells and abolish the microphonic response in control experiments, we added to the bathing solution 100 μM 3,5-diamino-6-chloro-N-(diaminomethylene)pyrazine-2-carboxamide (amiloride).

The cupula was either stimulated directly with a piezoelectric transducer or indirectly by surface waves. For direct stimulation, a high-voltage amplifier (Burleigh) was used to drive an open-loop piezoelectric stack (Burleigh) coupled to a glass fiber. Sinusoidally modulated sine wave trains were used to drive the amplifier. The glass fiber was placed against the cupula such that the translations of the fiber would deflect the cupula along the longitudinal axis of the neuromast. The cupula was slightly pre-loaded so that there would be net deflections in both directions. The movement of the piezoelectric transducer in response to specific voltage steps and sinusoidal voltage amplitudes of different frequencies was calibrated using a heterodyne interferometer (OFV501 and OFV3001, Polytec GmbH).

Indirect stimulation of the neuromasts was achieved using a air-puffing system as described in §3.1B. The system was secured to a computer-controlled

two-dimensional positioning system (Arrick Robotics) that was used to stimulate at different points on the surface of the water. Waveforms were monitored using a heterodyne interferometer whose laser beam was aimed 1 - 3 mm lateral to the fish's left eye. Experiments were controlled by a computer running LabView 8.5 (National Instruments). Microphonic and interferometric data were analyzed in Fourier space with programs written in Matlab (Mathworks).



4.1.B. *Ridge modifications*

The hydrodynamic environment of a fish's head was modified by the addition of ridges as described in §3.1.C. Successful experiments were

Figure 4.2 - Response under direct stimulation

A. The neuromasts are sensitive to displacements of less than one hundred nanometers up to a few micrometers. The responses are relatively linear over that range, although they may begin to saturate at the larger stimulations. Each line represents a different neuromast. There are no differences between the sensitivities of different neuromasts. This range of sensitivity is similar to that reported for behavioral responses.

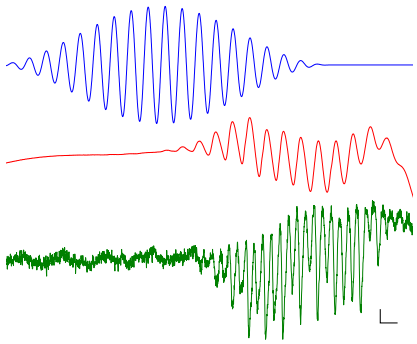
B. Neuromasts act as high-pass filters. Their sensitivity increases radically as the frequency rises from 25 Hz to about 100 Hz and then increases gradually up to about 200 Hz before beginning to fall gradually. Each line represents a different neuromast. The increased sensitivity at higher frequencies is unsurprising. Waves at those frequencies are more likely to be of biological origin [Lang 1980] and they also attenuate more rapidly with distance. There are no differences in the frequency sensitivities of different neuromasts.

performed on a total of 26 animals. Although it was also possible to remove an animal's normal ridges surgically, we found that damage to the nearby neuromasts precluded interpretation of the results.

4.2 Results

4.2.A. *Microphonic responses under direct stimulation*

Stimulating the cupula directly while recording from a neuromast allowed us to determine the response characteristics of neuromasts under well-controlled conditions (Figure 4.1). The results we obtained from the microphonic recordings were similar to those previously described from behavioral experiments and nerve recordings from *Aplocheilus*. Stimulating sinusoidally at 100 Hz, we saw responses for displacements commencing below 50 nm peak-to-peak stimuli and responding relatively linearly up to a few microns (Figure 4.2A). The response characteristics did not differ significantly between neuromasts.



We next stimulated at an equal amplitude of 2.6 µm peak-to-peak while systematically changing the frequency of stimulation and observed how the microphonic response changed with frequency (Figure 4.2B). The sensitivity of the

Figure 4.3 - Example of a response to indirect stimulation
The blue trace is the driving voltage sent to a air-puff stimulation system. The red trace is the displacement of the water surface in response to the stimulation. The green trace is the microphonic response to the surface waves. The stimulator drives a high-fidelity waveform that produces a clear microphonic response with twice the frequency of the wave. The horizontal calibration bar represent 7 ms. The vertical calibration bar represents 1 V for the driving voltage, 2 µm for the waveform displacement, and 10 µV for the microphonic response.

neuromast increases rapidly from 25 Hz to about 100 Hz, then increases gradually to around 200 Hz before beginning to fall. The behavior does not differ between neuromasts. Overall, our observations are very similar to the previously described nerve responsive of *Aplocheilus* [Bleckmann 1981B]. Although the *Aplocheilus* lateral line appears to have slightly reduced sensitivity to low-frequency stimuli relative to that of other fish [Montgomery 1992], overall its spectral sensitivity is within the conventional range for fish lateral lines [Weeg 2002].

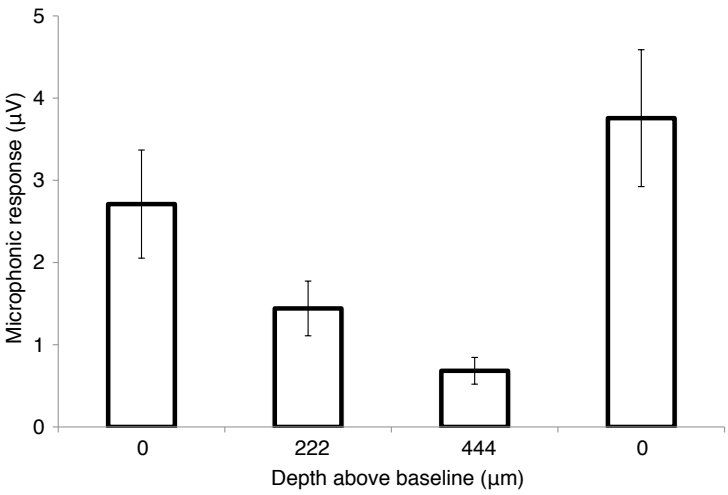


Figure 4.4 - Effect of water depth on microphonic response
Increasing the depth of water the fish's head above the baseline depth of about 200 μm reduces the amplitude of the microphonic response. This is likely due to the attenuation of the waveform amplitude with depth (see Figure 3.2). The wavelength of the 150 Hz wave used for stimulation is about 435 μm. The reduction in the amplitude of the microphonic response appears to be less dramatic than we would expect given the predicted attenuation of the waveform amplitude.

4.2.B. *Microphonic responses under indirect stimulation*

Next we developed a system for producing waves on the surface of the water. It was based on a system previously described by Bleckmann and colleagues [1981A] modified to send air puffs onto the surface of the water. By monitoring the displacement of the water surface with an interferometer we confirmed that this procedure faithfully produced sinusoidal

surface waves of the proper frequency. These waves were able to drive robust microphonic responses (Figure 4.3).

We utilized the indirect stimulation system to examine the effect on the microphonic response of increasing the water depth over the neuromasts. We found that the responses rapidly attenuated either when the water became too shallow or when it became too deep Figure 4.4). It is likely that the response disappeared when the water became too shallow because the surface waves were no longer able to reach the neuromasts. The attenuation as the water depth increased can be explained by the shallow penetration depth. Capillary waves attenuate exponentially with depth and are generally negligible by a depth of one wavelength, which, for the 150 Hz stimuli used for this experiment, equates to about 435 μm. The microphonic responses do not seem to attenuate quite as rapidly as we would expect given the increase in depth, perhaps suggesting that the hair cell responses are non-linear, as has been described in other systems [Hudspeth 2008].

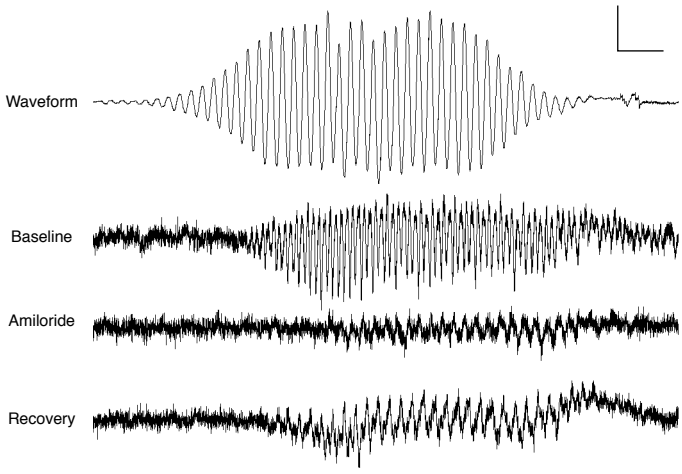


Figure 4.5 - Amiloride control
Amiloride, a drug that blocks the mechanotransduction channels of hair cells, should abolish any hair-cell based potentials. The addition of 100 μM amiloride eliminates most of the microphonic response. Washout of the amiloride leads to a partial recovery. The horizontal calibration bar represents about 33 ms. Recorded form neuromast LII3. The vertical calibration bar represents 2 μm for the waveform trace and 10 μV for the microphonic responses.

As a control, we added amiloride to the bathing solution. Amiloride blocks the transduction channel of hair cells and would abolish any hair-cell produced potentials. The addition of amiloride reversibly abolished the microphonic response (Figure 4.5).

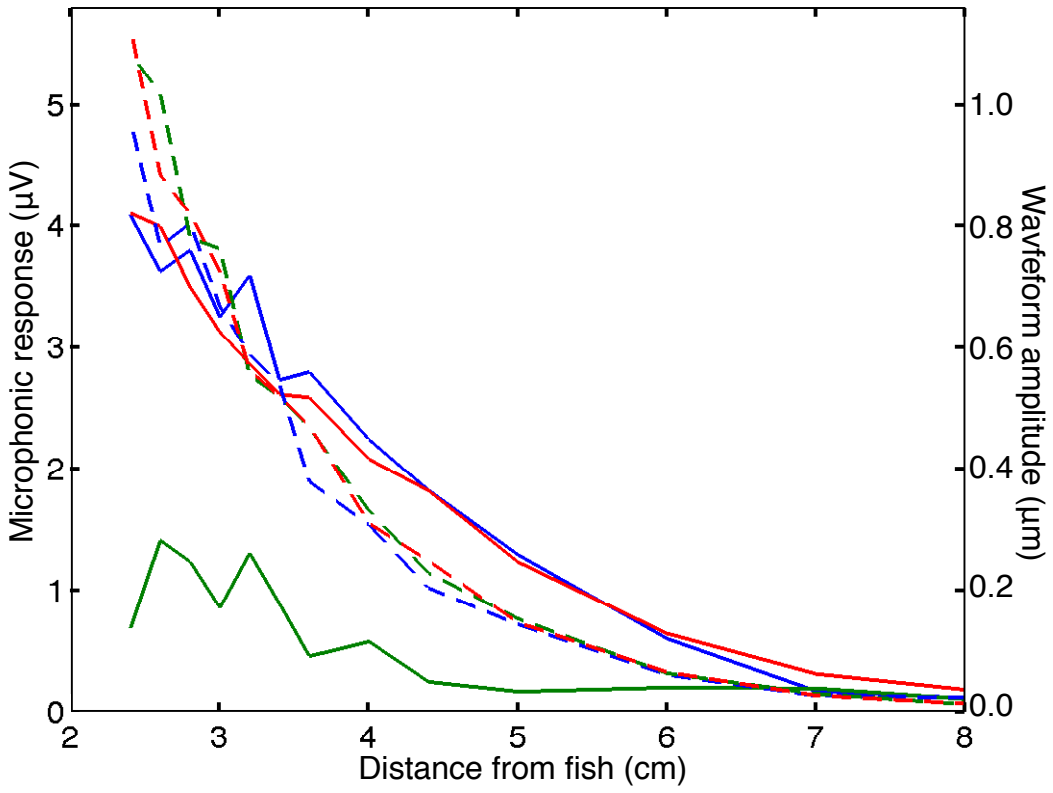


Figure 4.6 - Distance response
As the stimulus move further from the fish, the amplitude of the waves reaching the animal declines. The microphonic responses, shown as solid traces, also decline. The dashed traces are the waveform amplitudes. The three colors represent tests at three different angles: 10° to the left of the fish in red, 60° to the left of the fish in green, and 110° to the left of the fish in blue. The fish has a low sensitivity to waves from 60° to its left.

4.2.C. *Neuromast receptive fields: effect of distance*

Mounting the stimulation system on a two-dimensional positioning system allowed us to precisely and repeatedly change the position of the wave source. Using this system we were able to stimulate each neuromast from an array of different points on the water surface. By mapping how the sensitivity of a neuromast changes over the surface of the water, we defined the receptive field of each neuromast.

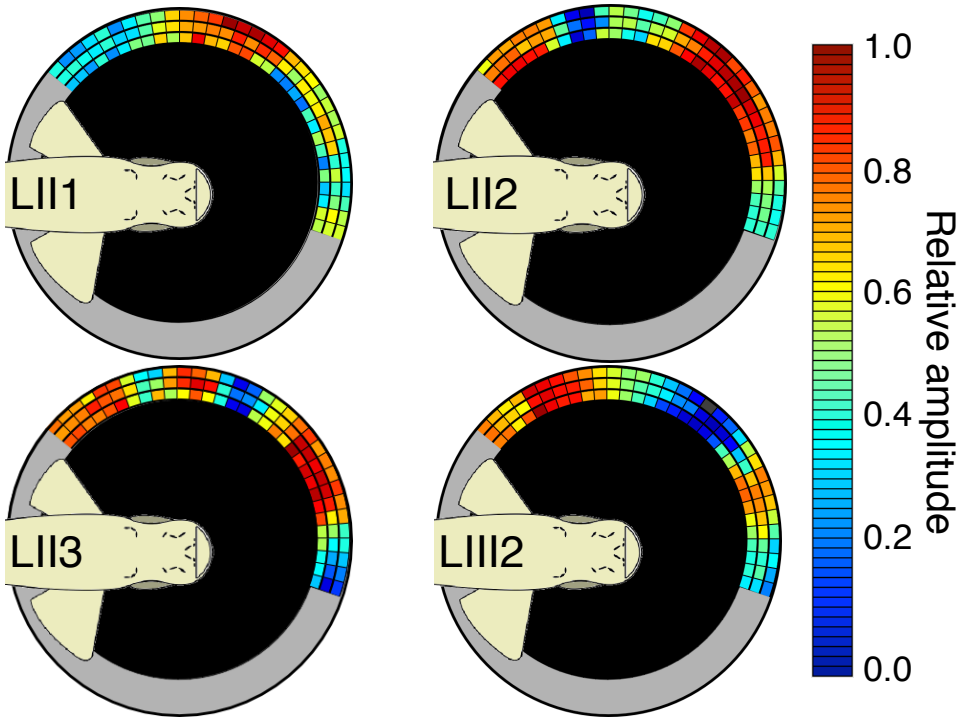


Figure 4.7 - Neuromast receptive fields
The spatial sensitivity of an individual neuromast was determined by recording microphonic potentials while stereotyped 150 Hz waves were generated at different points on three arcs around the head. Each neuromast has a unique receptive field, here portrayed to scale around diagrams of a fish's head. Red indicates a maximal response and blue a minimal response; grey areas were not sampled.

Systematically increasing the distance between the fish and the wave source while holding the angle between the fish and the source constant led to a relatively simple attenuation in the response of the neuromast (Figure 4.6). We

recorded the waves' amplitude at the head of the fish, allowing us to compare the attenuation of the waves with the attenuation of the response to those waves. Somewhat surprisingly, we observed that the microphonic responses attenuate less rapidly than do the waves. The wave-packet amplitude should attenuate exponentially as a function of the wavelength and the distance

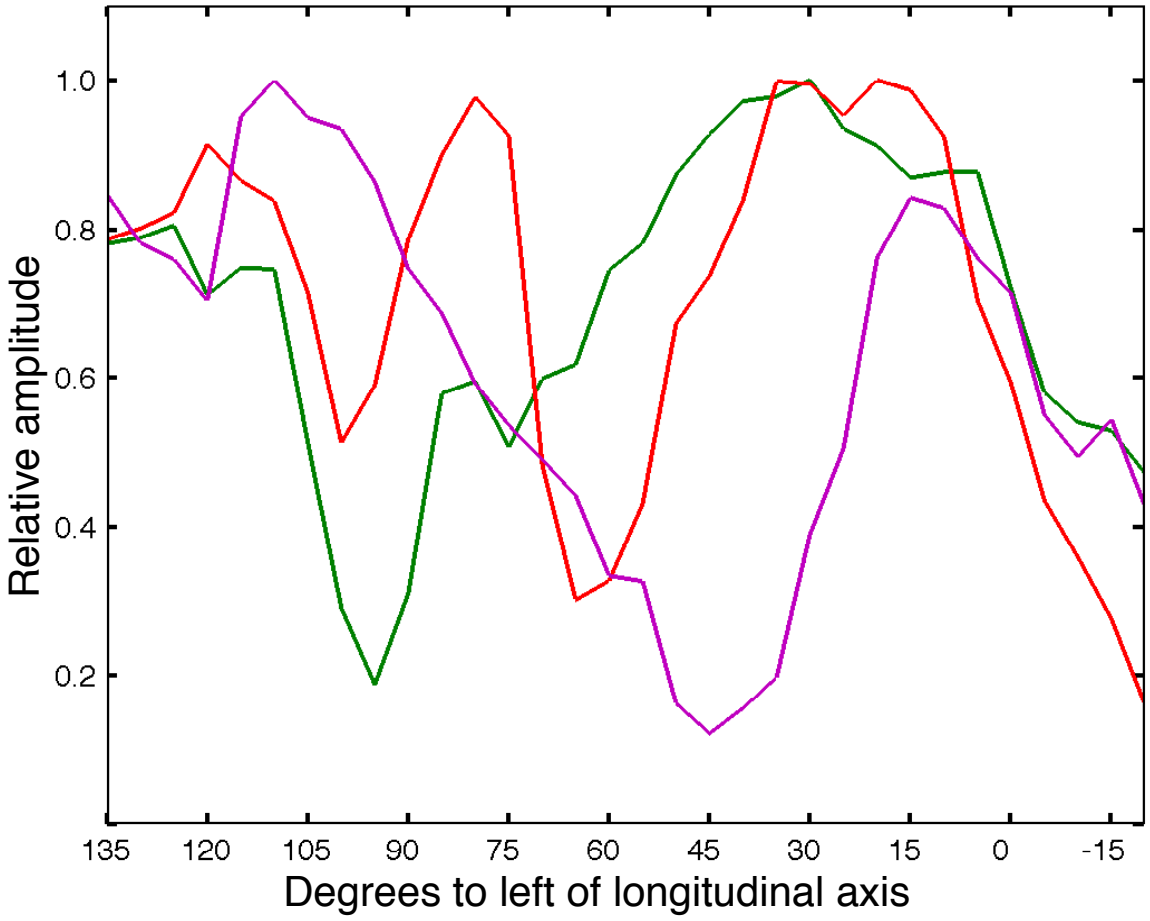


Figure 4.8 - Histograms of neuromast receptive fields
This is a different representation of the data from Figure 4.7 to accentuate the differences between the receptive fields of adjacent neuromasts: 0° is straight ahead, positive angles lie to the left of the fish, and negative angles occur to its right. The green trace is from neuromast LII2, the red from LII3, and the purple from LIII2. Not only are the peaks in different places, which would be expected given the differences in the orientations of those neuromasts, but the sharpness of the angular modulations are different, suggesting a role for the ridges in shaping the receptive fields.

traveled. As recorded by the interferometer, we observed that the waves' amplitudes do indeed attenuate exponentially (Figure 4.6). However, the amplitudes of the microphonic response to those waves attenuate more slowly. This observation that the hair-cell responses are non-linear suggests that the hair cells display compressive non-linearity, a hallmark of hair-cell amplification [Hudspeth 2008].

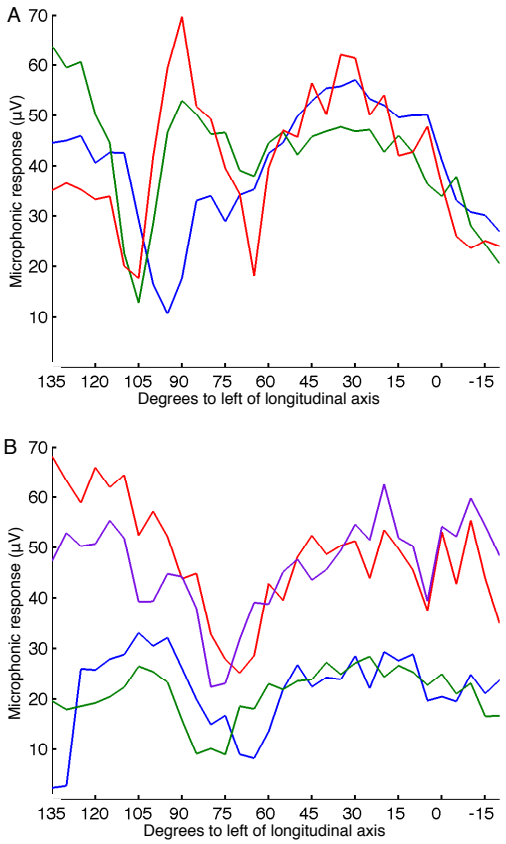


Figure 4.9 - Stability of receptive fields over time
A. The microphonic receptive field of neuromast LII2 from a single fish reveals some variation on three different days, probably owing to slight changes in the mounting angle of the fish. Nevertheless, the shape of the field is stereotyped.
B. Similar records represent neuromast LII3 from a single fish on four different days.

4.2.D. *Neuromast receptive fields: effect of angle*

We also recorded microphonic potentials from the neuromasts while systematically changing the angle between the longitudinal axis of the fish and the stimulus location, keeping the distance constant. This allowed us to determine the sensitivity of each neuromast as a function of source angle. We found that each neuromast has a unique angular receptive field, hereafter referred to as its receptive field, which may be relatively simple or may include multiple lobes of enhanced and diminished sensitivity (Figure 4.7). The receptive fields of

different neuromasts overlap, but also differ noticeably from each other (Figure 4.8).

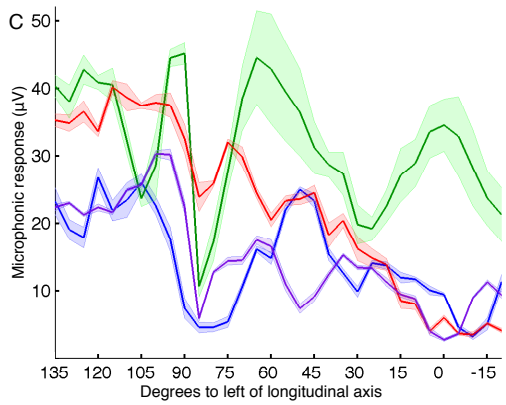


Figure 4.10 - Receptive field of neuromast LII2 in different fish
The receptive fields for neuromast LII2 from four different fish display some differences, but the general features most conspicuously the reduced sensitivity between 75° and 90° are maintained between animals. Each record, the average of five determinations, is bracketed by its standard error.

As expected if the receptive fields are essential for an animal's detection of targets, the receptive fields of individual fish are stable over time (Figure 4.9). There is some variation from day to day, but this is probably related to the fact that the fish's mounting position and angle likely differ slightly between experiments.

The receptive fields of the corresponding neuromasts in multiple fish differ in some specific characteristics, but maintain the same general features (Figure 4.10). For example, neuromast LII2 consistently displays an area of reduced sensitivity directly to the left of a fish, but the size of this notch varies between animals. This variability accords with the observation that, although the anatomy of the head is stereotyped across fish, there is individual variability in the exact structure. Developmental plasticity may permit a juvenile fish to learn the particular receptive fields of its own neuromasts.

The response of an isolated hair cell varies as the cosine of the angle between the direction of stimulation and the cell's axis of sensitivity [Shotwell et al. 1981]. The receptive fields display sharper angular sensitivity than expected from a simple cosine relation (Figure 4.11). The receptive fields also show changes in angular sensitivity that cannot be explained by angular

changes in the waves' amplitude, which tends to change gradually with angle (Figure 4.11). Neither the cosine modulation nor changes in the waveform amplitude can explain the sharp changes in angular sensitivity of the neuromasts, suggesting that something else is responsible for that phenomenon.

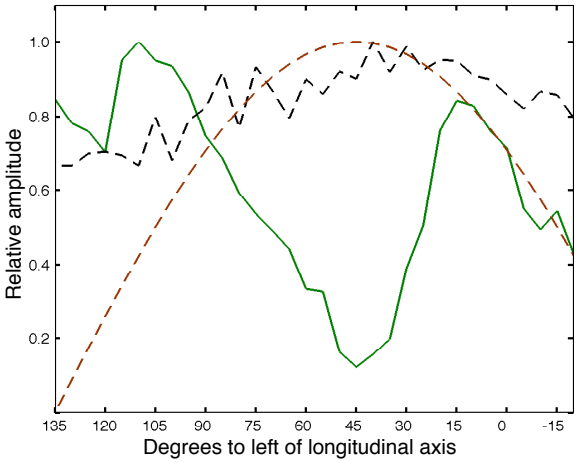


Figure 4.11 - Comparison of receptive field with waveform and cosine modulation
Not only do different neuromasts have distinct receptive fields, but these fields are modulated over smaller angles than either the amplitude of water movement (dotted black line) or a cosine function (dotted brown line). The receptive field of neuromast LIII2 is shown in green.

4.2.E. *Effect of a supplemental ridge*

We hypothesized that the sharp angular sensitivity seen in the receptive fields is generated by the ridges bordering the neuromast. To test this, we removed the ridges and attempted to observe an effect on the neuromast receptive fields. Unfortunately, despite trying multiple techniques, we were unable to remove the ridges without destroying the adjacent neuromast due to their proximity to each other (Figure 2.10). Instead we placed plastic supplemental ridges in the region between the ridges lateral to neuromasts LII2 and LII3. The supplemental ridges were similar in dimension and shape to the natural ridges, albeit more hemispherical and less flange-like (Figure 3.6).

The result of the modification was striking. Adding such a ridge reversibly altered the receptive fields of the adjacent neuromasts (Figure 4.12). Not only the magnitude of the response was affected: in many instances the

pattern of angular responsiveness changed dramatically. The addition of a supplemental ridge abolished the characteristic notch in sensitivity of neuromast LII2 to the left of the fish, strongly suggesting that the notch is produced by the ridge lateral to the neuromast (Figure 4.12A). The rostral portion of the receptive field was minimally affected, which is not surprising given that the

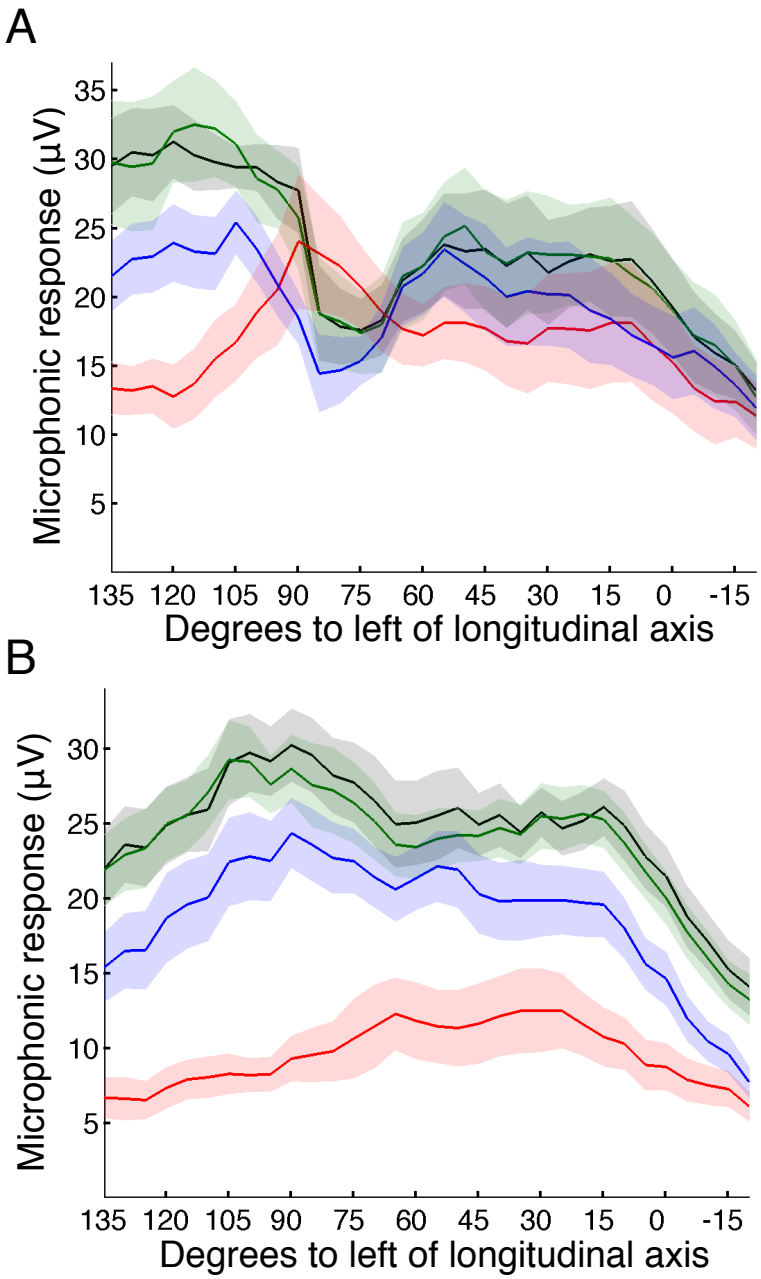


Figure 4.12 - Effect of the addition of a supplemental ridge

A. Adding a supplemental ridge lateral to neuromast LII3 alters the responsiveness of neuromast LII2. The black trace represents the control recording of the receptive field, the green trace the response after a sham operation, the red trace the effect of an added ridge, and the blue trace the recovery after removal of the artificial ridge. Note the effect of ridge addition on the characteristic notch in sensitivity at 60° - 90°. The shaded regions represent standard errors of the means for 12 animals.

B. The effect of the same added ridge on neuromast LII3 in 14 animals is most significant at the caudal end of the receptive field, as would be expected from the position of the supplemental ridge.

placement of the ridge should mostly affect waves reaching the neuromast from the caudal region. The effect of adding a ridge on neuromast LII3 was largely an attenuation of the sensitivity overall (Figure 4.12B. The attenuation was more severe in the caudal part of the field. Our hydrodynamic observations suggest that, whereas neuromast LII2 receives waves from both the anterior and posterior, neuromast LII3 mostly receives waves from its anterior side, which is where the supplemental ridge was placed. The ridge appears to have dramatically attenuated the waves reaching the neuromast. The large alteration of the receptive fields in response to a structure similar to the natural ridges suggests that the ridges themselves have a significant effect on water movement.

4.3. Significance

Although the response characteristics of the neuromasts are unremarkable, being similar to those of other fish and amphibian hair cells, they show remarkably complex receptive fields. These receptive fields agree qualitatively with the results of Müller in his neuromast ablation experiments [1981]. In those experiments, fish with only a single neuromast remaining would display an angular response that was independent of the target angle. The angle of this response depended on which neuromast remained. Those angular responses overlap well with the receptive fields we found. If neuromast LII1 was the only remaining neuromast, the fish's responses fell between 0° and 55° to the fish's left, which overlaps with the receptive fields we found for neuromast LII1, which tended to fall between about 20° and about 70° to the fish's left.

The structure of the receptive fields cannot be explained by the changes in the amplitude of the waves or by the angular sensitivity of the hair cells themselves. Adding a supplemental ridge to the head of the fish, however, can

change the structure of the receptive fields. This suggests that the natural ridges are involved in generating the form of the receptive fields, particularly the sharp changes in sensitivity that correspond with changes in the stimulation angle. The ridges appear to act as sensory antennae. They might focus wave energy from particular directions onto specific neuromasts and block energy from other directions, for example, thereby sharpening the spatial sensitivity of individual neuromasts. To gain further insight into the function of the ridges, and to understand the role of the receptive fields in the fish's behavioral response, we went on to investigate the effect of adding supplemental ridges on the fish's avisual prey-localization ability.

5. Behavior

Aplocheilus can hunt prey on the water surface in complete darkness using the mechanosensory lateral line atop its head. Each of the 18 sensory neuromasts in this cephalic array has a particular receptive field, the characteristics of which are defined to a significant degree by the fleshy ridges bordering them. Our hypothesis is that the ridges act as a sensory antenna by modulating the angular sensitivity of the neuromast they surround. The most penetrating test of our hypothesis is to examine the behavioral effect of modifications to the ridge structures.

Testing our hypothesis behaviorally assumes that the fish not only utilizes the information provided by the receptive fields for target localization, but that it depends on that information. The evidence that the fish are utilizing the receptive field information for target localization comes from experiments in which all neuromasts except one are removed. Under those conditions the fish no longer orients toward a target stimulus but rather turns to a particular angle. Because the turning angle depends on which neuromast remains the fish evidently assumes that a stimulus that predominantly stimulates that neuromast must originate at a particular angle. This is analogous to the use of interaural level differences (ILDs) by terrestrial vertebrates for sound localization. Birds and mammals use ILDs for sound localization on the basis that the diffraction of sound by the head and structures on the head depends on where the sound is coming from relative to the head. This produces an asymmetry in the sound level detected between the ears that the animal can use to determine the direction toward the sound source. [Köppl 2009]. Many vertebrates also use interaural time differences (ITD's) for sound localization. The use of ITD's for sound localization works on the principle that if a sound is directly in front of

the head it will arrive at both ears synchronously. However, if the sound is to one side, the sound will arrive at one ear before the other. The animal can measure this delay and use to to determine the angle toward the sound source. Given the speed of sound in air (about 340 $cm \cdot s^{-1}$), complex circuits are required to measure the microsecond delays in sound arrival between the ears [Köppl 2009]. *Aplocheilus* could use an analogous system by measuring the wave arrival times at different neuromasts and using the delays between neuromasts to determine the direction of the source. Given the slow propagation speeds of capillary surface waves, from around 36 $cm \cdot s^{-1}$ to 48 $cm \cdot s^{-1}$ at the relevant frequencies and the spacing between neuromasts, at least 200 μm, measuring the delay in activity between neuromasts would be hundreds of microseconds, which would not require complex circuitry to resolve as is necessary in many ITD systems [Köppl 2008]. Many higher vertebrates utilize both ITD and ILD information for sound localization and it seems possible that *Aplocheilus* use both cues as well.

We attempted to test the hypothesis that the ridges, by altering the receptive fields of the cephalic neuromasts, affect the fish's prey-localization ability. To address the hypothesis we developed a system for testing the fish's behavior. We found that the fish displayed no visual responses under infrared light. Rather than blinding the fish, we performed all experiments under infrared light, hereafter referred to as 'in darkness'. Most naïve fish, which have been fed in the light for their entire lives, readily hunt in darkness and over the course of a few days improve their performance. Past experiments have used the fish's angular response, distance response, and reaction time as measures of behavioral performance [Schwartz 1965, Schwartz 1971, Bleckmann 1981A, Bleckmann 1981B, Bleckmann 1981C, Bleckmann 1982, Müller 1982, Kaus 1987, Vogel 1987]. We extracted the same performance information using a fish-

tracking program. After a few days of testing, we tested our hypothesis by examining the effect of modifying the fish's head by adding a supplemental ridge.

5.1. Materials and methods

5.1.A. *Behavioral experiments*

Fish were trained to localize floating food pellets about 1 mm in diameter dropped onto the water's surface under infrared light. Behavioral experiments were then conducted by observers wearing light-amplifying goggles (Night Optics D2MV). Because the fish lacked their ordinary avoidance response to looming visual stimuli, they were evidently unable to see under these conditions. The addition of 500 μM amiloride to the tank, which blocks the transduction channels of hair cells, abolished behavioral responses. Videos of the fish behavior were recorded at 20 frames per second using a camera from Redlake Digital Imaging Systems.

5.1.B. *Analysis of behavioral experiments*

The videos were processed by subtracting the background, thresholding the resulting images, and tracking the fish's position and orientation with software written in Matlab. The positions of pellet targets were entered manually. Distances were calculated between the center of the fish's head and the pellet location and angles were calculated between the longitudinal axis of the fish and a line connecting the center of the head and the pellet.

5.1.C. *Ridge modifications*

The hydrodynamic environment of a fish's head was modified as described in §3.1.C. Successful experiments were performed on a total of 29 animals.

5.1.D. *Neuromast ablations*

Neuromasts were ablated using a cauterizing iron. Fish were anesthetized as above and under a dissecting microscope the hot filament was applied to individual neuromasts. Ablations were confirmed using 4-Di-2-Asp labeling (§2.1.B)

5.2. Results

Unconditioned *Aplocheilus* were stimulated in darkness with food pellets dropped individually onto the water's surface at a

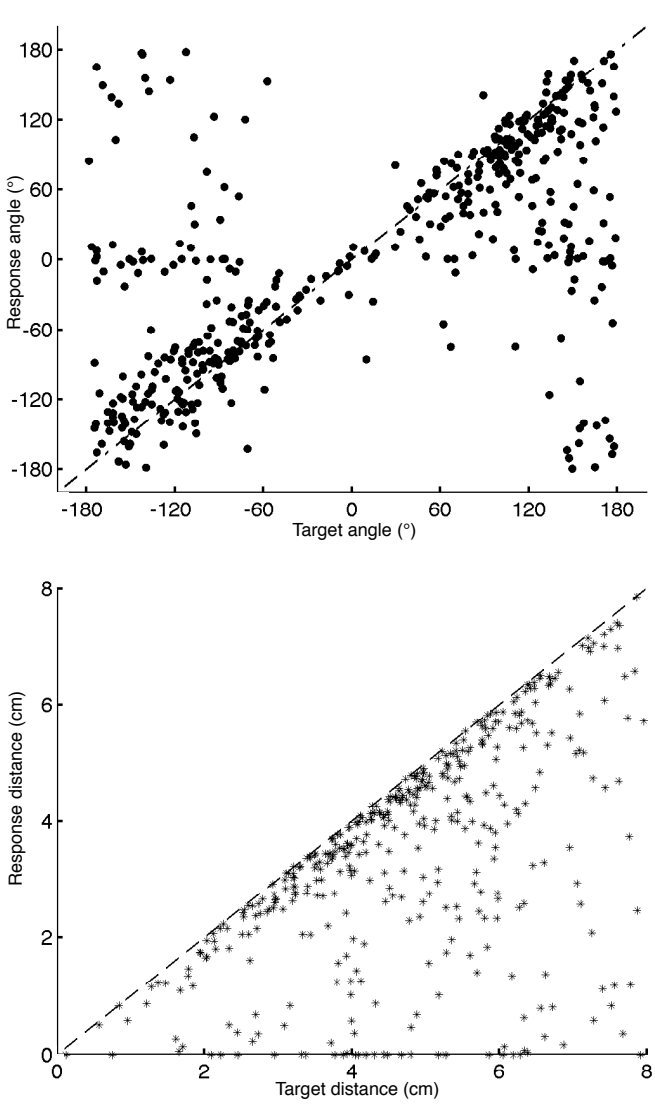
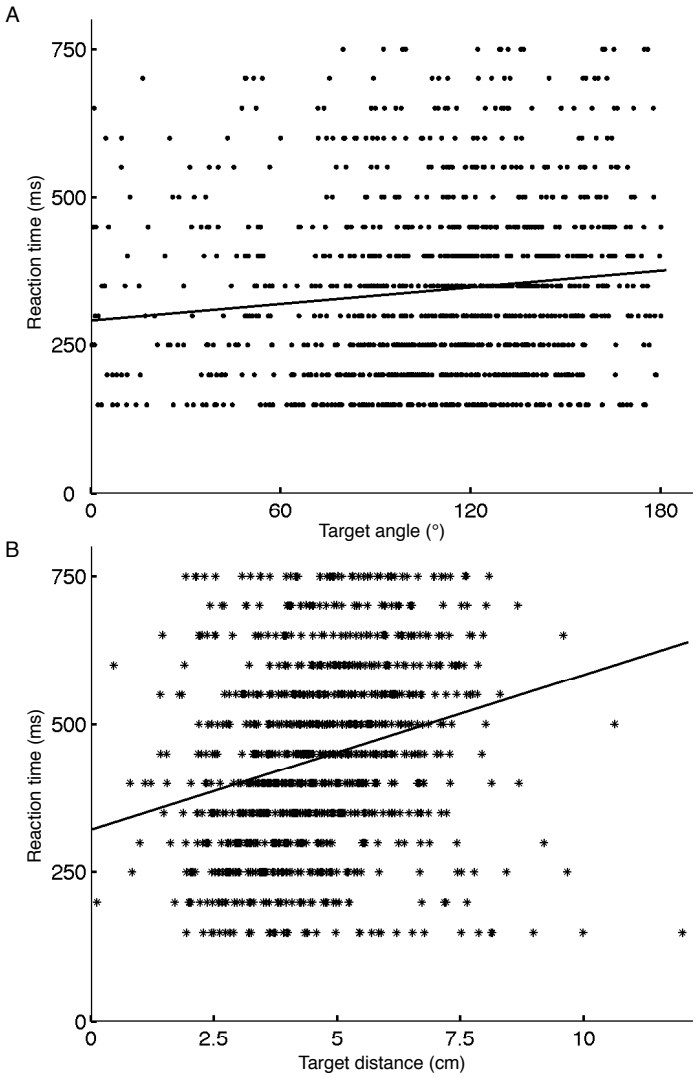
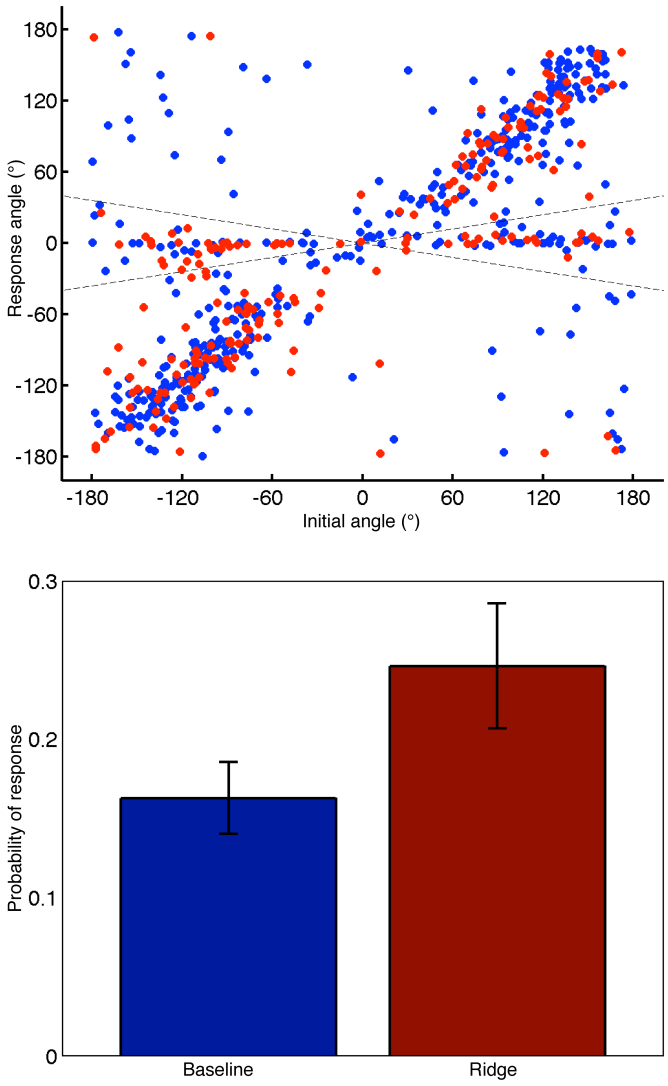


Figure 5.1 - Fish behavioral response
A. In darkness, the fish reliably turned to the target angle when a pellet was dropped on the water surface. Accurate responses are along the dotted line (Y=X). On some trials the fish did not respond. These failed responses are clustered along Y=0. Occasionally the fish turned in the wrong direction. These responses are in quadrants 2 and 4. Positive angles represent targets to the left and negative angles targets to the right of the fish.
B. The fish also reliably moved to the target distance. Again, failed responses can be seen along Y=0.



variety of angles with respect to the animal's initial orientation. The fish responded by rotation to the heading of the target pellet, followed by a relatively straight-line movement toward the target. Although a subset of fish were unable or unwilling to do so, the majority quickly became accustomed to the behavior despite previously being fed only in the light. Fish accurately determined both the distance and angle at

Figure 5.2 - Reaction time as a function of target angle and distance
A. In darkness, the fish's reaction time scaled linearly with the absolute value of the target angle ($p < 0.001$). For every 1° increase in angle, the reaction time increased by 0.46 ms. The vertical offset is 292 ms, which is higher than previously reported [Bleckmann 1981C]. In those experiments, however, minimal reaction times were investigated. We eliminated reactions below 100 ms from this analysis, which were likely to be trials where the fish was moving when the target was presented, and above 750 ms, which were clearly slower than the fish's capacity.
B. The fish's reaction time also scaled linearly with the target distance ($p < 0.001$). For each centimeter increase in target distance, the reaction time increased by 26 milliseconds. This suggests that the fish is responding with an invariant delay after the arrival of a wave traveling 38.24 $cm \cdot s^{-1}$, which indicates a capillary wave with a frequency of 120 Hz.



which a target pellet landed (Figure 5.1. The reaction time scaled with both distance and angle. It is unsurprising that the reaction time scaled with distance, as the waves have a limited speed, ranging from 36 cm·s^{-1} at 100 Hz to 48 cm·s^{-1} at 250 Hz. The rate of scaling with distance suggests that the reaction occurs after an invariant delay following the arrival of waves traveling 38.24 cm·s^{-1}, or about 120 Hz waves (Figure 5.2). The addition of a

Figure 5.3 - Behavioral impact of a supplemental ridge
A. Fish that have had the neuromasts of groups I and III ablated are still able to accurately estimate target angles (blue). The addition of a supplemental ridge lateral to neuromasts LII2 and LII3 does not have a noticeable effect on the fish's ability to estimate angle (red). However, there is a larger proportion of failed trials when a supplemental ridge is present. We defined failed trials as trials where the fish's response angle is less than 20% of the target angle as indicated by the dashed lines. **B**. The probability of failing to respond to a target stimulus is higher when a ridge is present. The error bars represent 95% confidence intervals calculated using bootstrapping. We randomly sampled 70% of the trials and found the proportion of failures 1 million times and used this to calculate the standard deviation.

supplemental ridge had no affect on the behavioral responses of these fish. However, if we bilaterally removed the neuromasts of groups I and III and then added a ridge (Figure 3.6), we did see an effect on the behavioral responses. There was a transient drop in performance when the neuromasts of group I and III were removed, but the fish recovered rapidly. When a supplemental ridge was subsequently added, the fish's performance dropped markedly (Figure 5.3). The specific effects we saw were a decreased probability of response and an increased reaction time. After adding a ridge probability of failure went from 0.16 to 0.25. The mean reaction time went from 444 milliseconds to 528 milliseconds, a statistically significant difference ($p = 0.0376$). We did not see a significant defect in either the fish's estimate of angle or of distance. Perhaps the addition of a supplemental ridge, which we know affects the receptive fields of the neuromasts, provides the fish with aberrant information. This aberrant information takes longer to parse but, presumably using interneuromast timing delays, the fish is still reliably able to estimate the target angle.

5.3. Significance

We have attempted to behaviorally test the hypothesis that the ridges are acting as sensory antennae that define the receptive field of the adjacent neuromasts. The test involved measuring a behavioral impairment in response to the addition of a supplemental ridge. Given that the addition of a supplemental ridge affects the receptive fields of the adjacent neuromasts, the lack of a behavioral impairment in animals with a normal complement of neuromasts suggests that there is considerable redundancy in the system. In an intact animal, it seems likely that the neuromasts of groups I and III compensate for deficits in those of group II. The observation that an animal

with only group II neuromasts is impaired when a supplemental ridge is added nearby suggests that the fish utilizes differences in receptive fields between neuromasts for making directional estimates of prey location. For estimating the direction toward a target, a fish may compare the magnitude of activity between neuromasts, which depends on their respective receptive fields. Providing another layer of redundancy, the animal may also compare the timing of activity, which is sensitive to the neuromasts' positions relative to the target and to each other. Given a propagation speed of approximately 40 $cm \cdot s^{-1}$ and a spacing between neuromasts of group II of about 500 μm, the wave arrival time difference between neuromasts could be 1.25 ms or more, a latency resolvable by the central nervous system.

The fish reacts very rapidly, within a few hundred milliseconds after the arrival of a wave. It is also attuned to relatively high-frequency waves, 250 Hz. The advantage of being sensitive to high frequency waves is that they travel quickly; however, they attenuate very rapidly. At some of the longer distances tested, it is remarkable that the fish were able to even detect the 250 Hz waves, as we were unable to measure them against the background noise. Hair cells in general have an impressive capacity to reliably detect signals in the midst of noise [Hudspeth 2008], and averaging across the many cells of each neuromast should improve the signal further. The behavioral impact of adding supplemental ridges further supports the hypothesis that the natural ridges are important for defining the receptive fields of the neuromasts.

6. Discussion

Using only its mechanosensory lateral line, *Aplocheilus lineatus* displays a remarkable capacity to localize prey in darkness [Schwartz 1965], a valuable ability for an insectivorous tropical fish in its native habitat. An untrained animal readily hunts in low light and darkness, suggesting that, in the wild, *Aplocheilus* continues to hunt as insect activity increases around dusk and into the night [Corbet 1960, Jones 1967]. The structure of the cephalic lateral-line apparatus used for the behavior is stereotyped across individuals, not only in the location and orientation of the neuromasts but also in the striking presence of the adjacent ridges.

A sensory antenna modifies the incoming stimulus in such as way that the central nervous system can generate a behaviorally appropriate percept. For example, the mammalian external ear introduces a spectral modification, called the pinna notch, in the sound entering the ear canal [Middlebrooks 1991]. Because the nature of this modification depends on the elevation at which the sound originates, the brain is able to use the notch to determine the elevation of the sound source. We propose an analogous role for the ridges on the head of *Aplocheilus*, which shape the receptive fields of the neuromasts by causing steep changes in sensitivity over small angles. A fish's angular resolution is greatest when the sensitivity of individual neuromasts to angular changes is maximal.

The anatomy of the head of *Aplocheilus* is highly specialized; there are a number of structures whose function is still unclear. Although we have supplied evidence that the function of the ridges is to restrict the receptive fields of the neuromasts, our initial analysis considered other roles for them as well. Concurrently with their role in defining the receptive fields, the ridges could be fulfilling other functions, such as protecting the neuromasts and cupulae from

damage. Systematic removal of the cupulae abolishes behavioral responses [Schwartz 1965]. The cupula is a gelatinous structure and is not robust to mechanical abrasion. The ridges could also be involved in detecting the fish's depth below the surface of the water. We have observed that, when hunting, the fish often de-wets a few of its ridges. The ridges are somewhat flexible and collapse when the fish is removed from water. We did not see any innervation within the ridges (Figure 2.9), but we cannot rule out the possibility that that they are sparsely innervated and we merely failed to encounter a nerve or that there are nerves at the base of the ridges or in the underlying scales that are sensitive to the tension in the ridge. There is also the possibility that the ridges can themselves detect waves, although we found no evidence to support this hypothesis given that we did not see any mechanosensory corpuscles in the ridges.

The large size and unique shape of the neuromasts is also puzzling. Our *a priori* expectation was that the axis of sensitivity would lie parallel to the short dimension of the neuromast, rather than to the longitudinal axis. The cupula would then act as a sail and all of the hair cells would depolarize in phase with each other. Instead, we found that the axis of sensitivity of the neuromast lies along its long axis. This introduces the possibility of a phase lag along the length of the neuromast, with the hair cells at the 'front' of the neuromast, those closer to the waves' origin, depolarizing hundreds of microseconds before the hair cells at the 'back.' The impedance of the cupula would define the extent of phase lag, specifically the ratio of its viscosity to its elasticity, and warrants further study. The surface of the water is a noisy environment. Although waves with biotic origins tend to have more high-frequency content than waves generated by abiotic stimuli such as wind [Lang 1980], the signal-to-noise ratio is still very small, with signal amplitudes approaching the level of thermal noise

for the frequencies of maximal sensitivity. The coupling of hair bundles by the cupula allows the fish to average responses across many cells, filtering out some of the noise. The strength of the coupling by the cupula depends on its stiffness. The stiffness of the cupula could be assessed either by stimulating it with a probe at one end and recording the motions with another probe at the opposite end or by point-labeling along its length, stimulating it with a probe at one end, and tracking the motion of the different points.

We observed that the ridges bordering each neuromast form channels around the neuromast. These channels seem to block some wave energy and to direct the water displacement along the neuromast's axis of sensitivity. There remain some questions regarding the hydrodynamics over the surface of the head. The behavior of surface waves depends on the depth of the water they are traversing. In deep water, gravity waves drive circular translations of water particles with no net displacement after the wave has passed. Capillary waves drive spiraled translations of the water particles, resulting in a net displacement (Figure 3.2). As the water depth approaches the wavelength of the wave, the particle trajectories become flatter, with the horizontal component becoming larger than the vertical component but with the overall displacement remaining the same [Lighthill 1978]. Gravity waves washing up onto a beach are a clear example of this phenomenon. An analogous activity may be occurring as capillary surface waves translate from the deep water around the fish to the shallow water over the fish's head. The fish's shallow depth may itself act as an effective amplification of the waves. The neuromasts are sensitive only to displacements parallel to the water surface. The shallow depth over the fish may function to bias the wave energy into that axis, increasing the amplitude of the waves deflecting the neuromasts a greater distance.

We were also interested in the possibility that the fish uses interference between waves as a indicator of target angle. The group I neuromast lie in a channel behind the upper lip. This channel runs from pores immediately caudal to the upper-lip joint and meets in the middle of the head. The function of the lip is a mystery, but we considered that it could be acting as a refractive antenna and interacting with the channel behind it. Waves coming from directly in front of the fish would travel around the lip, enter the pores on both sides of the head, and interfere in the channel. This could amplify small differences in the angle from which a wave enters, much as small vertebrates have air coupling the tympanic membranes of their two ears to amplify small differences in wave arrival times [Köppl 2009]. We attempted to observe this phenomenon, but were unable to acquire data of sufficient quality to either support or falsify the hypothesis.

By electrophysiologically recording from neuromasts while stimulating from different angles on the head we discovered that the individual neuromasts have unique receptive fields. Further, by adding a supplemental ridge to the head we were able to change the structure of those receptive fields. This supports the hypothesis that the natural ridges function as sensory antennae to sharpen the angular sensitivity of the neuromasts. It would be useful in the future to use electrophysiology is to assess nerve activity rather than hair-cell activity. Experiments making multi-unit extracellular recordings from the anterior lateral-line nerve suggest that nerves fire in a phase-locked manner with the peaks and troughs of a wave's displacement [Bleckmann 1981B, Topp 1983], corresponding to firing at twice the wave frequency. This indicates that nerves fire action potentials at each depolarization peak in the microphonic response. It would be worthwhile to investigate the receptive fields of individual nerve fibers and see how closely they follow the receptive fields of the

innervated neuromasts. The efferent neurites in the lateral line are generally thought to modulate the sensitivity of the system, such as hyperpolarizing the hair cells in anticipation of swimming motions [Roberts 1972, Sapède 2004]. Perhaps the efferent neurites innervating the cephalic lateral line fire in anticipation of the striking motions of *Aplocheilus*.

Given the very rapid response to surface waves, it seems likely that a particular combination of neuromast activity drives activation of a particular position on a spatial map in the brain. The activation of the map could then elicit an orientation response, much as is seen in higher vertebrates where activation of a region of the spatial map in the tectum drives a gaze-changing behavior to the point in space represented by the stimulated area [du Lac 1990]. The fish tectum also holds a topographical spatial map of visual space. Electrode stimulation of particular regions in the map drive eye movement to a point in visual space represented by that region [Luque 2004]. Higher-order processing of lateral-line informations occurs in a tectal nucleus called the torus semicircularis, which is homologous to the inferior colliculus. Within the torus semicircularis of weakly electric fish there is a somatotopic map of the electrosensory system, a system that is closely related to the lateral line [Heiligenberg 1988]. Other teleosts have a somatotopic representation of the lateral line in the hindbrain [Gompel 2001]. Higher vertebrates have spatial and auditory maps overlaid in the tectum [Gufreund 2002]. It seems possible that the fish could have a similar arrangement of visual and lateral-line maps. We hypothesize that there is a topographical map of the water surface in the torus semicircularis. To test this hypothesis we could record from the torus semicircularis while stimulating the water surface at different points. Once the area of sensitivity of a particular recording location is found, we could systematically shift the electrode to a different location and see if the area of

sensitivity also moved systematically. If we did find such a map, we could attempt to test whether the visual map and lateral-line based map are overlaid by determining if visual stimulation was also able to drive activity in that region. We could additionally investigate whether electrode stimulation in the region would drive eye movements to the receptive field area. Behaviorally, we could lesion areas of the brain and look for blind spots around the fish where it was insensitive to wave stimuli, visual stimuli, or both.

There is a great deal of potential for further study in this system. My own work has been to characterize the peripheral system, in particular how the interaction of the hydrodynamics of the system with the surface anatomy of the head affects the electrophysiological sensitivity of the lateral-line system, and the impact of those effects on the fish's behavioral abilities. The cephalic lateral line of *Aplocheilus* is a manifold of sensory antennae. The ridges are the most explicit antennae, modulating the receptive fields of individual neuromasts. Other antennae include the cupulae, which transfer water motions onto the hair bundles; the top of the head, which may act as a beach to amplify the horizontal component of waves; and the lip, which may serve as a refractive antenna.

The mechanisms of sensory transduction are remarkably conserved. In the visual system, the metazoan opsins are all derived from a single common ancestor [Land 1992]. The cellular photoreceptors are more diverse, with two main classes traditionally thought to occur in the two main classes of metazoans: the protostomes and the deuterostomes, which diverged at least 520 million years ago [Blair 2005]. The protostomes tend to have receptors in which the photopigment-bearing membrane is derived from microvilli, whereas the deuterostomes have receptors in which the photopigment-bearing membrane is derived from cilia. The microvillous receptors usually depolarize in response

light, while the ciliated receptors tend to hyperpolarize [Land 1992]. Regardless of the transduction mechanisms, most of the differences involved in visual acuity are not related to either the photoreceptive molecule or the transducing photoreceptor cell. The visual acuity of an animal is defined by the structures surrounding the photoreceptors: the sensory antennae. Similarly, among vertebrates, mechanosensation is accomplished through hair cells. While there are morphological differences between different hair cells, particularly in bundle morphology, which could be thought of as a cellular sensory antenna, the class of stimuli to which a hair cell responds is defined by antenna structures. For example, a hair cell of an ampulla crista is responsive to rotation of the head around a particular axis but unresponsive to linear acceleration. This is due to the fact that it is embedded in a circular fluid-filled canal. It seems possible that evolution can occur more rapidly in the antenna structures than in the transducing cells. Selection may act on transducing cells such that they are sensitive to stimuli at the noise threshold, as both photoreceptors and hair cells are [Ashmore 1980, Hudspeth 2008]. However, rather than modifying the transducing cells to be optimal for each class of stimuli, perhaps it has been more evolutionarily efficient — there are fewer deleterious pleiotropic effects — to modify the surrounding structures to optimize the acuity of the system. This would explain the diversity of sensory organs surrounding nearly identical sensory cells.

In conclusion, we have described the system of mechanosensitive neuromasts that underlie the ability of *Aplocheilus* to hunt in darkness. When stimulated with capillary waves on the water's surface, each neuromast displays a unique angular receptive field. The fleshy ridges that border each neuromast modulate the receptive fields of the cephalic neuromasts that the fish uses to detect surface waves. The addition of a supplemental ridge reversibly affects the

flow of water over the head, alters the neuromasts' receptive fields, and produces a measurable behavioral impairment. The ridges act as sensory antennae that shape the animal's perception of its world.

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