The Journal of Experimental Biology 208, 3441-3450
Published by The Company of Biologists 2005
doi:10.1242/jeb.01766

Sensitivity of the anterior lateral line to natural stimuli in the oyster toadfish, *Opsanus tau* (Linnaeus)

Lucy M. Palmer[1,2], Max Deffenbaugh[2,3] and Allen F. Mensinger[1,2,*]

[1]*Biology Department, University of Minnesota Duluth, Duluth, MN 55812, USA,* [2]*Marine Biological Laboratory, Woods Hole, MA 02543, USA* and [3]*ExxonMobil Upstream Research Company, PO Box 2189, Houston, TX 77252, USA*

*Author for correspondence (e-mail: amensing@d.umn.edu)

Accepted 28 June 2005

Summary

Inductive neural telemetry was used to record from microwire electrodes chronically implanted into the anterior lateral line nerve of the oyster toadfish, *Opsanus tau* (L.). The lateral lines of free-ranging toadfish were stimulated by the swimming movements of a prey fish (*Fundulus heteroclitus*), and the corresponding neural activity was quantified. Both spontaneously active and silent afferent fibers experienced an increase in neural firing as the prey approached the lateral line. Activity was evoked when the prey fish approached to within 8–12 cm of the neuromast, with increases in nerve firing rates directly correlated with diminishing distance. Thus, adult toadfish (28 cm standard length; 33 cm total length) were only able to detect mobile prey that approached within approximately 40% of their body length. Both spontaneously active and silent afferent fibers also experienced a dramatic increase in firing during predatory strikes, indicating that the fibers were not inhibited during rapid body movement. This study investigates, for the first time, the neural response of the anterior lateral line to prey stimuli in free-ranging fish.

Key words: lateral line, telemetry, prey, toadfish, *Opsanus tau*.

Introduction

To enhance the interpretation of their environment, fish and aquatic amphibians have evolved a lateral line system that detects displacements in the local water field. Neuromasts are the basic unit of the lateral line system and consist of bundles of mechanoreceptive hair cells, which are comprised of a single kinocilium and numerous stereovilli protruding into a gelatinous cupula. The hydrodynamic properties of the neuromast act as a mechanical filter for sensory hair cells (van Netten, 1991). Hair cells are grouped into neuromasts located either upon the skin surface (superficial neuromasts) or enclosed within subdermal canals (canal neuromasts). Canal neuromasts function as water acceleration detectors, and superficial neuromasts act to determine water velocity (Kroese and Schellart, 1992).

Behavioral and electrophysiological experiments have illustrated that the lateral line functions in schooling behavior (Partridge and Pitcher, 1980), rheotaxis (Montgomery et al., 1997) and localization of underwater objects (Weissert and von Campenhausen, 1981). The lateral line has also been shown to receive water displacements generated by moving prey (Saunders and Montgomery, 1985; Montgomery and Macdonald, 1987; Montgomery et al., 1988; Bleckmann and Topp, 2003; Pohlmann et al., 2004). The great diversity in fish body plan and swimming strategies can generate extremely complex hydrodynamic trails consisting of unpredictable distributions of local particle activity, alternating eddies and slightly deformed vortex rings (Blickhan et al., 1992; Muller, 1996). Information about the direction and temporal scale of fish movement can be contained in these trails (Hanke et al., 2000), which can persist in the water column for several minutes (Hanke and Bleckmann, 2004). However, since the chain of vortices generated by fish movement is difficult to recreate and/or present during standard neurophysiological preparations, the activity of the lateral line in response to free-swimming prey has been difficult to quantify. Instead, vibrating spheres have been used historically as stimuli for the lateral line (Wubbels et al., 1993; Muller, 1996; Coombs, 1999; Kanter and Coombs, 2003). While instrumental in determining neuromast characteristics (frequency and directional sensitivity), pure stationary dipole-like stimuli are rarely encountered in nature.

The detection of biologically relevant stimuli must often be accomplished during self-generated movement (e.g. swimming, ventilation). Recent studies conducted by Palmer et al. (2003) indicate that the primary afferents of the anterior lateral line were stimulated during swimming and ventilatory movements. Various studies have shown that lateral line filtering during intense stimuli may be performed in higher brain regions. Tricas and Highstein (1990, 1991) identified efferent modulation of lateral line activity when toadfish

viewed live prey, and Montgomery and Bodznick (1994) indicated that the lateral line medullary nuclei contain an adaptive filter capability that cancels input consistently associated with an animal's own movements.

The operating range of the lateral line system has been reported to be one to two body lengths (Denton and Gray, 1983; Kalmijn, 1988; Coombs, 1999; Braun and Coombs, 2000). However, to accurately quantify the range and sensitivity of the lateral line to natural stimuli, neural activity must be monitored from an unconstrained teleost in quasi-natural settings. Recording neural activity from free-swimming fish has been complicated by the need for electrode stability and/or a suitable telemetry device. Terrestrial telemetry modes such as infrared light or radio waves are rapidly attenuated in seawater and are ineffective. The development of an inductive neural telemetry system allows the recording of neural responses from free-ranging toadfish (Mensinger and Deffenbaugh, 1998, 2000; Palmer and Mensinger, 2004). This study reports the activity of the lateral line in response to free-swimming prey.

Materials and methods

Animal care

Adult toadfish [~33 cm total length (TL); 28±1.4 cm standard length (SL); 675±46 g wet mass; means ± S.E.M.] of either sex were obtained from the Marine Biological Laboratory (MBL), Woods Hole, MA, USA. Fish were housed in large flow-through seawater tanks maintained at 20°C and fed squid and bait fish. All animal care and experimental procedures conformed to institutional animal care protocols.

Microwire electrode

Microwire electrodes consisting of three strands of insulated 20 μm-diameter 10% platinum/iridium wire (Sigmund Cohn Corp., Mt Vernon, NY, USA) were custom fabricated for each implantation. Each microwire strand was affixed to hard silver-plated copper multistranded wire (25 μm diameter) with conductive silver paint (Silver Print Paint, GC Electronics, Rockford, IL, USA). The multistranded wire was attached to silver wire (320 μm) that terminated into a multipin underwater connector. The anterior portion of the microwires was threaded through a 1 mm length of polymide tubing (180 μm outer diameter; A-M Systems Inc., Carlsborg, WA, USA) to maintain the recording sites in proximity. Any exposed wire/connections were encased in medical device adhesive (Loctite 3341; Henkel Loctite Corp., Rocky Hill, CT, USA) and cured with ultraviolet light (ELC #660; Electro-lite Corp., Danbury, CT, USA). The impedance of each electrode channel was determined with an impedance-test unit (FHC; Bowdoinham, ME, USA) using 1 kHz input frequency. Only electrodes with impedances between 0.5 and 1.2 MΩ were used.

Electrode implant

Fish were anesthetized by immersion in 0.005% tricaine (3-aminobenzoic acid ethyl ester; Sigma, St Louis, MO, USA) and paralyzed with an intramuscular injection of a 0.01% solution of pancuronium bromide (600 μg kg^{-1}; Sigma). An incision was made through the dorsal musculature overlying the sagittal crest, and the muscle was retracted. A small craniotomy was performed lateral to the sagittal crest and posterior to the transverse crest to expose the anterior ramus of the anterior lateral line nerve. The electrode was inserted into the nerve just prior to its exit from the braincase. Potentials were differentially amplified (Dagan, Minneapolis, MN, USA) and monitored on a portable computer using Chart5 for Windows software (ADInstruments, Colorado Springs, CO, USA). The two channels that provided the highest fidelity signal were chosen for the experiments. Once a candidate fiber was located, the fish was left undisturbed for 30 min to ensure fiber stability.

Cyanoacrylate gel (Pacer Technology, Rancho Cucamonga, CA, USA) was used to affix the electrode to the skull and seal the craniotomy. The muscle was restored to its original position, and the muscle, faschia and epidermis were individually sutured to provide a watertight seal over the craniotomy and around the transdermal electrode lead. The differential amplifier was disconnected from the electrode, and the cylindrical telemetry tag (15 mm×38 mm, diameter × length; 8 g) was inserted into the waterproof electrode connector. The tag was sutured parallel to the dorsal fin on the dorsal surface of the fish.

Neural recordings

Chronic extracellular recordings from lateral line primary afferent fibers were obtained using an inductive telemetry system (Mensinger and Deffenbaugh, 1998, 2000; Palmer and Mensinger, 2004). In brief, the inductive telemetry system consists of a transmitter tag and receiver coils. The tag transmits the neural signal as a frequency-modulated magnetic field (90 kHz carrier, 20 kHz bandwidth), which is detected by receiver coils embedded in a recharging habitat and stage (RECHABS). To recharge the tag, the RECHABS produces an oscillating magnetic field (50 μT, 200 kHz), and the tag stores energy from this field in its capacitors. The RECHABS consists of a cylindrical habitat (12 cm×30 cm, internal diameter × length) that opens onto an octagonal stage (16 cm per side; Fig. 1). The RECHABS can both receive the telemetry signal from the tag and recharge the tag when the tag is within the habitat or up to a height of approximately 15 cm above the stage. The tag can be fully charged in less than 30 s and will provide telemetry for 5 min.

Immediately after surgery, the fish were placed in an opaque fiberglass experimental tank (90 cm×45 cm, width × length; 15 cm water depth) and left undisturbed for a minimum of 90 min. The innervated neuromast location was determined by monitoring neural activity while a small brush was gently run along the supraorbital or infraorbital lateral line. This allowed the innervated neuromast to be localized to within two or three end organs, or an area of approximately one cm^2.

Killifish (*Fundulus heteroclitus*; 6–8 cm SL) were used as prey. To maximize predator–prey encounters, a circular

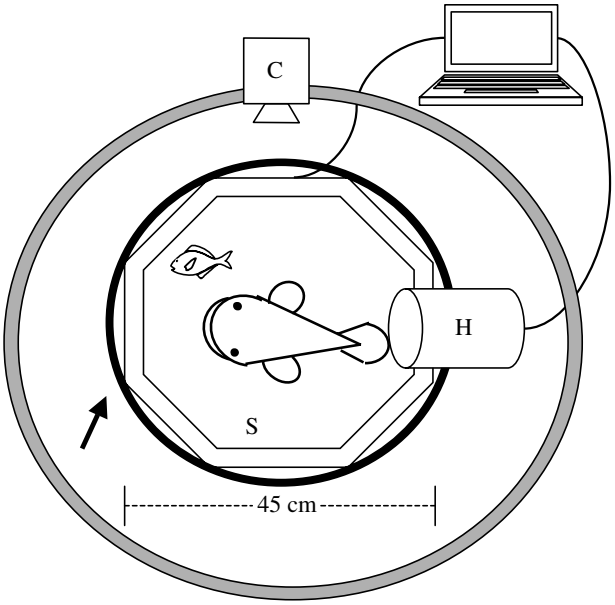

Fig. 1. Dorsal view of the experimental arena. The recharging habitat and stage (RECHABS) consists of the cylindrical habitat (H) and octagonal stage (S). Neural telemetry and tag recharging could transpire when the fish was in the habitat or over the stage. The black circle (arrow) represents an opaque barricade that restricted the prey and the toadfish to the stage area. Fish movements were recorded with an overhead video camera (C). Drawing is not to scale.

plastic barricade surrounded the stage and restricted the killifish to the RECHABS area. Prey that did not approach the toadfish within 15 min were anesthetized (0.0001% MS-222), and a barbless fish hook (size 6) attached to monofilament (1 kg test) was inserted between the premaxilla and maxilla bone of the killifish. Once the tethered *Fundulus* recovered normal swimming activity, they were placed in the experimental tank. Sufficient slack was maintained in the tether to allow normal swimming movements; however, the tether allowed the fish to be directed towards the toadfish when necessary.

The telemetry signals were recorded up to four days post electrode implantation and stored on a portable computer using Chart5 software and analyzed offline with Spike2 software (Cambridge Electronic Design, Cambridge, UK). Predator–prey interactions were simultaneously recorded on videotape (30 frames s^{-1}; Sony Digital Handycam, Sony Electronics USA, Oradell, NJ, USA) that was synchronized with the neural telemetry system and analyzed frame by frame using DV Shelf video frame capture and Scion Imaging software (Scion Corp., Frederick, MD, USA). Killifish movement alternated between caudal-fin-mediated forward swimming and stationary positioning *via* 2–3 Hz oscillation of the pectoral fins. Because the various fins and swimming motions of fish produce differing stimuli of varying intensity (Gibb et al., 1994; Drucker and Lauder, 2001), neural activity was only quantified when the prey hovered in the same location for greater than 500 ms. Prey distance was defined as the distance between the neuromast and the intersection point of the nearside killifish pectoral fin with its body axis.

A trial consisted of placing a free-swimming or tethered *Fundulus* into the arena and monitoring its swimming movements while concurrently recording toadfish lateral line activity for up to 10 min. An encounter consisted of a *Fundulus* hovering for >500 ms in the same position that was within 20 cm of the innervated neuromast during a trial. Five to 12 trials were conducted per toadfish, with up to 119 encounters recorded per trial. To compare activity between fibers, firing rates were normalized according to the maximum firing rate elicited by prey movements that was recorded in each trial.

Although the microwires often yielded multiunit activity, fiber discrimination was usually limited to a single unit that yielded the greatest action potential amplitude and was clearly discernible from other units. To verify that the same unit was consistently recorded during an experiment, individual fibers were distinguished using rigorous waveform analysis (Spike2) in addition to spike amplitude. During one implant, two units were discovered to be clearly distinguishable based on amplitude and waveform analysis, and both fibers were individually analyzed during the trial.

The streamlined telemetry tag only added 1% to toadfish body mass and its attachment did not have noticeable effects on behavior. Normal ventilation rates and equilibrium returned within 30 min of anesthetic withdrawal, and swimming activity resumed within two hours post-surgery. Recent work has shown that the sensitivity of the anterior lateral line to mechanical stimuli is restored within 90 min of anesthetic withdrawal (Palmer and Mensinger, 2004), and therefore experiments were not initiated until a minimum of two hours following anesthetic removal. During all trials, toadfish were monitored for abnormal physiological (respiration rate) and behavioral (sudden movement, tail contraction) changes before, during and after the application of the magnetic field, and no discernible effects were observed. Previous studies (Mensinger and Deffenbaugh, 1998, 2000; Palmer and Mensinger, 2004) demonstrated that the magnetic field does not affect neural activity or behavior in the toadfish.

Prey stimulus

Still-water trials were conducted with 8 cm SL *Fundulus* in a large rectangular tank (2.5 m×1.2 m×0.5 m) with water depth maintained at 20 cm. Water velocities generated by hovering *Fundulus* were calculated by digital particle tracking velocimetry. Fluid flow around the fish was illuminated by a horizontal laser sheet, 0.5 mm thick, and imaged from above with a high-resolution digital video camera (Kodak ES 1.0, 1008 pixels×1018 pixels). The flow was seeded with 20–40 μm-diameter neutrally buoyant fluorescent particles. For further details, refer to Anderson et al. (2001).

Statistical analysis

All statistical analysis was performed using GraphPad Software (San Diego, CA, USA) or SigmaStat for Windows

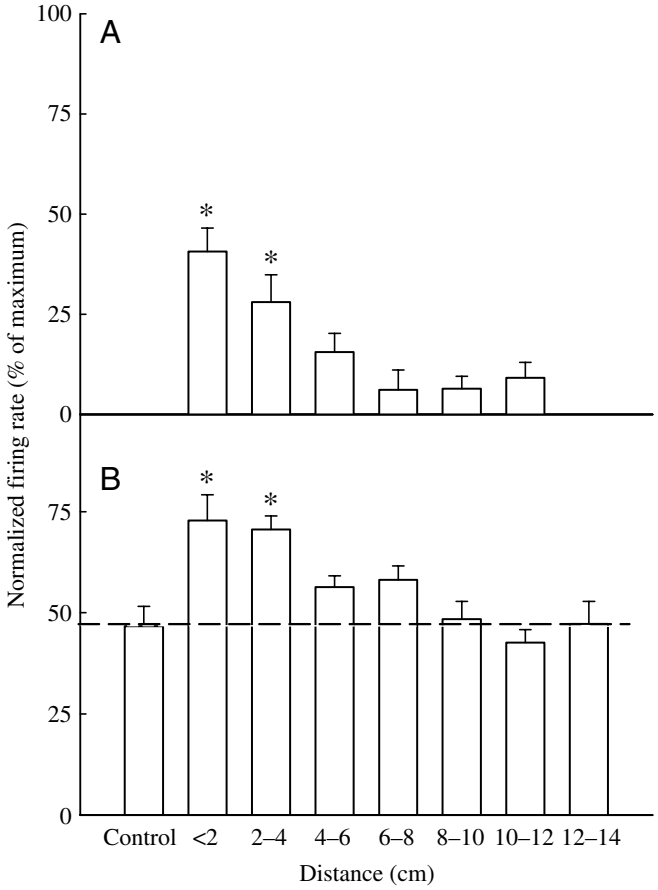

Fig. 2. The mean normalized neural firing rates of (A) silent (N=4) and (B) spontaneously active afferent fibers (N=5) of the anterior lateral line are plotted *versus* the distance of the innervated neuromasts to the prey. All firing rates were normalized according to the maximum firing rate recorded during each trial. Neural activity was analyzed only when the prey fish was at the same location for greater than 500 ms. All distances were measured from the insertion point of the nearside prey pectoral fin to the neuromast that was innervated by the recording. Asterisks indicate significantly different means from controls (ANOVA). The broken horizontal line in B represents the mean normalized spontaneous firing rate from spontaneously active fibers.

version 3.10 (Systat Software, Inc., Richmond, CA, USA). All data represent mean values ± 1 S.E.M. unless otherwise indicated. Fiber activity during an encounter was binned into 2 cm intervals (distance of *Fundulus* fin origin to innervated neuromast). As the range of activity for the silent fibers was moderate (0–17 Hz), a one-way analysis of variance (ANOVA) was used to determine differences in spike activity among the four fibers during prey encounters. For the spontaneous active fibers, firing rates were normalized according to the maximum firing rate elicited by prey movements that was recorded in each fiber during a trial. The resulting percentages were transformed using the arcsine function (Zar, 1984), and a one-way ANOVA was used to determine differences in the transformed data for fiber activity for the combined five spontaneous active fibers. Samples were tested for normality using the method of Kolmogorov and Smirnov. Bartlett's test was used to determine the use of parametric or non-parametric testing.

Results

Two types of lateral line fibers were identified. Silent fibers (N=4) did not display any spontaneous activity and were only activated by water movement. Their average sustained firing rate to prey movement ranged from 3 to 5 Hz, which was ~27% of the fibers' maximal evoked response. Spontaneous active fibers (N=5) were of the irregular type (Tricas and Highstein, 1991) and had an average discharge rate of ~47 Hz in the absence of stimulation. Prey movements stimulated these fibers to firing frequencies approximately 65% of their maximum firing rate.

As the distance between the prey and neuromast diminished, silent fibers were stimulated to fire (Fig. 2A). The greatest range at which prey stimulated a silent fiber was 11 cm. Spontaneous fibers showed similar characteristics, with activity significantly increasing (ANOVA, P<0.05) above resting background levels at prey distances of up to 4 cm from the neuromasts (Fig. 2B). Prey at distances between 4 and 8 cm stimulated firing rates above background levels; however, at distances greater than 8 cm, the presence of prey did not lead to elevated rates.

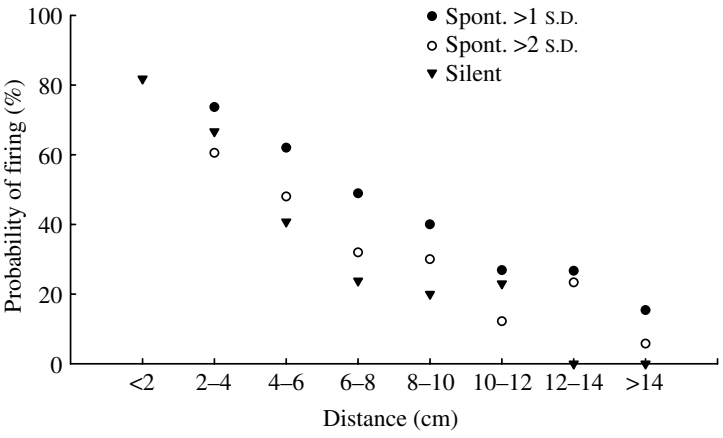

Fig. 3. The probability of a silent fiber (triangles) firing during a prey encounter and the probability of a spontaneously active fiber increasing its discharge rate one (filled circle) or two (open circle) standard deviations above its spontaneous discharge rate during a prey encounter is plotted *versus* prey distance. If the silent fiber fired during the event, it was considered stimulated, and the probability of the silent fibers firing was calculated as: (stimulated encounters/total encounters)×100. If the firing activity of the spontaneously active fibers firing increased one and/or two standard deviations above its mean resting discharge rate it was considered stimulated and the probability was calculated as: (stimulated encounters >1 S.D./total encounters)×100 and (stimulated encounters >2 S.D./total encounters)×100. The data represent the summary of all trials for each fiber class, and each point represents a minimum of 15 trials at each distance. Prey distance was binned into 2 cm segments.

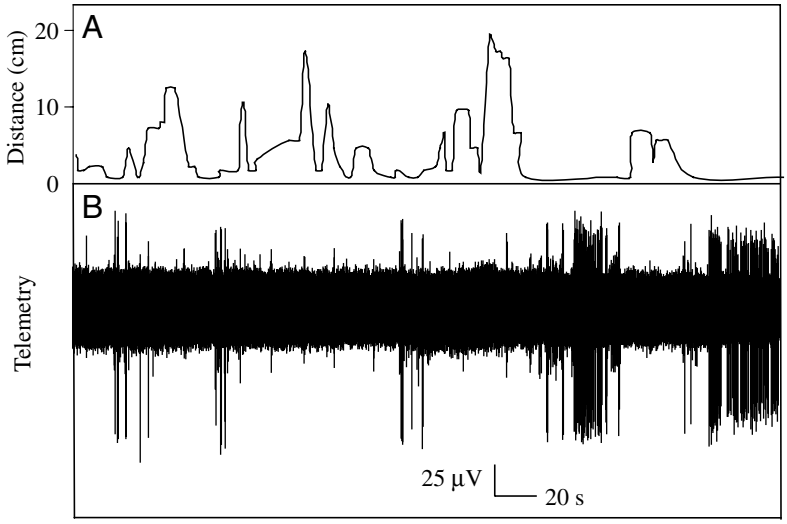

Fig. 4. The activity of a silent, afferent fiber innervating a superficial neuromast on the infraorbital lateral line, as monitored during prey movement. (A) The distance (cm) of the prey fish from the recorded neuromast. (B) The full neural waveform from the anterior lateral line nerve that was transmitted *via* inductive telemetry. Although multiunit activity is visible in the trace, only the fiber with the greatest amplitude of action potential was used for data analysis. The fiber had no spontaneous activity and exhibited maximum firing (~10 Hz) when the prey fish was within 1 cm of the neuromast.

Small fluctuations in action potential frequency may be more valuable for prey detection for the toadfish than statistically significant changes. For silent fibers, the probability of a silent fiber firing during an encounter was plotted *versus* prey distance (Fig. 3). Silent fibers fired greater than 60% of the time when prey was located within 4 cm of the neuromast. This probability decreased to approximately 20% between 6 and 12 cm, and prey located further than 12 cm failed to stimulate silent fibers. For spontaneous fibers, determining small fluctuations in action potential frequency was not as clear, as spontaneous discharge rates could drift by 5–10% during a trial. Therefore, Fig. 3 includes the probability of spontaneous fibers firing one and/or two standard deviations above their resting discharge rate during an encounter *versus* prey distance. Thus, even after including a less rigorous benchmark (1 S.D.), the probability of a spontaneous fiber reacting to the prey during an encounter continues to decline sharply with distance.

Fig. 4 shows the neural activity in a silent lateral line fiber correlated with the position of a free-swimming *Fundulus* during a 5 min trial. Both transient swimming and hovering evoked a neural response when the prey fish approached within 6 cm of the neuromast; however, movement outside this range did not induce firing. Activity for a silent anterior lateral line fiber innervating a superficial neuromast on the right infraorbital lateral line of a toadfish is illustrated in Fig. 5. The fiber was unresponsive at prey distances greater than 10 cm; however, activity was evoked (~5 Hz) when the prey approached to within 3.5 cm of the neuromasts. As the prey closed to within 1 cm, firing rate increased to ~10 Hz.

The neural response of a spontaneously active fiber and a silent fiber to hovering prey is illustrated in Fig. 6. Both fibers innervated superficial neuromasts located on the infraorbital line of the anterior lateral line. As prey approached within 8 cm, both fibers experienced an increase in neural activity.

The neural activity during predatory strikes was recorded in four fibers from three fish during 20 prey strikes. The firing

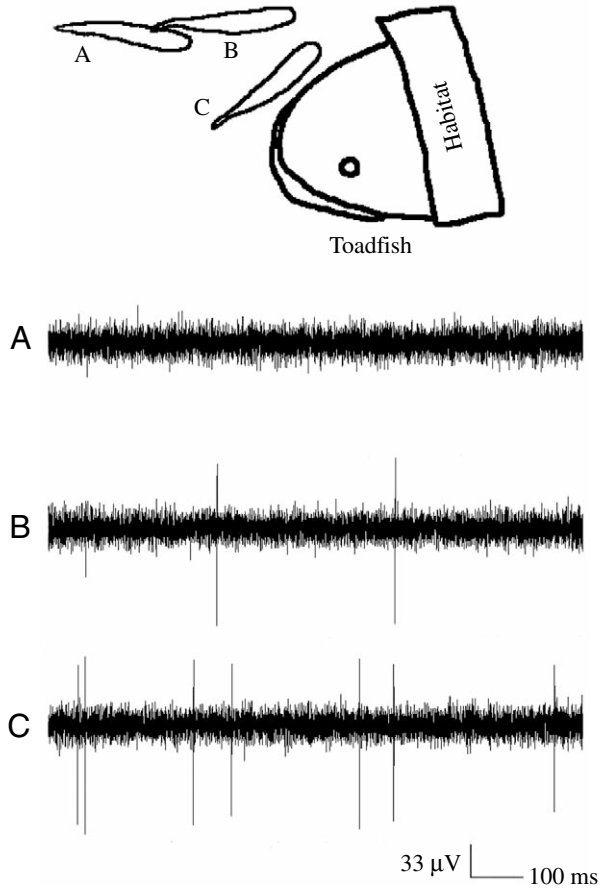

Fig. 5. Neural activity during the approach of a single prey fish. The diagram depicts the head of the toadfish projecting out of its habitat and the sequential positions of the approaching prey: (A) 10 cm; (B) 3.5 cm; (C) 1.0 cm. Images were reconstructed from single video frames. The letter next to the prey fish corresponds to neural activity from a superficial neuromast on the suborbital portion of the infraorbital lateral line. Although multiunit activity is visible in the trace, data analysis was restricted to the fiber with the greatest amplitude of action potential.

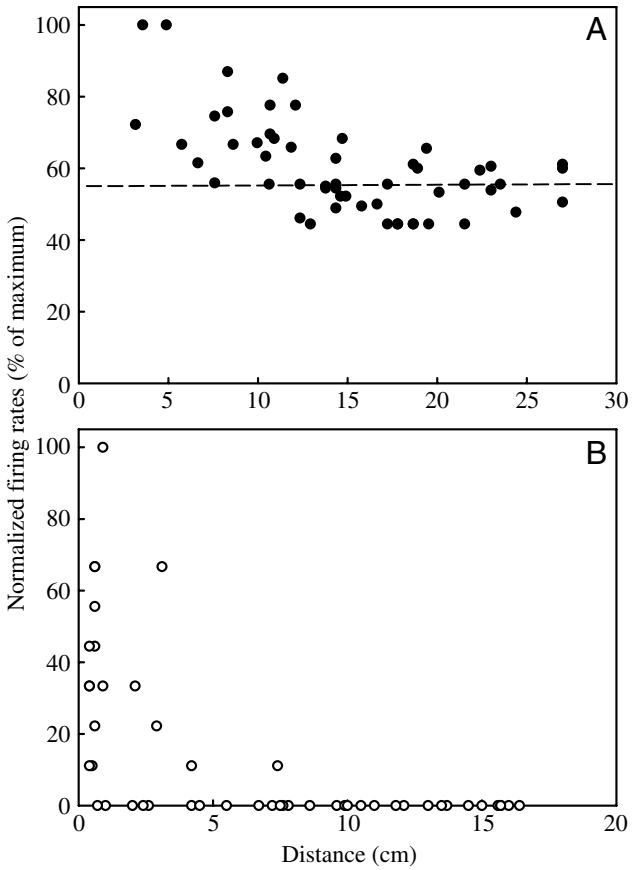

Fig. 6. Firing rates of (A) a spontaneously active afferent fiber and (B) a silent fiber in response to a prey fish located at variable distances from the neuromast. Both fibers innervated superficial neuromasts. All distances were measured from the insertion point of the nearside prey pectoral fin to the neuromast that was innervated by the recording. The broken horizontal line in A represents the mean spontaneous activity. All firing rates were normalized according to the maximum firing rate elicited by prey movements during each trial. Neural activity was analyzed only when the prey fish was at the same location for greater than 500 ms.

activity of the fibers increased during all strikes (Fig. 7). The spontaneously active fibers (N=3) experienced a 9-fold average increase in firing activity above resting discharge rates. These increases were much greater than the maximum firing activity evoked by prey movements in the same fibers. Neural activity from a single fiber during a prey strike is illustrated in Fig. 8. The killifish approached the toadfish at a constant velocity (2 cm s^{-1}) from the contralateral side of the neuromast, and the toadfish struck when the prey was directly in front of the mouth, 2 cm from the premaxilla (Fig. 8B). During the strike, the toadfish moved forward slightly (1 cm) and opened its mouth to engulf the prey fish. The prey was retained in the toadfish's mouth for 1300 ms before being expelled.

Both silent and spontaneously active fibers were observed to continually fire in phase with the toadfish's ventilation cycle. The fibers fired regularly with each ventilation cycle and were never observed to become habituated (Fig. 8).

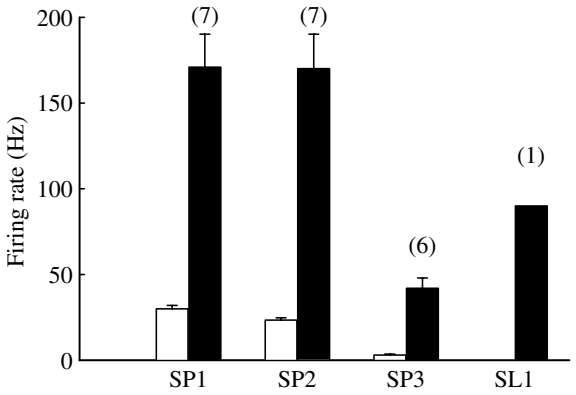

Fig. 7. Mean firing rate (± 1 S.E.M.) of four anterior lateral line afferents fibers [three spontaneous (SP) and one silent (SL)] immediately before (open) and during (filled) a toadfish prey strike. Numbers above the bars represent the number of prey strikes that were averaged for each fiber.

The water velocities generated by pectoral and caudal fin movement of 8 cm SL *Fundulus* were extremely complex (Fig. 9). Maximum water velocities generated by the pectoral fins during hovering were approximately 5 cm s^{-1}, with water displacement rapidly attenuating with distance from the body axis. Velocities between 2 and 3 cm s^{-1} were often detectable within 2–3 cm of the fin's insertion; however, at distances greater than 5 cm, water movement remained less than 1 cm s^{-1}.

Discussion

Oyster toadfish are benthic fish that inhabit most estuaries along the Atlantic Coast of the USA (Gudger, 1910). They are

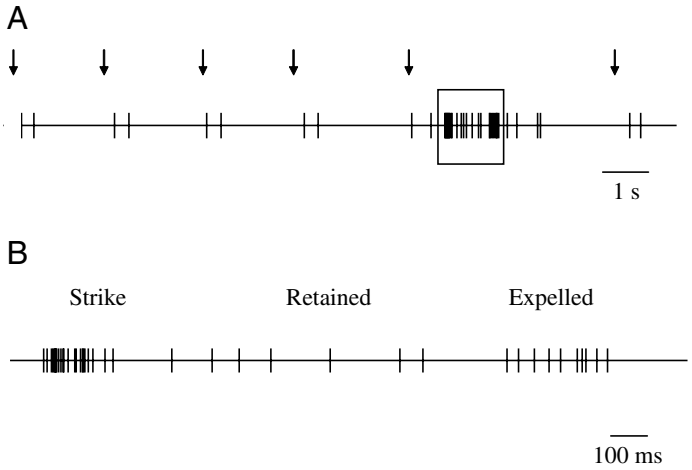

Fig. 8. Neural activity from an afferent anterior lateral line fiber before, during and after a prey strike. Vertical lines on the trace indicate individual action potentials from a single fiber that were discriminated based on spike amplitude. The arrows indicate initiation of opercular contraction for each ventilation cycle. The time during the strike, capture and subsequent expulsion of the prey is boxed in A, and this interval is expanded in B. The prey was retained in the mouth of the toadfish for approximately 1 s before being expelled.

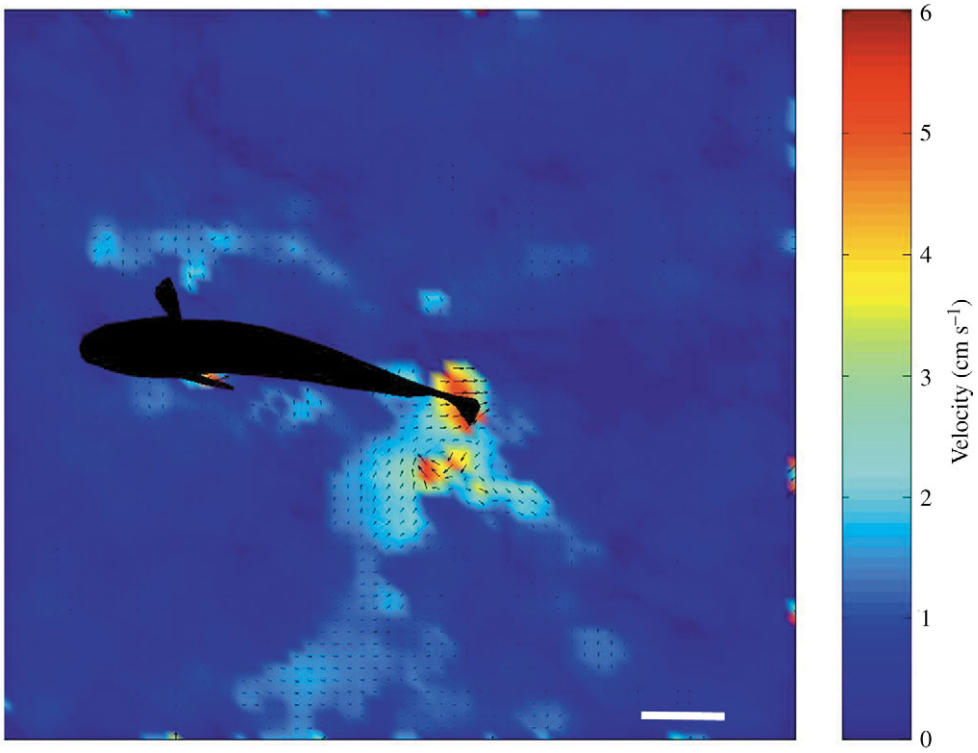

Fig. 9. The picture shows the water movements produced by an 8 cm SL *Fundulus heteroclitus* taken with a digital video camera at 1000×1000 pixel resolution. Each velocity vector represents the average of a 32 pixel×32 pixel window, and the center of each window is spaced 16 pixels apart. Velocities are presented by pseudo color images. Scale bar, 2 cm.

predominately ambush predators that prey largely upon crustaceans and small fish (Chrobot, 1959; Schwartz and Dutcher, 1963; McDermott, 1964; Phillips and Swears, 1979; Wilson et al., 1982; Price and Mensinger, 1999; Mensinger et al., 2003). Field and laboratory observations indicate that toadfish spend the majority of their time in small caves or crevices with their head facing outward and will often remain motionless prior to launching a ballistic strike at nearby prey. Therefore, with the exception of water currents generated by respiratory activity and prey strikes, there exists little self-generated interference for their lateral line.

As fish swimming combines the locomotion of several independent fin systems (Drucker and Lauder, 2001), the generated hydrodynamics can be complex. A swimming fish leaves a hydrodynamic trail in the water that can persist several minutes after its passage (Hanke et al., 2000; Hanke and Bleckmann, 2004). Experiments conducted by Enger et al. (1989) concluded that the lower-frequency accelerations resulting from the prey's motion are biologically of utmost importance in lateral line detection. The killifish was chosen for these experiments as it is natural prey for the toadfish (Chrobot, 1959). Its swimming behavior alternates between forward propulsion mediated by body and caudal fin movement, and stationary hovering using pectoral fin oscillation. Caudal fin movement generally creates greater water displacement and may consequently provide a larger stimulus for the lateral line. However, as toadfish predominantly strike at approaching prey, bow waves or forward fin (pectoral and/or pelvic) displacement may be more important than caudal movements or subsequent wakes. To limit the disparity in *Fundulus* swimming characteristics, data analysis was restricted to periods when prey were hovering in the same location for greater than 500 ms. Consequently, much of the data analysis characterizes lateral line activity in response to oscillation ($\leqslant$3 Hz) of the killifish pectoral fin.

Digital particle tracking velocimetry allowed analysis of the water velocities generated by hovering *Fundulus*. Retraction of the pectoral fins in hovering killifish produced maximum water velocities of approximately 5 cm s^{-1} within the arc traveled by the fin. These velocities rapidly attenuated with distance from the fin's origin. Velocities between 2 and 3 cm s^{-1} were often detectable within 3 cm of the fin; however, at distances greater than 5 cm from the fin's origin, water movement declined to less than 1 cm s^{-1} before fading into the background (0.1 cm s^{-1}). Caudal fin motion disrupted a larger volume of water over greater distances. However, as these deflections were lateral and posterior to the pectoral fin currents, there appeared to be little potential for constructive inference between the two sources. As data analysis was restricted to hovering, during which the caudal fin remained relatively stationary, pectoral fin movements were the primary stimulus source.

Previous studies have indicated the persistence of hydrodynamic trails several minutes following fish passage (Hanke et al., 2000; Hanke and Bleckmann, 2004). Slowly decaying wakes could complicate determining if the lateral line is reacting to current prey motion or residual wakes. Fig. 4 illustrates a typical trial during which the prey moved throughout the arena. The silent fiber ceased to fire when the prey moved greater than 10 cm away from the toadfish. If the

hydrodynamic trails continued to persist at a physiological level, the fiber should have continued to fire after the prey vacated the area. However, the previous studies examined wakes generated by rapidly swimming fish which would create greater and more persistent, water disturbance than *Fundulus*. Alternatively, the lateral line fibers could have become habituated to a persistence stimulus and ceased to respond. However, when lateral line fibers were stimulated for up to 60 s with continuous water flow, habituation was not evident (Palmer and Mensinger, 2004), indicating that a dispersing stimulus and not habituation was responsible for the return of lateral line activity to baseline levels.

Several studies have suggested that the lateral line detects prey up to a distance of 1–2 body lengths (Denton and Gray, 1983; Kalmijn, 1988; Coombs, 1999; Braun and Coombs, 2000). However, many lateral line studies have been performed with vibrating probes, which may generate a stronger stimulus than prey and do not reflect the complexity of water currents generated by natural stimuli. The neural telemetry system allowed lateral line activity to be monitored in the presence of free-swimming prey. The distance at which prey movement modulated the toadfish lateral line was shorter than the range usually proposed for other species. Both spontaneous and silent fibers of the toadfish anterior lateral line were stimulated by prey pectoral fin oscillation at distances from the sensory neuromast of less than half the toadfish body length. The detection range represented the distance between neuromast location and pectoral fin insertion; however, the nearest distance between predator and prey (often the head of the killifish and the body of the toadfish) was frequently 2 cm closer.

Caution must be applied in two areas of our data interpretation. As the recordings were restricted to individual fibers in a localized region of the anterior lateral line, it is possible that other regions may contain neuromasts more sensitive to prey movements. Alternatively, central integration from multiple neuromasts may function to increase resolution and sensitivity and project the detection distance further than an individual neuromast. Additionally, slight changes in firing frequency may provide important information for the fish but fail to be statistically significant, especially when averaged over different neurons and fish, as in the current study. For example, in the 4–8 cm range for spontaneous fibers, the fibers fired well above background discharges and undoubtedly provided information about the prey. However, using less rigorous statistical analysis (1 S.D. above spontaneous rate) did not greatly extend the maximum detection distance. It remains possible that small, transient fluctuations in lateral line activity that were outside our resolution ability encode for prey distance and extend detection distance. However, the response dynamics of the silent fibers, which did not fire in the absence of stimulus, provide support for the limited range of the lateral line as these fibers failed to fire until the prey approached within 11 cm.

Predator–prey interactions are also consistent with lateral line sensitivity recorded in toadfish. Although behavioral observations are limited to discerning when the predator visibly reacts to the prey rather than when detection occurs, they provide further support for close-range detection. Year one toadfish (8 cm SL; 10 cm TL) feeding on small guppies (2 cm SL) during daylight trials did not launch strikes at prey that were greater than 5 cm from the toadfish (Fig. 10; Price and Mensinger, 1999). These attacks were probably mediated by both visual and mechanical cues, and reaction distances under low light conditions would be predicted to be shorter and more accurate indicators of lateral line range. Ongoing studies in juvenile toadfish indicate that both reaction distance and attack range are less in the dark than the light (L. Lundeen and A. F. Mensinger, unpublished) and are consistent with studies by Enger et al. (1989), New and Kang (2000) and Richmond et al. (2004) that found that predatory fish without visual cues reacted to prey at distances of less than 50% of the predator's body length.

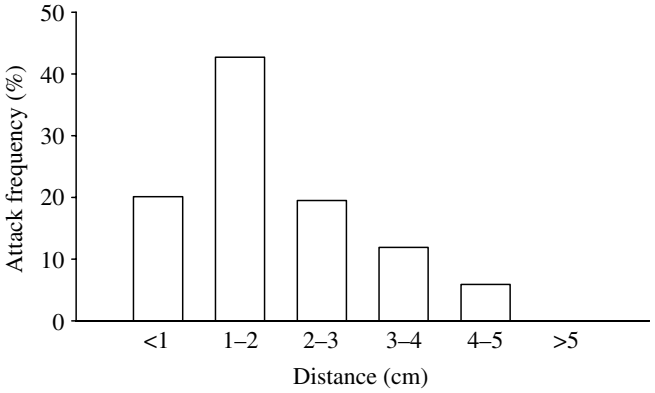

Fig. 10. The frequency of attacks by 8 cm SL toadfish at 2 cm SL guppies is plotted *versus* the distance between the two fish at the time the toadfish launched its attack. The total number of attacks analyzed was 78. Modified from Price and Mensinger (1999).

Although the lateral line can contribute significant information for prey localization, due to its short range it is evident that other systems are important in locating distant prey. The far hydrodynamic fields of moving objects are hypothesized to be mediated by the inner ear (Kalmijn, 1988). Additionally, behavioral and physiological experiments have illustrated that tactile, chemosensory, hydrodynamic and visual stimuli are capable of guiding prey capture (Montgomery et al., 2002).

The oyster toadfish possesses an anterior lateral line dominated by superficial neuromasts (Clapp, 1898). Although behavioral experiments in other species have indicated that prey detection and localization is mediated by canal neuromasts (Coombs et al., 2001), it appears that the afferent fibers innervating the superficial neuromasts of the toadfish anterior lateral line are responsive to the stimuli produced by prey. Consequently, although canal neuromasts may provide important prey localization information, superficial neuromasts are able to detect the low-frequency water displacements generated by prey and contribute to near-field prey detection.

Efferent innervation of lateral line hair cells has been hypothesized to inhibit afferent firing (Russell and Roberts,

1974) and prevent depletion of transmitter from lateral line hair cells during locomotion or rapid movements (Russell, 1971). Tricas and Highstein (1990) illustrated that the lateral line experienced transient inhibition when toadfish were allowed to view live *Fundulus* in an adjacent aquarium and that in a minority of fibers there was a decrease in neural activity in anterior lateral line afferent fibers during a predatory strike. However, recent studies have illustrated the ability of hair cells to release neurotransmitters for prolonged periods with little exhaustion (Moser and Beutner, 2000; Trussell, 2002). Evidence of efferent modulation (reduction or cessation of nerve activity) was not observed during any trial. The length of our trials and the inclusion of intermittent mechanosensory stimulation may have occluded our ability to detect efferent modulation. However, the lack of inhibition of toadfish neuromasts located near the operculum that were continually stimulated by opercular displacement (present study) or prolonged water current (Palmer and Mensinger, 2004) appears to indicate that efferent modulation was not common in our sample population.

Both silent and irregular fibers experienced a dramatic increase in firing during a predatory strike. It is possible that self-generated noise created during a predatory strike may be filtered by higher order neurons. Montgomery and Bodznick (1994) indicate that the lateral line medullary nuclei contain an adaptive filter capability that cancels inputs consistently associated with an animal's own movements. Further experiments are required to determine decisively whether the lateral line conveys self-regulatory information during a predatory strike.

In summary, the toadfish lateral line can detect transient water displacement generated by natural prey. The distance at which stimulation occurred was less than 40% of toadfish body length. This is the first study that investigates the neural response of the anterior lateral line to prey stimuli in free-ranging fish and highlights the importance of the lateral line in near-field prey detection.

The authors wish to thank Tom Hrabik and John Nicols for helpful criticisms of the manuscript, and Beth Giuffrida for her analysis assistance. We are especially indebted to Mark Grosenbaugh for his assistance with the digital particle tracking velocimetry. This work was supported by a University of Minnesota Grant in Aid and by the Minnesota Sea Grant College Program supported by the NOAA Office of Sea Grant, United States Department of Commerce, under grant No. NOAA-NA16-RG1046. The U.S. Government is authorized to reproduce and distribute reprints for government purposes, not withstanding any copyright notation that may appear hereon. This paper is journal reprint (512) of the Minnesota Sea Grant College Program.

References

Anderson, E. J., McGillis, W. R. and Grosenbaugh, M. A. (2001). The boundary layer of swimming fish. *J. Exp. Biol.* **204**, 81-102.

Bleckmann, H. and Topp, G. (2003). Surface wave sensitivity of the lateral line organs of the top minnow *Aplocheilus lineatus*. *Naturwissenschaften* **68**, 624-625.

Blickhan, R., Krick, C., Zehren, D., Nachtigall, W. and Breithaupt, T. (1992). Generation of a vortex chain in the wake of a subundulatory swimmer. *Naturwissenschaften* **79**, 220-221.

Braun, C. B. and Coombs, S. (2000). The overlapping roles of the inner ear and lateral line: the active space of dipole source detection. *Phil. Trans. R. Soc. Lond. B* **355**, 1115-1119.

Chrobot, R. J. (1959). The feeding habits of the toadfish (*Opsanus tau*) based on an analysis of the contents of the stomach and intestine. Master's thesis. University of Maryland.

Clapp, C. (1898). The lateral line system of *Batrachus tau*. *J. Morph*. **15**, 223-265.

Coombs, S. (1999). Signal detection theory, lateral-line excitation patterns and prey capture behaviour of mottled sculpin. *Anim. Behav.* **58**, 421-430.

Coombs, S., Braun, C. B. and Donovan, B. (2001). The orienting response of Lake Michigan mottled sculpin is mediated by canal neuromasts. *J. Exp. Biol.* **204**, 337-348.

Denton, E. J. and Gray, J. (1983). Mechanical factors in the excitation of clupeid lateral lines. *Proc. R. Soc. Lond. B.* **218**, 1-26.

Drucker, E. G. and Lauder, G. V. (2001). Locomotor function of the dorsal fin in teleost fishes: experimental analysis of wake forces in sunfish. *J. Exp. Biol.* **204**, 2943-2958.

Enger, P. S., Kalmijn, A. J. and Sand, O. (1989). Behavioral investigations on the functions of the lateral line and inner ear in predation. In *The Mechanosensory Lateral Line – Neurobiology and Evolution* (ed. S. Coombs, H. Munz and P. Gorner), pp. 265-285. New York: Springer-Verlag.

Gibb, A. C., Jayne, B. C. and Lauder, G. V. (1994). Kinematics of pectoral fin locomotion in the bluegill sunfish *Lepomis macrochirus*. *J. Exp. Biol.* **189**, 133-161.

Gudger, E. W. (1910). Habits and life history of the toadfish (*Opsanus tau*). *Bull. U.S. Bur. Fish.* **28**, 1071-1099.

Hanke, W. and Bleckmann, H. (2004). The hydrodynamic trails of *Lepomis gibbosus* (Centrarchidae), *Colomesus psittacus* (Tetraodontidae) and *Thysochromis ansorgii* (Cichlidae) investigated with scanning particle image velocimetry. *J. Exp. Biol.* **207**, 1585-1596.

Hanke, W., Brucker, C. and Bleckmann, H. (2000). The ageing of the low-frequency water disturbances caused by swimming goldfish and its possible relevance to prey detection. *J. Exp. Biol.* **203**, 1193-1200.

Kalmijn, A. J. (1988). Hydrodynamic and acoustic field detection. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 83-130. New York: Springer-Verlag.

Kanter, M. J. and Coombs, S. (2003). Rheotaxis and prey detection in uniform currents by Lake Michigan mottled sculpin (*Cottus bairdi*). *J. Exp. Biol.* **206**, 59-70.

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

McDermott, J. J. (1964). Food habits of the toadfish, *Opsanus tau* (L.), in New Jersey waters. *J. Penn. Acad. Sci.* **38**, 64-71.

Mensinger, A. F. and Deffenbaugh, M. (1998). Prototype rechargeable tag for acoustical neural telemetry. *Biol. Bull*. **195**, 194-195.

Mensinger, A. F. and Deffenbaugh, M. (2000). Anechoic aquarium for ultrasonic neural telemetry. *Phil. Trans. R. Soc. Lond. B* **355**, 1305-1308.

Mensinger, A. F., Price, N. N., Richmond, H. E., Forsythe, J. W. and Hanlon, R. T. (2003). Mariculture of the oyster toadfish: juvenile growth and survival. *N. Am. J. Aquacult.* **65**, 289-299.

Montgomery, J. C. and Bodznick, D. (1994). An adaptive filter that cancels self-induced noise in the electrosensory and lateral line mechanosensory systems of fish. *Neurosci. Lett*. **174**, 145-148.

Montgomery, J. C. and Macdonald, J. A. (1987). Sensory tuning of lateral line receptors in Antarctic fish to the movements of planktonic prey. *Science* **235**, 195-196.

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

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

Montgomery, J. C., Macdonald, F., Baker, C. F. and Carton, A. G. (2002). Hydrodynamic contributions to multimodal guidance of prey capture behavior in fish. *Brain Behav. Evol*. **59**, 190-198.

Moser, T. and Beutner, D. (2000). Kinetics of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse of the mouse. *Proc. Natl. Acad. Sci. USA* **97**, 883-888.

Muller, H. M. (1996). Indications for feature detection with the lateral line organ in fish. *Comp. Biochem. Physiol.* **114A**, 257-263.

New, J. G. and Kang, P. Y. (2000). Multimodal sensory integration in the strike-feeding behaviour of predatory fishes. *Phil. Trans. R. Soc. Lond. B* **355**, 1321-1324.

Palmer, L. M. and Mensinger, A. F. (2004). The effect of the anesthetic tricaine (MS-222) on nerve activity in the anterior lateral line of the oyster toadfish, *Opsanus tau*. *J. Neurophysiol.* **92**, 1034-1041.

Palmer, L. M., Giuffrida, B. A. and Mensinger, A. F. (2003). Neural recordings from the lateral line in free-swimming toadfish, *Opsanus tau*. *Biol. Bull.* **205**, 216-218.

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

Phillips, R. R. and Swears, S. B. (1979). Social hierarchy, shelter use, and avoidance of predatory toadfish (*Opsanus tau*) by the striped blenny (*Chasmodes bosquianus*). *Anim. Behav*. **27**, 1113-1121.

Pohlmann, K., Atema, J. and Breithaupt, T. (2004). The importance of the lateral line in nocturnal predation of piscivorous catfish. *J. Exp. Biol.* **207**, 2971-2978.

Price, N. N. and Mensinger, A. F. (1999). Predator–prey interactions of juvenile toadfish, *Opsanus tau*. *Biol. Bull.* **197**, 246-247.

Richmond, H. E., Hrabik, T. and Mensinger, A. F. (2004). Light intensity, prey detection and foraging mechanisms of age-0 year yellow perch. *J. Fish Biol*. **65**, 195-205.

Russell, I. J. (1971). The role of the lateral-line efferent system in *Xenopus laevis*. *J. Exp. Biol.* **54**, 621-641.

Russell, I. J. and Roberts, B. L. (1974). Active reduction of lateral-line sensitivity in swimming dogfish. *J. Comp. Physiol*. **94**, 7-15.

Saunders, A. J. and Montgomery, J. C. (1985). Field and laboratory studies of the feeding behaviour of the piper *Hyporhamphus ihi* with reference to the role of the lateral line in feeding. *Proc. R. Soc. Lond B* **224**, 209-221.

Schwartz, F. J. and Dutcher, B. W. (1963). Age, growth and food of the oyster toadfish near Solomons, Maryland. *Trans. Am. Fish. Soc*. **92**, 170-173.

Tricas, T. C. and Highstein, S. M. (1990). Visually mediated inhibition of lateral line primary afferent activity by the octavolateralis efferent system during predation in the free- swimming toadfish, *Opsanus tau*. *Exp. Brain Res.* **83**, 233-236.

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

Trussell, L. O. (2002). Transmission at the hair cell synapse. *Nature Neurosci*. **5**, 85-86.

van Netten, S. M. (1991). Hydrodynamics of the excitation of the cupula in the fish canal lateral line. *J. Acoust. Soc. Am.* **89**, 310-319.

Weissert, R. and von Campenhausen, C. (1981). Discrimination between stationary objects by the blind cave fish *Anoptichthys jordani* (Characidae). *J. Comp. Physiol.* **143**, 375-381.

Wilson, C. A., Dean, J. M. and Radtke, R. (1982). Age, growth rate and feeding habits of the oyster toadfish, *Opsanus tau* (Linnaeus) in South Carolina. *J. Exp. Mar. Biol. Ecol*. **62**, 251-259.

Wubbels, R. J., Kroese, A. B. and Schellart, N. A. (1993). Response properties of lateral line and auditory units in the medulla oblongata of the rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **179**, 77-92.

Zar, J. H. (1984). *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall.