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The directional hearing abilities of two species of bamboo sharks

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Summary

Auditory evoked potentials (AEPs) were used to measure the directional hearing thresholds of the white-spotted bamboo shark *Chiloscyllium plagiosum* and the brown-banded bamboo shark *Chiloscyllium punctatum* at four frequencies and seven directions, using a shaker table designed to mimic the particle motion component of sound. Over most directions and frequencies there were no significant differences in acceleration thresholds, suggesting that the sharks have omni-directional hearing abilities. Goldfish *Carassius auratus* were used as a baseline to compare a species with specialized hearing adaptations *versus* sharks with no known adaptations, and were found to have more sensitive directional responses than the sharks. Composite audiograms of the sharks were created from the average of all of the directions at each frequency and were compared with an audiogram obtained for *C. plagiosum* using a dipole stimulus. The dipole stimulus audiograms were significantly lower at 50 and 200 Hz compared to the shaker audiograms in terms of particle acceleration. This difference is hypothesized to be a result of the dipole stimulating the macula neglecta, which would not be stimulated by the shaker table.

Key words: directional hearing, auditory evoked potentials, elasmobranch, *Chiloscyllium plagiosum*, *Chiloscyllium punctatum*, acceleration.

Introduction

The ability to localize a sound in fishes is very important for the detection of prey and predators, and in some cases for communication. However, the physics of underwater sound present many problems for directional hearing in fishes. Sound travels approximately five times faster underwater compared to in air. The presence of an external ear for detecting sound (Batteau, 1967), as well as having widely separated ears allowing for the detection of time-of-arrival differences (Thompson, 1882), are adaptations that help land animals orient to a sound source. The ears of fishes, on the other hand, are very close together and have no external meatus. Also, most fishes can only detect lower frequency sounds (<1000 Hz), which have very long wavelengths (Fay, 1988). Higher frequencies (>1000 Hz) have very short wavelengths, which could potentially be used to determine directionality by the difference in phases detected between the ears, but only a few families of bony fishes can detect sounds at high frequencies (Astrup and Møhl, 1993; Astrup and Møhl, 1998; Mann et al., 1996; Mann et al., 1998; Mann et al., 2001). These differences present problems for fishes in trying to localize a sound source. However, the sensory hair cells of the inner ears are arranged in distinct patches with the same directional orientation (Flock, 1964; Popper, 1977) and the otoliths are also angled in different planes (Lu and Popper, 1998). There also appears to be a higher level of neural directional processing that has been found along the pathways between the auditory nerve and the brain of some species of bony fishes (Edds-Walton and Fay, 2002; Edds-Walton and Fay, 2003). These features allow for directional sensitivity along the axis of acoustic particle motion, but the extent and importance of this is only known in a few bony fishes and in no elasmobranchs.

Directional hearing abilities have been measured in a variety of teleost fishes, but have been largely ignored in elasmobranchs. One behavioral experiment (Nelson, 1967) showed that the lemon shark *Negaprion brevirostris* could differentiate between speakers with an error of only 9.5° at a distance of ~2.1 m. Sharks have also been attracted from large distances in response to high levels of erratically pulsed sounds in the field, most likely necessitating directional sensitivity (Nelson and Gruber, 1963; Richard, 1968; Myrberg, Jr et al., 1969; Nelson et al., 1969; Nelson and Johnson, 1972; Myrberg, Jr et al., 1972; Myrberg, Jr, 1978). Several researches have suggested that sharks should be able to detect and localize sounds using both their otoconia as well as the non-otolithic macula neglecta (Corwin, 1981; Corwin, 1989). Due to the dorsal/ventral polarization of the hair cells in the macula neglecta (Corwin, 1978; Corwin, 1981, Corwin, 1983; Barber et al., 1985), it has been hypothesized that elasmobranchs could detect sounds from above the head through the parietal fossa region using the macula neglecta, and from all directions using the otoconia in the saccule and utricle. This differential

detection could aid sharks in determining the location of a sound stimulus.

We have previously measured the hearing thresholds of two species of sharks using a dipole stimulus (mechanical shaker with a plastic ball attached to a metal rod) rather than the more commonly used monopole underwater speaker as the sound stimulus (Casper and Mann, 2007). We found that with the dipole stimulus located above the shark's head, significantly lower thresholds were obtained compared with monopole experiments (Casper and Mann, 2006). One hypothesis from this set of experiments was that sharks could better detect sounds from above the head than when the stimulus was anterior to the shark, supporting the idea of the macula neglecta being a specialized organ for detecting sounds (including hydrodynamic stimuli) above the shark.

A shaker table has been used for measuring directional hearing abilities in several species of teleosts (Fay, 1984; Lu et al., 1996; Fay and Edds-Walton, 1997a; Fay and Edds-Walton, 1997b; Lu et al., 1998; Edds-Walton et al., 1999; Ma and Fay, 2002; Edds-Walton and Fay, 2003). This method applies directional whole body accelerations to stimulate the inner ears of fishes. As the fish body is being shaken, structures of greater density than the surrounding tissues, such as the inner ear otoliths (or otoconia in sharks), lag relative to the rest of the fish body. This lag results in a shearing of the attached hair cells, thereby stimulating the auditory system. The shaker setup is unique in that it recreates the effects of a sound stimulus with only the particle motion component of the sound and no sound pressure.

The goal of these experiments was to determine (1) if sharks are better able to detect sounds from one particular direction, and (2) whether a dipole stimulus produces a stronger evoked potential response than whole-body acceleration. The directional hearing abilities of two species of sharks, the white-spotted bamboo shark *Chiloscyllium plagiosum* and the brown-banded bamboo shark *Chiloscyllium punctatum*, were measured using a shaker table. These two species were chosen due to their demersal life style, making them ideal for experiments in which they must remain motionless for long periods of time. Particle acceleration thresholds were measured for seven different directions and four different frequencies using auditory evoked potentials. Finally, hearing measurements were made using a dipole stimulus with *C. plagiosum* to compare thresholds to those obtained with whole-body acceleration. It was hypothesized that thresholds would be lower with the dipole stimulus because the macula neglecta, which is not mass-loaded, would not respond to whole body acceleration, but would to the dipole stimulus.

Materials and methods

Two juvenile *Chiloscyllium punctatum* Müller and Henle 1838 (16.2–18 cm total length) and four juvenile *Chiloscyllium plagiosum* Bennett 1830 (17–18.4 cm total length) were maintained in aquaria on 12 h:12 h light:dark cycles and fed squid. Two goldfish *Carassius auratus* Linnaeus 1758 (6 cm total length) were also run for comparison with the sharks. Hearing experiments were conducted at the University of South Florida, College of Marine Science and followed the guidelines for the care and use of animals approved by the Institutional Animal Care and Use Committee at University of South Florida protocol #2118.

Shaker table setup

The directional hearing experiments were performed on top of a vibration isolation table (Vibraplane 5602; Kinetic Systems, Boston, MA, USA) with four vibration, isolation mounts (Tech Products Corporation, Dayton, OH, USA; model #52512) underneath to minimize low frequency vibrations.

A fish was placed in an aluminum dish (20.5 cm diameter, 5 cm deep) and restrained with plastic fasteners that looped through mounting bases affixed to the bottom of the dish. The plastic fasteners were tight enough to stop any movements without affecting the breathing of the fish. As the shark's head was only 2 cm high, it was completely submerged below the water level. The dish was held in place by four custom-built electromagnetic shakers surrounding the outside of the dish, with a fifth, mechanical shaker positioned below the dish (mini-shaker type 4810; Brüel and Kjaer, Naerum, Denmark). The electromagnetic shakers were constructed from four rod-shaped magnets (#R2000D, Ni-Cu-Ni plated, 5 cm×1.2 cm; Amazing Magnets, Irvine, CA, USA), which were equal distances apart and were held in place by smaller disk-shaped magnets (1.4 cm diameter×0.4 cm thick) on the inside of the dish. The external rod magnets were held in the center of spools of coiled wire that were attached to stainless steel plates. The stainless steel plates were in turn attached to the vibration isolation table (Fig. 1).

Each electromagnetic shaker was connected to an 8 Ω power resistor to keep the coiled wire from overheating. Standard speaker wires connected the resistor and then led back to an amplifier. The four electromagnetic shakers were used to deliver stimuli in the horizontal (*X*–*Y*) plane. In order to drive the dish in the *Z* direction (up and down), the mechanical shaker was screwed into the isolation table below the dish. A nylon screw was threaded into the shaker and a small piece of neoprene was glued to the top of the screw. The bottom of the dish rested on the screw.

Calibration of the acceleration signals

Two dual-axis (*X* and *Y* directions) accelerometers (Dimension Engineering, Akron, OH, USA; ADXL320 buffered ±5 g accelerometer, 312 mV g^{-1} sensitivity) were glued perpendicular to each other to create one three dimensional accelerometer for calibrating the accelerations in the *X*, *Y* and *Z* directions (Fig. 2A). The accelerometer was attached to the bottom of the shaker dish with double-sided tape so that it would be exposed to the same accelerations as the dish and the fish. A laser vibrometer (CLV1000; Polytec, Waldbronn, Germany) was used to calibrate the accelerometer recordings.

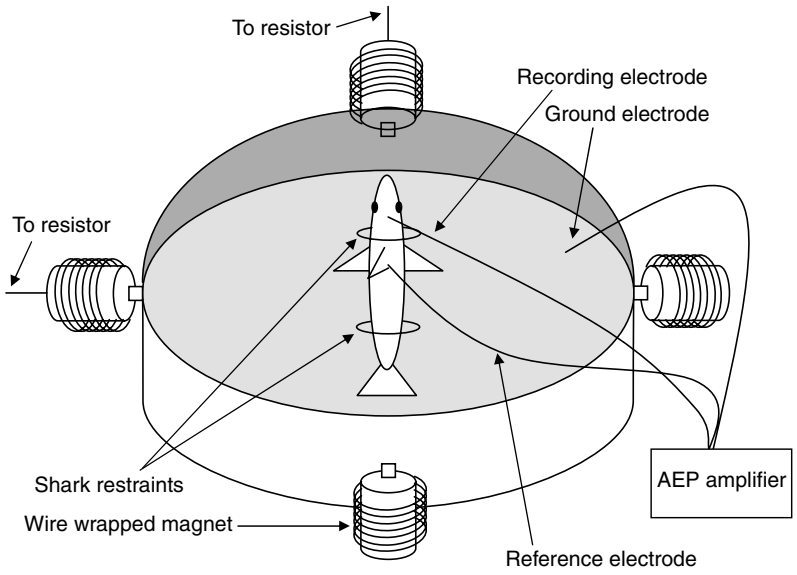

Fig. 1. Diagram of the directional shaker table setup. The fifth, mechanical shaker, which produces the up/down motion (*Z*-axis) of the dish, is located under the dish and not visible in this picture. Drawing not to scale.

Directional hearing threshold experiments

Hearing thresholds were measured using Auditory Evoked Potentials (AEP) and follow similar methods as previously (Casper and Mann, 2006; Casper and Mann, 2007). Wire electrodes (12 mm length, 28 gauge low-profile needle electrode; Rochester Electro-Medical, Inc., Tampa, FL, USA) were placed subdermally 1 cm posterior to the endolymphatic pores in sharks (recording electrode), in the dorsal musculature 3 cm anterior to the dorsal fin (reference electrode), and free in the water (ground electrode). In the goldfish, the recording electrode was placed above the cerebellum, the reference electrode was placed in the dorsal musculature, and the ground electrode was free in the water. The electrodes were connected to a TDT pre-amplifier (HS4, Tucker Davis Technologies, Gainesville, FL, USA), which was then connected by a fiber-optic cable to a TDT evoked potential workstation (System 2) with TDT BioSig software.

A MATLAB program was created to produce the accelerations while simultaneously recording the evoked potentials from the fishes. The program was designed to allow manipulations of both the amplitude and phase of the signal so that the accelerations were focused on the desired direction. The software displayed the time domain and frequency domain (Fast Fourier Transform; FFT) of the acceleration signal as well as the time and frequency domains of the AEP being recorded from the fish in order to monitor that the appropriate frequency was being presented and detected.

Frequencies tested included 20, 50, 100 and 200 Hz. Higher frequencies above this were tested (300, 400 and 1000 Hz) and yielded no AEPs. All accelerations were pulsed tones that were 400 ms in duration with a 100 ms cosine squared gated window. Signals were delivered at 2.22 presentations per second. Accelerations were attenuated in 6 dB steps, beginning at the highest level that could be generated at each frequency. The AEP waveforms were digitized at 25 kHz and averaged between 100–1000 times. More averages are needed as the signal moves closer to the threshold in order to pull the signal out of the AEP noise floor (Fig. 2B).

Seven different directions were tested for each species of shark. These include 0° (*X*-axis), 90° (*Y*-axis), 30°, 60°, up and down (*Z*-axis), and the directional vectors between *X*- and *Z*-axes and *Y*- and *Z*-axes.

A 2048-point FFT was used to analyze the AEP signals in the frequency domain. An AEP was determined to be present if the signal showed a doubling of the sound frequency (e.g. a 400 Hz peak when the signal played was 200 Hz) with a peak at least 3 dB above the AEP noise floor (Fig. 2C). The AEP noise floor is estimated from the AEP power spectrum with a window of 100 Hz around the doubling frequency (i.e. 50 Hz on each side of the peak). This frequency doubling occurs in all low frequency fish AEP testing (Mann et al., 2001; Egner and Mann, 2005; Casper and Mann, 2006; Casper and Mann, 2007).

Dipole hearing measurements

Hearing measurements were also conducted in *C. plagiosum* with a dipole stimulus. This species was chosen for the dipole hearing experiments because it was hardier than *C. punctatum* and could survive repeated testing. The methods and analysis follow the same methodology as in a previous dipole hearing experiment (Casper and Mann, 2007). In brief, the dipole stimulus consisted of a mechanical shaker (Brüel and Kjaer mini-shaker type 4810) with a stainless steel tube (27 cm long, 0.4 cm diameter) that was threaded at one end into the shaker and had a PVC ball (1.3 cm diameter) attached to the other end. Dipole hearing experiments were conducted in a sound isolation booth (2.44 m×2.44 m×2.23 m) in a large, fiberglass tank (1.96 m×0.95 m×0.60 m) with a water depth of 0.5 m. The tank sat on top of a wood pallet separated from the floor of the booth by four vibration isolation mounts (Tech Products Corporation model #52512).

Each subject was wrapped in a fine nylon mesh. These holders were tightened with metal binder clips that were tight enough to keep the shark from moving, but did not affect breathing. The shark was suspended by PVC pipe with a binder clip attached to one end. The PVC pipe was firmly attached to an aluminum bar held above the tank. The sharks were suspended 20 cm below the surface of the water. The electrodes and their placement were identical to the directional hearing experiments. The mechanical shaker (Brüel and Kjaer mini-shaker type 4810) was attached to another aluminum bar suspended independently from the experimental tank by PVC pipes attached to the walls of the booth.

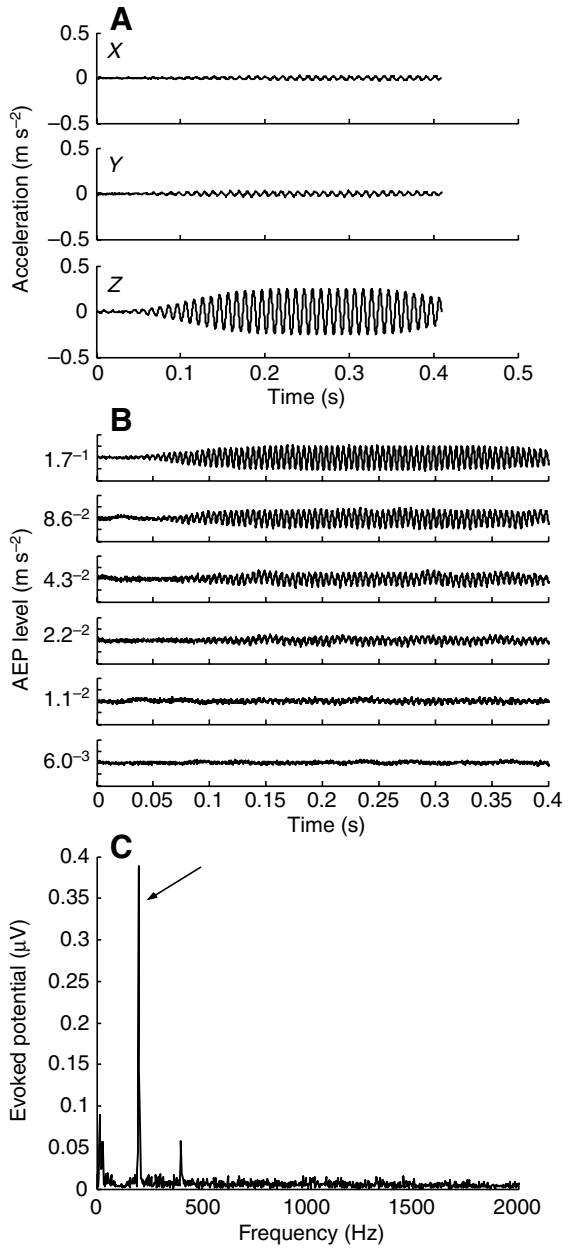

Fig. 2. (A) Acceleration raw signals for a stimulus directed in the *Z* direction (up/down) as recorded from the three-dimensional accelerometer. (B) Auditory evoked potentials (AEPs) from the white-spotted bamboo shark *Chiloscyllium plagiosum*, in response to a 100 Hz signal at six signal levels. As the signal is decreased in acceleration level (m s^{-2}) the AEP signal also decreases until it is lost in the noise at 6.0^{-3} m s^{-2}. (C) 2048-point Fast Fourier Transform (FFT) of the same AEP for the shark in response to a 100 Hz sound. The arrow indicates the frequency doubling peak, which occurs at 200 Hz.

BioSig software (Tucker-Davis Technologies) was used for the hearing experiments. All sounds were pulsed tones that were 50 ms in duration and shaped with a Hanning window (25 ms rise and fall time). Sounds above 20 Hz were delivered with a 70 ms presentation period (14 s^{-1}), while 20 Hz sounds had a 1000 ms presentation period (1 s^{-1}). Test frequencies ranged from 20 Hz to 200 Hz (20, 50, 100, 200 Hz). Sounds were attenuated in 6 dB steps, beginning at the highest level that could be generated at each frequency. The AEP waveforms were digitized at 25 kHz and averaged between 100 and 1000 times. Positive detection of the signals was determined using the same methods as in the directional hearing experiments (see above).

Following all hearing tests the fish was removed and replaced with a pressure/velocity probe (Uniaxial Pressure/Velocity Probe, Applied Physical Sciences Corporation, Groton, CT, USA) that was positioned where the head of the fish had been. The probe contained a velocity geophone (sensitivity 212 mV cm^{-1} s^{-1}, bandwidth 10–1 kHz) and a hydrophone (sensitivity: –176 dB re. 1 V/μPa, bandwidth 10–2 kHz), which could simultaneously record sound pressure and particle velocity. Calibration with the geophone was performed in all orientations [0° horizontal (*X*-axis), 90° horizontal (*Y*-axis) and vertical (*Z*-axis)] and all calibrations were computed as the Root Mean Square (RMS) for the magnitude of the three axes combined. The hydrophone was omni-directional and therefore did not need to be measured along different axes. Many researchers have suggested that the hair cells in the inner ear of fishes act as an accelerometer and therefore detect acoustic particle acceleration (Kalmijn, 1988; Fay and Edds-Walton, 1997a; Braun et al., 2002; Bass and McKibben, 2003). Therefore, all audiograms have hearing thresholds shown in units of particle acceleration (m s^{-2}). Particle velocity of tonal signals can be converted to acceleration with the following equation: acceleration= velocity×(2π×frequency). The acceleration thresholds are also given as a function of the magnitude of the three (*X*, *Y*, *Z*) directions measured. Background noise was also measured and was consistently below 10^{-7} m s^{-2}.

Data analysis

Particle acceleration thresholds were log transformed to satisfy assumptions of normality. A repeated-measures ANOVA was used to measure differences between species of sharks. Since no differences were detected, the species were pooled and a repeated-measures ANOVA was used to compare the differences between directions among each of the frequencies, and a Tukey *post-hoc* comparison was used if the ANOVA showed significant differences. The repeated-measures ANOVA with a Tukey *post-hoc* test was also used to test differences between the white-spotted bamboo thresholds obtained with the shaker and those obtained with the dipole stimulus over all frequencies tested.

Results

Particle acceleration thresholds were obtained from both species of bamboo sharks over all seven directions (Fig. 3). There was no significant difference between species of sharks (P=0.42), therefore the species were pooled together for testing differences between frequencies and directions. There was no

significant difference between directions for each of the individual sharks (P=0.06). There was a significant interaction among direction and frequency, but a Tukey *post-hoc* test revealed no significant difference among hearing thresholds at any of the directions tested for any of the species (P>0.05).

The thresholds of all directions at each frequency were averaged together to create a composite shaker audiogram for each of the species (Fig. 4). The sharks had their best thresholds at the lowest frequencies with increasing thresholds as the frequencies increased. The dipole audiogram for the *C. plagiosum* yielded significantly lower thresholds than audiograms acquired from the shaker stimuli (P=0.018). At 20 Hz and 50 Hz the dipole particle acceleration mean thresholds were more than 10 times lower than the particle acceleration thresholds obtained with the shaker. A Tukey *post-hoc* multiple comparisons test showed differences were statistically significant at 50 Hz (P=0.045) and 200 Hz (P=0.001), but not at 20 Hz and 100 Hz. Thresholds were lower for *C. auratus* at all frequencies except 100 Hz compared to the sharks.

Discussion

These directional hearing experiments are the first physiological measurements of directional hearing thresholds in elasmobranch fishes. These results suggest that the ear of *C. plagiosum* is an omnidirectional particle acceleration sensor (Kalmijn, 1988), as there were no significant differences among thresholds in each of the different directions (Fig. 3). These results are consistent with studies on hair cell polarities in elasmobranch fishes (Barber and Emerson, 1980; Corwin, 1981). An examination of the winter skate, *Raja ocellata*, showed a wide range of hair cell polarities depending on the endorgan (Barber and Emerson, 1980). The utricular macula had most cells in the anterior/posterior directions with some at varying degrees towards the dorsal/ventral directions. The saccular macula was predominantly in the dorsal/ventral directions with a few cells in the anterior/posterior directions. The lagenar macula showed varying angles towards the dorsal/ventral directions. It should be noted that the macular sensory area of each endorgan is not typically flat, but more often curved and angled in specific directions. This is particularly apparent in *Negaprion brevirostris*, in which the saccular macula was an S-shaped structure following the contours of the bottom of the saccule (Corwin, 1981). Based on this distinct shape it appeared that the hair cell polarizations of *N. brevirostris* cover all directions, which would contribute to successful directional hearing abilities.

The dipole hearing thresholds are significantly lower than the majority of other elasmobranchs (Banner, 1967; Kelly and Nelson, 1975; Casper and Mann, 2006; Casper and Mann, 2007). This result suggests that near-field sounds coming from above the shark should yield lower thresholds than other directions (previous monopole hearing experiments in elasmobranchs had sounds directed from the anterior). However, the whole-body acceleration data clearly show that

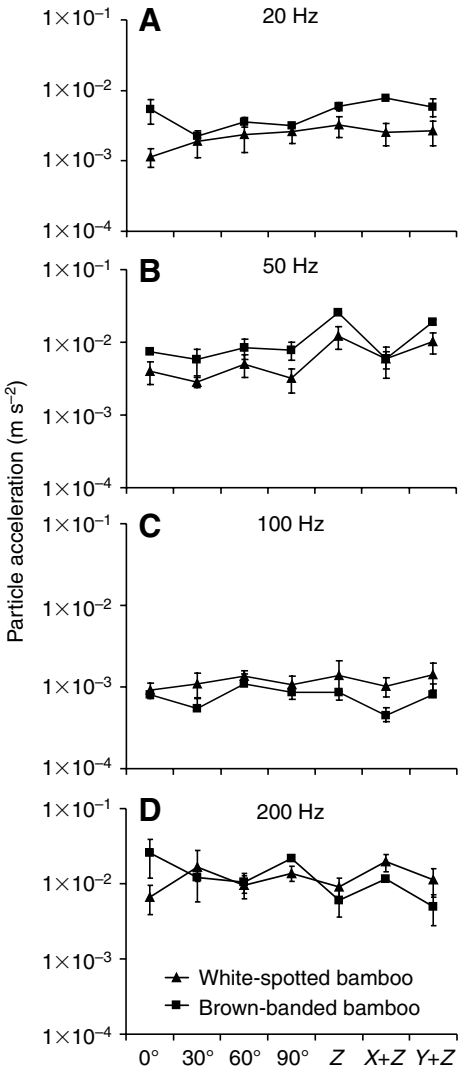

Fig. 3. Directional hearing thresholds for the white-spotted bamboo shark *Chiloscyllium plagiosum* (N=4) and the brown-banded bamboo shark *Chiloscyllium punctatum* (N=2), for each of the seven directions (see text) measured at (A) 20 Hz, (B) 50 Hz, (C) 100 Hz and (D) 200 Hz. Values are means ± s.e.m. There was no significant difference between any of the directions at any of the frequencies except at 50 Hz for interactions between the *Z* and 30° directions and the *Z* and 90° directions.

there is no specific direction that yields consistently lower hearing thresholds than the others (Fig. 3). The likely explanation for this involves the method of stimulation in each experiment. The directional hearing experiments use a shaker table to produce whole-body accelerations of the sharks. As the shark's body is being accelerated back and forth, structures of greater density than the surrounding tissues, such as the otoconia, lag relative to the rest of the shark body. This causes a shearing of the hair cells, thus stimulating the ear. This method of stimulation will only function as long as there is a density differential to create this lag. In the case of the macula neglecta, the hair cells are not mass-loaded with otoconia, but

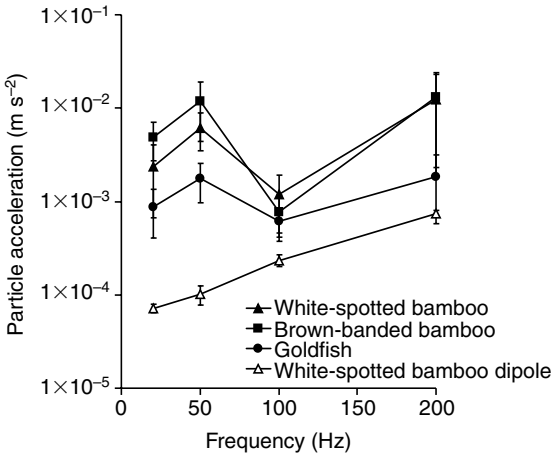

Fig. 4. Composite directional shaker audiograms of the white-spotted bamboo shark *Chiloscyllium plagiosum* (*N*=4), the brown-banded bamboo shark *Chiloscyllium punctatum* (*N*=2), and the goldfish *Carassius auratus* (*N*=2). These audiograms are compiled from the average of all of the thresholds at each of the directions for each frequency tested. Values are means ± s.e.m. Also plotted is the dipole audiogram for *C. plagiosum* (*N*=4) to compare responses from different stimuli. The dipole thresholds were significantly lower than the directional shaker thresholds at 50 and 200 Hz for *C. plagiosum*.

have a gelatinous cupula similar to the hair cells of the lateral line organs and semicircular canal cristae. This cupula likely would not be affected by the accelerations as its density is not large enough to create a lagging effect, and like the lateral line cupula, would need fluid flow in the posterior canal duct for movement to occur. Therefore, in the shaker experiments, it is highly likely that the sacculus, utricle and lagena were being stimulated, but the macula neglecta was not.

One of the conclusions drawn from the shark dipole hearing experiments (Casper and Mann, 2007) is that the dipole stimulus creates a strong, localized velocity fluid flow from the vertical movement of the plastic ball. This fluid flow would be directed towards the parietal fossa, where it would create a fluid flow in the posterior canal duct where the macula neglecta is located. Fluid flow within this canal across the cupula of the macula neglecta would cause a movement of the cupula, thereby shearing the hair cells and stimulating the endorgan. Based on the significantly lower thresholds observed in the dipole experiments, it appears that the macula neglecta is more sensitive than the other endorgans to localized flow (Fig. 4). However, if the macula neglecta is responding to particle velocity from fluid flow and the otoconia-based endorgans are responding to particle accelerations then there cannot be any direct comparison between thresholds. It is also possible that the dipole stimulated the lateral line. The thresholds from the vibrational hearing experiments (Fig. 4) are also closer to other monopole shark audiograms (Banner, 1967; Kelly and Nelson, 1975; Casper and Mann, 2006), suggesting that these experiments were only stimulating the otoconia.

Similar directional hearing experiments were conducted on the goldfish *Carrassius auratus*, which has specialized Weberian ossicles that transmit the sound pressure detected by the swim bladder as particle motion to the inner ears. However, because the shaker table does not produce an appreciable sound pressure, *C. auratus* should be only exposed to particle motion putting it on a level 'hearing' field as the sharks. Interestingly, *C. auratus* appears to have lower hearing thresholds at all frequencies, except 100 Hz, than the sharks, even though the swim bladder has been theoretically neutralized by the lack of sound pressure in the experiment. Two hypotheses for the lower thresholds could be mass loading by the Weberian ossicles, and the composition of the otoliths in *C. auratus versus* the otoconia in elasmobranchs. The otoliths in teleosts are generally composed of a solid calcium carbonate matrix, while elasmobranch otoconia are calcium carbonate, with exogenous siliceous material, in a gelatinous matrix. It has been suggested that ears with otoliths of a higher density are more sensitive to accelerations (Lychakov, 1990; Lychakov and Rebane, 2005). Therefore, the solid, dense otoliths of *C. auratus* should result in a more sensitive ear than the less dense, gelatinous otoliths of sharks. Elasmobranchs can add to the density of their otoconia through the passive uptake of exogenous particles through the endolymphatic ducts (Stewart, 1906; Nishio, 1926; Fänge, 1982; Vilches-Troya et al., 1984; Hanson et al., 1990; Lychakov et al., 2000), but it is doubtful that they would be able to compensate enough to equal the acoustic abilities of a solid structure like a dense otolith. The hearing of *C. auratus* was measured in another shaker table experiment (Fay, 1984) at 140 Hz, with thresholds ranging from 7.74×10^{-7} m s^{-2} for the most sensitive neurons to 7.74×10^{1} m s^{-2} for the least sensitive neurons. This range falls about the data obtained in the current experiment for *C. auratus* evoked potentials at 100 Hz at 6.14×10^{-3} m s^{-2}.

These experiments provide the first physiological evidence that elasmobranchs detect sounds from all directions. Similar thresholds were obtained at each of the directions tested, which suggests that the these sharks have omnidirectional ears, and this is further supported by previous anatomical studies on the inner ear hair cell polarities (Barber and Emerson, 1980; Corwin, 1981). Composite audiograms obtained from the average of all seven directions shows that the *C. auratus* had lower thresholds than *C. plagiosum* and *C. punctatum*. Based on the lower thresholds obtained from the dipole experiment with *C. plagiosum*, it is likely that the directional shaker only stimulated the acceleration-sensitive otoconia end organs (sacculus, utricle and lagena) of the inner ear and not the cupula-loaded macula neglecta, offering further evidence that the macula neglecta is most likely a velocity sensitive endorgan. These results are consistent with measurements showing that sharks are not as sensitive to sounds in the far-field, which would likely not stimulate the macula neglecta (Casper and Mann, 2006).

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