Field hearing measurements of the Atlantic sharpnose shark *Rhizoprionodon terraenovae*

B. M. Casper*† and D. A. Mann

College of Marine Science, University of South Florida, 140 7th Avenue South, St Petersburg, FL 33701, U.S.A.

(Received 24 November 2008, Accepted 5 October 2009)

Field measurements of hearing thresholds were obtained from the Atlantic sharpnose shark *Rhizoprionodon terraenovae* using the auditory evoked potential method (AEP). The fish had most sensitive hearing at 20 Hz, the lowest frequency tested, with decreasing sensitivity at higher frequencies. Hearing thresholds were lower than AEP thresholds previously measured for the nurse shark *Ginglymostoma cirratum* and yellow stingray *Urobatis jamaicensis* at frequencies <200 Hz, and similar at 200 Hz and above. *Rhizoprionodon terraenovae* represents the closest comparison in terms of pelagic lifestyle to the sharks which have been observed in acoustic field attraction experiments. The sound pressure levels that would be equivalent to the particle acceleration thresholds of *R. terraenovae* were much higher than the sound levels which attracted closely related sharks suggesting a discrepancy between the hearing threshold experiments and the field attraction experiments.

Key words: auditory evoked potential; field attraction; threshold.

INTRODUCTION

A wide range of experiments have been conducted to examine the hearing abilities of elasmobranchs. Field attraction experiments have found that certain species of sharks are attracted to low frequency (20–1000 Hz), erratically pulsed sounds from distances up to several hundred metres (Nelson & Gruber, 1963; Richard, 1968; Myrberg *et al*., 1969, 1972; Nelson *et al*., 1969; Nelson & Johnson, 1972; Myrberg, 1978). The sounds played in these experiments were probably at higher levels than most auditory stimuli that sharks would be exposed to in their natural environments (Richard, 1968; Myrberg, 1978; Kalmijn, 1988). Also, the intensity of the sounds was recorded in reference to the sound pressure component of sound, which is not what sharks can hear. Only some bony fishes with swimbladders and hearing specializations are able to detect sound pressure, whereas all others, including

*Author to whom correspondence should be addressed. Tel.: +1 727 631 2098; fax: +1 301 314 9358; email: bcasper@umd.edu

†Present address: Biology Department & Center for Comparative and Evolutionary Biology of Hearing, University of Maryland, 2217 Biology-Psychology Building, College Park, MD 20742, U.S.A.
elasmobranchs, can only detect the particle motion component of sound (acceleration, velocity and displacement). Without measuring acoustic particle motion it is not possible to characterize the signals to which the sharks respond.

The anatomy of the elasmobranch inner ear is well studied (Tester et al., 1972; Corwin, 1977) and the pathways by which sound travels from the environment to the ear have been hypothesized (Tester et al., 1972; Fay et al., 1974; Corwin, 1977, 1981). Elasmobranchs have two proposed methods of detecting sound: (1) the otolithic pathway, involves direct detection of the particle acceleration component of sound via the inner ear otoconia: the sacculus, utricle and lagena (though the lagena is primarily assumed to respond to angular accelerations and not serve an auditory purpose). The density of the shark’s body is approximately the same as the surrounding water; therefore, sound travels through the body until it comes in contact with a structure of differing density. The otoconia are denser than the surrounding tissues and lag in response to the sound causing a shearing of the sensory hair cells attached to these structures. This shearing causes stimulation of the hair cells and thus acoustic detection. (2) The non-otolithic pathway, involves the fourth inner ear endorgan, the macula neglecta. The macula neglecta differs in that it has a cupula overlying the sensory hair cells rather than an otoconia and thus does not have a mass-loaded structure of greater density for stimulation. It is believed that the macula neglecta is a particle velocity detector designed to detect sounds from above the shark’s head, based on the dors-ventral polarization of the sensory hair cells (Corwin, 1978, 1981, 1983; Barber et al., 1985; Lovell et al., 2007). The macula neglecta is located in the posterior canal duct in the dorsal portion of the ear just under an area of loose connective tissue, the parietal fossa. As an animal swims over the top of an elasmobranch’s head, it creates a strong velocity flow that travels through the parietal fossa and into the posterior canal duct via the fenestrae ovalis. This velocity flow causes the fluid in the posterior canal duct to move across the macula neglecta, moving the cupula and shearing the sensory hair cells.

Audiograms have also been acquired of several species of elasmobranchs (Kritzler & Wood, 1961; Olla, 1962; Banner, 1967; Nelson, 1967; Kelly & Nelson, 1975; Casper et al., 2003; Casper & Mann, 2006) and other ancient fishes (Lovell et al., 2005), though few were measured in terms of particle acceleration (Banner, 1967; Kelly & Nelson, 1975; Casper & Mann, 2006). Determining elasmobranch audiograms in terms of sound pressure can indicate the frequency ranges that these species can detect, but it provides no data as to how well they can detect these sounds, if they do not detect sound pressure. Also, the majority of audiograms have been measured from demersal elasmobranchs, except for the lemon shark Negaprion brevirostris (Poey) (Banner, 1967). The majority of sharks that were observed in the field attraction experiments were pelagic. Thus, the lack of hearing data on these species makes it difficult to compare elasmobranch hearing thresholds and the sound levels to which sharks were attracted.

The goals of this study were to measure field hearing thresholds of the piscivorous Atlantic sharpnose shark Rhizoprionodon terraenovae (Richardson) and relate the thresholds to other elasmobranch audiograms. Several members of the same genus, Rhizoprionodon porosus (Poey), were observed in the attraction experiments (Richard, 1968; Myrberg et al., 1969). Therefore, this audiogram will provide a relevant comparison to the sound levels used in the field attractions experiments and will represent the first time that elasmobranch hearing has been measured in the field.
MATERIALS AND METHODS

SHARK HEARING THRESHOLDS

Three juvenile *R. terraenovae* (39–50 cm total length, $L_T$) were caught with hook and line off the beach at Little Gasparilla Island, Florida, U.S.A. (26° 50' N; 82° 17' W) during July 2006. Upon capture, each shark was quickly transported in a cooler to a dock area where the hearing experiments were conducted. Hearing experiments followed the guidelines for the care and use of animals approved by the Institutional Animal Care and Use Committee at the University of South Florida protocol #2118. The auditory evoked potential (AEP) methods described follow a similar protocol as previous elasmobranch AEP tests (Casper & Mann, 2006).

Each subject was placed in stiff plastic mesh holders (2.54 cm x 2.54 cm mesh size). These holders were tightened with tie wraps that were tight enough to keep the fish from moving but did not affect breathing. Pieces of nylon rope were attached to either end of the plastic mesh and the fish was suspended in the water 2 m below a section of the dock (water depth = 3 m). The dock was 10 m from a mangrove fringed habitat. The substratum below the dock consisted of mud, with sparse sea grass. The water temperature was 32° C with a salinity of 34. The transducer (Aquasonic Tactile Sound Underwater Speaker AQ339, Clark Synthesis; www.clarksynthesis.com) was hung with nylon rope from a different area of the dock 2.75 m from the fish’s head.

Wire electrodes (12 mm length, 28 gauge low-profile needle electrode, Rochester Electro-Medical, Inc.; www.rochestermed.com) were placed subdermally 1 cm posterior to the endolymphatic pores (recording electrode), in the dorsal musculature 3 cm anterior to the dorsal fin (reference electrode) and free in the water (ground electrode). The electrodes were connected to a TDT pre-amplifier (HS4, Tucker Davis Technologies; www.tdt.com) which was then connected by a fibre-optic cable to a TDT System III evoked potential workstation with TDT BioSig software.

All sounds were pulsed tones that were 50 ms in duration and shaped with a Hanning window. Sounds above 20 Hz were delivered with a 70 ms presentation period (14 per s), while 20 Hz sounds had a 1000 ms presentation period (1 per s). Test frequencies ranged from 20 to 2000 Hz (20, 50, 100, 200, 300, 400, 800, 1000 and 2000 Hz). Sounds were attenuated in 6 dB steps beginning at the loudest level that could be generated at each frequency. The AEP waveforms were digitized at 25 kHz and averaged between 100 and 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.

A 2048-point fast Fourier transform (FFT) was used to analyse the AEP signals in the frequency domain. The entire 70 ms window was FFT-transformed, because for many of the lower frequencies that were tested the AEP signal took up the entire window. This was done at every frequency for the analysis to remain consistent. An AEP was determined to be present if the recorded 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. 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 & Mann, 2005; Casper & Mann, 2006). Thresholds were determined as the lowest sound level that produced an AEP.

Upon completion of the experiment, each fish was measured and released. For calibration a pressure and velocity probe (Uniaxial Pressure/Velocity Probe, Applied Physical Sciences Corporation; www.aphysci.com) was positioned in the same location where the head of the fish had been. The probe contained a velocity geophone (sensitivity 212 mV cm$^{-1}$s$^{-1}$, bandwidth 10 Hz to 1 kHz) and a hydrophone (sensitivity: $-176$ dB re 1 V $\mu$Pa$^{-1}$, bandwidth 10 Hz to 2 kHz), which could simultaneously record sound pressure and particle velocity (Table I). Calibration with the geophone was performed in all orientations [$0^\circ$ horizontal (x-axis), $90^\circ$ 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 inner ear of fishes act as an accelerometer and therefore detect acoustic particle acceleration (Kalmijn, 1988; Fay & Edds-Walton, 1997; Braun et al., 2009).
Table I. Example of the sound pressure levels measured by the pressure and velocity probe and the associated particle acceleration levels that were converted from the recorded particle velocities. These levels represent the loudest sounds which were produced by the underwater transducer at each frequency.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Sound pressure levels (dB re 1 μPa)</th>
<th>Particle acceleration (m s(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>83.3</td>
<td>1.3 \times 10^{-3}</td>
</tr>
<tr>
<td>50</td>
<td>115.3</td>
<td>2.3 \times 10^{-3}</td>
</tr>
<tr>
<td>100</td>
<td>112.9</td>
<td>3.7 \times 10^{-3}</td>
</tr>
<tr>
<td>200</td>
<td>111.5</td>
<td>9.8 \times 10^{-3}</td>
</tr>
<tr>
<td>300</td>
<td>108.3</td>
<td>6.4 \times 10^{-3}</td>
</tr>
<tr>
<td>400</td>
<td>117.7</td>
<td>1.9 \times 10^{-2}</td>
</tr>
<tr>
<td>500</td>
<td>115.4</td>
<td>1.3 \times 10^{-2}</td>
</tr>
<tr>
<td>600</td>
<td>101.3</td>
<td>1.0 \times 10^{-2}</td>
</tr>
<tr>
<td>800</td>
<td>110.0</td>
<td>3.3 \times 10^{-2}</td>
</tr>
<tr>
<td>1000</td>
<td>120.5</td>
<td>3.0 \times 10^{-2}</td>
</tr>
</tbody>
</table>

2002; Bass & 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 \times (2 \times \pi \times frequency). The acceleration thresholds are also given as a function of the magnitude of the three (x, y, z) directions measured.

RESULTS

SHARK HEARING THRESHOLDS

Rhizoprionodon terraenovae AEP showed frequency doubling where the frequency of the AEP was about twice the stimulus frequency as seen in previous AEP elasmobranch studies (Casper & Mann, 2006). The best sensitivity for this fish was at the lowest frequency, 20 Hz. The R. terraenovae audiogram had a similar shape as other elasmobranch audiograms with most sensitive hearing at low frequencies (20, 50 and 100 Hz) and increasing thresholds with increasing frequency (Fig. 1). Ambient noise recordings were consistently around 1 \times 10^{-3} m s\(^{-2}\) (Fig. 1) and were below the lowest thresholds detected by the fish.

DISCUSSION

The audiogram for R. terraenovae is the second audiogram recorded from the family Carcharhinidae in terms of particle motion and the first audiogram to be collected in the field for any species of elasmobranch. The first carcharhinid audiogram was determined by Banner (1967) for N. brevirostris (Fig. 2). Kritzler & Wood (1961) measured the hearing thresholds of the bull shark Carcharhinus leucas (Müller & Henle), also a Carcharhinid, using only a pressure hydrophone. These thresholds are only in terms of sound pressure and therefore cannot be interpreted in particle motion. Responses ranged from 100 to 1400 Hz referenced to an unspecified noise level.
The *R. terraenovae* audiogram shared a similar shape and frequency response with other elasmobranch audiograms that were obtained using AEP methods at ≥200 Hz (Casper & Mann, 2006) (Fig. 2). Below 200 Hz *R. terraenovae* had lower thresholds than previously measured AEP audiograms. The results, however, are different from audiograms obtained using classical conditioning methods (Banner, 1967; Kelly & Nelson, 1975). *Rhizoprionodon terraenovae* has lower thresholds than the horn shark *Heterodontus francisci* (Girard) (Kelly & Nelson, 1975) at all frequencies, although it should be noted that many of the lower frequencies tested in *H. francisci* were probably masked by ambient noise levels (Fig. 2). *Negaprion brevirostris* had lower thresholds than *R. terraenovae* at 20 Hz but higher thresholds at all other frequencies tested (Banner, 1967).

Previous research has suggested that the macula neglecta inner ear endorgan is a low frequency particle velocity detector in elasmobranchs (Casper & Mann, 2006).
Corwin (1978) found that the silky shark *Carcharhinus falciformis* (Müller & Henle) (reported species *menisorrah*) had a significantly larger macula neglecta with a greater number of sensory hair cells than any other species of elasmobranchs suggesting enhanced hearing abilities for more active, piscivorous elasmobranchs. If this pattern holds for *R. terraenovae* then this could help to explain the lower thresholds observed in this species at 100 Hz, and presumably lower frequencies, compared with demersal elasmobranchs such as the nurse shark *Ginglymostoma cirratum* (Bonnaterre) and yellow stingray *Urobatis jamaicensis* (Cuvier) which also have had hearing measured with AEP (Casper & Mann, 2006) and *H. francisci* using classical conditioning methods (Fig. 2). However, it is not clear whether simply increasing the number of hair cells will increase sensitivity. It should also be noted that the ambient noise levels in the *R. terraenovae* experiment were about three orders of magnitude higher than the *G. cirratum* and *U. jamaicensis* experiments ($c.1 \times 10^{-3}$ m$^{-2}$ s$^{-2}$ c.$1 \times 10^{-6}$ m s$^{-2}$), though this did not appear to affect the hearing thresholds as *R. terraenovae* had lower thresholds (Fig. 1).

Using the equation: $p = \rho c v$, where $p$ is pressure (Pa), $\rho$ is density of medium (1030 kg m$^{-3}$), $c$ is speed of sound in the medium (1500 m s$^{-1}$) and $v$ is particle velocity (m s$^{-1}$), the equivalent far-field sound pressure levels that would be associated with a given particle velocity level (determined from the particle acceleration values) can be estimated for the *R. terraenovae* thresholds (Fig. 3). These far-field pressure-based threshold estimates of *R. terraenovae* (Fig. 3) can be compared with the sound levels that were produced during the field attraction studies in which

![Fig. 3. The sound pressure needed to produce particle accelerations equivalent to the *Rhizoprionodon terraenovae* audiogram in a plane propagating wave (■) and this same audiogram shifted by a critical bandwidth to compare with broadband noise signals (□). The sound pressure levels of broadband noise used in two field attraction experiments in which members of the *Rhizoprionodon* genus were attracted are plotted for comparison, suggesting that *R. terraenovae* would not have been able to detect the sound stimuli being presented. Distances from the sound source to the hydrophone for measurements of sound pressure level were 1 m for Richard (1968) (○) and the *R. terraenovae* experiment, while they were made at 18.5 m for Myrberg *et al.* (1969) (×).](image-url)
R. porosus was observed (Richard, 1968; Myrberg et al., 1969). The R. terraenovae audiograms were shifted by an estimated critical bandwidth that is assumed to be 10% of the centre frequency, *i.e.* the threshold has been lowered by $10 \times \log$ (Yost, 2000; Egner & Mann, 2005), for direct comparison to the broadband noise stimuli used in the field attraction studies. Based on the sound pressure thresholds of R. terraenovae, it appears that the sound levels from the field attraction experiments were well below the hearing thresholds of the species (Fig. 3). A similar discrepancy was observed between measured hearing thresholds and the reported sound levels in which fish were observed to be attracted to in a previous experiment with G. cirratum (Casper & Mann, 2006).

There are several possible explanations for these observed discrepancies with R. terraenovae. It has been suggested that AEP measurements can underestimate hearing thresholds in fishes compared with behavioural training methods (Mann et al., 2001). This could help to explain the lower thresholds observed in *N. brevirostris* compared with R. terraenovae. Another possible explanation could be that other stimuli could have attracted fishes to the testing locations. In many of the test trials in one of the experiments (Richard, 1968), sharks appeared to be following other fishes into the test area. Several of these fishes, including snappers and groupers, have swimbladders and probably can detect lower level sounds than R. terraenovae. It has also been observed in many of the field attraction experiments that the sharks responded erratically with agitated behaviour close to the speakers, which was probably due to the strong electromagnetic fields being produced by these speakers. This erratic behaviour would provide another strong visual stimulus to which other sharks could respond from greater distances.

Future experiments could attempt to recreate the field attraction studies in an attempt to remove external stimuli that could also result in the attraction of the sharks. The underwater speaker can be shielded using a Faraday cage that would remove any electromagnetic field being produced doing the sound production. This electromagnetic field is probably what caused R. porosus to behave erratically as they approached the speaker. A second modification to the experiments would be to conduct them at night. This would presumably remove any visual stimulus that sharks outside the testing area could use to follow other sharks’ behaviours to the testing area. The movement patterns of the sharks could still be monitored using satellite or other tracking means. It would also be useful to measure the sound field created by the underwater speakers using a particle motion sensitive sensor as this is the component of sound that sharks detect. If the sharks are still observed in the testing area then this would provide further evidence that sharks are attracted by these underwater sounds.

We would like to thank J. Locascio and the residents of Little Gasparilla Island for assistance in obtaining the sharks. This research was partially funded by the University of South Florida Riggs Endowed Fellowship and the University of South Florida Tampa Bay Parrothead Fellowship. Thanks to M. Heupel for assistance in species identification.

**References**


© 2009 The Authors

Journal compilation © 2009 The Fisheries Society of the British Isles, *Journal of Fish Biology* 2009, 75, 2768–2776


