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RESEARCH ARTICLE

Underwater hearing in the loggerhead turtle (*Caretta caretta*): a comparison of behavioral and auditory evoked potential audiograms

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SUMMARY

The purpose of this study was to compare underwater behavioral and auditory evoked potential (AEP) audiograms in a single captive adult loggerhead sea turtle (*Caretta caretta*). The behavioral audiogram was collected using a go/no-go response procedure and a modified staircase method of threshold determination. AEP thresholds were measured using subdermal electrodes placed beneath the frontoparietal scale, dorsal to the midbrain. Both methods showed the loggerhead sea turtle to have low frequency hearing with best sensitivity between 100 and 400 Hz. AEP testing yielded thresholds from 100 to 1131 Hz with best sensitivity at 200 and 400 Hz (110 dB re. 1 μPa). Behavioral testing using 2 s tonal stimuli yielded underwater thresholds from 50 to 800 Hz with best sensitivity at 100 Hz (98 dB re. 1 μPa). Behavioral thresholds averaged 8 dB lower than AEP thresholds from 100 to 400 Hz and 5 dB higher at 800 Hz. The results suggest that AEP testing can be a good alternative to measuring a behavioral audiogram with wild or untrained marine turtles and when time is a crucial factor.

Key words: bioacoustics, auditory brainstem response, sound, Testudines, anthropogenic noise.

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INTRODUCTION

There are seven species of marine turtle globally, all threatened by increasing human presence in marine environments. In the Northwest Atlantic, loggerhead turtles (*Caretta caretta*) are threatened by interactions with commercial fisheries, including those using trawl nets, longlines and gillnets, as well as by direct harvesting, vessel collisions, marine debris, pollution, predation and the effects of beach development and erosion (National Marine Fisheries Service and US Fish and Wildlife Service, 2008).

There is growing concern about the effects of increasing anthropogenic noise and the potential impact of behavioral changes and physical harm to all marine animals (National Research Council, 2005; Richardson et al., 1995; Popper and Hastings, 2009). For marine vertebrates, we have the greatest amount of data on hearing in fishes and marine mammals, including audiograms (e.g. Wolksi et al., 2003; Finneran and Houser, 2006; Casper and Mann, 2006), short-term effects of air-guns on hearing (Kastak et al., 2005; Popper et al., 2005; Finneran et al., 2005; Lucke et al., 2009) and behavioral responses to sound (Nowacek et al., 2007).

The impacts of anthropogenic noise in the marine environment are poorly understood in marine turtles partly because of limited studies addressing their auditory ability. Much of what is known about marine turtle hearing is inferred from results obtained in terrestrial and semi-aquatic turtles. Auditory evoked potential (AEP) testing, in which electrodes are used to detect voltages generated by the brain in response to acoustic stimuli, supports findings that terrestrial turtles have low-frequency hearing with best sensitivity below 1 kHz (Adrian et al., 1938; Wever and Bray, 1931; Wever and Vernon, 1956a; Wever and Vernon, 1956b; Wever and Vernon, 1956c). Terrestrial turtles are also able to behaviorally respond to sound stimuli (Andrews, 1915; Patterson, 1966) and even learn complex behaviors like maze navigation in response to directional sound cues (Lenhardt, 1981).

Early observations of the marine turtle tympanic membrane left doubt that marine turtles were capable of hearing because of the thickness and rigidity of the membrane (DeBurlet, 1934). However, further anatomical investigations found that the marine turtle ear is capable of low-frequency aerial and bone conduction hearing (Wever, 1978; Lenhardt et al., 1985). Behavioral responses to seismic air guns in a closed canal have also been observed in free-swimming loggerhead turtles (O'Hara and Wilcox, 1990). Behavioral responses in the form of bodily movement, head retraction and limb extension were noted in response to vibrational stimuli in loggerhead and Kemp's ridley turtles (*Lepidochelys kempii*) (Lenhardt et al., 1983).

Electrophysiological testing of marine turtles has confirmed previous speculation that the ear is designed to detect low-frequency stimuli. Cochlear potentials in response to aerial and mechanical stimuli showed that the juvenile green turtle (*Chelonia mydas*) could detect frequencies between 60 and 1000 Hz with best sensitivity between 300 and 500 Hz (Ridgway et al., 1969). Hearing thresholds of juvenile and subadult loggerhead turtles measured by recording auditory brainstem responses elicited through vibrational clicks and tone bursts showed a hearing range from 250 to 1000 Hz with best sensitivity at 250 Hz, which was the lowest frequency tested (Bartol et al., 1999). Both of these studies were conducted with the animals

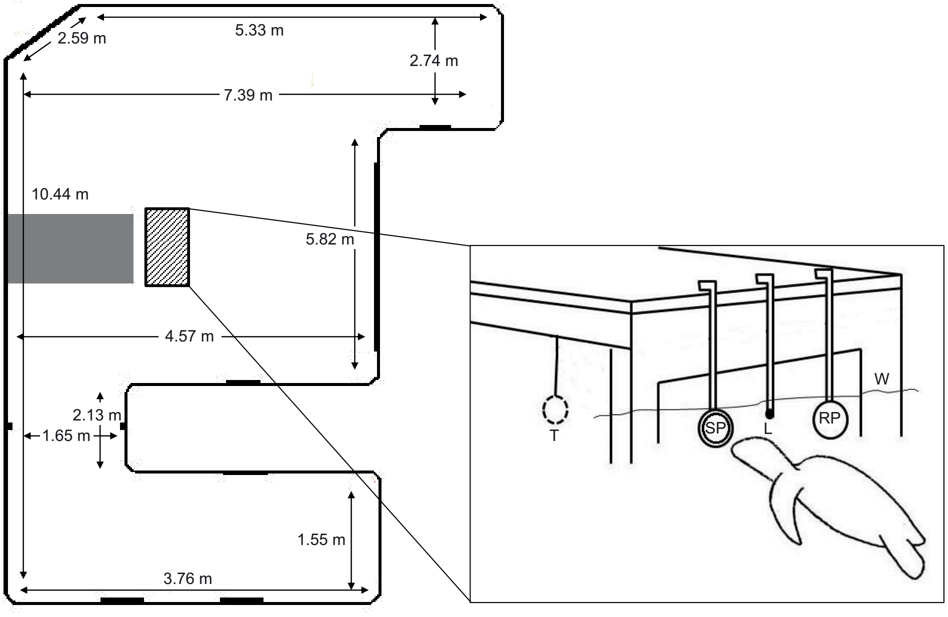

Fig. 1. Experimental design for the collection of behavioral audiogram data indicating locations of the transducer (T), located 1 m from subject, station paddle (SP), indicator light (L), response paddle (RP) and water level (W). Testing took place in the center of a 66,000 liter, irregularly shaped enclosure with the indicated dimensions.

removed from the water. The hearing of green and Kemp's ridley turtles measured with test subjects located at the water surface with stimuli presented from an aerial speaker found that green turtles were able to detect frequencies from 100 to 800 Hz with best sensitivity at 200–700 Hz, and Kemp's ridley turtles detected a narrower range of frequencies (100–500 Hz) with maximum sensitivity between 100 and 200 Hz (Bartol and Ketten, 2006).

The objective of this study was to measure the underwater audiogram of a fully submerged captive loggerhead turtle using both behavioral and AEP methods. Comparing behavioral and AEP audiograms from the same individual will help develop a better understanding of marine turtle hearing and ground-truth future results obtained through electrophysiological methods with animals that cannot be trained for the purposes of behavioral audiogram assessment.

MATERIALS AND METHODS

Subject

Behavioral and AEP data were collected from a single adult female loggerhead turtle *Caretta caretta* (Linnaeus 1758). The 31-year-old test subject weighed approximately 91 kg and was housed with a second adult female loggerhead of the same size and age at Mote Marine Laboratory and Aquarium in Sarasota, FL, USA. The subject was fed a mixed diet of approximately 680 g of squid and capelin in addition to vitamins and calcium supplements daily. Feeding took place twice daily and the subject typically consumed 60% of its food during behavioral testing sessions. Prior to behavioral audiogram testing, the subject spent 1 year undergoing basic husbandry training. This training conditioned the animal to voluntarily participate in medical procedures, including blood sampling and weight measurements. All methods and procedures were reviewed and approved by Mote Marine Laboratory IACUC (07-04-KM1) and the Florida Fish and Wildlife Conservation Commission (MTP #126).

Testing location

Testing was conducted in the habitat in which the subject lived. The test subject was housed in a 66,000 l, closed-system concrete tank. The water temperature was maintained between 25 and 28°C by a heating and cooling unit. The irregularly shaped tank had one large, acrylic viewing window (approximately 3×1 m) and five small viewing windows (approximately 0.5×0.5 m). Water depth was approximately 1.25 m throughout the entire enclosure. A training platform, from which testing was conducted, was suspended above the water (Fig. 1). A small portion of the tank could be separated by a PVC gate to isolate the second turtle in the enclosure and exclude behavioral interference during testing. Testing was conducted before aquarium visiting hours to reduce the amount of noise and disruption. Pumps operating on the testing tank and adjacent turtle tanks were turned off during testing to reduce ambient noise. It was necessary to leave life support systems for the nearby manatee and dolphin habitats running during testing, which may have contributed to background noise.

Training apparatus and equipment

Training was conducted from a platform in the center of the largest portion of the enclosure with 2 m between the test subject and the nearest tank wall. The response apparatus and transducer were suspended separately from the training platform (Fig. 1). Two acrylic paddles, a station and a response paddle, hung from the end of the platform nearest the middle of the tank. The response paddle was positioned 0.75 m to the right of the station paddle. The station paddle was used as a starting location for each trial. An LED light, potted in epoxy and clear PVC, was hung between the two paddles to indicate the start of a trial. A transducer (Aquasonic AQ39, Clark Synthesis, Littleton, CO, USA) was suspended from the middle of the platform, 1 m in front of the turtle at a depth of 0.5 m. During sound presentation, the turtle was positioned directly in line with the transducer, with the ear approximately 25 cm below the water's surface. The trainer was positioned above the test subject on the platform. Stimulus generation and recording were performed with hardware by Tucker-Davis Technologies (TDT) using SigGen and BioSig software (Tucker-Davis Technologies, Alachua, FL, USA).

Training and data collection

Training for the collection of the behavioral audiogram data began in September 2007. Operant conditioning utilizing positive reinforcement was used to establish all behaviors for both research and husbandry purposes. Training took 1 year and data collection began in September 2008. A go/no-go paradigm (Schusterman, 1980) was used to assess sound detection. During pre-trial periods and no-go trials, the turtle was trained to remain still with the beak positioned directly in front of the station paddle. During go trials, the turtle indicated stimulus detection by pressing the response paddle. An LED light was used to indicate the start of a trial. An overhead light indicated an actively running session. The overhead light was turned off to indicate an incorrect response and cue the turtle to return to the station to start the next trial. This light remained off at all times outside of training sessions.

The experimental setup required a trainer positioned on the research platform above the apparatus to ensure that the turtle was properly positioned in front of the station paddle and to reinforce the correct responses. To prevent trainer bias or cueing, the trainer wore headphones to mask experimental tones. On the opposite side of the tank, an assistant operated the TDT station to control stimulus presentation and to indicate correct or incorrect responses. The assistant indicated whether the subject was correct or incorrect using the overhead light. When left on, the subject was correct and received reinforcement. When turned off, the response was incorrect, no reinforcement was received and the animal returned to the station paddle for the next trial.

The turtle was trained to approach the station paddle on command and remain stationary. Once positioned and still, the trainer cued the assistant for the start of a randomized go (signal present) or no-go (signal absent) trial. The LED flashed for 1 s to indicate to the subject that the trial had started. During a go trial, a 2 s tonal stimulus immediately followed the LED signal. If detected by the subject, she swam to the response paddle and pressed it with her beak within 4 s of the termination of the tone. If correct, an immediate audible secondary reinforcer (a recording of a training clicker) was presented through the speaker followed by food primary reinforcement. After the food reinforcement, the subject returned to the station paddle for the start of the next trial. If incorrect (subject did not press the response paddle), the overhead light was turned off for 6 s, indicating an incorrect response. When the overhead light was turned on, the subject returned to the station paddle for the start of the next trial. For no-go trials, the subject refrained from pressing the response paddle for 6 s following the LED to indicate the absence of signal detection. If correct, the subject received reinforcement and the next trial began. If incorrect (turtle pressed the response paddle), the overhead light was turned off for 6 s and this was scored as a false alarm.

Daily sessions were initiated with warm-up blocks to gauge subject motivation and performance. Warm-up blocks consisted of four trials: two go trials and two no-go trials. Trial order was randomly assigned and the signal frequency and intensity were of known detectable level. The subject had to complete one warm-up block with at least 75% correct before testing began. If the first warm-up block was passed, testing began immediately. If it was not passed, an additional warm-up block was run with as many as four warm-up blocks taking place. If the fourth warm-up block was not passed, the day was considered for training purposes only, and the results were not counted towards threshold determination.

Stimulus levels were kept constant throughout a 10-trial block. Blocks were divided evenly into five go trials and five no-go trials. The order was counterbalanced based on Gellermann scales (Gellermann, 1933). A block was considered passed if the subject successfully responded to 60% or more of the go trials and the false alarm rate remained at or below 40%. Each successful block resulted in a 6 dB attenuation of the stimulus intensity until a block was failed. Upon the first failed block, the signal was increased by 6 dB until the next passed block. Each positive-to-negative or negative-to-positive change in intensity constituted a reversal. This modified staircase method (Schusterman and Balliet, 1971) continued in 6 dB steps, with one block serving as a step size, until a minimum of 10 reversals was achieved. Blocks were excluded from data analysis under three conditions: (1) the false alarm rate for the block exceeded 40%; (2) an interruption occurred mid-block, i.e. severe weather, human interference in the exhibit or loud outside noise; or (3) failure of warm-up trials, resulting in the testing session being used for training purposes only.

Threshold determination

Testing of each frequency was conducted over several weeks. Blocks resulting in reversals generally occurred over a period of days. Threshold was determined by averaging the intensities of blocks in which reversals occurred. Averages were calculated with an equal number of ‘failed’ reversals (reversals in which the subject did not pass the necessary go trial criteria) and ‘passed’ reversals (reversals which occurred when the subject passed the go trial criteria and intensity was decreased in the following block) (Fig. 2). These thresholds were calculated over 10–12 reversals. This process was repeated at 50, 100, 200, 400, 800 and 1131 Hz.

Sound pressure levels (SPLs) were measured and calibrated with a hydrophone (HTI-96 min, High Tech, Gulfport, MS, USA) placed at the turtle’s approximate tympanic location at the end of each test session. SPL recording during the test session was not possible because of the risk of damage from biting. A particle motion calibration was also carried out at each frequency with a six-hydrophone (HTI-96 min) array and three TDT RP2.1 modules sampling at 24,414 Hz. Each hydrophone was located on a frame so that pairs of hydrophones were 0.2 m apart. The center of the calibrated cube was located at the position of the turtle’s head during testing. The data were analyzed by first high-pass filtering with a six-pole Butterworth filter at 30 Hz to remove low-frequency noise. The time domain signals were then subtracted from each other in pairs (front–back, up–down and left–right) and the pressure gradient calculated as the root mean square level divided by the distance between hydrophones. The particle acceleration ($\mathrm{mm\,s^{-2}}$) was calculated as the pressure gradient divided by the seawater density ($1035\,\mathrm{kg\,m^{-3}}$). The particle acceleration magnitude was calculated by squaring each of the three directions, summing and taking the square root. The SPL at each location was not the same, so the particle acceleration was converted to the expected level that would be recorded in response to a received level of 100 dB re. 1 μPa.

AEP experimental setup

AEP testing was conducted in the same tank location and at the same depth used for behavioral testing. Two testing sessions were completed, one on 27 January 2009 and one on 24 July 2009. The January AEP testing session was conducted during the early stages of behavioral data collection and tested a wider range of frequencies. The July data set was collected near the end of behavioral testing and was limited to the same frequencies tested during behavioral testing.

During testing, the subject was placed on a canvas medical stretcher and secured to a restraint board to minimize movement during testing. The subject offered negligible resistance during testing, which minimized any noise detected in the response due to electrical impulses from muscle movement. Two animal handlers remained in the water with the animal to ensure that it remained positioned in front of the transducer, to assist if the

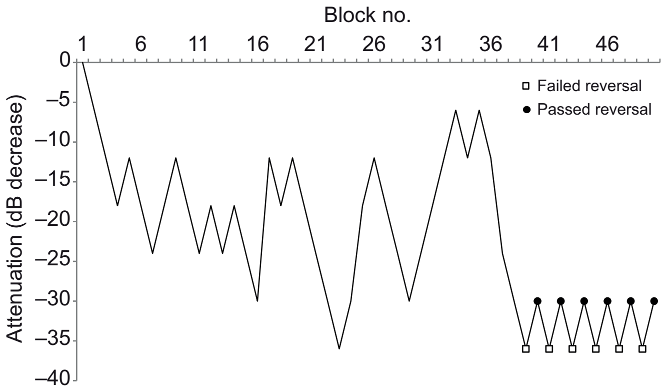

Fig. 2. Attenuations of all 10-trial blocks kept during collection of the threshold at 400 Hz for the behavioural audiogram, indicating the staircase used to determine the threshold. A total of 12 reversals were obtained and used for threshold calculation. These data were collected between 1 August and 14 September 2008.

animal became uncomfortable and to ensure that the animal could easily lift its head to breathe. Two 27 gauge, 12 mm, subdermal, stainless steel electrodes (Rochester Electro-Medical, Tampa, FL, USA) were inserted just beneath the skin to a depth of approximately 3 mm. The recording electrode was placed anterior to the frontoparietal scale on the top of the head in a caudally facing direction. The reference electrode was placed in the skin of the neck adjacent to the first marginal scute. The ground electrode was placed in the water near the subject. Exposed surfaces of the electrode that were not inserted into the skin were coated with enamel for insulation and the entire insertion point and electrode were sealed with petroleum jelly during testing to eliminate contact with the seawater.

AEP signal presentation

Stimulus generation was performed with the same TDT workstation and Aquasonic AQ39 transducer used for behavioral testing. Signals were amplified with an American Audio VLP300 amplifier (American Audio, Los Angeles, CA, USA). Auditory stimuli consisting of 50 ms, cosine-gated tone bursts with a 5 ms rise/fall time were presented at a rate of 11.0375 s^{-1}. Tone bursts were calibrated with an HTI-96 min hydrophone (sensitivity: –164±1 dBV μPa^{-1} from 2 Hz to 37 kHz) placed in the position of the subject's ear prior to the testing session using BioSig software. During January testing, the subject was tested at 50, 100, 200, 400, 600, 800, 1000, 1600 and 3200 Hz. During July testing, the subject was tested at 50,100, 200, 400, 800, 1131 and 1600 Hz.

Response detection

Tones were presented up to 1000 times at each SPL. Signals from the electrodes were passed through a digital biological amplifier (TDT DB4/HS4) and recorded over a 90.6 ms window using BioSigRP software (Tucker-Davis Technologies) at a sample rate of 24,412 Hz. Resulting AEPs were averaged for each frequency and SPL combination. To reduce testing time, if a clear AEP response was seen before 1000 averages, the program was advanced to the next SPL. Sound level was decreased in 6 dB steps for each frequency tested. Threshold was determined to be the level at which a clear signal was no longer detectable in the time domain waveform and a peak was no longer seen in the Fourier transform frequency spectra plots (Fig. 3). Analysis was conducted using BioSig, Excel (Microsoft, Redmond, WA, USA) and MATLAB (MathWorks, Natick, MA) software. Manual analysis was performed because analysis of the signal-to-noise ratio did not allow accurate automated threshold measurements in most cases because of the low-frequency electrical noise present in most signals. Two independent observers analyzed each frequency and waveform plot to ensure threshold determination reliability.

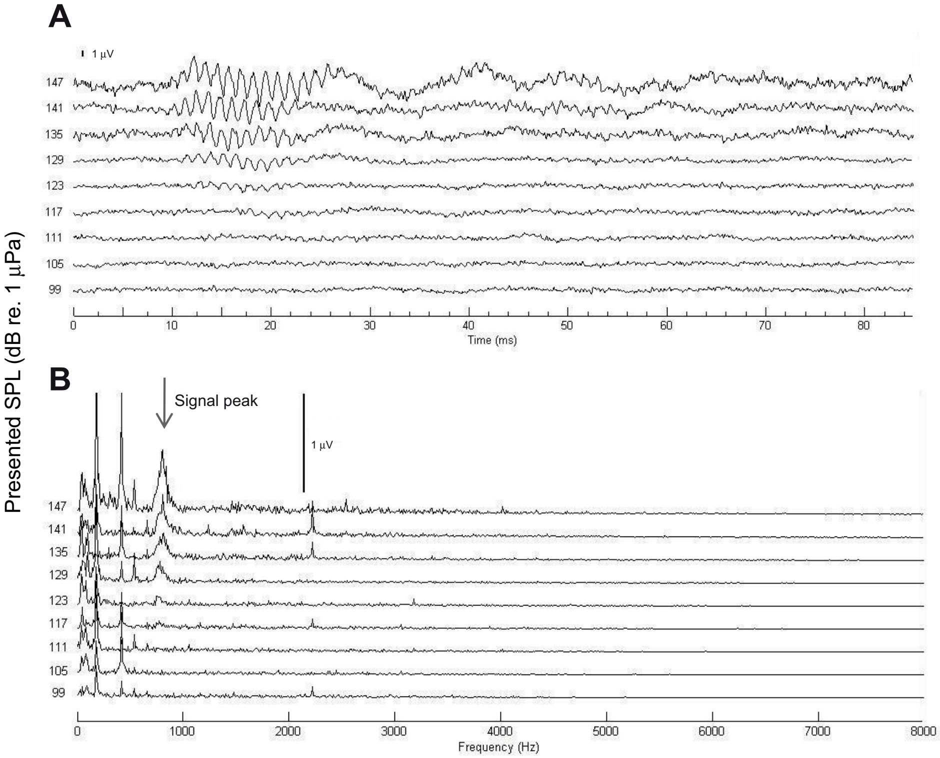

Fig. 3. Auditory evoked potential (AEP) waveform (A) and frequency spectra (B) measurements at 400 Hz. Sound pressure levels (SPLs) are indicated next to each signal. Waveforms are presented as raw output. AEP signals in power spectra are indicated with an arrow. Other spectral peaks are due to electrical mains noise (multiples of 60 Hz).

Table 1. Data collected from a single *Caretta caretta* subject during reversal blocks at 400 Hz for behavioral audiogram

Reversal	Date	Block of the day	Correct no. of go trials	Pass/Fail	False alarm rate for block (%)	SPL (dB re. 1 μPa)
1	3 September 2008	2/4	1/5	Fail	20	101
2	3 September 2008	3/4	3/5	Pass	0	108
3	3 September 2008	4/4	2/5	Fail	20	101
4	4 September 2008	1/4	5/5	Pass	40	108
5	4 September 2008	2/4	2/5	Fail	0	101
6	4 September 2008	3/4	4/5	Pass	20	108
7	5 September 2008	2/4	2/5	Fail	40	98
8	5 September 2008	4/4	4/5	Pass	40	104
9	13 September 2008	2/4	2/5	Fail	20	104
10	13 September 2008	3/4	4/5	Pass	20	110
11	13 September 2008	4/4	0/5	Fail	20	104
12	14 September 2008	1/1	4/5	Pass	0	109
Threshold						106

Five go trials and five no-go trials were presented in each block. The number correct out of five go trials is presented along with the false alarm rate (percentage incorrect out of five no-go trials). Sound pressure levels (SPLs) were recorded at the end of each training session and some variability occurred even among similar intended presentation levels, likely because of tank characteristics and hydrophone placement.

To rule out artifacts, a deceased loggerhead was tested with the same equipment and software. Access to deceased turtles was limited to hatchlings from nearby nesting beaches that were provided by Mote Marine Laboratory's stranding and investigations program. A hatchling (4.5 cm straight carapace length) was tested at all frequencies to rule out the presence of artifacts in the signal responses of live turtles.

RESULTS

Behavioral audiogram

Behavioral thresholds were collected over a period of 1 year. A total of six frequencies were tested and thresholds were determined for five frequencies. Each frequency took approximately 4–6 weeks with reversals occurring over a 10–15 day period.

To illustrate threshold determination, Table 1 summarizes data collected in all of the reversal blocks used to calculate the 400 Hz threshold and the SPL of each reversal. Passed blocks are indicated as those in which the correct detection rate within the block is equal to or greater than three out of five (60%) correctly detected go trials and the false alarm rate is at or below 40%. A total of 436 blocks were used for testing all frequencies, of which 59.4% (259) were kept. The majority of discarded blocks were due to high false alarm rates or were training blocks with failed warm-ups. False alarm rates ranged from 14.3% (1131 Hz) to 24.5% (200 Hz) when averaged over all kept blocks for each frequency. Average false alarm rates during reversal blocks were 25% (50 Hz), 32% (100 Hz), 25% (200 Hz), 20% (400 Hz) and 18.3% (800 Hz). Variability in the presented SPL between each passed and failed block averaged 7 dB over all frequencies with the most amount of variability occurring at best sensitivity (100 Hz, average variability of 9.7 dB).

The subject responded to tones from 50 to 800 Hz. The amount of testing time and effort, calculated by the number of blocks kept, are indicated in Table 2 along with the calculated threshold for each frequency tested. The resulting audiogram is a curve with best sensitivity of 98 dB re. 1 μPa occurring at 100 Hz (Fig. 4). Thresholds from 200 to 400 Hz were within 10 dB of best sensitivity. Sensitivity decreased sharply above 400 Hz with an increase of 42 dB between 400 and 800 Hz and the subject was unable to detect any tones when tested at 1131 Hz at the loudest level generated by the equipment (138 dB re. 1 μPa). The threshold at 50 Hz (110 dB re. 1 μPa) was slightly above best sensitivity, with a less dramatic decrease in sensitivity occurring at the lower frequency range than at the higher end.

AEP audiograms

AEP waveforms obtained from averaged responses to tone pip stimuli were present at approximately 7–10 ms after tone initiation. Responses consisted of a waveform that was twice the presented frequency. The resulting thresholds from both AEP testing sessions and their combined averages are listed in Table 3. Additionally, noise spectrum levels are listed for each testing session. There was minimal difference in calculated thresholds between the two sessions with a maximum difference of only 2 dB (Fig. 5). During the January testing session, best sensitivity was at 200 Hz with a threshold of 110 dB re. 1 μPa. During the July testing session, maximum sensitivity occurred at 200 and 400 Hz with a threshold of 109 dB re. 1 μPa. No detectable threshold was observed at 50 Hz in either session. Average combined thresholds from both sessions were within 2 dB between 100 and 400 Hz, with sensitivity decreasing by 25 dB between 400 and 600 Hz and decreasing to 143 dB re. 1 μPa at 800 Hz. The highest detected frequencies were 1000 Hz during January testing and 1131 Hz during July testing. Testing was conducted beyond these frequencies but peaks and waveforms were only visible at the highest presented SPL. The signals at these high frequencies are attributed to artifacts because

Table 2. Summary of collection effort for behavioral data and resulting thresholds for each frequency tested

Frequency (Hz)	Start date	End date	No. blocks	No. kept	% Kept	No. reversals	Threshold (dB re. 1 μPa)
50	27 July 2009	24 August 2009	73	50	68	12	110
100	3 April 2009	5 May 2009	67	41	61	10	98
200	26 September 2008	24 November 2008	157	80	51	12	103
400	1 August 2008	14 September 2008	71	52	73	12	106
800	12 May 2009	8 June 2009	61	29	48	12	148
1131	22 July 2009	24 July 2009	7	7	100	0	n/a

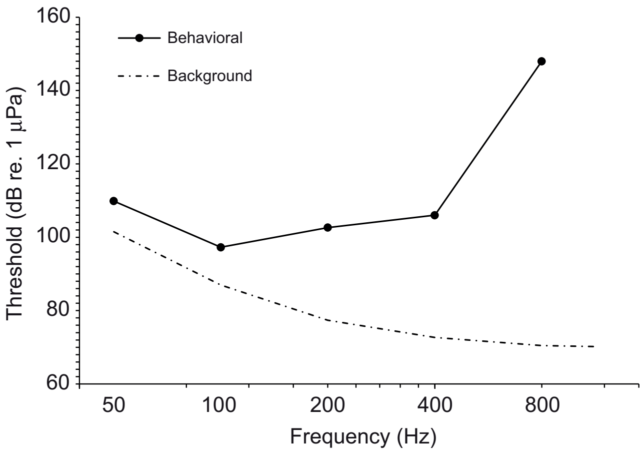

Fig. 4. Underwater behavioral audiogram and accompanying background noise. Background noise is presented as spectrum level (dB re. 1 μPa^2 Hz^{-1}).

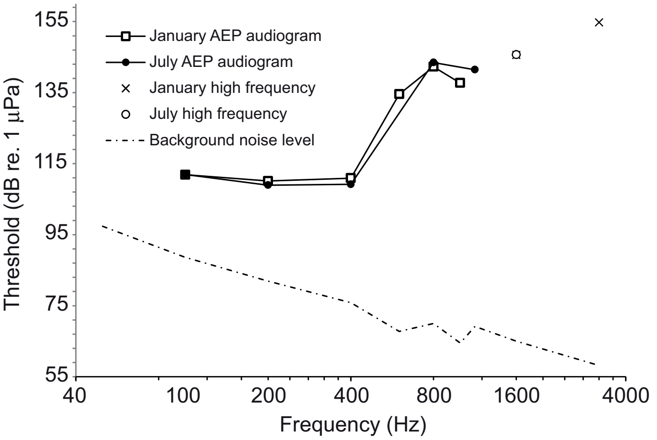

Fig. 5. Underwater AEP audiograms from two different testing sessions and average ambient noise spectrum level. During the January test session, measurements were taken at 50, 100, 200, 400, 600, 800, 1000, 1600 and 3200 Hz. During the July session, measurements were taken at 50, 100, 200, 400, 800, 1131 and 1600 Hz. Thresholds beyond 1000 Hz in January and 1131 Hz in July are believed to be due to artifact. Background noise levels are presented in spectrum level (dB re. 1 μPa^2 Hz^{-1}).

they lack onset delay and they were also produced in control tests run in a dead loggerhead turtle.

When testing the deceased loggerhead hatchling, signals were present at the highest presented SPLs at all frequencies tested with the exception of 50 Hz. These signals appeared 1–2 ms after stimulus presentation and the waveforms directly resembled stimulus waveforms. These signals disappeared with a 6 dB decrease in SPL.

The particle acceleration in each of three orthogonal directions is presented in Table 4. These measurements show that the greatest particle acceleration was in the vertical direction, which is expected because the surface acts a pressure release. The front–back direction, which was in line with the speaker, had the next highest level of particle acceleration. The magnitude of the particle acceleration was similar between frequencies.

DISCUSSION

Behavioral analysis

Initial training attempts with the test subject proved to be difficult. Husbandry training, designed to train the test subject to participate in health management procedures such as blood collection and mass determination, occurred for 1 year prior to the initiation of research training. Throughout this initial training, the subject was slow and inconsistent to perform learned behaviors. Only after months of repetition did behavioral consistency become evident and even after prolonged training, behavior was sometimes erratic and unpredictable. Such behavior is often seen in the conditioning of reptiles (Suboski, 1992). For this reason, we designed the modified staircase method for collecting behavioral data, using blocked trials instead of single trials to account for reversals. Analyzing performance in blocks of 10 trials smoothed the erratic trial-by-trial performance of the subject.

In addition to difficulty with erratic behavior within a session, the subject showed some seasonal behaviors that affected performance. Adult loggerhead turtles go through seasonal changes in behavior and appetite due to breeding cycles. Female loggerheads typically migrate from foraging grounds to breeding areas a few weeks to a few months prior to nesting season (Limpus et al., 1992; Schroeder et al., 2003). The subject was regularly observed going through periods of appetite loss and lack of interest in training or other daily behaviors during the months of December to March. Deterioration in performance was noted in October 2008 during testing of 200 Hz. Performance in testing sessions ceased completely in December and did not return until March 2009. Testing of 200 Hz

Table 3. Thresholds and background noise spectrum level at each test frequency from two separate AEP testing sessions and combined mean values

	January 27		July 24			
Frequency (Hz)	Threshold (dB re. 1 μPa)	Background noise (dB re. 1 μPa^2 Hz^{-1})	Threshold (dB re. 1 μPa)	Background noise (dB re. 1 μPa^2 Hz^{-1})	Mean threshold (dB re. 1 μPa)	Mean background noise (dB re. 1 μPa^2 Hz^{-1})
50	†	96	†	99	†	97
100	112	85	112	91	112	89
200	110	77	109	84	110	82
400	111	71	109	78	110	76
600	135	68	*	*	135	68
800	142	66	143	72	143	70
1000	138	64	*	*	138	64
1131	*	*	141	69	141	69
1600	146	64	146	66	152	65
3200	155	58	*	*	155	58

†, no signal detected; *, frequency not tested.

Table 4. Particle acceleration (mm s^{-2}) for a received level of 100 dB re. 1 μPa

Frequency (Hz)	Front–Rear	Up–Down	Left–Right	Magnitude
50	0.31	0.59	0.09	0.67
100	0.35	0.55	0.07	0.65
200	0.3	0.72	0.05	0.78
400	0.24	0.74	0.02	0.78
800	0.19	0.54	0.07	0.58
1131	0.3	0.89	0.07	0.94

took significantly more blocks than any other frequency, likely because of seasonal shifts in appetite and behavior.

False alarm rates, the frequency of incorrect responses on signal-absent trials, ranged from 18.3 to 32%. High false alarm rates could result in biased thresholds. A tendency to respond in the absence of a signal could result in lower thresholds. In this case, the subject showed an equal tendency to respond when no signal was present as she did to refrain from responding to signals that were within her auditory range. Therefore, the high false alarm rate was balanced by similar behavior in signal-present trials.

AEP analysis

AEP testing measures the small electrical impulses that are generated by the nerve cells in response to electrical signals from the stimulation of hair cells in the auditory system by an acoustical stimulus. In this study and in studies of other species, including fish, a frequency doubling effect is seen when measuring AEPs (Casper and Mann, 2006; Casper and Mann, 2007; Egner and Mann, 2005). This is likely because of the orientation of hair cells within the ear, so that certain cells fire on the compression phase of a sound wave and others fire on the rarefaction phase, resulting in a doubled response. These doubled responses were seen at all frequencies tested.

One of the difficulties associated with AEP testing is the subjective nature of threshold determination. Threshold determination at very low frequencies was made even more difficult by the high intensity of electrical background noise at low frequencies. Analysis of fast Fourier transforms of the signals indicated large peaks between 0 and 150 Hz, making the signal-to-noise ratio too low to detect a stimulus response at 50 Hz. Additionally, behavioral results indicate that the threshold at 50 Hz is 110 dB re. 1 μPa. The loudest SPL that testing equipment could produce during AEP testing of 50 Hz was 119 dB re. 1 μPa. Because AEP thresholds at low frequencies were 6–7 dB higher than behavioral thresholds, it is possible that we were not able to present a signal with an SPL sufficient enough to produce an AEP.

AEP testing was conducted at frequencies beyond the maximum hearing range determined by behavioral testing. During behavioral testing, the turtle showed no response to signals played at 1131 Hz at levels of 138 dB re. 1 μPa. During AEP testing, a response was detected during the presentation of 1131 Hz at 141 dB re. 1 μPa. Lower levels yielded no AEPs. Additional testing was conducted at 1600 and 3200 Hz during the first session and 1600 Hz during the second session. During analysis of these frequencies, a visible waveform was apparent in the response at the highest presented SPL. These waveforms were attributed to artifact, likely from direct electrode detection of the electromagnetic field of the transducer. To validate this, signal onset delay was studied at all frequencies. At zero attenuation, AEP responses appeared between 7 and 10 ms after signal initiation for frequencies below 1600 Hz. At 1600 and 3200 Hz, response signals appeared between 1 and 2 ms after signal initiation. This 1–2 ms delay can be accounted for by the delay produced by the filters in the amplifier. In addition to validating true detections with response onset times, results were examined in a deceased turtle. A dead loggerhead hatchling was studied using the same methods as used for the adult test subject. Responses were present at the highest presented SPL for all frequencies with the exception of 50 Hz. These responses were visible between 1 and 2 ms after signal presentation. Responses in the live turtle at lower frequencies did not occur until 7–10 ms after presentation. For these reasons, the responses at 1600 and 3200 Hz may be attributed to artifact and not actual detection.

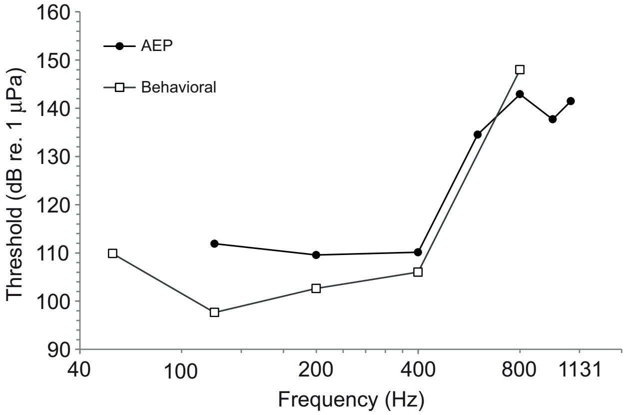

Fig. 6. Comparison of underwater behavioral and mean AEP audiograms. AEP threshold values are averaged over two testing sessions.

Comparison of behavioral and AEP audiograms

Hearing abilities are far better understood in marine mammals than in marine turtles. Access to multiple species of captive marine mammals and the relative ease in which they can be conditioned to participate in research tasks have allowed studies exploring the differences between behavioral and electrophysiological methods of auditory testing in the same individual (Finneran and Houser, 2006; Houser and Finneran, 2006; Schlundt et al., 2007; Szymanski et al., 1999; Wolski et al., 2003; Yuen et al., 2005). Each of these studies yielded a slight to significant difference in the results obtained from the two testing methods, with behavioral thresholds typically lower than AEP thresholds, particularly at lower frequencies. When parameters such as testing environment and stimulus presentation are kept similar between the two methods, the difference in the calculated thresholds decreases (Houser and Finneran, 2006).

In the present study, thresholds were determined through both AEP and behavioral methods at 100, 200, 400 and 800 Hz (Fig. 6). Thresholds differed by a maximum of 14 dB at 100 Hz and a minimum of 4 dB at 800 Hz. A threshold was not detected at 50 Hz during AEP testing but behavioral data indicated a threshold of 110 dB re. 1 μPa. AEP testing elicited responses at higher frequencies whereas behavioral responses indicated a complete lack of detection beyond 800 Hz. Audiogram curves followed similar shapes for both methods of testing. Both curves showed substantial decreases in sensitivity at frequencies higher than 400 Hz.

Behavioral thresholds were lower than AEP thresholds between 100 and 400 Hz and higher than AEP thresholds at 800 Hz. These differences may be caused by multiple factors. One important difference between the behavioral audiogram and the AEP testing is the duration of the signal. In the behavioral audiogram the test signal was 2 s in duration, whereas in the AEP audiogram the test signal was a 50 ms tone pip repeated approximately 11 times per second. Therefore, our results can be used as a transfer function between an AEP audiogram collected under these conditions and a behavioral audiogram collected with traditional longer stimuli. Natural variability in responses and behavior because of the unpredictable nature of marine turtles could also have led to these differences. In addition, there was potential variability in perceived signal SPL because of variations in the acoustic environment attributed to proximity to the reflective water surface. Another possibility is that during AEP testing, at low frequencies, the background electrical noise is naturally higher, making detection of small AEPs more difficult. Differences in AEP and behavioral thresholds may also result from the different systems involved in signal processing. AEP thresholds are determined by analyzing electrical signals from a single part of the auditory system whereas behavioral responses require the function of the entire auditory system and are based upon the performance and motivation of the test subject. Any combination of these factors can result in slight to significant differences in the results obtained from the two testing methods.

Noise spectrum level and masking

There is a strong possibility that noise masking occurred during low-frequency testing, resulting in higher thresholds. Critical ratio data are lacking in turtles and must be inferred from other species. Critical masking ratios, defined as the difference between the SPL of the tone at threshold and the spectrum level of masking noise, are poorly defined in reptiles. The bullfrog (*Rana catesbeiana*) aerial critical masking ratio at 100 Hz is 29 dB (Megela Simmons, 1988). Thresholds measured in the loggerhead turtle were 98 dB determined behaviorally and 112 dB determined through AEP testing. Noise spectrum levels were 87 and 89 dB, respectively, resulting in a difference of 10 and 23 dB. If critical ratios are similar to those of the bullfrog, masking is likely occurring. At 50 Hz, the behaviorally determined threshold was only 8 dB above the noise spectrum level. It is possible that masking was occurring during both methods of testing, and it is crucial to develop a better understanding of critical masking ratios in marine turtles.

We recommend conducting future testing in a quieter environment in which outside noise could be further reduced during testing. Samuel et al. (Samuel et al., 2005) measured noise levels in a known loggerhead, Kemp's ridley and green turtle habitat in New York during peak levels of human activity and periods of low activity. Results indicated SPLs as high as 113 dB re. 1 μPa between 200 and 700 Hz during peak activity. When recorded overnight, with naturally lower levels of human activity, noise levels were still around 83 dB re. 1 μPa. These results indicate that there is a strong likelihood that marine turtle hearing abilities are being masked by high levels of anthropogenic noise. This is especially true in shallow, coastal areas where noise levels, particularly those in the low-frequency range, are higher than in open-ocean areas because of coastal construction, a higher number of commercial and recreational vessels, and the presence of seismic exploration. These coastal areas provide key habitats for juvenile and subadult turtles as well as nesting and mating adults. Although the true function of hearing in marine turtles remains poorly understood, it is possible that aural sensing may assist in danger avoidance, beach location or navigation. If masking is occurring because of the presence of high levels of anthropogenic noise in the low-frequency range, natural behavior and survival may be affected.

Comparison with other turtle hearing studies

Frequency ranges detected by the loggerhead turtle were similar to those found in green turtles and Kemp's ridley turtles by Bartol and Ketten (Bartol and Ketten, 2006). Threshold levels are difficult to compare because that study utilized a mechanical vibrator attached directly to the tympanum to relay stimuli through the bone and the results of vibrational testing (presented in dB re. 1 g r.m.s.) cannot be easily compared with results presented in SPL. However, the frequency ranges are similar to those found in the present study. Similarly, Ridgway et al. (Ridgway et al., 1969) found green turtle sensitivity to be 60–1000 Hz with a peak between 300 and 500 Hz. Each of these studies indicates that marine turtles have hearing abilities that detect low-frequency sound with a narrow band of greatest sensitivity. A recent study of *Trachemys scripta elegans* utilized underwater auditory brainstem response measurements and laser vibrometry to reveal peak sensitivity of 400–600 Hz (Christensen-Dalsgaard et al., 2012). The present study confirms the results of previous electrophysiological tests through behavioral test comparisons. It would be beneficial to collect behavioral audiograms in other marine turtle species.

This study showed that an audiogram collected through operantly conditioned behavioral methods was similar to that measured with AEP testing, which can be conducted in just a few hours on an untrained animal. Additional testing with increased sample sizes as well as additional age classes may be needed to account for any ontogenetic changes in auditory function, behavior or habitat usage. Although behavioral testing methods provide accurate measures of threshold determination, AEP testing is beneficial when time is a factor and multiple individuals need to be tested. With continued improvement in threshold estimations through electrophysiological testing, a more rapid and thorough understanding of marine turtle auditory abilities will aid in future assessments of the impacts of anthropogenic noise and the function of sound in the marine turtle environment.

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