Acoustic Ecology of Sea Turtles: Implications for Conservation

by

Wendy Erin Dow Piniak

Marine Science and Conservation
Duke University

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David A. Mann

Dissertation submitted in partial fulfillment
of the requirements for the degree of Doctor
of Philosophy in Marine Science and
Conservation in the Graduate School
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ABSTRACT

An understanding of sensory ecology, how animals receive and respond to their environment, can be a powerful tool for the conservation of endangered species because it can allow us to assess the potential success of actions designed to mitigate particular threats. We have a general understanding of how sea turtles perceive and respond to certain visual, magnetic, and chemical cues, but we understand very little about how they perceive and respond to acoustic cues. This dissertation explores the acoustic ecology of sea turtles, focusing on their auditory capabilities, responses to acoustic stimuli and the implications of this knowledge for their conservation. I measured the underwater and aerial hearing sensitivities of juvenile green (*Chelonia mydas*), hatchling leatherback (*Dermochelys coriacea*), and hatchling hawksbill (*Eretmochelys imbricata*) sea turtles by recording auditory evoked potential responses to tonal stimuli. Green turtles detected tonal stimuli between 50 and 1,600 Hz underwater (maximum sensitivity: 200-400 Hz) and 50 and 800 Hz in air (maximum sensitivity: 300-400 Hz), leatherbacks detected tonal stimuli between 50 and 1,200 Hz underwater (maximum sensitivity: 100-400 Hz) and 50 and 1,600 Hz in air (maximum sensitivity: 50-400Hz), and hawksbills detected tonal stimuli between 50 and 1,600 Hz in both media (maximum sensitivity: 200-400 Hz). Sea turtles were more sensitive to aerial than underwater stimuli when audiograms were compared in terms of sound pressure, but they were more sensitive to underwater stimuli when audiograms were compared in terms of sound intensity. I also examined the behavioral responses of loggerhead sea turtle (*Caretta caretta*) to simulated low frequency acoustic deterrent devices (ADDs) and found that these turtles exhibited a mild, aversive response to these sounds. This finding indicates that low frequency tonal ADDs have the potential to warn sea turtles of the presence of fishing gear and suggest that field tests of ADDs are warranted. Finally, I conducted a comprehensive review of our knowledge of the acoustic ecology of sea turtles, examined the sources of marine anthropogenic sound sea turtles are able to detect, evaluated the potential physiological and behavioral effects of anthropogenic sound, identified data gaps, and made recommendations for future research.

DEDICATION

To my parents and Greg for their unfailing love and support.

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ACKNOWLEDGEMENTS

I am immensely grateful to the many people who have supported me throughout my graduate studies, helped to make my research and this thesis a reality, and who have in both small and large ways affected the person and scientist I have become.

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INTRODUCTION

Understanding how marine animals perceive and respond to their environment is critical to understanding their ecology. Sensory ecology is a relatively new discipline that explores the relationship between sensory physiology and ecology (Bowdan & Wyse 1996, Dusenbery 1992). Studies of sensory ecology from zooplankton to whales have provided new and valuable insights into the types of information available in marine environments, how that information is detected by animals, and how and why animals respond to this information (e.g. Johnson et al. 2009, Lohmann et al. 2008, Marshall et al. 2003, Nevit 2008). Knowledge of sensory ecology can be a powerful tool for the conservation of endangered or threatened species because it can help to assess the potential success of actions designed to mitigate particular threats.

Sea turtles are ancient marine reptiles and have existed for over 100 million years (Hirayama 1997). Seven species exist: leatherback (*Dermochelys coriacea*); hawksbill (*Eretmochelys imbricata*); green (*Chelonia mydas*); loggerhead (*Caretta caretta*); Kemp's ridley (*Lepidochelys kempii*); olive ridley (*Lepidochelys olivacea*); and flatback (*Natator depressus*) sea turtles. With the exception of the flatback sea turtle, for which insufficient data exist to assess their status, and olive ridleys which are classified as vulnerable, all species of sea turtles are classified as critically endangered or endangered on the International Union for Conservation of Nature Red List of Threatened Species (IUCN 2012). Sea turtle populations have experienced severe declines due to direct harvest, incidental capture in fisheries and the loss of foraging and nesting habitats

(National Research Council 1990). Many populations now exist only as remnants, incapable of fulfilling their former ecological roles (Bjorndal & Bolten 2003, Bjorndal & Jackson 2003). Sea turtles are found in nearly all temperate and tropical marine environments and are highly migratory, often travelling great distances between developmental, foraging, and nesting habitat. To conduct these remarkable feats of navigation, sea turtles take advantage of visual, magnetic, chemical and auditory cues.

We understand how some species of sea turtles perceive and respond to certain visual, magnetic, and chemical cues (Lohmann et al. 1997, Bartol & Musick 2003, Southwood et al. 2008), but we understand very little about how they perceive and respond to sound. The biological significance of hearing in sea turtles remains largely unstudied, but it seems likely that they use sound in navigation, to locate prey, avoid predators and in general environmental awareness.

We lack systematic measurements of levels of ambient sound around the globe, but it is generally agreed that such levels are increasing as sources of anthropogenic sound become more widespread and intense (Andrew et al. 2002, Hildebrand 2009, Ross 1993). Sound in the ocean comes from many natural and anthropogenic sources and overall sound levels are a combination of these sources. Natural acoustic sources include: waves, wind, and rain at the ocean's surface; seismic activity (such as earthquakes); sea ice movement; and marine animals. Anthropogenic acoustic sources include: ship traffic; mineral exploration (seismic air guns or sonar); drilling and extraction; sonar; explosives; industrial construction; and acoustic deterrent and harassment devices. Sounds produced by these sources differ in intensity (decibels), frequency (Hertz, or cycles per second), and wavelength (meters), and their persistence in the marine environment depends on these factors and the nature of the environment (water temperature, depth, benthic substrate etc.). Low frequency sounds, such as those produced by some sonars, shipping, and oil and gas exploration and extraction have long wavelengths and travel furthest in the ocean environment.

Recent increases in the intensity of anthropogenic sound underscore the importance of understanding the effects of sound on sensitive marine species. Such concerns have led to increased research on potential physiological and behavioral effects of anthropogenic sound on marine mammals and fish (for summaries see: National Research Council 2000, 2003, 2005, Nowacek et al. 2007, Popper & Hastings 2009, Richardson et al. 1995), but research on sea turtles has lagged behind, because our fundamental knowledge of their auditory sensitivities and responses is lacking. Therefore, my dissertation explores the acoustic ecology of sea turtles, focusing on their auditory capabilities, responses to acoustic stimuli, and the implications of this knowledge for their conservation.

My first three chapters investigate amphibious hearing in sea turtles. The anatomy and morphology of the sea turtle ear has been well described (Wever 1978, Hetherington 2008), but the functional morphology of the sea turtle ear is not well understood. As a consequence, much of our knowledge is inferred from studies of terrestrial and semi-aquatic turtles (Christensen-Dalsgaard et al. 2012, Lenhardt 1981, Lenhardt 1982, Lenhardt & Harkins 1983, Patterson 1966, Wever 1956a, b, c, Wever 1978). Lenhardt et al. (1983, 1985) suggest the sea turtle ear is adapted for hearing via bone conduction in water and is a poor aerial receptor, but Hetherington (2008) suggests a more typical tympanic pathway. It remains unclear whether sea turtle ears respond to acoustic pressure, particle motion, or both. Previous physiological and behavioral studies of hearing have shown that green, loggerhead, and Kemp's ridley sea turtles are able to detect low frequency (<2,000 Hz) acoustic tonal and vibratory stimuli in both air and water (Bartol 1999, Bartol & Ketten 2006, Martin et al. 2012, Ridgway et al. 1969). However no data exist on the auditory capabilities of hatchling, leatherback, or hawksbill sea turtles, and there have been no comparisons of underwater and aerial hearing sensitivity. I measured the aerial and underwater hearing sensitivities of juvenile green (Chapter I), hatchling leatherback (Chapter II), and hatchling hawksbill (Chapter III) sea turtles by recording auditory evoked potential (AEP) responses to tonal stimuli. AEPs are electrical responses produced by the central auditory nervous system in response to stimulation by sound that is detected by the ear (Yost 2007, Au & Hastings 2008). I developed methodologies for recording underwater AEPs, collected the first measurements of sea turtle hearing in reference to particle acceleration, and made the first comparisons of aerial and underwater hearing sensitivity via pressure and intensity.

In Chapter IV I examine the behavioral responses of sea turtles to acoustic stimuli to assess whether acoustic deterrent devices (ADDs) have the potential to reduce sea turtle by-catch in fishing gear. By-catch poses a significant threat to many populations of sea turtles because their populations are particularly sensitive to high rates of removals. By-catch of sea turtles occur in both industrial and artisanal fisheries and in a variety of gear types, including: longlines; gillnets; trawls; traps; and pots (Epperly et al. 2007, Finkbeiner et al. 2011, Lewison et al. 2004, Moore et al. 2009, Peckham et al. 2007, Wallace et al. 2010). Mitigation measures, such as gear modification and/or time-area closures, are not available for all types of fishing gear and are particularly lacking for gill net fisheries. ADDs, also known as pingers, are low-intensity sound sources that have been used to reduce the by-catch of marine mammals in some gillnet fisheries (see Nowacek et al. 2007 for a review), but their efficacy with sea turtles has not been evaluated. Previous assessments have dismissed the potential of ADDs to reduce sea turtle by-catch because of the similar hearing sensitivities of sea turtles and fishes and the expectation that, even if the devices were effective in reducing the by-catch of turtles, ADDs would also reduce the catch of target species. However flatfish have limited low-frequency hearing and it may be possible to design an ADD signal that deters sea turtles, but not flatfish. To determine if sea turtles are deterred by simulated ADDs, I examined the behavioral responses of loggerhead sea turtles to a tonal stimuli of 300 Hz in a tank environment.

In my last chapter, I place the results of my research in a comprehensive review of our knowledge of the acoustic ecology of sea turtles, determine the sources of marine anthropogenic sound sea turtles are able to detect, evaluate the potential physiological and behavioral effects of anthropogenic sound, identify data gaps, and make recommendations for future research.

CHAPTER I:

HEARING IN THE JUVENILE GREEN SEA TURTLE (*CHELONIA MYDAS*): A COMPARISON OF UNDERWATER AND AERIAL HEARING USING AUDITORY EVOKED POTENTIALS

ABSTRACT

Sea turtles spend much of their life in aquatic environments, but critical portions of their life cycle, such as nesting and hatching, occur in terrestrial environments, suggesting that it may be important for them to detect sounds in both air and water. In this study I compared underwater and aerial hearing sensitivities in five juvenile green sea turtles (*Chelonia mydas*) by measuring auditory evoked potential (AEP) responses to tone pip stimuli. Green turtles detected acoustic stimuli in both media, responding to underwater signals between 50 and 1,600 Hz and aerial signals between 50 and 800 Hz, with maximum sensitivity between 200 and 400 Hz underwater and 300 and 400 Hz in air. Green turtles are slightly more sensitive to aerial stimuli when mean underwater and aerial hearing sensitivities are compared in terms of pressure, although they are able to detect a wider range of frequencies underwater. When sensitivities are compared in terms of sound intensity, juvenile green turtles are more sensitive to underwater stimuli.

INTRODUCTION

Sea turtles do not appear to vocalize or use sound for communication, but are able to detect (Ridgway et al. 1969, Bartol et al. 1999, Bartol & Ketten 2006, Martin et al. 2012) and respond to acoustic stimuli (O'Hara & Wilcox 1990, McCauley et al. 2000, DeRuiter et al. 2012). The biological significance of hearing in sea turtles remains largely unstudied, but they may use sound for navigation, locating prey, avoiding predators, and general awareness. Sea turtles spend much of their life underwater, but they breathe at the air-water interface and critical portions of their reproductive cycle (egg laying and hatching) take place on land. Thus, it may be important that sea turtles be able to detect sound in both underwater and aerial environments.

Sea turtles lack external pinnae or ear canals and, like other Testudines, their ear is covered by an extension of facial tissue called the tympanum. Unlike terrestrial turtles and tortoises, however, marine turtles have a thick layer of subtympanal fat and connective tissue (Wever 1978). The middle ear is surrounded by bone, filled with air, and connected to the throat via the Eustachian tube. The sea turtle ossicular mechanism is comprised of the extracolumella and the columella (stapes). The mushroom shaped, cartilaginous extracolumella lies beneath the tympanum and is connected by ligaments to the columella. The collumella (stapes) is a long, thin, curved bone, encased in a narrow, bony channel, which extends medially into the middle ear, through the fluid-filled pericapsular recess to the oval window, where it expands to form a large, cone-shaped footplate (Wever 1978). Small, fibrous stapedosaccular strands, which are unique to turtles and hypothesized to relay vibrational energy, connect the stapes and oval window to the saccule (Lenhardt et al. 1985, Wever 1978, Wever & Vernon 1956a). Inward and outward movement of the columella causes movement of fluid in the pericapsular recess, stimulating hair cells located on the basilar membrane and limbus of the cochlea (Wever 1978).

The functional morphology of the sea turtle ear remains poorly understood and despite previous anatomical research it is still unclear whether sea turtle ears respond to pressure, particle motion, or both. Observational studies of the thick tympanum in marine turtles found little tympanic displacement in response to sound, and concluded that turtle ears had little capacity for impedance matching in air (Lenhardt et al. 1985). Computerized tomography of sea turtle subtympanal fat revealed that it is similar to the fat found in the middle ears of marine mammals and birds. The density of these fats is consistent with sound speeds in seawater, suggesting the subtympanal fat layer may act as a low-impedance channel for conduction of underwater sound to the middle and inner ears (Ketten 2008). Lenhardt et al. (1983, 1985) proposed that the sea turtle ear is adapted for hearing via bone conduction in water and is a poor receptor in air, suggesting that the whole body serves as a receptor while the turtle is underwater. However, evidence derived from research on freshwater turtles suggests a more typical tympanic middle ear pathway for sound in sea turtles (Hetherington 2008). Research on freshwater aquatic turtles has shown that aerial and vibrational stimuli produce different audiograms and that turtles are more sensitive to aerial, rather than vibrational stimuli (Lenhardt & Harkins 1983, Patterson 1966, Christensen-Dalsgaard et al. 2012). Removal or cutting of the columella drastically reduced aerial hearing sensitivity, but only slightly reduced vibrational hearing sensitivity (Patterson 1966). Both auditory and vibrational stimuli appear to be processed by the auditory system, likely combining to create a single electrophysiological response (Lenhardt & Harkins 1983).

Marine turtle ears are hypothesized to be adapted for underwater sound detection, but very little is understood about their underwater hearing sensitivity, or how their underwater hearing sensitivity compares with their sensitivity in air. Several electrophysiological and behavioral studies demonstrated that sea turtles are able to detect low-frequency acoustic stimuli. Ridgway et al. (1969) collected the first measurements of sea turtle hearing sensitivity by recording cochlear response potentials to aerial and vibrational stimuli in three juvenile green turtles. Turtles responded to aerial stimuli between 50 and 2,000 Hz and vibrational stimuli between 30 and 700 Hz, with maximum sensitivity between 300 and 500 Hz for both stimuli. These authors suggested that the "useful" frequency span of the green turtle ear was between 60 and 1000 Hz. More recent measurements of sea turtle hearing sensitivity have been made by recording auditory evoked potentials (AEPs). AEPs are an electrical response produced by the central auditory nervous system after stimulation by sound detectable by the ear (Yost 2007, Au & Hastings 2008). Bartol et al. (1999) measured short latency AEPs (auditory brainstem responses) in juvenile loggerhead sea turtles (*Caretta caretta*) in response to two types of vibrational stimuli: low-frequency clicks and tone bursts delivered directly to the tympanum. They measured a mean click threshold of -10.8 dB re: 1g rms ± 2.3 dB SD and an effective hearing range from tone bursts from 250 to 750 Hz with maximum sensitivity at 250 Hz, the lowest frequency tested (Bartol et al. 1999). AEP measurements of hearing sensitivity in partially submerged sea turtles in response to aerial stimuli found Pacific sub-adult green turtles respond to stimuli between 100 and 500 Hz, with highest sensitivity between 200 and 400 Hz (Bartol & Ketten 2006). In the same study, Atlantic juvenile green turtles responded to stimuli between 100 and 800 Hz, with highest sensitivity between 600 and 700 Hz, and juvenile Kemp's ridley sea turtles (*Lepidochelys kempii*) responded to stimuli between 100 and 500 Hz with maximum sensitivity between 100 and 200 Hz (Bartol & Ketten 2006). A single adult loggerhead sea turtle responded to underwater stimuli between 50 and 800 Hz with best sensitivity at 100 Hz using behavioral response techniques and between 100 and 1,131 Hz with best sensitivity between 200 and 400 Hz using AEP techniques (Martin et al. 2012).

In the present study I measured the underwater and aerial hearing sensitivity of juvenile green turtles in reference to both pressure and particle acceleration. I also compared, for the first time, underwater and aerial hearing in marine turtles.

MATERIALS AND METHODS

Sea turtles

I measured the hearing thresholds of five Atlantic juvenile green turtles underwater and in air, by recording auditory evoked potentials (AEPs) at the Animal Care Center at the University of British Columbia in Vancouver, BC, Canada. Turtles averaged 34 kg in weight (range: 26 - 38 kg), 65 cm in curved carapace length (range: 60 - 67 cm) and 56.5 cm in curved carapace width (range: 53 - 60 cm).

Auditory evoked potential measurements

Underwater experimental setup

To prevent muscle movement that would mask AEPs, I lightly restrained the turtles by encasing them in a cloth bag before testing. Their heads were left exposed so the turtles could breath. I completely submerged turtles to a depth of at least 10 cm (measured at the location of the ear) below the surface in a cylindrical fiberglass tank (2 m in diameter and 1.5 m in depth). I submerged an amplified speaker (AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Inc., Littleton, Colorado USA; amplfier: Hafler P1000, Rockford Corporation, Tempe, Arizona USA) 40 cm away from and level with the turtle's ear. During data collection, water temperatures were approximately 22 ºC.

Aerial experimental setup

I isolated turtles from noise and vibrations, lightly restrained them using a cloth bag to prevent excessive movement, and placed them on an angled resting board with their head free. I suspended an amplified speaker (AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Inc., amplfier: Hafler P1000, Rockford Corporation) 80 cm directly in front of the turtle and level with the turtle's ear. To reduce the possibility of a vibratory response during in the air trials, the speaker was suspended on an elastic cord and positioned on a separate table. During data collection, air temperatures were approximately 21 ºC.

Auditory evoked potential measurements with anesthesia

I collected underwater AEPs on two turtles using anesthesia and for one turtle I collected underwater AEPs with and without anesthesia. Anesthesia was induced with medetomidine 50 μg/kg and ketamine 5 mg/kg combined and injected intravenously into the dorsal cervical sinus. Turtles were intubated with a specially designed a double-cuffed endotracheal tube with both proximal and distal cuffs forming a watertight seal and preventing the cuff from slipping in the trachea. I ventilated the turtles manually with a 1.5 L ambu bag at a rate of two breaths in quick succession every two to three minutes, with additional ventilations during gaps in the AEP collection. Ventilation rate and volume were based on reported respiratory rates of green turtles, observations of voluntary respirations of manually restrained turtles, and calculation from reported tidal volumes (39 ml/kg). At the completion of AEP measurements anesthesia was reversed with atimpamezole 0.25 mg/kg half IV and half IM (see Harms et al. 2009 for further anesthesia details). To evaluate the efficacy of using anesthesia as a restraint for the collection of AEPs, I compared resulting audiograms and venous blood gas values before and after the procedures on two anesthetized and unanesthetized turtles.

Signal generation and recording of auditory evoked potentials

To record AEP signals, I inserted needle electrodes (27 ga, 12 mm in length, Rochester Electro-Medical, Inc., Lutz, Florida USA) subdermally on the top of the head under the frontal scale (recording electrode); in the deltoid muscle of the neck (reference electrode); and either in the deltoid muscle of the shoulder (air: ground electrode) or seawater (water: ground electrode). I used an Evoked Potential Workstation (Tucker-Davis Technologies, Inc. Alachua, Florida USA) and laptop computer with SigGenRP and BioSigRP software (Tucker-Davis Technologies, Inc.) to generate tonal stimuli and recorded AEP responses from the electrodes at a sampling rate of 24,412 Hz. I amplified signals from the electrodes using a digital biological amplifier (Tucker-Davis Technologies, Inc.) and filtered the signals to remove sound outside the frequencies of interest (high pass: 50 Hz; low pass: 5 kHz; band reject: 60 Hz). Electrode impedances were less than 3 kΩ. I presented pulsed sinusoidal tonal stimuli, 50 ms in length, shaped with a Hanning window, at a rate of 13 s^{-1}, with alternating phase. I recorded responses to frequencies between 50 and 3,200 Hz, and attenuated stimuli in 6 dB steps beginning at the highest level that could be generated at each frequency and attenuating until no further AEP signal could be identified (after up to 1,000 AEP signal averages). To increase the number of recordings for each individual, I advanced to the next reduced sound pressure level if an AEP response was detected before 1,000 signal averages. I paused recordings when the turtles moved or lifted their heads to breathe to ensure I made all measurements with the head in the same position in the acoustic field.

Calibration

I calibrated the sound field using a hydrophone (HTI96-min, High Tech, Inc. Gulfport, Mississippi USA; sensitivity: -164 dBV/µPa; 0.02-30 kHz) placed at the location where the turtle's head was placed, but with the turtle absent. The hydrophone response in air was later calibrated against a sound level meter (330-2050, RadioShack, Fort Worth, Texas USA) (hydrophone aerial sensitivity: -126 dBV/20 µPa: 50-3,200 Hz). Calibrations were made using two RP2.1 modules and BioSigRP (Tucker-Davis Technologies, Inc.) which repeatedly played the signal at the same rate used while recording AEPs, and simultaneously recorded the hydrophone signal at sampling rate of 24,414 Hz. In water this procedure accounts for reverberation in the tank, as opposed to calibrating with long duration tones. I measured the underwater pressure gradient using the same hydrophone to record the pressure signal at six locations in three orthogonal directions 5 cm from the location of the pressure measurement. I calculated the pressure gradient by subtracting the time-locked recordings in the time domain and dividing by the distance between recording locations (0.1 m). The particle acceleration was calculated by dividing the pressure gradient by the density of the water (1,032 kg/m^3). I measured the background noise level using FieldLog (custom software, David Mann, University of South Florida) at a sampling rate of 24,414 Hz using the RP2.1 with the HTI-96 min, and analyzed background noise frequency spectra using MATLAB (version 7.14, MathWorks, Inc. Natick, Massachusetts 01760, USA).

Data Analyses

Because the presence of low-frequency background and electrical noise caused inaccuracies in automated threshold detection, I performed threshold analyses manually, a method commonly used in hearing investigations using AEPs (e.g. Casper & Mann 2006, Egner & Mann 2005, Martin et al. 2012, Mooney et al. 2010). I used BioSipRP (Tucker- Davis Technologies, Inc.) and Matlab software (MathWorks, Inc.) to make visual inspections of AEP responses in the time domain and analyzed the presence or absence of AEP signals using 2,048-point fast Fourier transforms (FFTs) in the frequency domain (Fig. 1). An AEP was determined to be present if the signal showed a peak twice that of the stimulus frequency (e.g. a peak at 600 Hz when the stimulus presented was 300 Hz) at least 6 dB above the noise floor 100 Hz on either side of the peak. I defined threshold as the lowest sound level at which a peak in the FFT was recorded.

RESULTS

Auditory evoked potential waveform characteristics

The AEP waveforms recorded from averaged responses to pulsed tonal signals increased in latency and decreased in amplitude as I attenuated the stimuli (Fig. 1a). Recorded AEP waveforms were twice the frequency of the presented tonal stimuli (Fig. 1b), and AEP levels (μV) decreased with decreasing sound pressure levels in both air and water (Fig. 2).

Underwater audiograms

Juvenile green turtles responded to signals between 50 and 1,600 Hz in water, with maximum sensitivity between 200 and 400 Hz (Tables 1 and 2, Fig. 3); sensitivity decreased sharply above 400 Hz. The lowest pressure sensitivity recorded was 85 dB re: 1 μPa-rms at 300 Hz (turtle L2). The lowest particle acceleration sensitivity recorded was 1.7 x 10-4 m/s^2 at 300 Hz (turtle L2). I found variation among individuals in frequency threshold levels and highest frequency of response. Pressure threshold level differences among individuals varied between <1 and 19 dB, but up to 6 dB of this variability could be due to the step size used during the AEP measurements. Particle acceleration threshold differences were quite small at frequencies below 400 Hz (< 0.001 m/s^2) but differences increased with increasing frequency (0.085 m/s^2 at 800 Hz). All turtles responded frequencies between 50 and 800 Hz, but only three responded to 1,600 Hz. Background noise levels were <75 dB re: 1 μPa at 50 Hz, <63 dB re: 1 μPa at 300 Hz, and decreased as frequency increased.

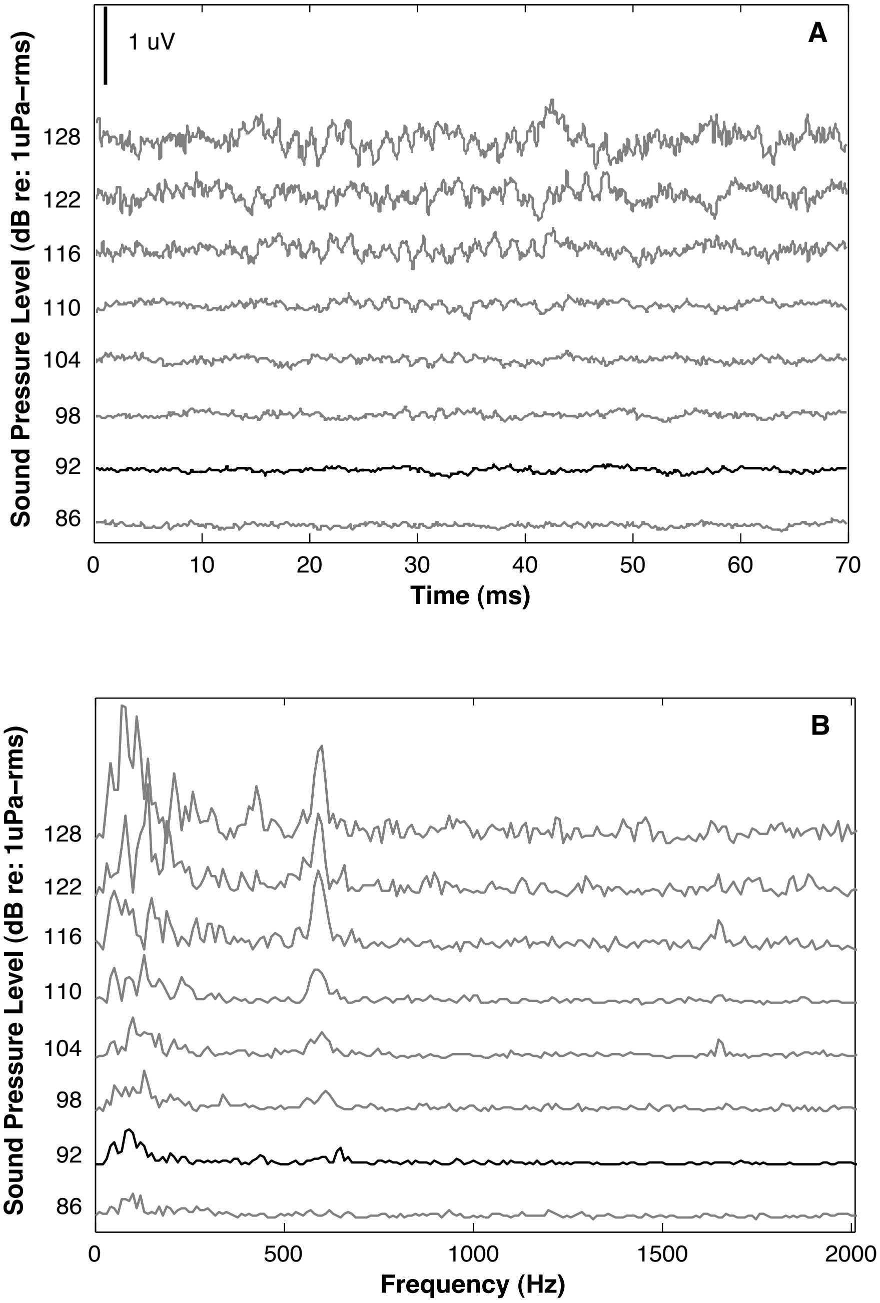


Figure 1. a. Underwater auditory evoked potential waveforms recorded from a juvenile green sea turtle (*Chelonia mydas*, L4) and corresponding stimuli levels in response to an underwater signal of 300 Hz. b. 2048-point fast Fourier transforms of recorded auditory evoked potentials (presented in a.) showing peak at twice the frequency presented (600 Hz). Threshold level is presented in black (92 dB re: 1 µPa-rms).

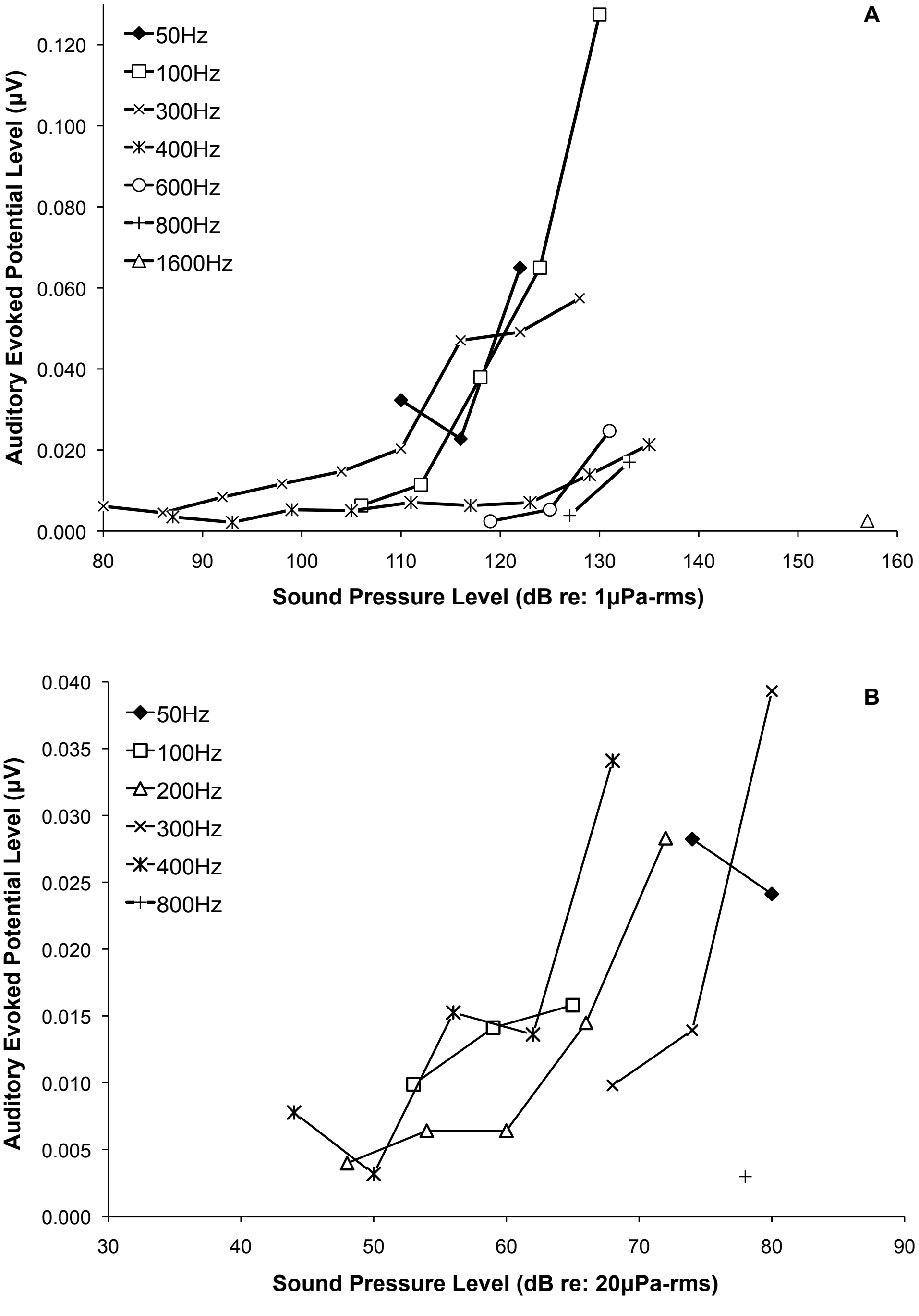


Figure 2. Juvenile green sea turtle (*Chelonia mydas*) underwater (a) and aerial (b) input-output functions of AEP level (µV) as a function of stimulus sound pressure level.

Table 1. Underwater pressure thresholds (dB re: 1 µPa-rms) for individual juvenile green sea turtles (*Chelonia mydas*), and mean thresholds for all turtles combined.

Turtle	**Frequency (Hz)**								
ID	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1600**	**3200**
R1	101	99	95	97	93	116	141	146	•
L2	95	94	87	85	88	121	140	>150	>146
R3	104	98	102	91	95	127	137	>155	>147
L3 A	106	99	95	•	101	•	137	150	>151
L4	110	106	•	92	99	125	127	157	•
L4 A	101	99	93	104	110	130	130	152	>152
Mean[a]	102	99	95	93	96	123	137	150	NA

"A" denotes use of anesthesia during collection of auditory evoked potentials

- *denotes a frequency not tested*

[a] *Only one value for L4 (mean of L4 and L4 A) was used for this calculation*

Table 2. Underwater particle acceleration thresholds (m/s^2) for individual juvenile green sea turtles (*Chelonia mydas*), and mean thresholds for all turtles combined.

Turtle	**Frequency (Hz)**							
ID	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1600**
L2	1.77 x 10^{-3}	1.49 x 10^{-3}	3.2 x 10^{-4}	1.7 x 10^{-4}	2.9 x 10^{-4}	1.47 x 10^{-2}	1.06 x 10^{-1}	>2.83 x 10^{-1}
R3	1.82 x 10^{-3}	9.4 x 10^{-4}	1.58 x 10^{-3}	3.65 x 10^{-4}	1.87 x 10^{-4}	2.35 x 10^{-2}	7.85 x 10^{-2}	>5.72 x 10^{-1}
L4	2.42 x 10^{-3}	3.89 x 10^{-3}	•	7.92 x 10^{-4}	1.01 x 10^{-3}	1.45 x 10^{-2}	2.1 x 10^{-2}	5.42 x 10^{-1}
Mean	2.12 x 10^{-3}	2.11 x 10^{-3}	9.51 x 10^{-4}	4.42 x 10^{-4}	4.96 x 10^{-4}	1.76 x 10^{-2}	6.86 x 10^{-2}	5.42 x 10^{-1}

- *denotes a frequency not tested*

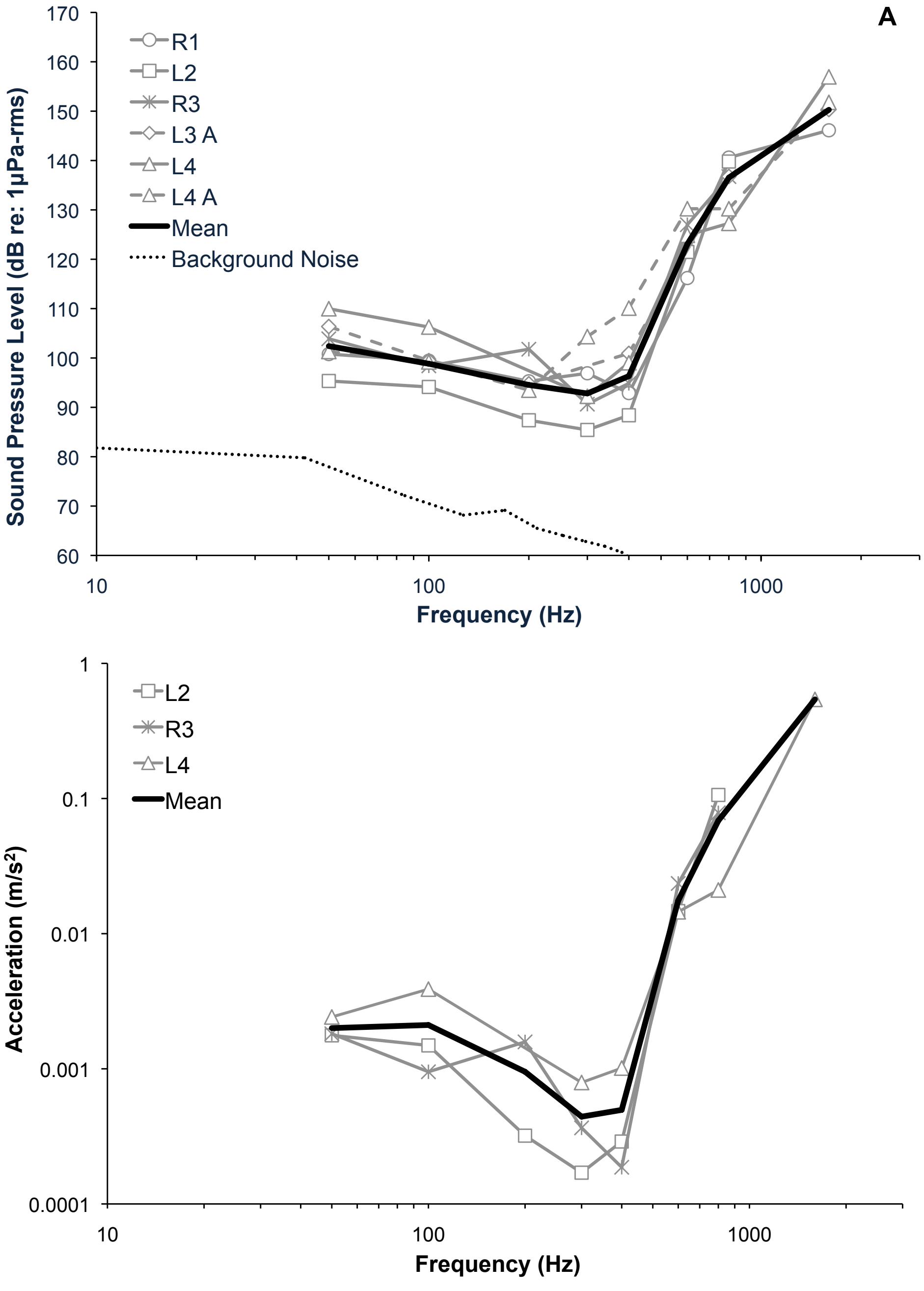


Figure 3. Underwater audiograms for juvenile green sea turtles (*Chelonia mydas*) in terms of pressure (a) and particle acceleration (b). "A" denotes the use of anesthesia when recording auditory evoked potentials. The hearing sensitivity of L4 was measured twice, with and without anesthesia (mean calculation uses the mean of these two measurements). Spectrum level background noise is represented by the dotted line (dB re: 1 µPa/√Hz).

Aerial audiogram

Juvenile green turtles responded to signals between 50 and 800 Hz in air, with maximum sensitivity at 400 Hz (Table 3, Fig. 4); sensitivity decreased sharply above 400 Hz. The lowest pressure sensitivity recorded was 44 dB re: 20 µPa-rms at 300 Hz (for turtle L3). I found variation among individuals in frequency threshold levels and highest and lowest frequency of response. I found variation among individuals ranging from <1 to 18 dB re: 20 µPa in air, however up to 6 dB of this variability could be due to the step size used in the AEP measurements. Four turtles responded to 800 Hz, and two turtles responded to 50 Hz. Background noise levels were <50 dB re: 20 µPa at 50 Hz, <28 dB re: 20 µPa at 300 Hz, and decreased with increasing frequency.

Table 3. Aerial pressure thresholds (dB re: 20 µPa-rms) for individual juvenile green sea turtles (*Chelonia mydas*), and mean thresholds for all turtles combined.

Turtle ID	**Frequency (Hz)** **50**	**100**	**200**	**300**	**400**	**600**	**800**	**1600**	**3200**
R1	>76	67	60	56	50	71	78	>61	>53
L2	80	59	60	62	50	75	72	>60	>49
R3	>80	65	66	68	56	69	78	>60	•
L3	>72	70	66	50	44	73	>78	>63	>58
L4	80	65	66	68	56	•	78	>60	•
Mean	80	65	64	60	51	72	77	NA	NA

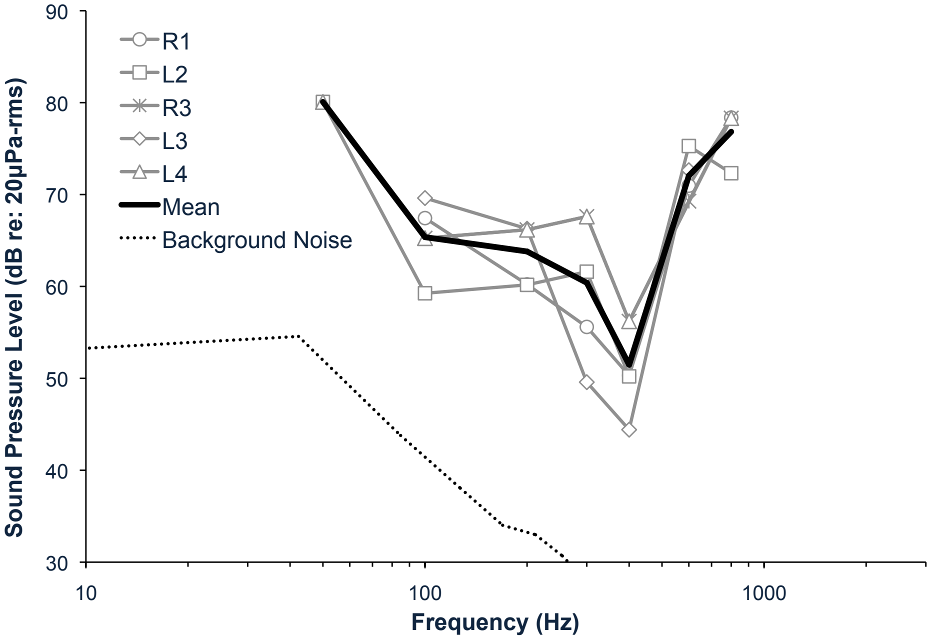


Figure 4. Aerial audiograms for juvenile green turtles (*Chelonia mydas*). Spectrum level background noise is represented the dotted line (dB re: 20 µPa/√Hz).

Auditory evoked potentials using anesthesia

The sample size was too small to perform an inferential statistical analysis, but audiograms for the two anesthetized turtles did not differ greatly from those tested without anesthesia and I found no differences in AEP waveform characteristics or latency periods. For the turtle (L4) tested both with and without anesthesia, differences between resulting thresholds varied between 3 to 12 dB, with lower anesthesia threshold levels at 50, 100 and 1,600 Hz, and higher threshold levels 300-800 Hz. Up to 6 dB of the difference in threshold levels could be due to the step size used during AEP collection. Additionally some of the difference could be due to variability in background noise levels during data collection. Some turtles resisted manual restraint (L3), rendering the collection of AEPs impossible. For these turtles anesthesia was less stressful as a restraint method, as evidenced by their better blood oxygen and lower lactate levels (see Harms et al. 2009 for details).

DISCUSSION

Green sea turtle hearing sensitivity

Juvenile green turtles have a narrow range of low frequency hearing underwater and in air. Turtles responded to underwater signals between 50 Hz to 1,600 Hz, with maximum sensitivity between 200 and 400 Hz. In air turtles responded to a narrower range of frequencies, between 50 and 800 Hz, with maximum sensitivity between 300 and 400 Hz. My underwater sound pressure threshold levels and frequencies of maximum sensitivity are similar to those measured by Bartol & Ketten (2006) for partially submerged Pacific sub-adult green turtles, but I found a greatly expanded hearing range (Ketten & Barrol: 100-500 Hz, current study: 50-1,600 Hz). The frequencies of maximum sensitivity and hearing range underwater are not consistent with those measured by Bartol & Ketten (2006) for partially submerged juvenile Atlantic green turtles. This difference may be due to variation in submergence levels (partially versus fully submerged), stimulus (aerial versus underwater) and/or population specific variability in hearing sensitivity. Ridgway et al. (1969) measured responses to cochlear potentials and not AEPs, so it is difficult to compare threshold levels in air, but the maximum sensitivity found using both techniques were similar. Unlike Ridgway I did not detect hearing sensitivity above 800 Hz in air, perhaps because my stimulus level was not high enough to elicit a response. The maximum aerial sound pressure level at 1,600 Hz was 63 dB re: 20 µPa-rms, to which the turtles did not produce a detectable AEP.

Green sea turtle AEP waveforms exhibited a frequency-doubling response at all frequencies tested, which has also been observed in other studies of fish, invertebrates and sea turtle AEPs (Casper & Mann 2006, Egner & Mann, 2005, Martin et al. 2012, Mooney et al. 2010). In fish and invertebrates it has been hypothesized that the doubling effect is due to differing hair cell orientation on the sensory epithelium of the otolith sac in the inner ear, causing some hair cells to fire during the compression phase of a sound wave and others to fire on the rarefaction phase, resulting in a doubled response. Sea turtle inner ears have cochleae, rather than otoliths, but a differing orientation of limbic and basilar membrane hair cells, as found in freshwater turtles (*Chysemys scripta elegans*: Wever 1978), could cause a similar double firing and doubled response.

It is challenging to evaluate responses to low frequency stimuli with AEP techniques. Peak background and electrical noise levels occur at very low frequencies (<200 Hz), so it can be difficult to differentiate low frequency peaks in the FFT caused by AEP presence from those caused by background noise. My determined thresholds at low frequencies are likely conservative. Background noise in this study likely masked low frequency stimuli, also resulting in higher thresholds for low frequencies where background noise was <20 dB lower than threshold levels. Critical ratios, or the difference between sound level for a barely audible tone and the spectrum level of background noise at a nearby frequency (Yost 2007), have not been examined in turtles. Given the prevalence of low frequency natural and anthropogenic sound in marine environments, I believe that future investigations of masking and critical ratios would be extremely useful.

Anesthesia technique and effects of anesthesia

We (Harms et al. 2009) developed a safe and effective technique to anesthetize sea turtles underwater to allow the collection of underwater AEPs without myogenic artifact (the technique is described in detail in Harms et al. 2009). Anesthesia was helpful to eliminate myogenic artifact in turtles that were not amenable to manual restraint, but chemical restraint was not required for all turtles. Manual restraint was superior to anesthesia for turtles that did not resist restraint due to better venous blood oxygenation and acceptable AEPs without the possibility of drug effects, but anesthesia was superior to manual restraint for turtles that resisted restraint, which exhibited marked lactic acidosis and for which AEP collection was not possible (Harms et al. 2009). I found differences (<12 dB) at several frequencies in the audiograms for the turtle subjected to both chemical and manual restraint techniques, but I cannot determine whether these differences were due to the anesthetic, the presence of the endotracheal tube's air sacs, and/or data collection parameters (e.g. 6 dB step size, variability in background noise levels). I recommend further research to determine if anesthesia has a significant effect on the measured hearing sensitivity of sea turtles, because this technique may be required to collect AEPs in many juvenile and adult sea turtles.

Comparison of underwater and aerial hearing sensitivities

The overall patterns of underwater and aerial audiograms of juvenile green turtles were similar, but the range of sensitivity and frequencies of maximum sensitivity were different. Importantly, I conclude that previous conjecture that the sea turtle is a poor receptor in air does not hold true. When thresholds were adjusted for reference pressures, green turtles appeared to be more sensitive to sound pressure in air, particularly at higher frequencies (Fig. 5a). Below 400 Hz, hearing sensitivity thresholds are quite similar. Aerial mean sound pressure level thresholds were lower (range: 5-34 dB) for all frequencies except for 50 Hz, where the mean aerial sound pressure level threshold was 4 dB higher than the underwater sound pressure threshold level.

Because of differences in characteristic impedances in the two media, sound intensity in water is approximately 35 dB greater than sound energy in air (under the assumptions of a plane wave in the far field). Taking this into account, when thresholds were adjusted for reference pressure and differences in impedance in the two media, underwater sound intensity level thresholds were lower (range: 2-39 dB), and green turtles appeared more sensitive to sound intensity levels underwater, particularly at frequencies below 400 Hz (Fig. 5b).

We still do not understand whether sea turtle ears respond to pressure, particle motion, or both, so it is difficult to determine whether it is more appropriate to compare hearing using pressure or intensity. Christensen-Dalsgaard et al. (2012) found better aerial sensitivity (5-12 dB) when comparing audiograms relative to sound pressure and better underwater sensitivity when evaluating intensity in the red-eared slider (*Trachemys*

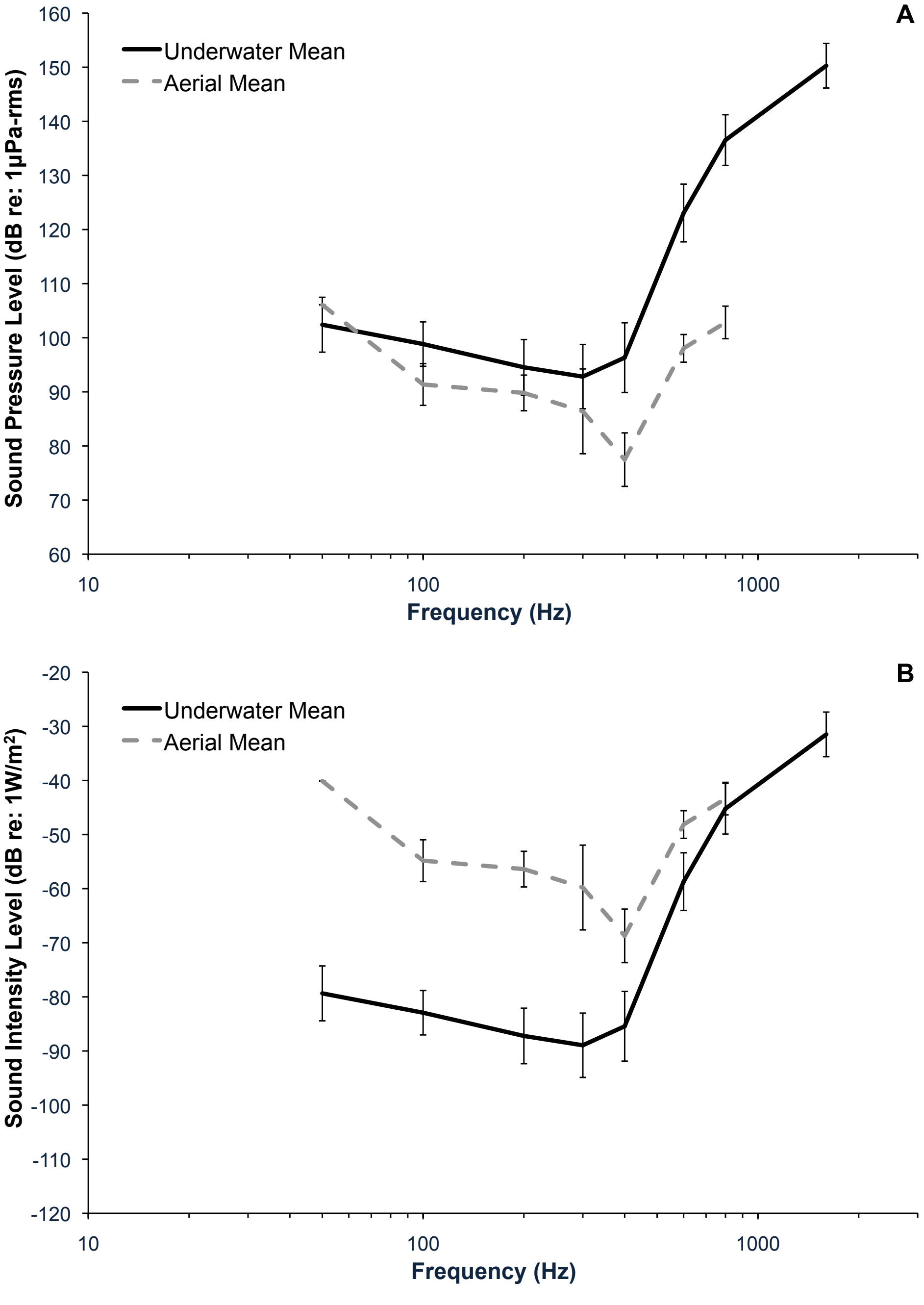


Figure 5. Comparison of mean (± 1 SD) underwater and aerial audiograms for juvenile green sea turtles (*Chelonia mydas*) in terms of pressure (a) and intensity (b).

scripta elegans). These authors hypothesized that the air-filled middle ear resonates with the underwater sound field and it is these pulsations that cause the extracollumela and columella to move, not the displacement of the tympanum. Lenhardt et al. (1983, 1985) proposed that the sea turtle ear is specialized for bone conduction, but Hetherington (2008) suggested a more standard tympanic middle ear path, given that middle and inner ears are encased in bone, restricting sound input to the oval window, further noting that marine turtles lack a heavy, inertially sensitive stapedial footplate. Bony encasing of the ear may minimize bone conduction of sound to the inner ear by restricting sound reception to the tympanum and preventing the collapse of the air-filled middle ear during deep dives, thus allowing sea turtles to hear at depth (Hetherington 2008). Christensen-Dalsgaard et al. (2012) also concluded that a specialization in bone conduction is unlikely given that low particle velocities in aquatic environments would elicit small vibrations causing an ear specialized for bone conduction to respond only to high-intensity sound levels at close ranges. The presence of an air-filled middle ear suggests that pressure likely plays some role in detection of acoustic stimuli. As a sea turtle dives, air in the lungs is pushed into the reinforced trachea, which connects to the middle ear cavity via the Eustachian tube. If the air cavity in the middle ear is compressed under pressure during deep dives and sea turtles are sensitive to only sound pressure, hearing sensitivity is likely to decrease dramatically at depth. It is possible that sea turtles detect and respond to both pressure and particle motion (via bone conduction or vibratory hearing), or that one component is detected at very low frequencies and another component is at detected higher frequencies. Experiments that are able to spatially separate acoustic pressure and intensity are needed in order to determine which component of sound sea turtles detect.

CONCLUSION

I conclude that juvenile green turtles can detect low-frequency acoustic stimuli both underwater and in air. The biological significance of hearing in sea turtles remains poorly understood, but as low-frequency sounds are most prevalent and travel the farthest in the marine environment there may be some advantage to sea turtles specializing in low-frequency sound detection. As acoustic stimuli may provide important environmental cues for sea turtles, further research is needed to determine how sea turtles behaviorally or physiologically respond to sounds in their environment.

Chapter II:

LEATHERBACK SEA TURTLE HEARING SENSITIVITY OVERLAPS WITH ANTHROPOGENIC SOUND

ABSTRACT

Rising levels of anthropogenic sound have created growing concern about the impact of sound on many marine vertebrates. Much of the habitat of critically endangered leatherback sea turtles (*Dermochelys coriacea*) overlaps with sound-producing activities. I measured the hearing sensitivity of leatherback hatchlings underwater and in air by recording auditory evoked potentials. Leatherbacks detected sounds in both media, responding to stimuli between 50 and 1,200 Hz underwater and 50 and 1,600 Hz in air, with maximum sensitivity between 100 and 400 Hz underwater and 50 and 400 Hz in air. Leatherback hearing sensitivity overlaps with the frequencies of many anthropogenic sources, including seismic airgun arrays, drilling, low-frequency sonar, shipping, pile driving, and wind turbines. My findings suggest that leatherbacks are able to detect the sounds produced by many human activities, and highlight the need to investigate potential physiological and behavioral impacts of these sounds.

INTRODUCTION

Our understanding of the effects of anthropogenic sound on sea turtles remain largely unknown because we understand so little about their hearing abilities. Green (*Chelonia mydas*), loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles can detect low-frequency tonal or vibratory stimuli in air and underwater

(Table 4), but no data exist on the hearing capabilities of critically endangered (IUCN 2012) leatherback sea turtles (*Dermochelys coriacea*). Leatherbacks have the largest latitudinal range of all sea turtles, foraging at high latitudes and migrating to nest in low latitudes (Eckert et al. 2012). Sound-producing human activities, such as oil and gas exploration and extraction, shipping, construction and sonar, occur globally in leatherback nesting and foraging habitats. In the present study I measured the hearing sensitivity of leatherback sea turtles.

MATERIALS AND METHODS

Auditory evoked potential measurements

I measured the hearing thresholds of 12 hatchling leatherback sea turtles underwater or in air by recording auditory evoked potentials (AEPs). AEPs are electrical responses produced by the central auditory nervous system after stimulation by sound detectable by the ear (Yost 2007). I collected hatchlings (mean weight: 44.8 g; mean curved carapace length: 63.2 mm; mean curved carapace width: 54.1 mm) emerging from nests at Matura Beach, Trinidad and Tobago. Before testing, I isolated hatchlings from noise and vibrations and lightly restrained them in elastic wrap. To further reduce myogenic artifacts, I lightly sedated hatchlings using midazolam at 2 or 3 mg/kg injected in the dorsal cervical sinus.

For underwater measurements, I submerged turtles 14 cm (range: 13.2-14.5 cm; measured at the location of the ear) in a high-density cylindrical polyethylene tank (94 cm in height and 40.6 cm in diameter) placed on 15 cm of Styrofoam. I suspended an

Table 4. Measurements of sea turtle hearing sensitivity.

Species	Technique	Stimulus	Media	Range (Hz)	Max Sensivitiy (Hz)	Sample Size	Source
Chelonia mydas							
juvenile	cochlear potential	aerial vibration	air	100-1000 30-700	300-500 300-500	3	Ridgway et al. 1969
juvenile (Atlantic) sub-adult (Pacific)	ABR	aerial	partially submerged	100-800 100-500	600-700 200-400	2 6	Bartol & Ketten 2006
Caretta caretta							
juvenile	ABR	vibration	air	250-1000	250	35	Bartol et al. 1999
adult	AEP behavior	underwater	water	100-1131 50-800	100-400 100-400	1	Martin et al. 2012
Lepidochelys kempii							
juvenile	ABR	aerial	partially submerged	100-500	100-200	2	Bartol & Ketten 2006

AEP: Auditory evoked potential; ABR: Auditory brainstem response amplified speaker (AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Inc.; amplifier: Servo 120A, Samson Technologies, Inc.) 5 cm from the bottom of the tank, at a distance that averaged 61.2 cm (range: 59.7-62.3 cm) from the turtle's ear. I collected AEP measurements in 45-60 second intervals, allowing the hatchling to breathe after each interval. Seawater temperatures averaged 26.8 ºC (range: 25.9-28.5 ºC). For aerial measurements I placed hatchlings on vibration-reducing foam and suspended an amplified speaker (speaker: DI 6.5R Definitive Technology; amplifier: Servo 120A) 40 cm in front of the turtle, level with its ear. Air temperatures averaged 27.6 ºC (range: 26.4-29.8 ºC).

I used an Evoked Potential Workstation (Tucker-Davis Technologies, Inc.) and laptop computer with SigGenRP and BioSigRP software (Tucker-Davis Technologies, Inc.) to generate tonal stimuli and record AEP responses. I inserted needle electrodes (27 ga, 6 mm in length, Rochester Electro-Medical, Inc.) subdermally above the brain (recording); in deltoid muscle of the shoulder (reference); and beneath the skin under the rear of the carapace (ground). I amplified and filtered signals from the electrodes using a digital biological amplifier (gain: 20; RA16LI/Medusa, Tucker-Davis Technologies, Inc.) (high pass: 50 Hz, low pass: 5 kHz, band reject: 60 Hz). Electrode impedances were less than 1 kΩ. I presented pulsed tonal stimuli shaped with a Hanning window, 50 ms in length, at a rate of 11 s^{-1} with alternating phase. I recorded AEP responses to frequencies between 50 and 1,600 Hz, and attenuated tones in 6 dB steps beginning at the highest level that could be generated and attenuating until no AEP signal was detected (after 1,000 signal averages). I paused recordings when turtles moved or lifted their heads to ensure all measurements were made with the head in the same position. I released hatchlings back to the nesting beach 24 hours after collection.

Calibration

I calibrated the sound field and measured background noise using a hydrophone (HTI96-min, High Tech, Inc.; sensitivity: -164 dBV/µPa; 0.02-30 kHz) underwater and a microphone (M31, LinearX Systems, Inc; sensitivity: -117 dBV/20 µPa; 0.1-10 kHz) in air placed at the location of the center of the turtle's head with the turtle absent. I made calibrations using two Evoked Potential Workstation RP2.1 modules and BioSigRP, which repeatedly played the signal at the same rate used while collecting AEPs, and simultaneously recorded the hydrophone signal at 24,414 Hz. I recorded background noise using FieldLog (David Mann, University of South Florida) at 24,414 Hz using the RP2.1 module and analyzed background noise frequency spectra using MATLAB (version 7.14, MathWorks, Inc.). To ensure AEP signals were not the result of electrical artifacts, I also collected control AEP measurements from a dead hatchling.

Data analyses

Turtle movement and low-frequency background and electrical noise did not allow automated threshold detection, so I performed manual threshold analyses. I analyzed AEP signals in the time and frequency domains using BioSigRP and MATLAB. I used a 2,048-point fast Fourier transform (FFT) to analyze AEP signals in the frequency domain. An AEP was considered present if the signal showed a peak twice that of the stimulus frequency at least 6 dB above the noise floor 100 Hz on either side of peak in the frequency domain. I defined threshold as the lowest sound level at which a peak in the FFT was recorded. To generate audiograms, I plotted the threshold for each frequency tested (Excel: version 12.3.3 Microsoft Corporation).

RESULTS

AEP waveforms increased in latency and decreased in amplitude as I attenuated the stimuli (Fig. 6a) and were twice the frequency of the presented tonal stimuli (Fig. 6b). Underwater AEP waveforms appeared 17-20 ms after stimulus presentation. The duration of underwater experiments did not allow for full audiograms to be collected for each individual, so I recorded responses to 3-5 frequencies per hatchling and averaged threshold levels to create one audiogram. Leatherbacks responded to underwater signals between 50 and 1,200 Hz with maximum sensitivity between 100 and 400Hz (Table 5, Fig 7a). The lowest sensitivity recorded was 84 dB re: 1 μPa-rms at 300 Hz. Background noise levels were <45 dB re: 1 μPa at 50 Hz, <35 dB re: 1 μPa at 300 Hz, and decreased with increasing frequency. Underwater frequency threshold level differences ranged from <1 to 18 dB among individuals, but up to 6 dB of this variability could be due to the step size used during measurements. All individuals responded to 600 Hz, but only four responded to 800 Hz and two responded to 1,200 Hz. No hatchlings responded to 1,600 Hz at a level of 128-129 dB re: 1μPa.

Aerial AEP waveforms appeared 13-16 ms after stimulus presentation. Leatherbacks responded to aerial signals between 50 and 1,600 Hz, with maximum sensitivity between 50 and 400 Hz (Table 6, Fig. 7b). The lowest sensitivity recorded was

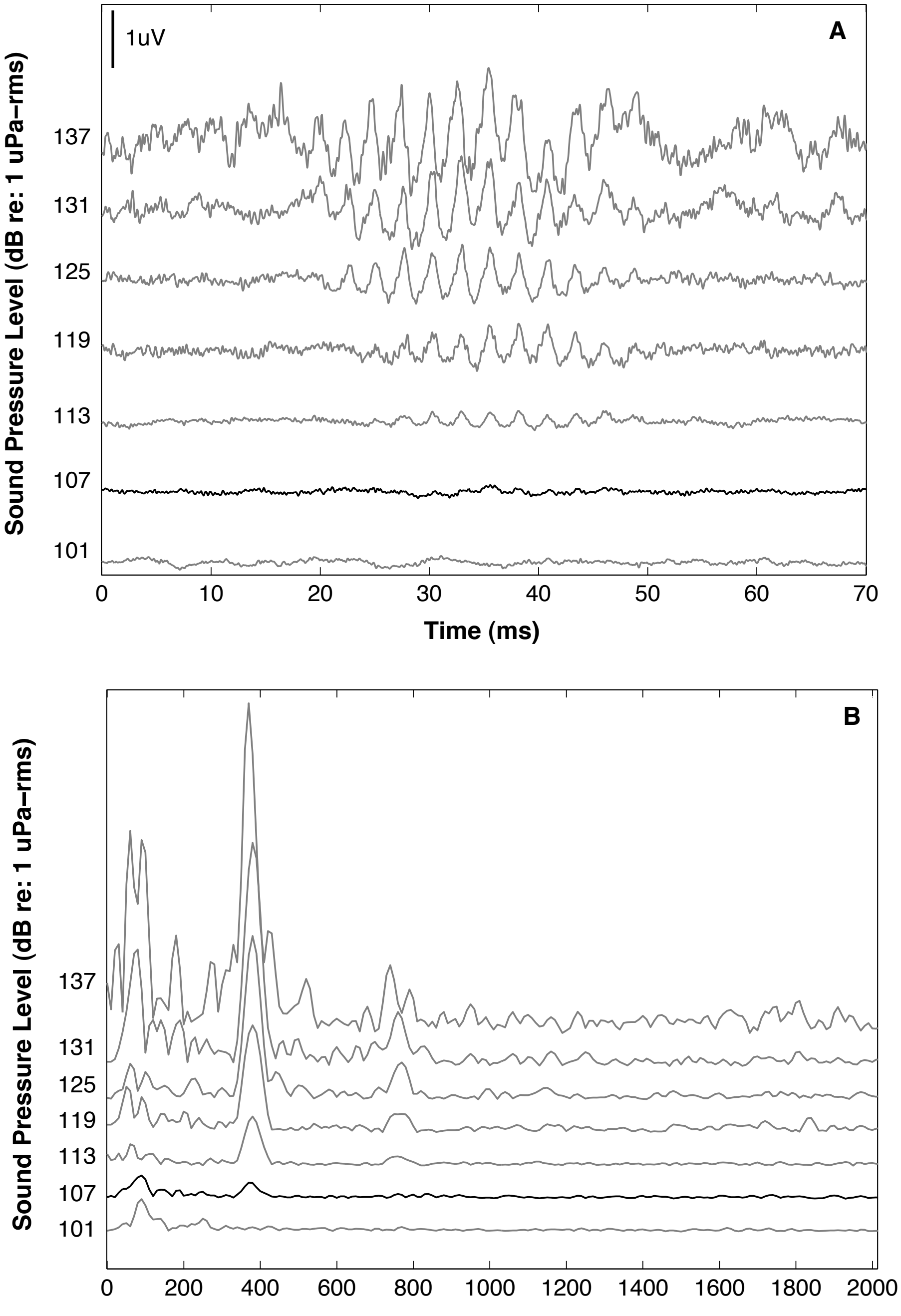


Figure 6. a. Underwater auditory evoked potential waveforms recorded from a hatchling leatherback sea turtle (*Dermochelys coriacea*) and corresponding stimuli levels in response to an underwater signal of 200 Hz. b. 2,048-point FFTs of recorded AEPs (presented in a.) showing peak at twice the frequency presented (400 Hz). Threshold level is presented in black (107 dB re: 1 μPa-rms).

Table 5. Underwater thresholds (dB re: 1 µPa-rms) for leatherback sea turtle hatchlings (*Dermochelys coriacea*), and mean thresholds for all turtles combined. Frequencies tested with no detected auditory evoked potential response are presented with > “highest sound pressure level presented” (dB re: 1 µPa-rms).

Turtle	**Frequency (Hz)**								
ID	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1200**	**1600**
Dc11	•	•	•	84	•	116	•	•	>129
Dc12	•	•	101	•	•	•	>135	•	•
Dc13	•	•	•	•	111	•	131	•	•
Dc14	125		•	•	117	•	•	•	>129
Dc15	•	106	•	101	•	•	135	>142	•
Dc16	108	•	115	•	•	134	•	•	•
Dc17	123	105	•	•	123	•	137	•	•
Dc18	•	•	107	•	•	122	134	136	>128
Dc19	•	106	•	93	113	•	•	142	•
Dc20	•	104	•	95	•	128	•	>141	•
Dc21	125	•	104	•	•	134	>137	>141	•
Mean	120	105	107	93	116	127	134	139	>128

- *denotes a frequency not tested*

Table 6. Aerial thresholds (dB re: 20 µPa-rms) for individual leatherback sea turtle hatchlings (*Dermochelys coriacea*). Frequencies tested for which no auditory evoked potential was recorded are presented with > “highest sound pressure level presented” (dB re: 20 µPa-rms).

Turtle	**Frequency (Hz)**								
ID	**50**	**200**	**300**	**400**	**600**	**800**	**1000**	**1200**	**1600**
Dc1	74	74	73	82	91	92	>105	>110	>110
Dc2	81	86	73	88	•	98	•	•	110
Dc3	75	74	67	82	98	>97	•	•	•
Dc4	81	80	67	82	92	97	104	110	110
Dc6	81	80	75	78	•	93	104	•	110
Dc7	81	85	73	86	92	>97	103	•	>109
Dc8	81	85	74	87	•	97	•	•	111
Mean	79	81	72	84	93	95	104	110	110

- *denotes a frequency not tested*

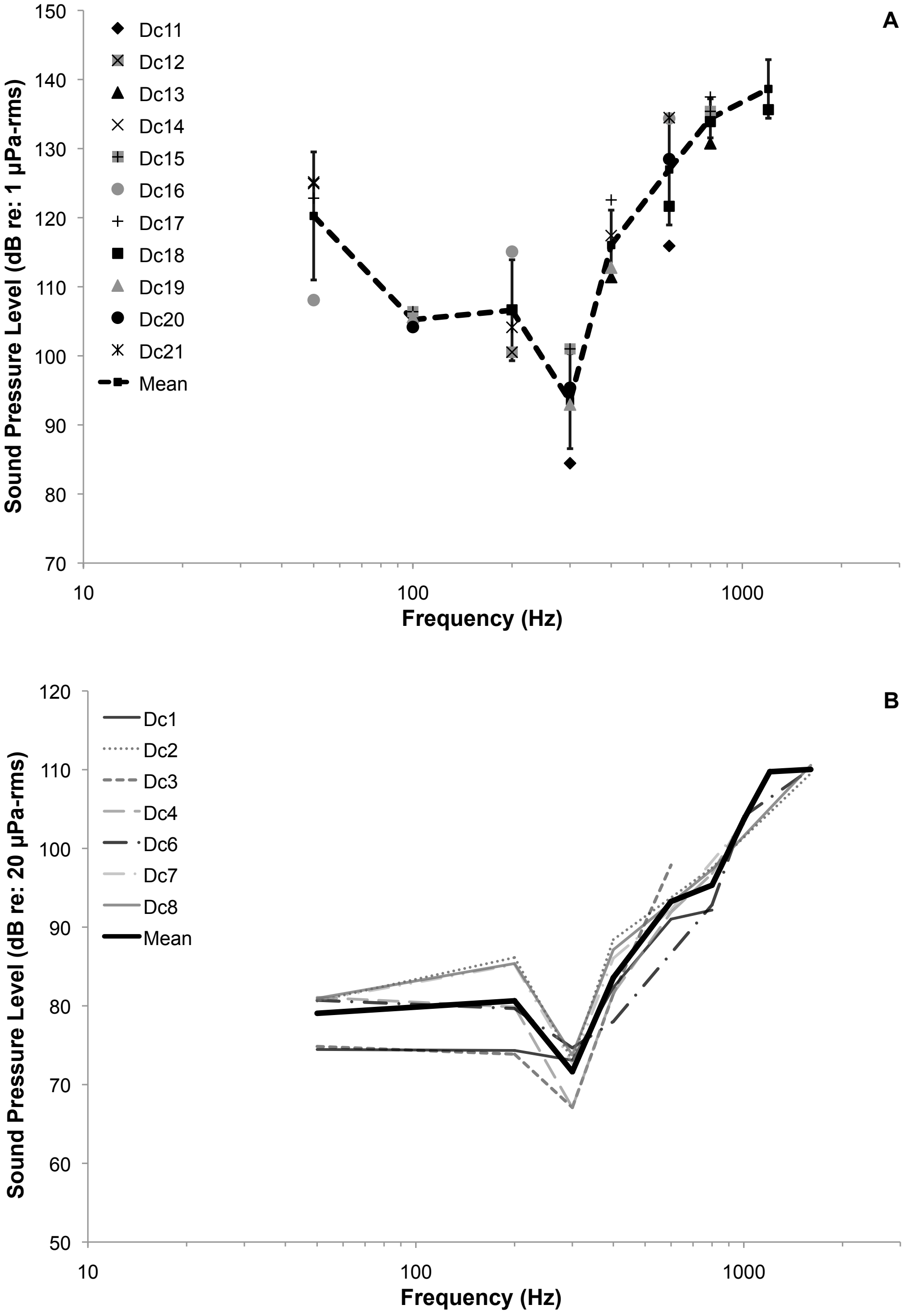


Figure 7. (a) Underwater audiogram (n = 11) (mean thresholds ± 1 SD) for hatchlings. (b) Aerial audiograms (n = 7) for individual hatchlings and mean audiogram for all hatchlings.

67 dB re: 20 µPa-rms at 300 Hz. Background noise levels were <0 dB re: 20 µPa at 50 Hz and decreased precipitously with increasing frequency. Aerial frequency threshold level differences ranged from <1 to 12 dB among individuals, but up to 6 dB of this variability could be due to the step size used during AEP measurements. All individuals tested responded to 600 Hz in air, but only five responded to 800 Hz, and four responded to frequencies >1,000 Hz. I did not to test above 1,600 Hz because I did not want to present turtles with potentially damaging levels of sound.

DISCUSSION

Auditory evoked potential measurements

Leatherback sea turtle hatchlings detected low-frequency tonal stimuli underwater and in air. I recorded similar frequencies of hearing, but the hatchlings I studied responded to much lower sound pressure levels than the adult loggerhead sea turtles studied by Martin et al. (2012), who used similar methodologies to measure underwater AEPs (loggerhead: 110 dB re: 1 µPa at 200-400 Hz, Martin et al. 2012; leatherback: max: 84 dB, mean: 93 dB re: 1 µPa-rms at 300 Hz). These differences could reflect variation among individuals, species, and/or age-classes.

A challenge in using AEPs to measure hearing sensitivity is the determination of thresholds. Peak noise levels occur at low frequencies, so it can be difficult to distinguish low-frequency peaks in the FFT caused by AEP from those caused by noise; thus estimates of low-frequency thresholds are often conservative. Critical ratios (Yost 2007) have not been examined in sea turtles, but noise levels in this study were very low in both media (>60 dB below thresholds in air and >50 dB below thresholds underwater) and were unlikely to have masked thresholds. I was able to detect AEP responses to 50 Hz in air, because of low signal-to-noise ratio or electrical interference, but I was unable to record responses at 100 Hz. I could not test below 50 Hz, but the flat shape of the audiogram below 200 Hz in air and the threshold level of 50 Hz in water suggest that leatherbacks may be able to detect sound below 50 Hz in air and underwater. It is also possible that higher stimuli levels may have elicited AEP responses at higher frequencies (≥1,200 Hz in water and ≥1,600 Hz in air).

Leatherback sea turtles and anthropogenic sound

High intensity sounds can cause physiological trauma and even death in some vertebrates (Richardson et al. 1995). No data exist on the physiological effects of anthropogenic sound on sea turtles, but other vertebrates exposed to such extreme stimuli may experience temporary or permanent auditory sensitivity threshold shifts. Noise can also mask important acoustic cues, but no information exists on masking in sea turtles. Cumulative effects of exposure are not well understood, but repeated exposures can cause habituation or sensitization, thus amplifying long-term physiological harm.

The underwater hearing sensitivity of leatherbacks overlaps with low-frequency anthropogenic sound sources such as: seismic airgun arrays, 5-300 Hz, 260 dB re: 1 μPa (Turner et al. 2006); offshore drilling, 700-1,400 Hz, 184 dB re: 1 μPa (Blackwell et al. 2004); low-frequency military sonar, 100-500 Hz, 215 dB re: 1 μPa (Anonymous 2007); pile driving (900 kJ hammer), 100-1,000 Hz, 209 dB re: 1 μP-peak: (Reyff 2007); cargo vessels (173 m in length, 16 knots), 40-100 Hz, 192 dB re: 1 µPa (NRC 2003); and wind turbines (wind speed 13 m-s, 180 Hz), 60-300 Hz, 151 dB re: 1 µPa: Wahlberg & Westerberg, 2005).

I recommend future studies investigate the physiological (critical ratios and temporary and permanent threshold shifts) and behavioral effects of exposure to these sound sources. As the temporal and spatial overlap of leatherback sea turtle habitat and anthropogenic sound varies depending on the environment and the anthropogenic sound source, it would be useful to examine the response of leatherbacks to a variety of anthropogenic sound sources, including those produced continuously over large areas (e.g. shipping) and especially those produced discretely and with high intensity in small areas (e.g. pile driving and sonar).

CHAPTER III:

UNDERWATER AND AERIAL HEARING IN HATCHLING HAWKSBILL SEA TURTLES (*ERETMOCHELYS IMBRICATA*)

ABSTRACT

Sea turtles inhabit both aquatic and terrestrial environments as hatchlings and small juveniles, so it may be important for them to detect sound in both media. I measured the underwater and aerial hearing sensitivities of hatchling hawksbills sea turtles (*Eretmochelys imbricata*) by recording auditory evoked potential (AEP) responses to tonal stimuli. Hawksbills detected acoustic stimuli in both air and water, responding to underwater and aerial signals between 50 and 1,600 Hz, with maximum sensitivity between 200 and 400 Hz. In terms of pressure, hawksbills were slightly more sensitive to aerial stimuli, but when sensitivities were compared in terms of sound intensity, they were more sensitive to underwater stimuli. My findings suggest that hawksbills are able to detect low-frequency natural and anthropogenic sounds in the marine environment, and emphasize the need to investigate how sea turtles use such acoustic cues and respond to sources of anthropogenic noise.

INTRODUCTION

Sea turtles perceive and respond to visual, magnetic and chemical cues (e.g. visual: Crognale et al. 2008, Levenson et al. 2004, Wang et al. 2007, Wang et al. 2010, Young et al. 2012; magnetic: Avens et al. 2003, Fuxjager et al. 2011, Lohman et al. 2001, Lohmann et al. 2004; chemical: Endres et al. 2012, Grassman & Owens 1987, Manton et al. 1972, Owens et al. 1982), but we understand very little about how they perceive and respond to acoustic stimuli. The anatomy of the sea turtle ear is fairly well described (Wever 1978, Hetherington 2008), but the functional morphology of the sea turtle ear is not well understood and, in particular, it is unclear whether sea turtle ears respond to acoustic pressure, particle motion, or both (Chapter I).

Electrophysiological and behavioral studies of hearing have demonstrated that green (*Chelonia mydas*), loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*) and leatherback (*Dermochelys coriacea*) sea turtles detect low frequency acoustic and vibratory stimuli underwater and in air. Vibratory and aerial tones elicited cochlear responses in juvenile green turtles between 50 and 2,000 Hz (Ridgway et al. 1969). Studies of auditory evoked potential (AEP) responses to aerial tones documented hearing between 100 and 500 Hz in juvenile greens, 100 and 800 Hz in subadult greens, 100 and 500 Hz in juvenile Kemp's ridleys partially submerged (Bartol & Ketten 2006). In loggerheads, vibratory stimuli elicited AEP responses between 250 and 750 Hz (Bartol et al. 1999) and underwater tones elicited behavioral responses to frequencies between 50 and 800 Hz and AEP responses between 100 and 1,131 Hz (Martin et al. 2012).

Elsewhere in this thesis I have reported that juvenile green turtles detect tonal stimuli between 50 and 1,600 Hz underwater (maximum sensitivity: 200-400 Hz) and 50 and 800 Hz in air (maximum sensitivity: 300-400 Hz) (Chapter I) and hatchling leatherbacks detected tonal stimuli between 50 and 1,200 underwater (maximum sensitivity: 100-400 Hz) and 50 and 1,600 in air (maximum sensitivity: 50-400Hz) (Chapter II). But no data exist on the auditory capabilities of hawksbill sea turtles

(*Eretmochelys imbricata*) and very little amphibious hearing data have been collected from hatchlings of any species (see Chapter II). Hawksbills are listed as critically endangered by the International Union for Conservation of Nature (IUCN 2012). Like other sea turtles, hawksbills may use sound for navigation, avoiding predators and general environmental awareness. For example, hawksbills may locate coral reefs, by following acoustic cues, as has been hypothesized for larval reef fish and invertebrates (Simpson et al. 2005, Stanley et al. 2012, Tolimieri et al. 2004) or use the sounds of crashing waves to locate suitable nesting beaches. Hawksbills travel great distances between developmental, foraging, and nesting habitats (Plotkin 2003). Hatchlings emerge from the nest, crawl down to the sea and find their way offshore to oceanic developmental habitats. To conduct these remarkable feats of navigation, sea turtles likely use visual, magnetic, chemical and auditory cues. Once in the oceanic environment, hawksbill hatchlings live at the air-water interface, foraging in floating seaweed (Musick & Limpus 1997). All sea turtle hatchlings and young juveniles spend much of their lives at the interface between underwater and aerial environments, suggesting that it may be important for them to detect sound in both media.

In the present study, I made the first measurements of underwater and aerial hearing sensitivities of hawksbill sea turtles in reference to both pressure and particle motion. I also compared aerial and underwater hearing sensitivities in relation to the sound pressure and sound intensity.

MATERIALS AND METHODS

Sea turtles

I measured the hearing sensitivity of 10 hatchling hawksbill sea turtles in water or air by recording auditory evoked potentials (AEPs) in Barbados. Hatchlings, collected after emergence from several different nests, averaged 15 g in weight (range: 11-17 g), 41.4 mm in straight carapace length (range: 39.2-43.1 mm), 30.3 mm in straight carapace width (range: 29.3-32 mm), 44.7 mm in curved carapace length (range: 41-47 mm), and 42 mm in curved carapace width (range: 39-46 mm). I released all hatchlings back at their nest sites at dusk within 24 hours of their collection.

Auditory evoked potential measurements

Underwater experimental setup

To reduce masking of AEP signals caused by myogenic artifacts I lightly restrained hatchlings by wrapping them in cloth elastic wrap. I completely submerged turtles 10 cm (measured at the location of the ear) below the surface in a 50-gallon high-density polyethylene cylindrical tank. I suspended an amplified speaker (AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Inc.; amplifier: Servo 120A, Samson Technologies, Inc.) approximately 10 cm from the bottom of the tank and 52 cm from the hatchling's ear. I submerged hatchlings using a T-bar constructed of polyvinyl chloride (PVC) pipe, which rested on the top of the tank. To reduce vibrations from the tank, I wrapped the T-bar with additional wrap and towels. To ensure hatchlings did not come in contact with the T-bar, I suspended hatchlings with an extended piece of elastic wrap and a metal clip. I used clips to hold the T-bar in place on the sides of the tank, and used precise markings on the pipe itself to ensure I raised and lowered the hatchlings to the same location after each breath. I collected AEP measurements in two-minute submergence intervals and allowed the hatchling to breathe after each interval. Submergence intervals were calculated by monitoring the respiratory rates (0.2-0.3 breaths m^{-1}) of hatchlings in the testing arena prior to the start of experiments. I chose two-minute submergence intervals (roughly half their observed normal dive times in the tank) to ensure the hatchlings were well ventilated during the underwater experiments. If a turtle appeared to desire a breath (e.g. rear flipper movement, raising head, appearance of an air bubble at either naris), I raised it to the surface regardless of the intended submergence interval. Seawater temperatures in the experimental tank averaged 26.3 ºC.

Aerial experimental setup

Before testing, I isolated hatchlings from noise and vibration and lightly restrained them by wrapping them in cloth elastic. I placed an amplified speaker (XS-MP1610W, Sony Corporation, Minato-ku, Tokyo, Japan) 20 cm directly in front of the turtle, level with the turtle's ear. To reduce the possibility of a vibratory response during these trials, I placed the entire setup on vibration-reducing foam. Air temperatures averaged 28.2 ºC during data collection.

Signal generation and AEP recording

To collect AEP signals, I inserted needle electrodes (27 ga, 12 mm in length, Rochester Electro-Medical, Inc., Lutz, Florida USA) subdermally under the frontal scale on top of the head (recording electrode); in the deltoid muscle of the neck (reference electrode); and either in the deltoid muscle of the shoulder (air: ground electrode) or seawater (water: ground electrode). Underwater electrodes were covered with Vaseline to reduce electrical noise. I employed an Evoked Potential Workstation run by laptop computer with SigGenRP and BioSigRP software (Tucker-Davis Technologies, Inc. Alachua, Florida USA) to simultaneously generate tonal stimuli and record AEP responses at a sampling rate of 24,412 Hz. I amplified signals from the electrodes using a digital biological amplifier (gain: 20; RA16LI/Medusa. Tucker-Davis Technologies, Inc.) and filtered sound outside the AEP signal frequencies (high pass: 50 Hz; low pass: 5 kHz; band reject: 60 Hz). Electrode impedances were less than 3 kΩ. I presented 50 ms pulsed sinusoidal tonal signals, shaped with a Hanning window, over an 85 ms presentation period (13 s^{-1}) with alternating phase. I recorded averaged AEP responses to frequencies between 50 and 1,600 Hz, and attenuated tones in 6 dB steps beginning at the highest level that could be generated at each frequency and attenuating until no further AEP signal could be detected (up to 1,000 signal averages). To reduce the length of the experiment, if an AEP signal was detected before 1,000 averages I advanced to the next reduced sound pressure level. I paused recordings whenever the turtles lifted their heads to breath or moved to ensure I made all measurements with the head in the same position in the acoustic field.

Calibration

I calibrated the sound field and measured background noise using a hydrophone (HTI96-min, High Tech, Inc.; sensitivity: -164 dBV/μPa; 0.02-30 kHz) in water and a microphone (M31, LinearX Systems, Inc; sensitivity: -117 dBV/20 μPa; 0.1-10 kHz) in air placed at the location of the center of the turtle's head. I made calibrations using the Evoked Potential Workstation with two RP2.1 modules and BioSigRP (Tucker-Davis Technologies, Inc.) which played the signal repeatedly at the same rate used during AEP recordings and simultaneously recorded the signal from the hydrophone at sampling rate of 24,414 Hz. I measured the underwater pressure gradient using the same hydrophone by recording pressure signals at six locations in three orthogonal directions 5 cm from the location of the original pressure measurement. I calculated the pressure gradient by subtracting the time-locked recordings in the time domain and dividing by the distance between recording locations (0.1 m). The particle acceleration was calculated by dividing the pressure gradient by the density of water (1,035 kg/m^3). I measured the background noise level using FieldLog (custom software, David Mann, University of South Florida) at a sampling rate of 24,414 Hz using the RP2.1, and analyzed background noise frequency spectra using MATLAB (version 7.14, MathWorks, Inc. Natick, Massachusetts 01760, USA). To ensure recorded AEP signals were not the result of electrical artifacts, I collected AEP measurements from a dead hatchling (found in the field after death from natural causes) at all frequencies using the same experimental setup.

Data Analyses

The presence of low-frequency background and electrical noise did not allow us to use automated threshold detection, so I manually analyzed threshold levels (e.g. Casper & Mann 2006, Egner & Mann 2005, Martin et al. 2012, Mooney et al. 2010). I made visual inspections of the AEP signal in the time domain using BioSigRP, and used a 2,048-point fast Fourier transform (FFT) to analyze the AEP signals in the frequency domain in BioSigRP and MATLAB. I considered an AEP to be present if the FFT of the signal had a peak at twice that of the presented stimulus frequency and was at least 6 dB above the AEP noise floor 100 Hz on either side of the doubling frequency. I defined threshold as the lowest sound pressure level at which a peak in the FFT was recorded. I plotted individual frequency thresholds to produce audiograms for each individual hatchling using Excel (version 12.3.3 Microsoft Corporation).

RESULTS

Auditory evoked potential waveform characteristics

Hatchling hawksbill sea turtle AEP waveforms increased in latency and decreased in amplitude as I attenuated the stimuli (Fig. 8a). Recorded AEP waveforms were twice the frequency of the presented tonal stimuli (Fig. 8b) and AEP levels (µV) decreased with decreasing sound pressure levels in both air and water (Fig. 9).

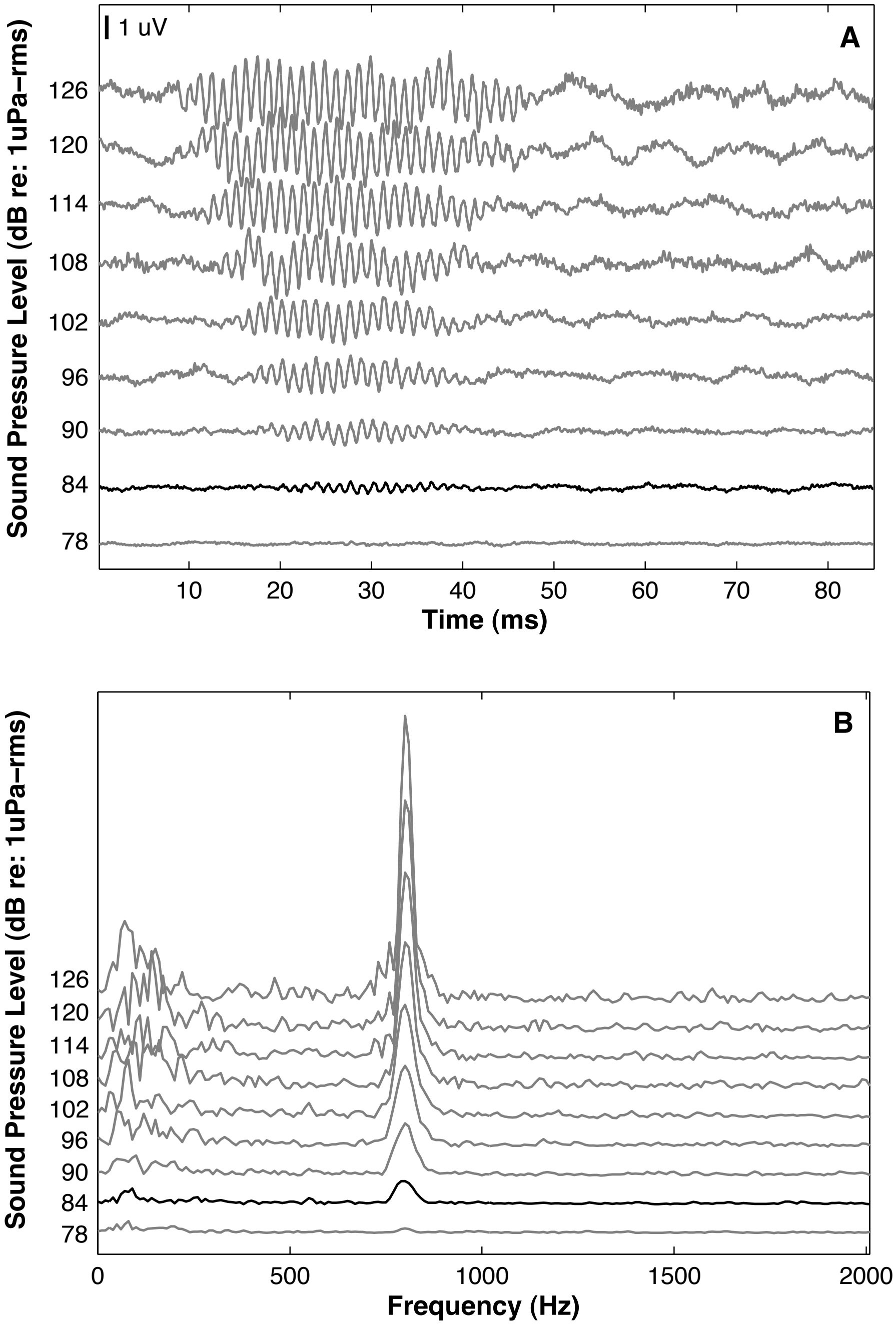
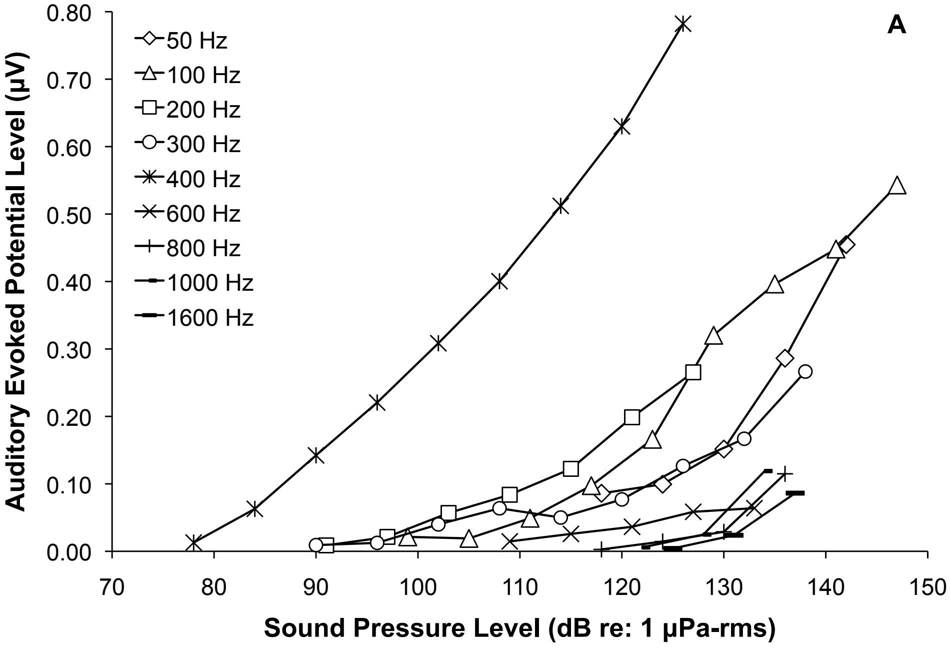


Figure 8. a. Underwater auditory evoked potential waveforms recorded from a hatchling hawksbill sea turtles (*Eretmochelys imbricata*, Ei6) and corresponding stimuli levels in response to an underwater signal of 400 Hz. b. 2048-point fast Fourier transforms of recorded auditory evoked potentials (presented in a.) showing peak at twice the frequency presented (800 Hz). Threshold level is presented in black (84 dB re: 1 μPa-rms).



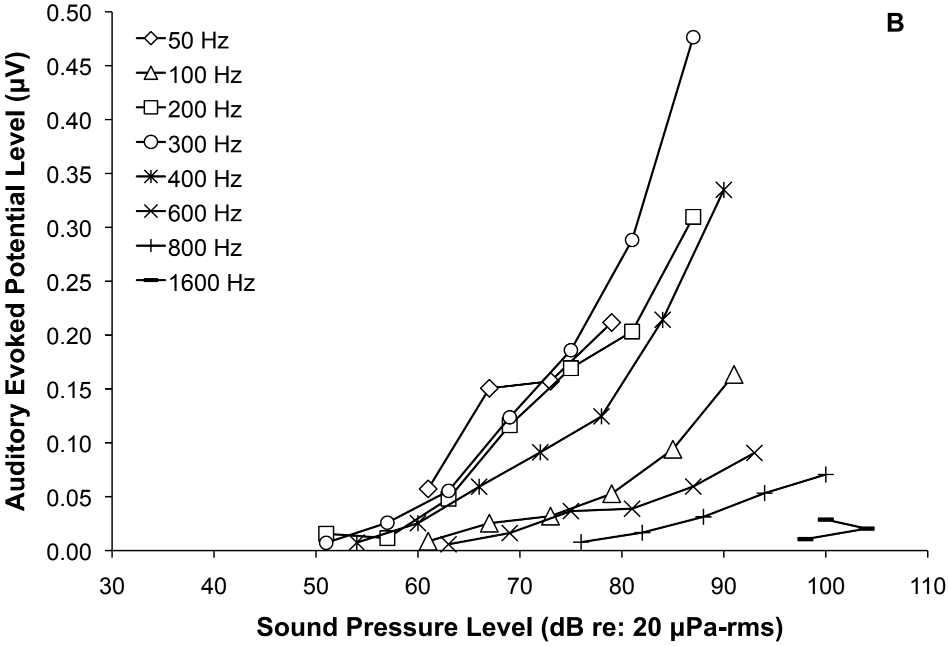


Figure 9. Hatchling hawksbill sea turtle (*Eretmochelys imbricata*) underwater (a: Ei6) and aerial (b: Ei17) input-output functions of AEP level (µV) as a function of stimulus sound pressure level.

Underwater audiograms

Hatchling hawksbills responded to underwater signals between 50 and 1,600 Hz, with maximum sensitivity between 200 and 400 Hz (Tables 7 and 8, Fig. 10); sensitivity decreased sharply at frequencies above 400 Hz. The lowest pressure sensitivity recorded was 84 dB re: 1 μPa-rms at 400 Hz (turtle Ei6). The lowest particle acceleration sensitivity was 4.22 x 10-4 m/s^2 at 300 Hz (turtles Ei3, Ei4, and Ei10). Pressure threshold level differences among individuals varied between <1 and 20 dB, although up to 6 dB of this variability could be due to the step size used during AEP measurements. Particle acceleration threshold differences were quite small at frequencies ≤400 Hz (< 0.002 m/s^2) but increased with frequency (0.034 m/s^2 at 800 Hz). All turtles responded to frequencies between 50 and 1,600 Hz. AEP signals were detected from the dead hatchling underwater at 1,000, 1,600 and 3,200 Hz. At 1,000 Hz I only detected signals at the loudest level. At 1,600 Hz I detected signals at two highest levels presented. AEP signal levels acquired from live turtles were always higher than levels acquired from the dead turtle at the same level. Because AEP response levels at 3,200 Hz were similar in both alive and dead hatchlings I did not include these data in my analyses. Background noise levels were <88 dB re: 1 μPa at 50 Hz, <57 dB re: 1 μPa at 300 Hz, and decreased with increasing frequency.

Table 7. Underwater pressure thresholds (dB re: 1 µPa-rms) for individual hatchling hawksbill sea turtles (*Eretmochelys imbricata*), and mean thresholds for all turtles combined.

Turtle	Frequency (Hz)								
ID	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1000**	**1600**
Ei3	121	110	96	93	89	101	121	127	128
Ei4	127	109	89	85	87	106	124	129	132
Ei6	124	105	97	96	84	109	124	128	131
Ei7	129	122	108	98	93	121	132	131	132
Ei10	129	116	96	92	87	109	120	125	132
Mean	126	113	97	93	88	109	124	128	131

Table 8. Underwater particle acceleration thresholds (m/s^2) for individual hatchling hawksbill sea turtles (*Eretmochelys imbricata*), and mean thresholds for all turtles combined.

Turtle	Frequency (Hz)								
ID	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1000**	**1600**
Ei3	6.34 x 10^{-3}	1.19 x 10^{-3}	4.22 x 10^{-4}	1.99 x 10^{-3}	3.83 x 10^{-3}	4.32 x 10^{-3}	2.33 x 10^{-2}	3.13 x 10^{-2}	3.47 x 10^{-2}
Ei4	6.34 x 10^{-3}	1.19 x 10^{-3}	4.22 x 10^{-4}	1.99 x 10^{-3}	3.83 x 10^{-3}	8.62 x 10^{-3}	2.33 x 10^{-2}	3.13 x 10^{-2}	3.47 x 10^{-2}
Ei7	6.34 x 10^{-3}	2.38 x 10^{-3}	1.68 x 10^{-3}	1.99 x 10^{-3}	3.83 x 10^{-3}	1.72 x 10^{-2}	4.66 x 10^{-2}	3.13 x 10^{-2}	3.47 x 10^{-2}
Ei10	6.34 x 10^{-3}	1.19 x 10^{-3}	4.22 x 10^{-4}	9.98 x 10^{-4}	1.92 x 10^{-3}	4.32 x 10^{-3}	1.17 x 10^{-2}	1.57 x 10^{-2}	3.47 x 10^{-2}
Mean	6.34 x 10^{-3}	1.49 x 10^{-3}	7.36 x 10^{-4}	1.74 x 10^{-3}	3.35 x 10^{-3}	8.62 x 10^{-3}	2.62 x 10^{-2}	2.74 x 10^{-2}	3.47 x 10^{-2}

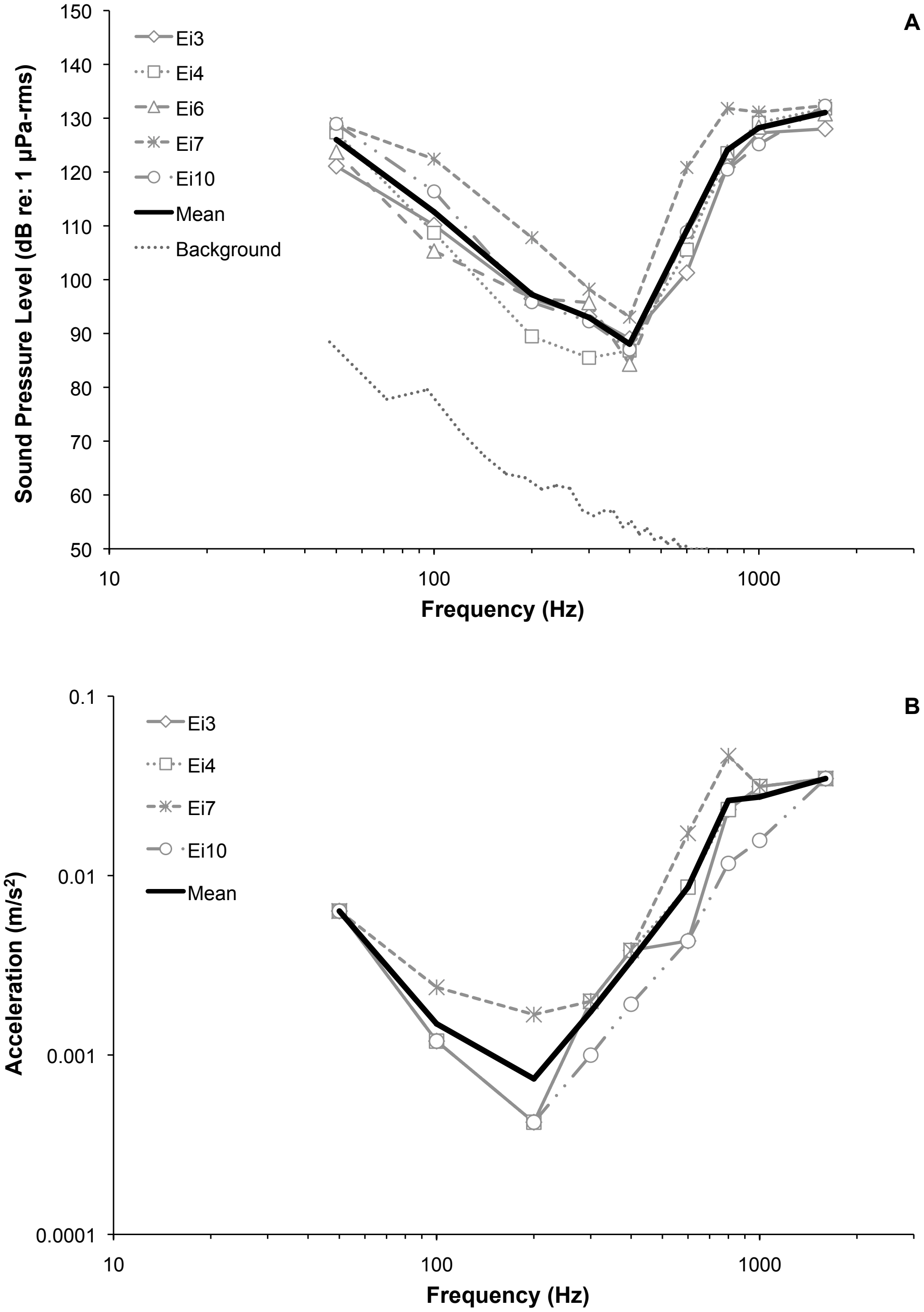


Figure 10. Underwater audiograms for hatchling hawksbill sea turtles (*Eretmochelys imbricata*): pressure (a) and particle acceleration (b). Spectrum level background noise is represented by the dotted line (dB re: 1 μPa/√Hz).

Aerial audiogram

Hatchling hawksbills responded to signals in air between 50 and 1,600 Hz, with maximum sensitivity between 200 and 400 Hz (Table 9, Fig. 11); sensitivity decreased sharply at frequencies above 400 Hz. The lowest pressure sensitivity recorded was 51 dB re: 20 µPa-rms at 300 Hz (for turtle Ei1). Individuals varied in their pressure threshold levels, ranging from <1 to 18 dB re: 20 µPa in air, though up to 6 dB of this variability could be due to the step size used in the AEP measurements. One hatchling tested at 3200 Hz did not respond to a sound pressure level of 103 dB re: 20 µPa. No AEP signals were detected from the dead hatchling in air. Background noise levels were <51 dB re: 20 µPa at 50 Hz, <33 dB re: 20 µPa at 300 Hz, and decreased with increasing frequency.

Table 9. Aerial pressure thresholds (dB re: 20 µPa-rms) for individual hatchling hawksbill sea turtles (*Eretmochelys imbricata*), and mean thresholds for all turtles combined.

Turtle ID	**Frequency (Hz)** 50	100	200	300	400	600	800	1600
Ei1	•	69	56	51	64	•	86	95
Ei13	63	63	57	57	54	70	95	104
Ei15	75	75	69	69	60	82	89	104
Ei16	68	68	68	62	53	81	89	103
Ei17	67	67	57	57	60	69	82	104
Mean	68	68	61	59	58	75	88	102

- *denotes a frequency not tested*

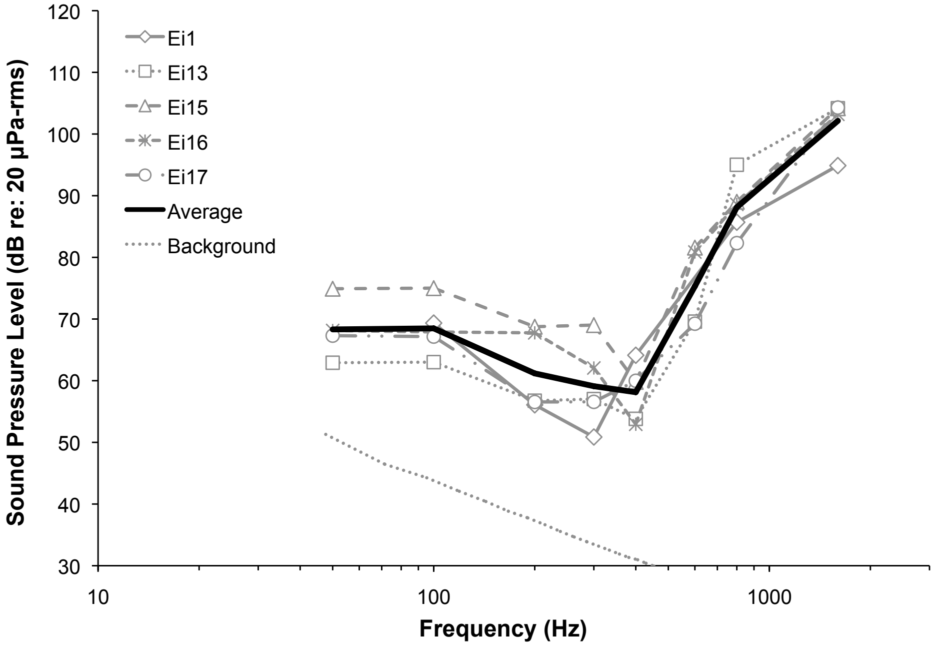


Figure 11. Aerial audiograms for hatchling hawksbill sea turtles turtles (*Eretmochelys imbricata*). Spectrum level background noise is represented the dotted line (dB re: 20 μPa/√Hz).

DISCUSSION

Hawksbill sea turtle hearing sensitivity

Hawksbill sea turtle hatchlings have a narrow range of low frequency hearing sensitivity in both air and water. Hawksbills detected tonal stimuli between 50 Hz to 1,600 Hz, with maximum sensitivity between 200 and 400 Hz in both media. It is difficult to compare my results with previous studies because of differences in methodologies, but my results are comparable to recent studies using similar methodologies (Chapter I, Chapter II, Martin et al. 2012). The ranges of hearing for all species are fairly similar (low frequencies <1,600 Hz), however I found differences in underwater sound pressure and particle acceleration and aerial sound pressure thresholds among species (see Tables 9-12). These differences in hearing sensitivity may be attributed to variation among individuals, species, and/or age-class. Future research on multiple size and age classes is needed to determine sea turtles experience ontogenetic shifts in hearing sensitivities.

Critical ratios, or the difference between sound level for a barely audible tone and the spectrum level of background noise at a nearby frequency (Yost 2007), have not been examined in turtles. However, background noise in this study may have masked some low frequency stimuli, resulting in higher thresholds for low frequencies. I could not test frequencies below 50Hz, but the flat shape of the aerial audiogram below 100 Hz suggests that hawksbills may be able to detect sound below 50 Hz in air. I was unable to detect AEP responses above 1,600, but it is possible that higher stimuli levels (>104 dB re: 20 μPa-rms in air and >132 dB re: 1 μPa-rms in water) could elicit AEP responses at higher frequencies.

Table 10. Mean underwater pressure thresholds (dB re: 1 µPa-rms) determined by recording auditory evoked potentials to underwater tonal stimuli for hatchling hawksbill (*Eretmochelys imbricata*, n = 5), juvenile green (*Chelonia mydas*, n = 5) (Chpater I), hatchling leatherback (*Dermochelys coriacea*, n = 11) (Chapter II), and adult loggerhead (*Caretta caretta*, n =1) (Martin et al. 2012) sea turtles.

Turtle	**Frequency (Hz)**											
Species	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1000**	**1131**	**1200**	**1600**	**3200**
Hawksbill	126	113	97	93	88	109	124	128	•	•	131	•
Green	102	99	95	93	96	123	137	•	•	•	150	>152
Leatherba	120	105	107	93	116	127	134	•	•	139	>128	•
Loggerhe	>119	112	110	•	110	135	143	138	141	•	>152	>155

• *denotes a frequency not tested*

Table 11. Underwater particle acceleration thresholds (m/s^2) for hatchling hawksbill (*Eretmochelys imbricata*, n = 4) and juvenile green (*Chelonia mydas*, n = 3) (Chapter I) sea turtles.

Turtle	**Frequency (Hz)**								
Species	**50**	**100**	**200**	**300**	**400**	**600**	**800**	**1000**	**1600**
Hawksbill	6.34 x 10^{-3}	1.49 x 10^{-3}	7.36 x 10^{-4}	1.74 x 10^{-3}	3.35 x 10^{-3}	8.62 x 10^{-3}	2.62 x 10^{-2}	2.74 x 10^{-2}	3.47 x 10^{-2}
Green	2.12 x 10-3	2.11 x 10-3	9.51 x 10-4	4.42 x 10-4	4.96 x 10-4	1.76 x 10-2	6.86 x 10-2	•	5.42 x 10-1

• *denotes a frequency not tested*

Table 12. Mean aerial pressure thresholds (dB re: 20 μPa-rms) determined by recording auditory evoked potentials to underwater tonal stimuli for hatchling hawksbill (*Eretmochelys imbricata*, n = 5), juvenile green (*Chelonia mydas*, n = 5) (Chpater I), and hatchling leatherback (*Dermochelys coriacea*, n = 7) (Chapter II) sea turtles.

Turtle Species	**Frequency (Hz)**										
	50	**100**	**200**	**300**	**400**	**600**	**800**	**1000**	**1200**	**1600**	**3200**
Hawksbill	68	68	61	59	58	75	88	•	•	102	•
Green	80	65	64	60	51	72	77	•	•	>63	>58
Leatherba	79	•	81	72	84	93	95	104	110	110	•

- *denotes a frequency not tested*

Comparison of underwater and aerial hearing sensitivities

The overall patterns of underwater and aerial audiograms of hawksbill sea turtle hatchlings were similar. When I adjusted for reference pressures, hawksbills appeared to be more sensitive to sound pressure in air, particularly at lower frequencies (Fig. 12a). Aerial mean sound pressure level thresholds were lower (range: 3-32 dB) for all frequencies. At frequencies ≥400 Hz, hearing sensitivity thresholds were similar and differences in MTLs were <10 dB. These results are similar to those reported for juvenile green sea turtles in that their aerial sound pressure thresholds were lower than underwater thresholds, but larger differences between the two audiograms were found at higher, rather than lower frequencies in green sea turtles (Chapter I).

When I adjusted thresholds for reference pressure and differences in impedance in the two media (under the assumptions of a plane wave in the far field), underwater sound intensity level thresholds were lower than aerial thresholds (range: 4-33 dB) and hawksbills appeared more sensitive to sound intensity levels underwater, particularly at frequencies ≥400 Hz (Fig. 12b). Again these results are similar to those reported for

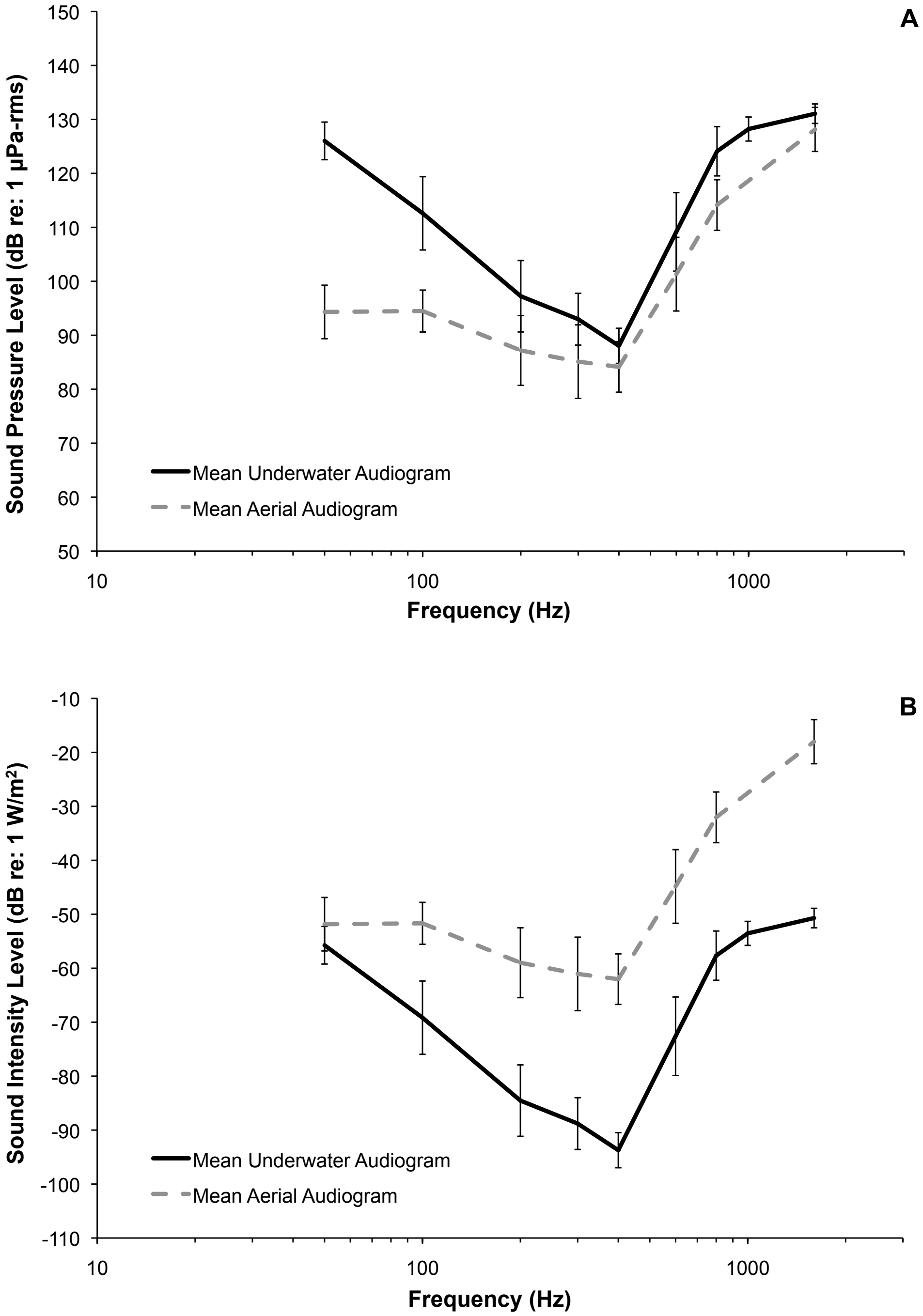


Figure 12. Comparison of mean (± 1 SD) underwater and aerial hearing sensitivities for hatchling hawksbill sea turtles (*Eretmochelys imbricata*) taking into account reference pressures (a) and the characteristic impedance differences of the two media (intensity) (b).

juvenile green sea turtles, but larger differences between the two audiograms were found at lower, rather than higher frequencies in green sea turtles (Chapter I).

CONCLUSION

Hawksbill sea turtle hearing sensitivity overlaps with much of the low-frequency natural and anthropogenic sound found in aerial and underwater environments, suggesting that hawksbills are able to detect these sounds. Further research is needed elucidate the biological significance of hearing in these sea turtles, and particularly to document the role of sound in behavioral responses, navigation and habitat use.

CHAPTER IV:

LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) BEHAVIORAL RESPONSES TO ACOUSTIC DETERRENT DEVICES

ABSTRACT

The by-catch in fishing gear poses a serious threat to many populations of sea turtles throughout the world's oceans. Acoustic deterrent devices (ADDs or pingers) have been used to reduce the by-catch of marine mammals in some fisheries, but their efficacy has not been evaluated for sea turtles. Past assessments have dismissed the potential of ADDs to reduce sea turtle by-catch because of the similar hearing sensitivities of sea turtles and fishes and the subsequent expectation that, even if the devices were effective in reducing the by-catch of turtles, ADDs would reduce the catch of target species. However, in fisheries for flatfish, which have very poor low frequency hearing, ADDs could be detectible by turtles, but not the target species. In such cases ADDs could have the potential to reduce the by-catch of sea turtles without reducing the catch of target species. I examined the behavioral responses of loggerhead sea turtles (*Caretta caretta*) to a simulated low frequency tonal ADD (300 Hz, 152 dB re: 1 μPa-rms) in a tank environment. My results indicate that loggerheads have a mild, aversive response to ADDs. This response was manifested by orienting away and increasing their distance from the ADD. My results suggest that low frequency tonal ADDs have the potential to warn sea turtles of the presence of fishing gear and suggest that field tests of ADDs are warranted.

INTRODUCTION

By-catch, or the incidental capture of non-target species in fishing gear, is one of the primary challenges facing marine resource managers. This is especially true when long-lived species, such as sea turtles, are taken incidentally in a fishery. By-catch of sea turtles occurs in industrial and artisanal fisheries and in a variety of gear types, including: longlines; gillnets; trawls; traps; and pots (Epperly et al. 2007, Finkbeiner et al. 2011, Lewison et al. 2004, Moore et al. 2009, Peckham et al. 2007, Wallace et al. 2010). By-catch poses a significant threat to many populations of sea turtles, because these animals have low reproductive rates and delayed maturation and, as a consequence, are particularly sensitive to high rates of removals. Accordingly, the development of by-catch reduction mitigation techniques is a research priority for sea turtle conservation (Hamann et al. 2010). Certain mitigation techniques, such as turtle excluder devices in trawl fisheries and circle hooks in longline fisheries, have reduced sea turtle by-catch (Epperly 2003, Read 2007), but such approaches are not available for all types of fishing gear and particularly not for gill net fisheries.

Acoustic deterrent devices (ADDs), also known as pingers, are low-intensity sound sources that have been used to reduce the by-catch of some marine mammals in gill net fisheries (see Nowacek et al. 2007 for a review), but their efficacy has not been evaluated for sea turtles. Very little is understood about the behavioral responses of sea turtles to underwater sound and there are no published reports of sea turtle responses to tonal stimuli. Visual signals have been evaluated for mitigation of sea turtle by-catch in some fisheries (Gilman et al. 2010, Wang et al. 2010), but such approaches are not effective in turbid or tannic waters. In contrast, low-frequency sound travels long distances underwater and therefore holds potential promise as a mitigation strategy with fisheries in such environments.

Past assessments have dismissed the potential of ADDs to reduce sea turtle by-catch because of the similar hearing sensitivities of sea turtles and fishes and the subsequent expectation that, even if the devices were effective in reducing the by-catch of turtles, ADDs would reduce the catch of target species. Sea turtles are known to detect low-frequency sounds, generally below 2,000 Hz (Chapters I-III, Bartol et al. 1999, Bartol & Ketten 2006, Martin et al. 2012, Ridgway et al. 1969). Most fish are able to detect sounds from 50 Hz to 500-1,500 Hz, but a few species, including clupeids and some alosids with specialized anatomical structures, may be able to detect sounds above 100 kHz (Popper & Hastings 2009).

Flatfish, such as flounders and plaice, however, lack the swim bladder that enhances hearing in other species of fish and have a very limited range of low-frequency hearing sensitivity, often less than 200 Hz (Chapman & Sand 1974, Karlsen 1992). Thus, in fisheries where flounder is the target species, it may be possible to design an ADD signal that is detectable by sea turtles, but not fish. In such cases, ADDs have the potential to successfully reduce sea turtle by-catch without reducing target catches.

To determine whether ADDs have the potential to deter sea turtles from certain types of fishing gear, such as flounder gill nets, I examined the behavioral response of juvenile loggerhead sea turtles (*Caretta caretta*) to short duration, low-frequency tonal sounds, similar to those produced by ADDs.

MATERIALS AND METHODS

Loggerhead Sea Turtles

During the summer of 2010 and the spring of 2011, I collected twelve loggerhead sea turtles caught in pound nets in Back Sound, North Carolina USA. These loggerheads averaged 61.9 cm in curved carapace length, 59.5 cm in curved carapace width, 58.7 cm in straight carapace length, 50.7 cm in straight carapace width, and 28.5 kg in weight (Table 13). I allowed each turtle to acclimate to the tank for 18-24 hours before testing. All turtles were released less than 48 hours after collection near their collection location. One turtle, Cc7, did not acclimate to the tank and swam in continuous circles, so I did not include data from this individual in the analyses. I tested each of the remaining eleven turtles once (ten in the morning and one in the afternoon) and ten of these individuals twice. I attempted to conduct the second tests 24 hours after the first test, but when space in holding tanks was limited, some tests occurred in the afternoon following a morning test (Cc3, Cc5, Cc8, and Cc9), or in the morning following a test in the previous afternoon (Cc4) (Table 13).

Acoustic deterrent device experimental setup

I exposed each sea turtle to a simulated low-frequency acoustic deterrent device in a cement tank. The tank measured 4.88 m long, 1.82 m wide, and 0.61 m deep, and was lined with a tarpaulin to prevent the turtles from rubbing against the cement walls. The walls of the tank were 20 cm thick and the tank was submerged in the ground to a depth of approximately 30 cm. Six sandbags held the tarp in place and a standpipe, 8 cm in

Table 13. ID, date retrieved from pound net, time of entry to test tank, test dates and times, and morphological dimensions of loggerhead sea turtles (*Caretta caretta*).

Turtle ID	Date Collected	Time in tank	Test 1 Date	Test 1 Time	Test 2 Date	Test 2 Time	CCL (cm)	CCW (cm)	SCL (cm)	SCW (cm)	Depth (cm)	Weight (kg)
Cc1	6/2/10	10:00	6/3/10	10:20	6/4/10	9:50	58.2	56	55.5	46.6	23.3.	24.6
Cc2	6/3/10	12:00	6/4/10	9:54	6/5/10	N/A	56.5	58.6	52.8	49.5	24.9	24.8
Cc3	6/4/10	14:00	6/5/10	9:23	6/6/10	16:16	54.7	55.5	51.7	43.1	22.3	22.7
Cc4	6/5/10	13:00	6/6/10	15:37	6/7/10	9:32	63.6	60.3	60.5	50.6	25.5	29.3
Cc5	6/6/10	11:30	6/7/10	9:41	6/8/10	16:38	71	67.1	65.6	53.4	26.7	40.6
Cc6	6/7/10	11:00	6/8/10	8:32	6/9/10	8:16	62	61.2	57.8	50.9	26.5	N/A
Cc8	6/8/10	11:00	6/9/10	9:02	6/10/10	15:30	59.1	50.6	62.7	61.6	23	27.6
Cc9	6/9/10	11:00	6/10/10	9:12	6/11/10	15:00	62.3	59.8	57.3	49.8	23.2	24.4
Cc10	6/10/10	11:00	6/11/10	10:00	6/12/10	9:56	60.4	60.2	56.4	48.8	23.2	26.2
Cc11	6/11/10	11:00	6/12/10	10:05	6/13/10	9:35	64.2	60.4	60.2	51.3	26.2	36
Cc12	6/12/10	11:00	6/13/10	10:30	6/14/10	9:30	68.8	64.8	64.7	51.7	23.4	N/A

CCL - curved carapace length; CCW - curved carapace width; SCL - straight carapace lenth; SCW - straight carapace width

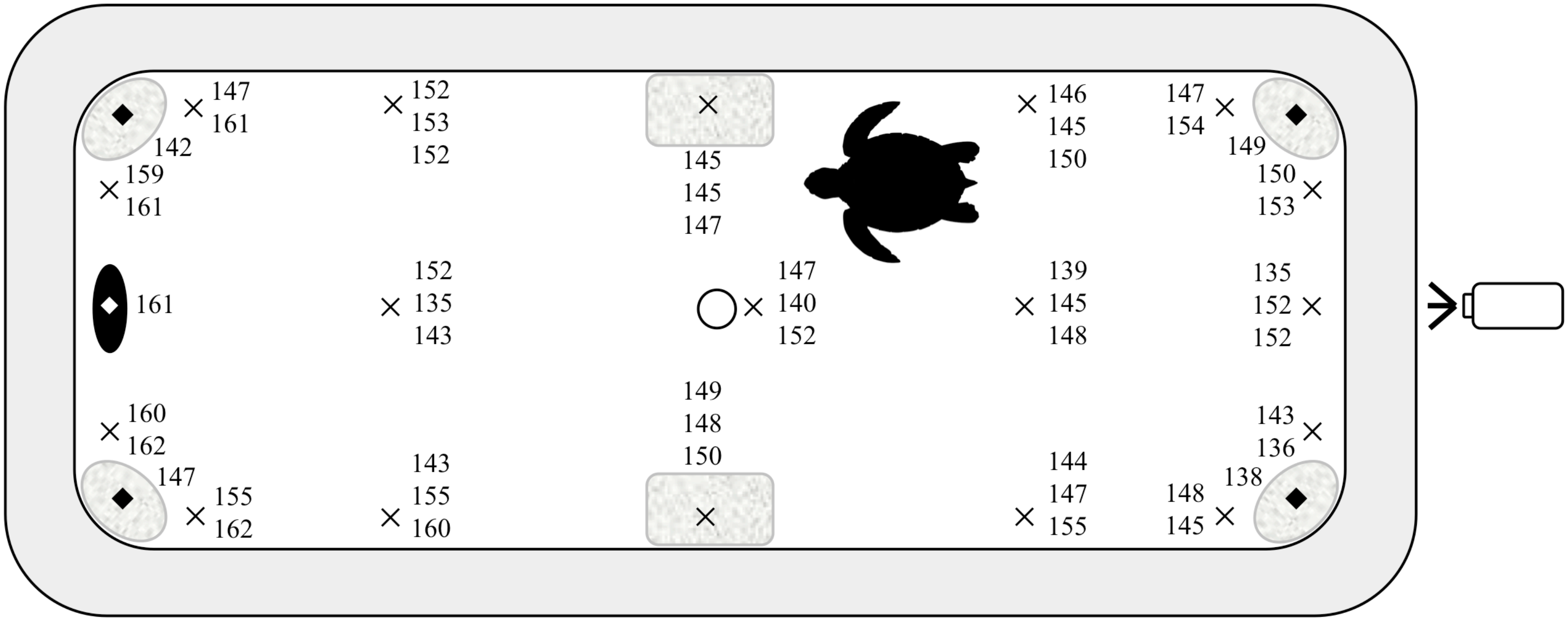


Figure 13. Experimental tank set up. The black oval represents the speaker. × represents a bottom and middle level sound field calibration location, ◆ represents a top level sound field calibration location. Decibel levels for each location are presented for the speaker opposite the camera scenario and organized vertically by level (top, middle, and bottom level).

diameter, allowed flow of sea water when experiments were not in session (Fig. 13). I submerged an amplified speaker (AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Inc. Littleton, Colorado USA; amplifier: Servo 120A, Samson Technologies, Inc. Hauppauge, New York USA) in the middle of the water column, equidistant from both sides of the tank, and 10 cm from the rear wall at one end of the tank (Fig. 13). For the turtles which were tested twice, I alternated the location of the speaker, placing it at the opposite end of the tank of the previous test. Each experiment was divided into three 30-minute periods: pre-exposure; exposure; and post-exposure. The pre- and post-exposure periods were both silent. During the exposure period I played a 300 Hz tone (500 ms in length, cosine-gated with a 10 ms rise-fall time, 152 dB re: 1 μPa-rms at 1 m) every 10 or 15 seconds. I chose this frequency and sound pressure level because it fell with the range of best hearing sensitivity for loggerhead sea turtles (Bartol et al. 1999, Martin et al. 2012) and cannot be detected by flatfish (Chapman & Sand 1974, Karlsen 1992). I used an Evoked Potential Workstation with a RP2.1 module (Tucker-Davis Technologies, Inc. Alachua, Florida USA) and laptop computer with RPvdsEx software (Tucker-Davis Technologies, Inc.) to create a program that generated and automatically played the ADD signal. Tank temperatures averaged 28 °C in 2010 and 23 °C in 2011.

Visual observers recorded the position of the sea turtle in the tank and water column (upper, middle, or bottom) and the activity of the sea turtle (traveling or resting) at point samples taken every 30 seconds. In addition, I recorded each experiment, from speaker setup to removal, using a stationary, elevated video camera (Sony Handycam DCR-SR68, Sony Corporation, Minato-ku, Tokyo, Japan).

Sound field calibration

Distance cannot be used as a proxy of decreasing sound pressure levels in tanks due to sound reflections from the tank walls, so I calibrated the sound field of the tank by making measurements of the ADD signal using a hydrophone (HTI96-min, High Tech, Inc.; Gulfport, Mississippi USA sensitivity: -164dBV/μPa; 0.02-30 kHz) and a Microtrack II recorder (M-inMusic - Audio, Inc. Cumberland, Rhode Island USA, sampling rate: 96 kHz). I made recordings in 15 locations in the upper (10 cm beneath the surface) portion of the water column and 18 locations in the middle (30 cm beneath the surface) and bottom (5 cm above the bottom of the tank) portions of the water column (Fig. 13). I calculated the ADD signal sound pressure level (dB re: 1 μPa-rms) at each location using Audition (version 2.0, Adobe Systems, Inc. San Jose, California USA).

Data analysis

Visual observations and analysis of the video recordings allowed me to record the following parameters at each point sample: activity (traveling or resting); location in the water column (bottom, middle, or upper); location in the tank with respect to the speaker (speaker side or non-speaker side of the tank); relative distance to the speaker (categorized as 1, 2, 3 or 4; 1 being the quadrant closest to the speaker and 4 being the quadrant farthest from the speaker); heading (toward, parallel, or away from the speaker); and received level (dB re: 1 μPa-rms) based on the location of the turtle's head in the tank and the water column and data from the tank calibration exercise.

To explore the effect of behavioral responses at varying time steps, I made statistical comparisons of each behavioral parameter using data from: (1) 10 minutes prior and 10 minutes after the onset of the ADD signal and (2) 30 minutes prior and 30 minutes after the start of the signal. I used chi-square tests to determine if there was a difference in behavior after exposure to the ADD signal by comparing expected (10 and 30 minutes prior to ADD initiation) and observed (10 and 30 minutes after ADD initiation) values for each parameter. To test the hypothesis that turtles responded to the ADD signal by moving to quieter locations in the tank, I compared the means of all received levels (derived from the calibration exercise in dB re: 1 μPa-rms) 10 and 30 minutes prior to ADD initiation with those 10 and 30 minutes after ADD exposure using a Wilcoxon Signed-Rank Test in JMP (JMP Pro 10.0, SAS Institute, Inc, Cary, North Carolina, USA). I considered a p-value less than 0.05 statistically significant.

RESULTS

In the first test, comparing observations made 10 minutes prior with those made 10 minutes following the start of the ADD signal, loggerhead sea turtles oriented away from the speaker ($p = 0.013$), spent more time on the side of the tank opposite the speaker ($p = 0.017$), and increased their distance from the speaker ($p < 0.001$). However, they exhibited no significant change in activity ($p = 0.131$) or location in the water column ($p = 0.154$). Loggerheads also changed their location in the sound field ($p = 0.014$), spending more time in slightly louder locations. This latter finding was largely driven by the behavior of two turtles (Cc8 and Cc11) for which received levels during exposure were up to 27 and 28 dB higher than in the pre-exposure control. When comparing the 30 minute time periods, the turtles spent more time resting (p = 0.029) at the bottom of the tank or breathing at the surface (p = 0.020). They oriented away from the speaker (p < 0.001), spent more time on the side of the tank opposite the speaker (p < 0.001) and increased their distance from the speaker (p < 0.001), but they did not change their location in the sound field (p = 0.476).

Table 14. Test 1 aggregate behavioral state (travelling or resting) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Traveling	**Resting**	**X^2: p-value**
Observed (During: 10 min)	101	119	0.131
Expected (Before: 10 min)	90	130	
Observed (During: 30 min)	297	363	***0.029***
Expected (Before: 30 min)	325	335	

Table 15. Test 1 aggregate location in the water column (bottom, middle, or upper) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Bottom	**Middle**	**Upper**	**X^2: p-value**
Observed (During: 10 min)	168	47	5	0.154
Expected (Before: 10 min)	167	52	1	
Observed (During: 30 min)	507	141	12	***0.020***
Expected (Before: 30 min)	497	157	6	

Table 16. Test 1 aggregate hearing (toward, away, or parallel to the speaker) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Toward	**Away**	**Parallel**	**X^2: p-value**
Observed (During: 10 min)	49	112	59	***0.013***
Expected (Before: 10 min)	64	112	44	
Observed (During: 30 min)	151	363	146	***<0.001***
Expected (Before: 30 min)	222	295	143	

Table 17. Test 1 aggregate location in the tank with respect the speaker (non-speaker or speaker side of the tank) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Non-speaker	**Speaker**	**X^2: p-value**
Observed (During: 10 min)	123	97	***0.017***
Expected (Before: 10 min)	140	80	
Observed (During: 30 min)	456	204	***<0.001***
Expected (Before: 30 min)	403	257	

Table 18. Test 1 aggregate distance to speaker (1 = closest quadrant to speaker and 4 = farthest quadrant from speaker) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Distance to Speaker				**X^2: p-value**
	1	**2**	**3**	**4**	
Observed (During: 10 min)	62	35	36	87	***<0.001***
Expected (Before: 10 min)	37	44	49	90	
Observed (During: 30 min)	135	71	118	336	***<0.001***
Expected (Before: 30 min)	147	108	163	242	

In the second test, comparing observations made 10 minutes prior with those made 10 minutes following the start of the ADD signal, loggerheads spent more time on the bottom of the tank ($p = 0.047$), spent more time on the side of the tank opposite the speaker ($p < 0.001$), and increased their distance from the speaker ($p < 0.001$), but did not change their activity ($p = 0.278$), orientation ($p = 0.068$), or their location in the sound field ($p = 0.4766$). When comparing the 30 minute time periods, loggerheads spent more time on the side of the tank opposite the speaker ($p < 0.001$) and increased their distance from the speaker ($p < 0.001$), but did not change their activity ($p = 0.741$), location in the water column ($p = 0.655$), orientation ($p = 0.235$), or location in the sound field ($p = 0.117$).

Table 19. Test 2 aggregate behavioral state (travelling, resting) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Traveling	**Resting**	**X^2: p-value**
Observed (During: 10 min)	52	148	0.278
Expected (Before: 10 min)	59	141	
Observed (During: 30 min)	261	339	0.741
Expected (Before: 30 min)	257	343	

Table 20. Test 2 aggregate location in the water column (bottom, middle, or upper) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Bottom	**Middle**	**Upper**	**X^2: p-value**
Observed (During: 10 min)	184	12	4	***0.047***
Expected (Before: 10 min)	177	21	2	
Observed (During: 30 min)	489	86	25	0.655
Expected (Before: 30 min)	497	81	22	

Table 21. Test 2 aggregate hearing (toward, away, or parallel to the speaker) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Toward	**Away**	**Parallel**	**X^2: p-value**
Observed (During: 10 min)	67	79	54	0.068
Expected (Before: 10 min)	70	89	41	
Observed (During: 30 min)	192	283	125	0.235
Expected (Before: 30 min)	190	268	142	

Table 22. Test 2 aggregate location in the tank with respect the speaker (non-speaker or speaker side of the tank) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Non-speaker	**Speaker**	**X^2: p-value**
Observed (During: 10 min)	216	24	***<0.001***
Expected (Before: 10 min)	181	59	
Observed (During: 30 min)	470	130	***<0.001***
Expected (Before: 30 min)	431	169	

Table 23. Test 2 aggregate distance to speaker (1 = closest quadrant to speaker and 4 = farthest quadrant from speaker) counts for juvenile loggerhead sea turtles (*Caretta caretta*) 10 and 30 minutes before and after ADD signal onset and associated chi-square statistic p-values.

	Distance to Speaker				**X^2: p-value**
	1	**2**	**3**	**4**	
Observed (During: 10 min)	14	10	45	131	***0.006***
Expected (Before: 10 min)	46	13	40	101	
Observed (During: 30 min)	67	63	135	335	***<0.001***
Expected (Before: 30 min)	99	70	112	319	

Despite these significant responses at an aggregate level, I observed a high degree of variation in the responses of individual turtles. Some turtles responded almost immediately to initiation of the ADD signal (Fig. 14, Cc8, test 1), but other turtles, particularly when resting prior to the start of the signal, seemed to ignore the ADD completely (Fig 15. Cc2, test 1), or wait for long periods before responding (Fig 16. Cc10, test 2). A few turtles appeared to be attracted to the speaker (Fig. 17, Cc11, test 1).

DISCUSSION

The objective of this experiment was to determine if turtles responded to a simulated ADD signal in a manner that was consistent with an aversive reaction. If so, perhaps field trials of ADDs in a field setting using real nets would be warranted. My results provide evidence that, in aggregate, loggerhead sea turtles respond to low frequency tonal signals, such as those produced by ADDs. My findings agree with those of Samuel (2004), who demonstrated that green (*Chelonia mydas*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles responded to low frequency sweeps by resting on the

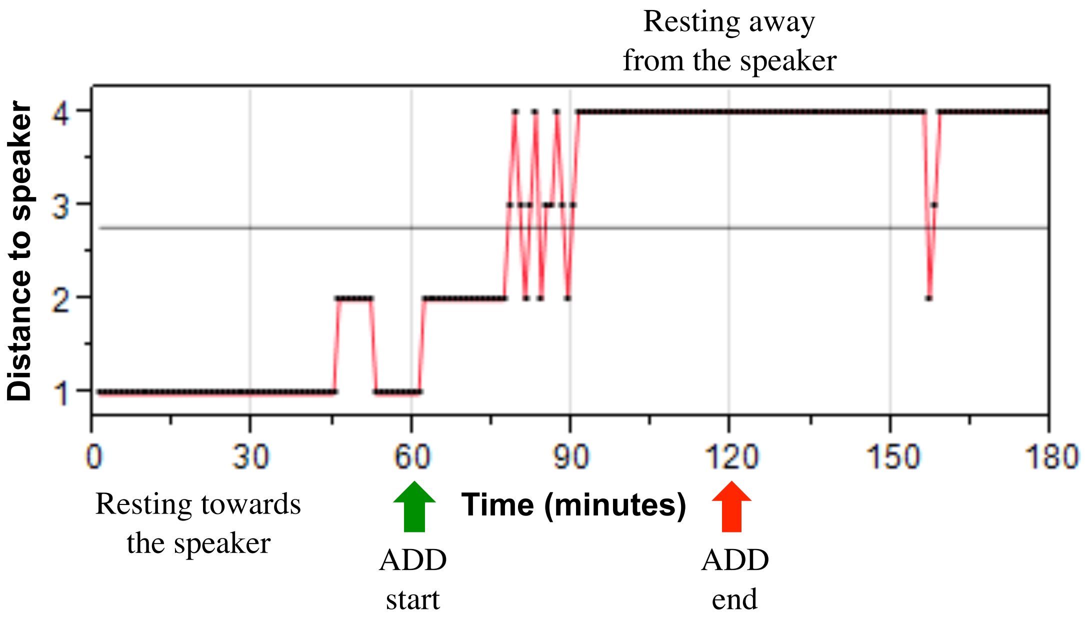


Figure 14. Turtle ID - Cc8 (test 1): Time series of turtle distance to speaker (1 = closest quadrant to speaker and 4 = farthest quadrant from speaker) showing aversive response to ADD initiation.

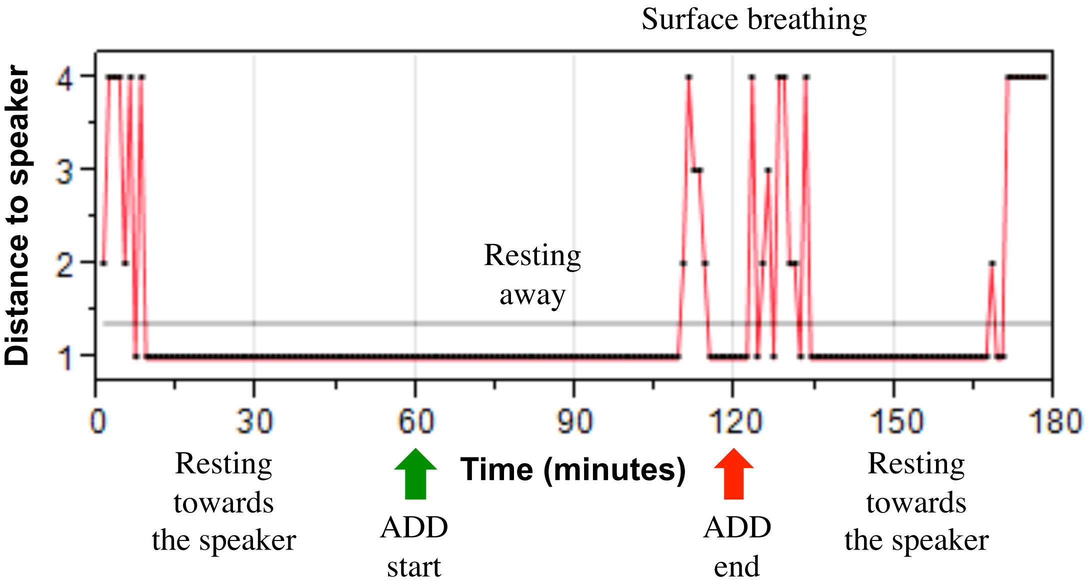


Figure 15. Turtle ID - Cc2 (test 1): Time series of turtle distance to speaker (1 = closest quadrant to speaker and 4 = farthest quadrant from speaker) showing no response to ADD stimuli.

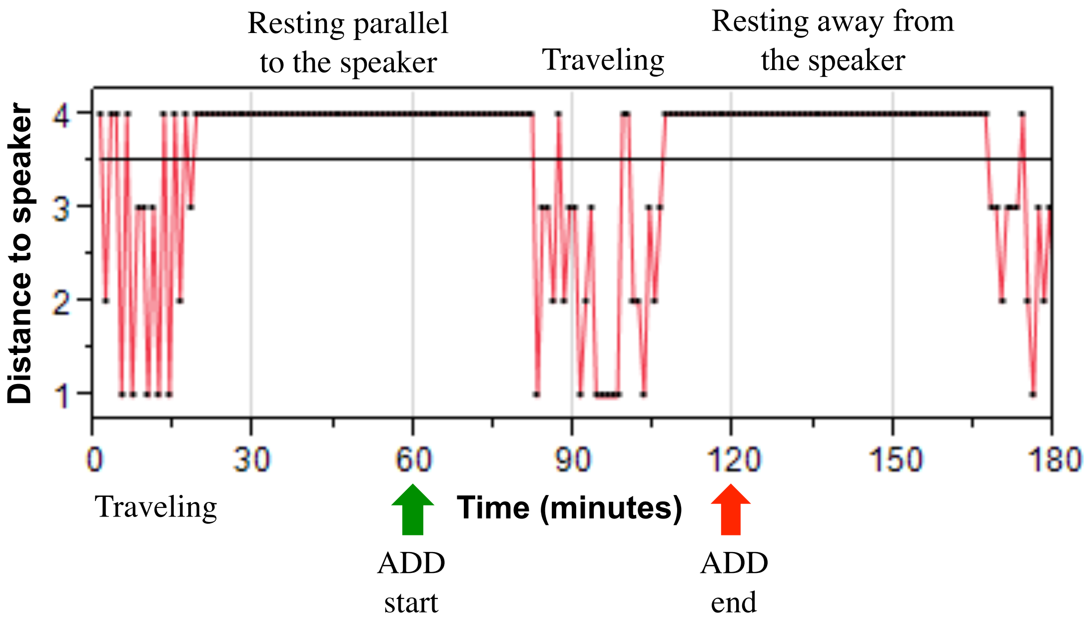


Figure 16. Turtle ID - Cc10 (test 2): Time series of turtle distance to speaker (1 = closest quadrant to speaker and 4 = farthest quadrant from speaker) showing potential delayed response to the ADD stimuli.

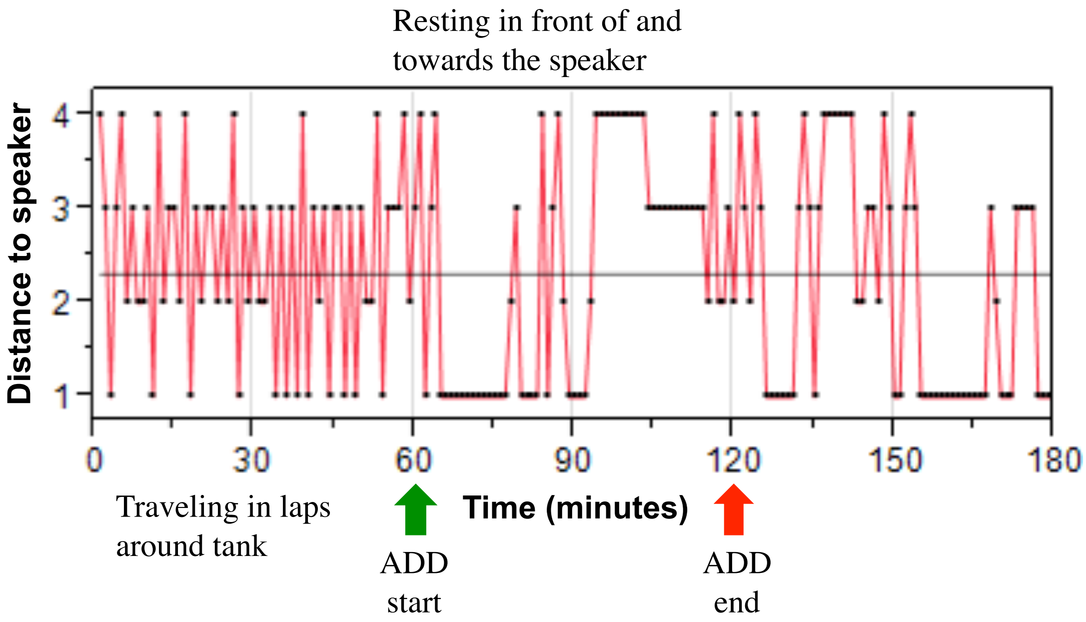


Figure 17. Turtle ID - Cc11 (test 1): Time series of turtle distance to speaker (1 = closest quadrant to speaker and 4 = farthest quadrant from speaker) showing attraction to speaker after ADD initiation.

bottom and increasing submergence time. I did not observe strongly aversive responses, such as those observed in some small cetaceans and pinnipeds (Bowles & Anderson 2012, Kastelein et al. 2000). Instead, loggerheads exhibited a mildly aversive response and slowly changed their behavior after exposure to the sound source. Loggerheads exhibited responses in the first 10 minutes after exposure and many of these responses were maintained over the entire 30-minute experimental period. I did record a small number of startle responses (head jerks coupled with rapid swimming away from the speaker), but these responses were typically elicited only during when the turtle's head was next to the speaker or the turtle's body was in direct contact with the speaker.

Laboratory studies of behavioral response are useful for obtaining baseline data, but the results obtained from such studies must be validated by field experiments. Behavioral responses are often context specific, and responses, particularly to acoustic stimuli, are known to vary due to variation in individual hearing sensitivity, behavioral state, age and sex, and the context, novelty, and movement of the sound (Ellison et al. 2011, National Research Council 2003, 2005). I observed a considerable amount of variability in the type and degree of behavioral response and much this variability could be due to one or more of these factors.

ADDs will not be effective deterrents over long periods if animals become tolerant of or habituated to the stimulus signal, or attracted to the stimulus because of a perceived benefit (e.g. the "dinner bell" concept, Carretta & Barlow 2011). In the present study loggerheads continued to respond to a simulated ADD stimulus by maintaining their distance from the speaker during their second exposure, but it is unclear whether or not sea turtles will habituate, tolerate, or become sensitized to ADD signals over longer periods.

I would like to emphasize that my results should be interpreted conservatively due to the artificial nature of the testing environment. The dimensions of the tank restricted the behavioral responses of the turtles I studied. For example, turtles could not "escape" from the speaker by continuous swimming away from the sound source, as they might in a more natural environment. My analyses could also have been influenced by the behavioral state of each turtle immediately prior to initiating the experiment. The most common behavioral response to ADD initiation was to move away from the speaker. However, if turtles were already in quadrant 4, farthest from the speaker prior, it would be difficult for them to respond in a way that could be detected by my analytical methods. Due to signal reflections caused by the tank walls, the acoustic field varied over short distances in the tank and increasing distance from the speaker did not always correlate with decreasing dB levels (Fig. 13). The delayed responses exhibited by some turtles (ex. Fig. 16) could be due to periodic changes in behavior, rather than due to a response to the ADD. Additionally, due to my limited sample size, I did not test multiple frequencies, types of tonal signals (e.g. frequency sweeps), or amplitudes. It is possible that alternate ADD stimuli may elicit more aversive responses in sea turtles.

My results suggest that low frequency tonal ADDs have the potential to reduce sea turtle by-catch in certain flatfish fisheries using stationary gear such as gill nets or pound nets, however these results must be verified under natural field conditions. One possible method for testing the efficacy of ADDs would be to conduct an experiment similar to the one described here in an open water pen. This experiment would be strengthened by increasing the sample size of turtles and by varying the acoustic stimuli (frequency and presentation rate) to determine the most aversive acoustic signal. Another possible method to examine the efficacy of ADDs for turtles and ensure they do not reduce catch of target species, would be to attach ADDs to fishing gear and set them on a 24-hour on, 24-hour off duty cycle, allowing for the collection of data on sea turtle and fish catch rates on relatively comparable fishing days with and without the presence of the ADD. In cases where this design does not provide enough statistical power because individual net/set interactions with sea turtles are very low, testing the response of turtles and fish separately (allowing for catch of turtles in areas where high interactions are likely) can also provide useful results (e.g. Wang et al. 2010).

CONCLUSIONS

Fisheries interactions are one of the most serious conservation threats to sea turtle populations globally (Wallace et al. 2011). My results suggest that low frequency tonal ADDs have the potential to deter sea turtles from or warn them of the presence of static fishing gear, such as gill nets. I conclude that field tests should be conducted to determine whether ADDs are a viable mitigation strategy for reducing sea turtle by-catch without reducing target catch, especially in flounder gill net fisheries.

CHAPTER V:

THE ACOUSTIC ECOLOGY OF SEA TURTLES: CURRENT KNOWLEDGE, DATA GAPS, AND FUTURE RESEARCH DIRECTIONS

INTRODUCTION: SEA TURTLES AND MARINE SOUND

Recent increases in the intensity of anthropogenic sound underscore the importance of understanding the effects of sound on sensitive marine species. Such concerns have led to increased research examining the potential physiological and behavioral effects of anthropogenic sound on marine mammals and fish (for summaries see: National Research Council 2000, 2003, 2005, Nowacek et al. 2007, Popper & Hastings 2009, Richardson et al. 1995), but research on sea turtles has lagged behind because of lacking fundamental knowledge of their auditory sensitivities and behavioral responses. In this chapter I review the state of our knowledge on the acoustic ecology of sea turtles, examine the sources of marine anthropogenic sound sea turtles are able to detect, evaluate the potential physiological and behavioral effects of anthropogenic sound, identify data gaps, and make recommendations for future research.

Seven species of sea turtle exist worldwide, including the leatherback (*Dermochelys coriacea*), hawksbill (*Eretmochelys imbricata*), green (*Chelonia mydas*), loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), olive ridley (*Lepidochelys olivacea*), and flatback (*Natator depressus*) sea turtles. Sea turtle populations have experienced severe declines due to direct harvest, incidental capture in fisheries, and the loss of foraging and nesting habitats (National Research Council 1990).

With the exception of the flatback sea turtle, for which insufficient data exist to assess their status, and olive ridley sea turtles which are classified as vulnerable, all other species are classified as critically endangered or endangered on the International Union for Conservation of Nature Red List of Threatened Species (IUCN 2012). Sea turtles can be found in nearly all temperate and tropical, coastal and offshore habitats, and are highly migratory, travelling great distances between developmental, foraging and reproductive habitats. To conduct these remarkable feats of navigation, sea turtles take advantage of visual, magnetic, chemical, and auditory cues in their environment.

We understand how some species of sea turtles perceive and respond to certain visual, magnetic, and chemical cues (e.g. visual: Crognale et al. 2008, Levenson et al. 2004, Wang et al. 2007, Wang et al. 2010, Young et al. 2012; magnetic: Avens et al. 2003, Fuxjager et al. 2011, Lohman et al. 2001, Lohmann et al. 2004; chemical: Endres et al. 2012, Grassman & Owens 1987, Manton et al. 1972, Owens et al. 1982), but we understand very little about how they perceive and respond to sound.

Marine sound is produced by many natural and anthropogenic sources, and in any particular location it is the combination of these sounds that creates the soundscape. Natural acoustic sources include: waves, wind, and rain at the ocean’s surface; seismic activity (such as earthquakes); sea ice movement; and marine animals. Anthropogenic acoustic sources include: ship traffic; mineral exploration (seismic airguns or sonar); drilling and extraction; sonar; explosives; industrial construction; and acoustic deterrent and harassment devices. Sounds produced by these sources differ in intensity (decibels), frequency (Hertz, or cycles per second), and wavelength (meters), and their persistence in the marine environment depends on these parameters and the nature of the environment (water temperature, depth, benthic substrate etc.). Low-frequency sounds, such as those produced by some sonars, shipping, and seismic exploration have long wavelengths and travel furthest in the ocean environment. Although we lack systematic measurements of the levels of ambient and anthropogenic sound around the globe, it is generally agreed that levels of marine sound are increasing as sources of anthropogenic sound have become more widespread and intense (Andrew et al. 2002, Hildebrand 2009, Ross 1993).

Marine animals can be affected by marine sound both physiologically and behaviorally. High-intensity sounds can cause physiological trauma and even death in some vertebrates (National Research Council 2000, 2003, 2005, Nowacek et al. 2007, Popper & Hastings 2009, Richardson et al. 1995, Southall et al. 2007). Vertebrates exposed to intense acoustic stimuli may experience temporary or permanent auditory sensitivity threshold shifts (TTS and PTS) or damage to the tissues of the ear. TTS and PTS are temporary or permanent increases in the threshold level of audibility for the ear at a particular frequency or frequencies (Yost 2007). For example, pinnipeds experienced an average TTS of 4.6-4.9 dB when presented with stimuli 55-75 dB above sensation level (Kastak et al. 1999), and noise generated by airguns during seismic surveys has been found to damage hair cells in the inner ears of the pink snapper (McCauley et al. 2003). Though often difficult to investigate and interpret, noise can cause physiological stress responses in marine animals (Rolland et al. 2012, Romano et al. 2004, Wright et al. 2007). For example, seismic water gun exposure caused stress-induced changes in blood levels of nueral-immune parameters (such as levels of norepinephrine, epinephrine, dopamine, aldosterone, and monocytes) in beluga whales and bottlenose dolphins (Romano et al. 2004). Noise can also mask important acoustic cues. Measurements of critical ratios, or the difference between sound level for a barely audible tone and the spectrum level of background noise at a nearby frequency (Yost 2007), have shown that masking occurs, usually increasing with increases in frequency in marine mammals and fish (e.g. Scholik & Yan 2001, Southall et al. 2000). Unlike the acute effects of physiological damage, the cumulative or chronic effects of increased stress and acoustic masking are difficult to quantify on individual and population levels (Clark et al. 2009).

Behavioral responses are often context specific, and responses are known to differ due to variation in individual hearing sensitivity, behavioral state, age and sex, and the context, novelty, and movement of the sound (Ellison et al. 2011, National Research Council 2003, 2005). The effects of these behavioral responses can be short or long lasting and can affect one individual or an entire population. If avoidance is a response, animals may forage less or use more energy while traveling or migrating to avoid the sound source. Frequent loss of energy could delay reproduction or shorten life span, thus decreasing the fitness of the entire population, and/or making the population more susceptible to predators and diseases. Cumulative effects of exposure and behavioral response are not well understood, however over time, repeated exposures can cause sensitization (increased behavioral responses due to animals learning that a stimulus has significant consequences) or habitation (gradual decline of behavioral responsiveness due to animals learning that a stimulus does not have significant consequences) (Richardson et al. 1995).

SEA TURTLE BIOACOUSTICS

The sea turtle ear

Sea turtles lack an outer ear, external pinnae, or ear canal. Like all terrestrial turtles and tortoises, the sea turtle ear is covered by an extension of the facial tissue called the tympanum, and both the middle and inner ears are encased in bone (Wever 1978). Acoustic energy is transmitted through the tympanum and a thick layer of subtympanal fatty tissue to the columella, or stapes, located in an air-filled middle ear. The thin columella forms a cone-shaped footplate, which expands throughout the oval window. Stapedosaccular strands, found only in turtles, connect the oval window and stapes to the saccule and are hypothesized to relay vibrational energy to the saccule (Lenhardt et al. 1985, Wever 1978, Wever & Vernon 1956a). Inward and outward movement of the stapes causes movement of fluid in the pericapsular recess of the inner ear, stimulating hair cells located on the basilar membrane and limbus of the cochlea (Wever 1978).

The functional morphology of the sea turtle ear remains poorly understood and despite previous anatomical research it is unclear whether sea turtle ears respond to pressure, particle motion, or both. Lenhardt et al. (1983, 1985) proposed that due to the sea turtle’s thick tympanum the sea turtle ear is adapted for hearing via bone conduction in water and is a poor aerial receptor, suggesting the whole body serves as a receptor underwater and that sound passes through bones and soft tissue to stimulate the inner ear directly. However, aspects of the sea turtle ear morphology and evidence from freshwater turtle research suggest a more typical tympanic middle ear pathway (Hetherington 2008). Recent studies of sea turtle subtympanal fat using computerized tomography revealed that it is similar to the fat found in the middle ears of marine mammals and birds. The density of these fats is similar to the density of seawater, suggesting the subtympanal fat layer may act as a low-impedance channel for conduction of underwater sound to the middle and inner ears (Ketten 2008). Research on freshwater aquatic turtles has shown that aerial and vibrational stimuli elicit different audiograms and that turtles are more sensitive to aerial, rather than vibrational stimuli (Lenhardt & Hawkins 1983, Patterson 1966). Removal or cutting of the columella drastically reduced aerial hearing sensitivity, but only slightly reduced vibrational hearing sensitivity (Patterson 1966). Christensen-Dalsgaard et al. (2012) propose that the air-filled middle ear of turtles resonates with the underwater sound field and it is these pulsations that cause the extracollumela and columella to move, not the displacement of the tympanum. Auditory and vibrational stimuli both appear to be processed by the auditory system and electrophysiological responses to acoustic stimuli, particularly underwater stimuli, are likely a combination or summation of the responses to all stimuli present (Lenhardt & Hawkins 1983).

Sea turtle hearing

The biological significance of hearing in sea turtles remains largely unstudied, but it seems likely that they use sound for navigation, to locate prey, to avoid predators, and for general environmental awareness. Sea turtles do not appear to use sound for communication, but leatherback sea turtles have been recorded making low-frequency sighs or grunt-like sounds up to 1,200 Hz (maximum energy from 300-500 Hz) while nesting, however these sounds appear to be connected with respiration (Mrosovsky 1972, Cook & Forest 2005).

Because turtles lack a visible external ear and did not often respond to acoustic stimuli during early behavioral experiments, research prior to the mid-1900s, primarily focused on freshwater aquatic and terrestrial turtle hearing capabilities, provided contradictory results (Wever 1978). More recent studies have shown that turtles are able to detect and respond to acoustic stimuli. Recent electrophysiological measurements of cochlear and auditory evoked potentials (AEPs) and behavioral studies of hearing have demonstrated that green, loggerhead, Kemp's ridley, hawksbill, and leatherback sea turtles detect low-frequency acoustic and vibratory stimuli underwater and in air (see Table 1 for a summary of the studies presented below). Cochlear and AEPs are electrical responses produced by the central auditory nervous system after stimulation by sound detectable by the ear (Yost 2007, Au & Hastings 2008).

Ridgway et al. (1969) made the first measurements of sea turtle hearing sensitivity by recording cochlear response potentials to aerial and vibrational stimuli in three juvenile green turtles. Turtles responded to aerial stimuli between 50 and 2,000 Hz and vibrational stimuli between 30 and 700 Hz, with maximum sensitivity between 300 and 500 Hz for both stimuli. They determined that 2,000 Hz was the upper limit for observation of cochlear potentials without injury, and suggested that the useful frequency span of sensitivity of the green turtle ear was between 60 and 1,000 Hz.

Table 24. Summary of sea turtle hearing studies.

Species	Technique	Stimulus	Media	Hearing Range (Hz)	Max Sensivitiy (Hz)	Sample Size	Source
Green turtle (*Chelonia mydas)*							
juvenile	cochlear potential	aerial vibration	air	100-1000 30-700	300-500 300-500	3	Ridgway et al. 1969
juvenile (Atlantic) sub-adult (Pacific)	ABR	aerial	partially submerged	100-800 100-500	600-700 200-400	2 6	Bartol & Ketten 2006
juvenile	AEP	aerial underwater	air water	50-800 50-1600	300-400 200-400	5	Chapter I
Loggerhead (*Caretta caretta)*							
juvenile	ABR	vibration	air	250-1000	250	35	Bartol et al. 1999
adult	AEP Behavior	underwater	water	100-1131 50-800	100-400 100-400	1	Martin et al. 2012
Kemp's ridley (*Lepidochelys kempii*)							
juvenile	ABR	aerial	partially submerged	100-500	100-200	2	Bartol & Ketten 2012
Hawksbill (*Eretmochelys imbricata*)							
hatchling	AEP	aerial underwater	air water	50-1600 50-1600	200-400 200-400	10	Chapter II
Leatherback (*Dermochelys coriacea*)							
hatchling	AEP	aerial underwater	air water	50-1600 50-1200	50-400 100-400	10	Chapter III

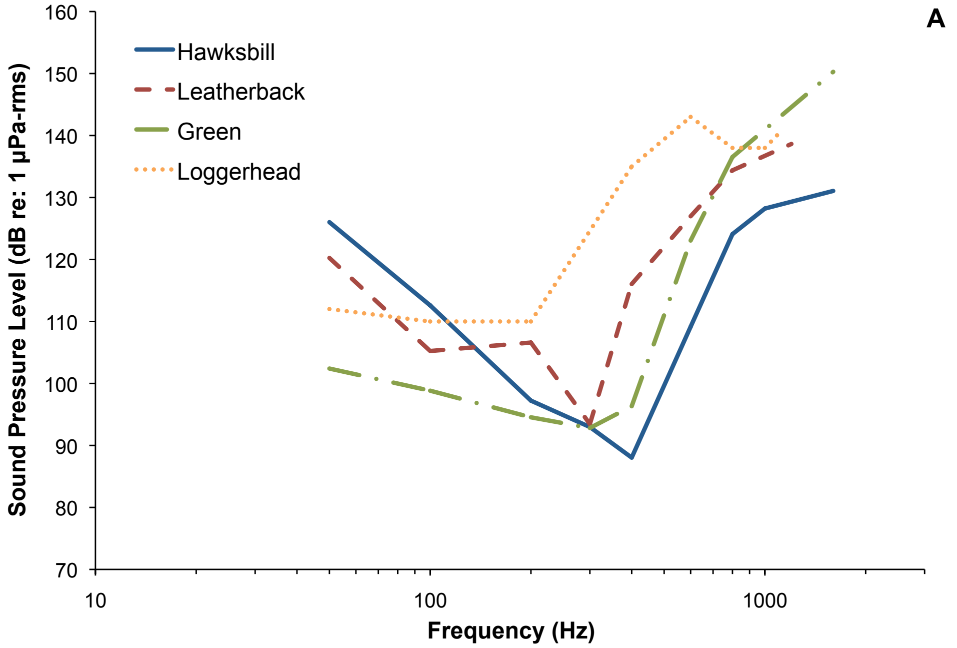
Bartol et al. (1999) measured the hearing of 35 juvenile loggerhead sea turtles by collecting short latency AEPs (auditory brainstem responses, or ABRs), recorded in response to two types of vibrational stimuli: low-frequency clicks and tone bursts delivered directly to the tympanum using a mechanical vibrator. They measured a mean click threshold of -10.8 dB re: 1g rms ± 2.3 dB SD and an effective hearing range from tone bursts from 250 to 750 Hz. The most sensitive frequency was the lowest frequency tested, 250 Hz, with a mean threshold of -23.3 dB re: 1 g rms ± 2.3 dB SD.

Bartol and Ketten (2006) measured ABRs in two juvenile and six sub-adult green sea turtles, and two juvenile Kemp's ridley sea turtles by recording ABR responses from partially submerged turtles in response to aerial tonal stimuli. Sub-adult Pacific green turtles responded to stimuli between 100 and 500 Hz, with maximum sensitivity between 200 and 400 Hz, while juvenile Atlantic green turtles responded to stimuli between 100 and 800 Hz, with maximum sensitivity between 600 and 700 Hz. Kemp's ridleys responded stimuli between 100 and 500 Hz, with maximum sensitivity between 100 and 200 Hz.

Martin et al. (2012) examined the underwater hearing sensitivity of one adult loggerhead by collecting behavioral and AEP measurements in response to pulsed tonal underwater stimuli. The loggerhead responded to underwater stimuli between 50 and 800 Hz with best sensitivity at 100 Hz using behavioral techniques and 100 and 1,131 Hz with best sensitivity between 200 and 400 Hz using AEP techniques.

Elsewhere in this thesis I report the results of my investigations of underwater and aerial sea turtle hearing sensitivity measured by recording AEPs in response to underwater and aerial pulsed tonal stimuli. I demonstrated that juvenile green turtles detect acoustic stimuli between 50 and 1,600 Hz underwater (maximum sensitivity: 200-400 Hz) and 50 and 800 Hz in air (maximum sensitivity: 300-400 Hz) (Chapter I), hatchling leatherbacks detect acoustic stimuli between 50 and 1,200 underwater (maximum sensitivity: 100-400 Hz) and 50 and 1,600 in air (maximum sensitivity: 50-400 Hz) (Chapter II), and hawksbill detect acoustic stimuli between 50 and 1,600 Hz in both media (maximum sensitivity: 200-400 Hz). I presented audiograms derived by measurements of sound pressure and particle acceleration, and found that when I compared underwater and aerial audiograms in terms of sound pressure, greens and hawksbills appeared to be more sensitive to aerial stimuli, but when I compared them in terms of sound intensity, greens and hawksbills appeared to be more sensitive to underwater stimuli.

It is difficult to compare hearing sensitivity thresholds or audiograms between studies employing different methodologies, however recent studies included in this thesis and presented by Martin et al. 2012, use similar methodologies and found similar low frequency ranges of hearing sensitivity and relatively small differences in sound pressure and acceleration threshold levels among sea turtle species (for species comparisons see: Figs. 17 and 18).



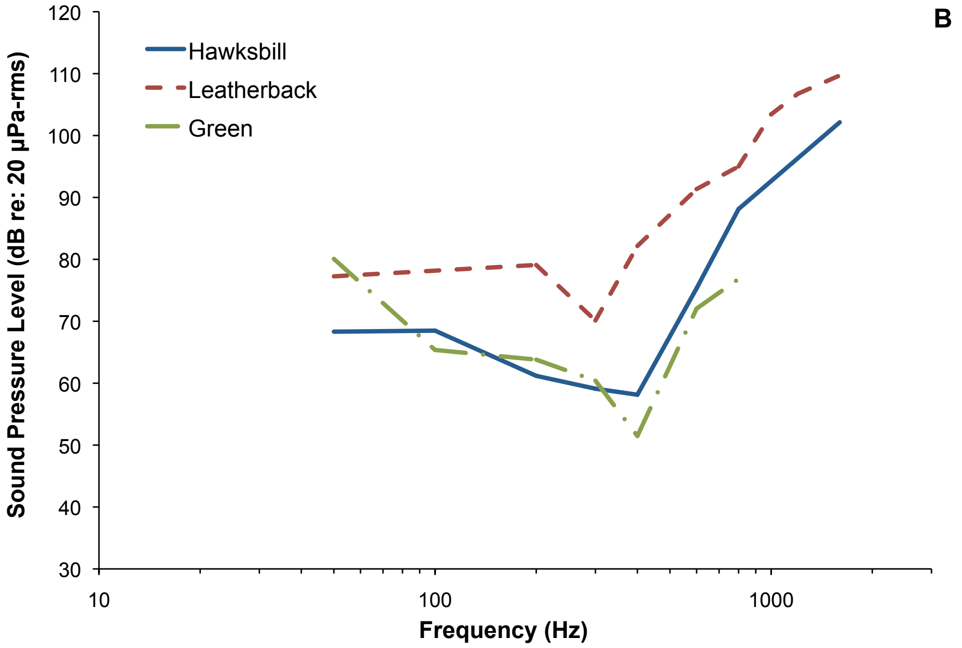


Figure 17. a. Fully submerged underwater (a) and aerial (b) sound pressure audiograms for juvenile green (*Chelonia mydas*, Chapter I), hatching leatherback (*Dermochelys coriacea*, Chapter II), hatchling hawksbill (*Eretmochelys imbricata*, Chapter III), and adult loggerhead (*Caretta caretta*, Martin et al. 2012) sea turtles determined by recording auditory evoked potential responses to tonal stimuli.

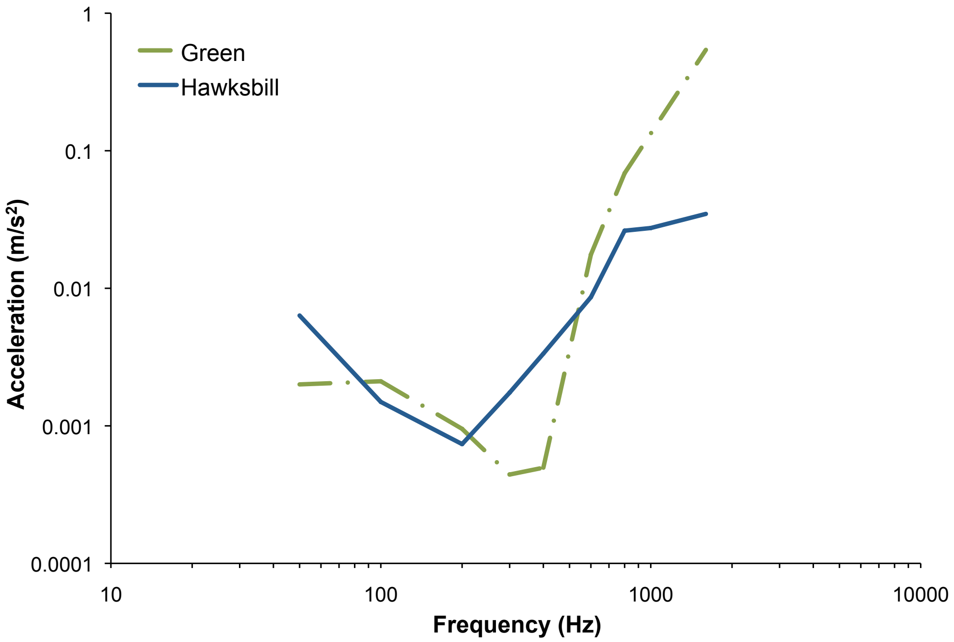


Figure 18. Particle acceleration audiograms for green (*Chelonia mydas*, Chapter I) and hawksbill (*Eretmochelys imbricata*, Chapter III) sea turtles determined by recording auditory evoked potential responses to tonal stimuli.

SEA TURTLES AND ANTHROPOGENIC SOUND

Because sea turtles are highly migratory species, sound events in one area have the potential to impact not only the turtles that use that habitat to reproduce and forage, but also those simply "passing through". As sea turtles can be found in nearly all temperate and tropical and coastal and offshore habitats, the potential for temporal and spatial overlap between sea turtle habitat and marine anthropogenic sound is vast. A detailed comprehensive review of the sources of natural and anthropogenic sound are outside the scope of this review, however such reviews are readily available (see Hildebrand 2009, Nowacek et al. 2007, Richardson et al. 1995). A comparison of the hearing range and sensitivities of sea turtles with the frequencies and sound pressure levels produced by many sources of marine anthropogenic sound suggests that sea turtles are able to detect much of the intense and prevalent low-frequency sound in the ocean, such as those produced by oil and gas exploration and extraction, low frequency naval sonar, pile driving, shipping, and operating wind turbines (Fig. 19).

The temporal and spatial overlap of leatherback sea turtle habitat and anthropogenic sound varies depending on the environment and the sound source. Within categories of sound source types, sound pressure levels and sound propagation can vary greatly depending on the configuration of the source, its location in the water column, and environmental variables such as water depth and bottom type. Received levels can also vary depending on the receiver's location in the water column and in relationship to the source.

Oil and gas exploration and extraction presently occurs in many important sea turtle nesting and foraging habitats and generates high-intensity low-frequency sounds within sea turtle hearing ranges. In a report prepared for the International Association of Oil and Gas Producers Exploration and Production Sound and Marine Life Joint Industry Project, sea turtles are shown to be present in 11 of the 13 oil and gas industry offshore interest areas (Thorson et al. 2005). Drilling from stationary platforms occurs over long time scales, however oil and gas exploration is not a continuous activity. Even though exploration is not a continuous activity recent studies have shown that airgun sounds can be detected many thousands of miles away from the exploration site (e.g. Nieukirk et al. 2012). There is potential however, to avoid times of the year when sea turtles are most

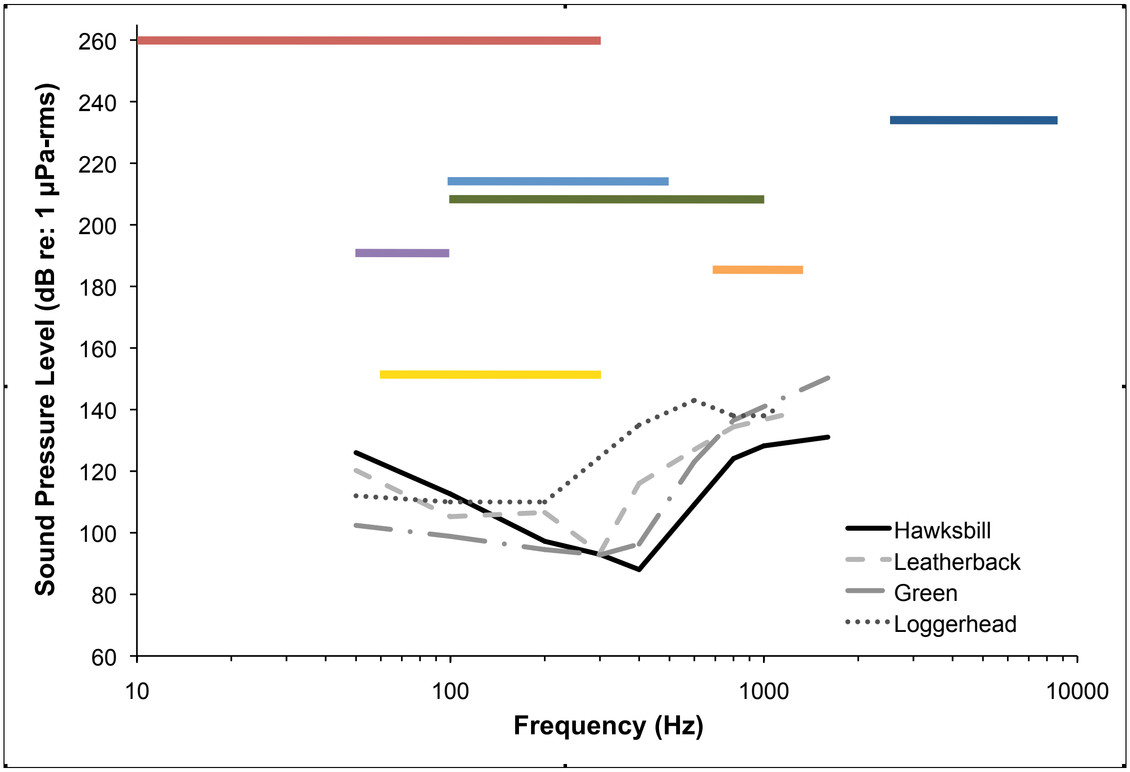


Figure 19. Comparison of sea turtle underwater audiograms to the frequency bandwidth of peak sound pressure levels of sounds produced by marine anthropogenic sources: seismic airgun arrays (red), 5-300 Hz, 260 dB re: 1 µPa (Turner et al. 2006); offshore drilling (orange), 700-1,400 Hz, 184 dB re: 1 µPa (Blackwell et al. 2004); low-frequency military sonar (light blue), 100-500 Hz, 215 dB re: 1 µPa (Anonymous 2007); mid-frequency military sonar (dark blue), center frequencies 2,600 and 3,600 Hz, 235 dB re: 1 µPa (US Navy AN/SQS-53C) and center frequencies 6,800 and 8,200 Hz, 223 dB re: 1 µPa (US Navy AN/SQS-53): Evans & England 2001); pile driving (900 kJ hammer) (green), 100-1,000 Hz, 209 dB re: 1 µP-peak: (Reyff 2007); cargo vessels (173 m in length, 16 knots) (purple), 40-100 Hz, 192 dB re: 1 µPa (NRC 2003); and wind turbines (wind speed 13 m-s, 180 Hz) (yellow), 60-300 Hz, 151 dB re: 1 µPa: Wahlberg & Westerberg 2005).

likely to be in exploration areas in order to avoid sea turtle exposure very intense sounds close to exploration activities.

Naval sonar often occurs in designated areas or ranges, however many of these operation areas cover extensive geographic ranges and provide habitat for sea turtles.

While sea turtles appear to be capable of detecting low-frequency sonar, current sea turtle hearing research indicates that peak sound pressure level frequencies of mid-frequency sonar are out the range of sea turtle hearing sensitivity, and perhaps would be more so if we applied weighting functions to sea turtle audiograms as has been done with many species of marine mammal (Southall et al. 2007). However, sea turtle hearing sensitivity in response to high-intensity sounds, such as that produced by mid-frequency sonar, (estimated to be 235 dB re: 1 μPa, Evans & England 2001) has not been measured, and it is possible that turtles are able to detect these higher frequencies at increased sound pressure levels.

Pile driving occurs over small spatial and relatively short temporal scales (depending on the construction activity) and produces high-intensity low-frequency sounds that can be detected by sea turtles. Source levels depend on hammer type and diameter, type of pile, and sediment type (Reyff 2007). Like oil and gas exploration, pile driving sounds have the potential to travel long distances in the ocean environment, however it may be possible to avoid pile driving during times when sea turtles are most likely to be present in the area of construction.

The construction of offshore wind farms (using pile driving) is an example in which pile-driving activity has the potential to have relatively longer temporal scales. However, even once wind farms are constructed, wind turbines generate continuous, moderate levels of low-frequency sound that can be detected by sea turtles. Depending on environmental variables, sea turtles are unlikely to be able to detect these sounds at large distances away from the farm, however wind farms have the potential to disrupt sea turtle behavior or habitat use depending on their placement.

Shipping noise is a combination of the relatively continuous sound generated by large ocean tankers and more intermittent sounds generated by local inshore boat traffic. The frequency and sound pressure level of individual vessels varies widely by overall size, and engine and propeller size and configuration. The low-frequency noise created by commercial shipping can be heard in every ocean of the world and can be detected by sea turtles. Areas of high-use by commercial and recreational vessels (e.g. inshore ports and shipping lanes) overlap with sea turtle reproductive and foraging habitat. Samuel et al. (2005) recorded levels of up to 113 dB re: 1 μPa (200-700 Hz) in juvenile loggerhead, green and Kemp's ridley sea turtle habitat in the Peconic Bay Estuary system in Long Island, New York. While these levels may not directly damage hearing, they may mask important auditory cues and/or affect behavior and habitat use.

Physiological and behavioral studies of sea turtles and anthropogenic sound

Very little data exist on the physiological impacts of anthropogenic sound on sea turtles, however one study (Kilma et al. 1988) examines the impact of explosions on sea turtles. Kilma et al. 1988 made observations of sea turtles at sites where explosives were used to remove offshore petroleum drilling platforms. They conducted a preliminary experiment to determine impact zones for sea turtles by holding juvenile Kemp's ridley and loggerhead sea turtles in steel cages underwater at four distances (229, 366, 549 and 915 m) away from the detonation of four 23 kg charges. Received levels were estimated via models to be 221, 217, 213, and 219 dB respectively. However these source levels should be evaluated carefully as Vaida et al (2008) reviewed these models and resulting source levels and determined them to be inaccurate because the charges were buried and not in the water column as modeled. Two Kemp's ridleys and two loggerheads at 366 m and one loggerhead at 915 m were found unconscious after charge explosions. One Kemp's ridley at 229 m had a prolapsed cloaca and all loggerheads had an abnormal pink coloring of the skin at the base of the throat and flippers. Kilma et al. (1988) also observed an increase in the number of sea turtle strandings after explosive removal of platforms.

Similarly, very little data exist on the behavioral responses of sea turtles to anthropogenic sound, however several studies have examined the behavioral response of sea turtles to seismic airguns. O'Hara and Wilcox (1990) attempted to create a sound barrier using seismic airguns to deter loggerhead sea turtles from the entrance of a Florida Power & Light cooling water canal. They found that two air guns (a 165 cm^3 capacity Bolt 600B and a 13 cm^3 capacity 542 popper) at a depth of 2 m, presented every 15 seconds were an effective deterrent for a distance of 30 m. They recorded erratic turtle behavior, with some loggerheads moving away from airguns and to the surface, while others moved to locations directly below the airguns. They predicted the airguns produced sound levels of 200 dB re: 1μPa at 1 m (25-1,000 Hz), however, these sound pressure levels may be misleading as the authors did not measure received levels or map sound field in order to take into account reflections of sound by the canal walls (possibly creating areas of high-intensity sound and "dead" zones).

Moein et al. (1995) (as presented in Bartol & Musick 2003) measured behavioral and physiological responses of juvenile loggerhead sea turtles to airguns in a net enclosure in the York River in Virginia (USA), to evaluate the effectiveness of airguns as a sea turtle deterrent on hopper dredges. The airguns produced frequencies between 100-1,000 Hz at three levels (175, 177, 179 dB) every 5 seconds for 5 minutes. Details about the operational pressures of the airguns and deployment depth were not presented, and the turtle received level was not measured. Juvenile loggerheads avoided the airguns (up to 24 m) during the first exposure, but appeared to habituate to following exposures and ceased to distance themselves from the airguns after three exposures. Physiological measurements showed increase stress levels, however the effects of handling were not measured, so stress increases could not be attributed completely to airgun exposure. Pre- and post-hearing threshold measurements showed a temporary decrease in hearing sensitivity after airgun exposures, however hearing was not measured throughout the trials, so it is unclear whether the observed habituation was due to a TTS in hearing sensitivity.

McCauley et al. (2000) examined the environmental impacts of marine seismic surveys in Australia. They recorded the behavior of one loggerhead and one green sea turtle suspended in an open water cage when approached a single air gun (Bolt 600B, 20 in^3 chamber). Both turtles exhibited increased swimming behavior at received sound pressure levels above 166 dB re: 1 μPa-rms (approximately 2 km from the approaching airgun). Above 175 dB re 1 μPa-rms (approximately 1 km from the approaching airgun) the sea turtles' behavior became erratic, possibly indicating that they were in a stressed or agitated state.

Weir (2007) made observations of green and hawksbill sea turtles during a ten-month seismic survey off the coast of Angola. Weir observed no significant behavioral response to airgun arrays (when compared to control "array off" behavior), but did observe responses to the presence of ships. Weir hypothesized that the response to the presence of ships before airgun sounds may have been due to the placement of the array, which was towed 300 m behind the ship, causing turtles to encounter the ship before the array. While there was no statistical difference in mean distance from ship, roughly two times more turtles were sighted when the arrays were off, however these results may have been impacted by improved sea states during array off observations. Most importantly, Weir indicated that shipboard observations for sea turtles are challenging and less effective in Beaufort states >1.

Hazel et al. (2009) evaluated the response of green turtles to approaching vessels at slow, moderate or fast speeds. They found that turtles were more likely to flee vessels traveling at slow speeds than those travelling at moderate to high speeds. Turtles also avoided fast approaching vessels at significantly shorter distances than turtles that fled from vessels approaching slowly. They did not measure received level, however the authors hypothesized that vessel direction would be difficult to determine underwater and that turtles might have habituated to vessel sound.

DeRuiter and Larbi Doukara (2012) made visual observations of loggerhead sea turtle surface diving behavior during seismic airgun surveys in the Mediterranean Sea off the coast of Angola. The seismic array produced source levels of 252 dB re: 1 µPa-peak. They found that 57% of turtles dove in response to airgun firing. They estimated the received levels of diving turtles using spherical spreading and three hull-mounted hydrophones. All observed dives occurred relatively close to the ship at high estimated exposure levels (at an estimated received level of 191 dB re: 1 µPa-peak at 130 m and 175 dB re: 1 µPa-peak at 830 m). Because no control (non airgun firing) observations were made, authors were not able to conclude that airguns alone (and not visual sightings or presence of the ship) caused dive response, however they observed several “startle” responses directly following airgun firing.

Previously in this thesis I described a study to evaluate the behavioral responses of juvenile loggerhead sea turtles to low-frequency tonal acoustic deterrent devices (ADDs, or pingers) (Chapter IV). I examined behavioral responses to tonal stimuli of 300 Hz (500 ms in length, cosine-gated with a 10 ms rise-fall time, 152 dB re: 1 µPa-rms at 1 m) played every 10 or 15 seconds in tank and found that loggerheads significantly changed their behavior by heading away from and increasing their distance from the sound source. These results should be interpreted conservatively due to the artificial nature of the tank testing environment, however my results suggest that low-frequency tonal ADDs have the potential to deter sea turtles from or warn them of the presence of fishing gear and suggest that field tests of ADDs are warranted.

CONCLUSIONS

Recent research has progressed our understanding of the underwater hearing sensitivities in sea turtles, and some investigations have been made to determine the behavioral responses of sea turtles to several sources of anthropogenic sound, however many fundamental gaps remain in our knowledge of the acoustic ecology of sea turtles. Current research indicates that all species of sea turtle for which we have hearing data are able to detect low-frequency stimuli (50-2,000 Hz) in either underwater and/or aerial environments, and that sea turtles are able to detect and behaviorally respond low-frequency sounds in their laboratory and natural environments.

Data gaps and recommendations for future research

Notwithstanding recent advances in or our knowledge of sea turtle hearing capabilities, we still lack a fundamental understanding of the functional morphology of the sea turtle ear, particularly which component of sound sea turtle ears detect, pressure, particle motion, or both and which parts of the ear are responsible for detecting sounds. Experiments that are able to spatially separate acoustic pressure and intensity are needed in order to determine which component(s) of sound sea turtles detect. We still lack hearing sensitivity data for olive ridley or flatback sea turtles and several age classes for the species for which hearing sensitivity has been measured. It has been hypothesized that sea turtle hearing sensitivity may change with age, either due to changes in anatomy (e.g. size of the middle ear) or loss of sensitivity due to aging ear structures and hair cells. Future hearing studies should focus on filling in these species and age-class data gaps.

Because sea turtles spend much of their time conducting dives or at depth, it would be valuable to examine if hearing sensitivity changes under pressure. Additionally due to the wide latitudinal range of sea turtles, particularly leatherbacks, it would be useful to determine whether hearing sensitivity is affected by changes in temperature. Finally, in order to make more informed comparisons of sea turtle hearing sensitivity and anthropogenic sound, sea turtle audiograms should be weighted, similarly to marine mammal audiograms (Southall et al. 2007).

With the exception of one study on the effects of explosives (Kilma et al. 1988), no data exist on the physiological impacts of sound. There is a critical need for investigations on the physiological effects of anthropogenic sound, such as: determining the levels of sound required to induce temporary and permanent threshold shifts; determining if high-intensity sounds cause ear tissue trauma; measuring critical ratios to determine levels of acoustic masking; and determining if intense or prolonged sound causes a stress response in sea turtles. Studies of masking may be particularly important as masking of low-frequency sounds may affect sea turtles who likely use prevalent low-frequency sounds in their environment for navigation, to locate foraging areas, or find suitable nesting habitat.

By far most behavioral response studies conducted thus far have examined the response of sea turtles to airguns, with the vast majority of those evaluating the behavioral responses of loggerhead or green sea turtles. However because the received levels were not characterized, many of these studies are inconclusive. In order to mitigate potential affects of anthropogenic sound, controlled exposure experiments to determine the received sound levels that cause behavioral responses in individual turtles of all species and age classes are needed. These studies should focus on measuring behavioral responses to low-frequency anthropogenic sounds such as: seismic airguns, pile driving, low frequency sonar, and shipping. In addition to quantifying responses to anthropogenic sounds there is a need to examine baseline behavioral responses to natural acoustic stimuli. This information is vital to determining if current mitigation methods (ramping up, shut down based on received level, and time-area closures) are effective and to create effective exposure criteria for sea turtles.

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Publications

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