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Underwater hearing sensitivity of the leatherback sea turtle (*Dermochelys coriacea*): Assessing the potential effect of anthropogenic noise.

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ABOUT THE COVER

Underwater leatherback sea turtle (*Dermochelys coriacea*) hatchling. Photo credit:

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SUMMARY

Rising levels of anthropogenic noise throughout the world's oceans have created growing concern about the impact of sound on many marine species. Sea turtles do not appear to vocalize or use sound for communication, but may use sound for navigation, locating prey, avoiding predators, and general environmental awareness. Endangered leatherback sea turtles (*Dermochelys coriacea)* have the largest latitudinal distribution of all sea turtles, foraging in high-latitude sub-polar waters and nesting on low-latitude tropical beaches. Much of their habitat overlaps with sound-producing activities, exposing them to anthropogenic sounds such as: oil and gas exploration and extraction, shipping, construction, and sonar.

To determine if leatherbacks are capable of detecting these sounds, we measured the hearing sensitivity of hatchlings in water (n=11) or air (n=12) by recording auditory evoked potentials (AEPs). AEPs are produced by the synchronous discharge of neurons in the auditory pathway of the central auditory nervous system after acoustic stimulation detectable by the ear. Before testing, we isolated hatchlings from noise and vibrations and lightly restrained them to prevent movement that would mask AEP signals. To further reduce myogenic artifacts, we sedated (underwater: n=11; air: n=7) or anesthetized (air: n=5) hatchlings. For underwater measurements, we submerged hatchlings 14 cm and presented stimuli with an underwater speaker (Clark Synthesis, Inc. AC339), calibrated with a hydrophone (High Tech, Inc. HTI-96-MIN). We recorded AEPs during 45-60 second intervals, raising hatchlings to the surface to breathe between intervals. For aerial measurements, we placed hatchlings on foam pads to reduce the opportunity for detection of vibratory stimuli, and presented stimuli with an aerial speaker (Definitive Technology, Inc. DI6.5R), calibrated with a microphone (LinearX Systems, Inc. M31). An Evoked Potential Workstation run by laptop computer with SigGenRP and BioSigRP software (Tucker-Davis Technologies, Inc.) generated stimuli and recorded AEP responses. Using a three-electrode array, we recorded responses to 50 ms pulsed tonal stimuli between 50 and 1600 Hz, beginning at the loudest producible level and attenuating in 6 dB steps until no AEP response could be detected. We determined that an AEP response was present if the recorded signal showed a peak in the frequency domain twice that of the stimulus frequency and ≥6 dB above the noise floor 100 Hz on either side of the peak. We defined threshold as the lowest level we detected an AEP response. We monitored hatchling respiratory and heart rates throughout the experiment and measured blood gas values at the completion of the experiment.

Results showed that leatherback sea turtle hatchlings are able to detect sounds underwater and in air, responding to stimuli between 50 and 1200 Hz in water and 50 and 1600 Hz in air, with maximum sensitivity between 100 and 400 Hz in water (84 dB re: 1 μPa-rms at 300 Hz) and 50 and 400 Hz in air (62 dB re: 20 μPa-rms at 300 Hz). These represent the first measurements of leatherback hearing sensitivity and, like other species of sea turtle for which hearing has been measured, they appear to have a relatively narrow, low-frequency range of hearing sensitivity. Sedation or anesthesia proved to be a successful technique for facilitating the collection of AEPs. Anesthesia had little effect on measured hearing sensitivity with average thresholds for anesthetized hatchlings <7 dB lower (more sensitive) than those not anesthetized. Anesthesia may have improved our ability to detect AEPs by reducing internal body noise, and increasing the signal to noise ratio. Leatherback hearing sensitivity overlaps with the frequencies and source levels produced by many anthropogenic sources, including seismic airgun arrays, drilling, low-frequency sonar, shipping, pile driving, and operating wind turbines, suggesting that leatherbacks are able to detect the sounds produced by these activities, and highlighting the need to investigate their potential physiological and behavioral impacts.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS, ACRONYMS, AND SYMBOLS

ABR	auditory brainstem response
AEP	auditory evoked potential
C	Celsius
CCL	curved carapace length
CCW	curved carapace width
dB	decibel
FFT	fast Fourier transform
g	gram
IM	intramuscular
IV	intravenous
kg	kilogram
µg	microgram
µPa	micropascal
min	minute
mg	milligram
mm	millimeter
mm Hg	millimeter of mercury
mmol L^{-1}	millimoles per liter
ms	milliseconds
PCV	pack cell volume
pCO_2	partial pressure of carbon dioxide
pO_2	partial pressure of oxygen
PVC	polyvinyl chloride
rms	root mean square
s	second
SCL	straight carapace length
SCW	straight carapace width
SD	standard deviation
SPL	sound pressure level
TC	temperature corrected
WIDECAST	Wider Caribbean Sea Turtle Conservation Network

INTRODUCTION

Rising levels of anthropogenic noise throughout the world's oceans have created growing concern about the impact of sound on many marine species. While very little data exist on the underwater hearing abilities of sea turtles or the potential physiological and behavioral effects of sound on sea turtles, some evidence exists that sea turtles are able to detect (Bartol et al. 1999, Bartol and Ketten 2006, Martin et al. 2012, Ridgway et al. 1969) and behaviorally respond to acoustic stimuli (DeRuiter and Doukara 2012, McCauley et al. 2000, Moein et al. 1995, O'Hara and Wilcox 1990). While the biological significance of sound for sea turtles is largely unknown, they may use sound for navigation, locating prey, avoiding predators, and general environmental awareness. Increases in marine anthropogenic sound combined with the endangered and threatened status of all species of sea turtles occurring in United States waters (NMFS 2012) highlight the importance of understanding the effects of marine anthropogenic sound on these marine reptiles.

SEA TURTLE EAR MORPHOLOGY

Sea turtles lack an outer ear, external pinnae, or ear canal. 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, 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 and Vernon 1956). Inward and outward movement of the stapes 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 is poorly understood and debated. Computerized tomography of sea turtle subtympanal fatty tissue has shown it has a density similar to that of water, which may decrease sound attenuation from the environment to the middle ear, and suggests that the sea turtle ear may be well adapted for underwater sound conduction (Ketten 2008). Lenhardt et al. (1982, 1985) suggest the sea turtle ear is adapted for hearing via bone conduction in water and is a poor aerial receptor, proposing the whole body serves as a receptor with sound passing through bones and soft tissue to stimulate the inner ear directly. However, some aspects of the sea turtle ear morphology and evidence from freshwater turtle research suggest a more typical tympanic middle ear pathway (Hetherington 2008). Freshwater turtle hearing research has shown that aerial and vibrational stimuli produce different audiograms and that turtles are more sensitive to aerial, rather than vibrational stimuli (Lenhardt and Hawkins 1983, Patterson 1966). Removal or cutting of the columella drastically reduced aerial hearing sensitivity, but only slightly reduced vibrational hearing sensitivity (Patterson 1966). 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 and Hawkins 1983). Sea turtles likely use both acoustic and vibratory stimuli to acoustically monitor their environment.

SEA TURTLE HEARING

Largely due to their accessibility and small size, turtle hearing research prior to the early 1900's focused on behavioral studies of freshwater aquatic and terrestrial turtles. Because acoustic stimuli differed between studies and turtles did not often respond to all acoustic stimuli presented, these early studies provided contradictory results, with some researchers concluding that turtles were unable to detect sound (Wever 1978). It wasn't until the mid 1900s that electrophysiological research definitively showed that turtles were able to detect acoustic stimuli Patterson 1966, Wever and Vernon 1956). Due to their larger size and inaccessibility, research on sea turtle hearing proved to be more challenging and did not begin until the mid 1900s. Ridgway et al. (1969) collected the first measurements of sea turtle hearing sensitivity by using both aerial and vibrational sound stimuli between 50 and 2000 Hz to collect measurements of the cochlear response potential of three juvenile green sea turtles (*Chelonia mydas*). Turtles responded to aerial stimuli between 100 and 1000 Hz and vibrational stimuli between 30 and 700 Hz, with maximum sensitivity between 300 and 500 Hz for both stimuli with a rapid decline in sensitivity in lower and higher frequencies. They found 2000 Hz was the upper limit for observation of cochlear potentials without injury and suggested the practical hearing range of the green turtle did not exceed 1000 Hz.

More recent measurements of sea turtle hearing sensitivity have been made by recording auditory evoked potentials (AEPs). AEPs are an electrical response of the central auditory nervous system after stimulation by sound detectable by the ear, and can be recorded using electrodes (Yost 2007, Au and Hastings 2008). This technique is a rapid, non-invasive method for measuring hearing sensitivity in non-communicative species and has been successfully used to generate audiograms in many species of marine mammals, fish and invertebrates (Casper and Mann 2006, Mann et al. 2005, McCauley et al. 2003, Mooney et al. 2010, Nachtigall et al. 2004).

Bartol et al. (1999) measured the hearing of 35 juvenile loggerhead sea turtles (*Caretta caretta*), 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 threshold in response to click stimuli of -10.8 dB re: 1g rms ± 2.3 dB SD, and a hearing range from tone bursts from 250 Hz to 750 Hz. The most sensitive threshold was the lowest frequency tested, 250 Hz, with a mean threshold of -23.3 dB re: 1g rms ± 2.3 dB SD (Bartol et al. 1999).

Bartol and Ketten (2006) measured ABRs in two juvenile and six sub-adult green sea turtles, and two juvenile Kemp's ridley sea turtles (*Lepidochelys kempii*) partially submerged (ear submerged, with top of head and portions of carapace in air) using an 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 greens 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 (Bartol and Ketten 2006).

Dow Piniak et al. (2012) developed techniques to measure fully submerged underwater sea turtle hearing and recorded AEPs underwater and in air in five juvenile green sea turtles. Underwater measurement techniques included the development of underwater anesthesia protocols for sea turtles (Harms et al. 2009). Green sea turtle AEP signals exhibited a frequency-doubling signature similar to that seen in fish. Juvenile green sea turtles responded to stimuli between 50 and 1600 Hz in water and 50 and 800 Hz in air, with ranges of maximum sensitivity

between 50 and 400 Hz in water and 300 and 400 Hz in air. In both media, sensitivity decreased sharply after 400 Hz (Dow Piniak et al. 2012). Martin et al. (2012) used similar underwater methodologies to record AEPs in one adult loggerhead and recorded responses to frequencies between 100 and 1131 Hz with greatest sensitivity between 200 and 400 Hz. Both studies using this newly developed methodology found that green and loggerhead sea turtles responded to a broader and higher range of frequency sensitivity than previously reported by Bartol et al. (1999), Bartol and Ketten (2006), and Ridgway et al. (1969) in air and at the water's surface.

LEATHERBACK SEA TURTLES AND SOUND

Leatherbacks sea turtles (*Dermochelys coriacea*) are morphologically and physiologically unique among sea turtles. They are extremely large (average weight - 300-400 kg: Eckert et al. 2012) soft bodied, with no hard protective shell, and live primarily in oceanic waters (Boulon et al. 1996; Eckert 2002; James and Herman 2001). Leatherbacks have the largest latitudinal range of all sea turtles, regularly foraging in high-latitude seas with water temperatures as low as 6 °C and migrating to nest on low latitude tropical beaches (Eckert 1987, Eckert and Eckert 1988, Eckert et al. 2006, Eckert 2006, James and Mrosovsky 2004, James et al. 2005, James et al. 2005). Primary Western Atlantic nesting beaches for leatherback sea turtles within United States jurisdiction occur along the eastern coast of Florida (Stewart and Johnson 2006), the U.S. Virgin Islands (Boulon et al. 1996, Eckert 1987, Eckert et al. 1989) and Puerto Rico (Eckert et al. 1989, Tucker and Frazer 1994). Foraging areas within United States jurisdiction include the entire United States Atlantic exclusive economic zone (Eckert et al. 2006, Turtle Expert Working Group 2007) and the Pacific coasts of California, Oregon and Washington (Benson et al. 2007, Eckert and Dutton 2000).

No data exist on the hearing capabilities of leatherback sea turtles. Leatherback sea turtles are listed as Endangered under the United States Endangered Species Act (NMFS 2012) and classified as Critically Endangered by the International Union for Conservation of Nature (IUCN 2012). Marine anthropogenic sound such as oil and gas exploration and extraction, shipping, construction, and sonar is produced in leatherback sea turtle nesting and foraging habitats both within United States jurisdiction and globally. Given these spatial and temporal overlaps and the known potential impacts of marine anthropogenic sound on the physiology and behavior of marine species, determining the hearing capabilities of leatherbacks and identifying the sound sources they are able to detect is critical to the formation of research designed to determine if anthropogenic sound has physiological or behavioral impacts on sea turtles and the creation of appropriate mitigation strategies. The objectives of this research were to measure the underwater and aerial hearing sensitivity of hatchling leatherback sea turtles using AEP techniques, and to determine the overlap of the sounds produced by marine anthropogenic sources (seismic airguns, drilling, pile driving, shipping, wind mills, sonar etc.) and the sounds that can be detected by leatherback sea turtles.

METHODOLOGY

LEATHERBACK SEA TURTLES

By recording auditory evoked potentials, we measured the hearing thresholds of 23 hatchling leatherback sea turtles either underwater or in air in Matura, Trinidad, Trinidad and Tobago. For comparative purposes, we collected blood from an additional six hatchlings. We

collected hatchlings at dusk from several different nests just after nest emergence at Matura Beach. While no genetic variability in hearing sensitivity was expected, collecting hatchlings from several different nests ensured that we measured hearing in a representative sample of hatchlings. We housed hatchlings in a quiet, dark room adjacent to the testing room and kept at the ambient environmental temperature. Hatchlings averaged 44.8 g in weight, 59.7 mm in straight carapace length, 41.6 mm in straight carapace width, 63.2 mm in curved carapace length, and 54.1 mm in curved carapace width (Table 1). After testing, we isolated hatchlings in small, shallow buckets to ensure individual identification and monitor recovery. We released all hatchlings at Matura Beach at dusk 24 hours after collection.

AUDITORY EVOKED POTENTIAL MEASUREMENTS

Underwater experimental setup

Before testing, we lightly restrained the hatchlings by wrapping them in elastic veterinary wrap to reduce excessive movement. For underwater measurements, we completely submerged turtles to an average of 14 cm (range: 13.2-14.5 cm; measured at the location of the ear), below the surface of the water in a high-density polyethylene cylindrical tank. To reduce vibrations with the cement floor, we placed the barrel-shaped tank (94 cm in height and 40.6 cm in diameter at the top and bottom) on 15 cm of Styrofoam. We grounded the tank the using a copper wire. An amplified speaker (speaker: AQ339 Aquasonic Underwater Speaker, Clark Synthesis, Inc., Littleton, Colorado 80125, USA; sensitivity: 158 dB/uPa/m ±10dB; 0.2-17 kHz; amplifier: Servo 120A, Samson Technologies, Inc. Hauppauge, NY 11788) was suspended using string approximately 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. During data collection, seawater temperatures in the experimental tank averaged 26.8 ºC (range: 25.9-28.5 ºC)

We 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, we wrapped the T-bar with additional veterinary wrap and towels. To ensure hatchlings did not come in contact with the T-bar, we suspended hatchlings from the T-bar using an extended piece of veterinary wrap and a metal clip. Clips held the T-bar in place on the sides of the tank, and along with precise markings on the pipe itself, ensured that we raised and lowered the hatchlings to the same location after each breath. We collected AEP measurements in 45-60 second submergence intervals, after which we brought the hatchling to the surface to breathe. Submergence intervals were determined based on spontaneous respiratory rates and respiratory patterns of midazolam-sedated hatchlings in air, reported respiratory rates and patterns for hatchling leatherbacks (mean: 1.45, range: 0.39-2.80 min^{-1}: Price et al. 2007), and response to submergence. If the turtle showed any signs of desiring a breath (e.g. rear flipper movement, raising head, appearance of an air bubble at either naris), we raised it to the surface regardless of the intended submergence interval. At the end of the planned 45-60 second submergence interval (adjusted individually based on response to submergence), we brought the turtle to the surface to breathe whether or not it showed an indication of desiring a breath, and we did not submerge it again until it took at least one breath and had resumed the typical respiratory pause. Underwater tests did not exceed 60 minutes.

Table 1.

Research activity, date of activity, weight, (in grams) and carapace (straight carapace length (SCL), straight carapace width (SCW), curved carapace length (CCL), curved carapace width (CCW)) measurements (in millimeters) for the 23 leatherback sea turtle hatchlings (*Dermochelys coriacea*) for which we collected auditory evoked potential (AEP) measurements ("A" denotes used of anesthesia), and six hatchlings for which we collected blood.

Turtle ID	Activity	Date	Weight (g)	SCL (mm)	SCW (mm)	CCL (mm)	CCW (mm)
Dc1	AEP Air	16-Jun-12	43.9	60	40	64	54
Dc2	AEP Air	16-Jun-12	44.2	59	41	63	52
Dc3	AEP Air	16-Jun-12	43.7	59	41	64	54
Dc4	AEP Air	16-Jun-12	45.7	62	41	62	53
Dc6	AEP Air	17-Jun-12	41.1	58	40	60	51
Dc7	AEP Air	17-Jun-12	42	60	41	62	54
Dc8	AEP Air	17-Jun-12	42.4	60	42	62	54
Dc9	AEP Air (A)	18-Jun-12	48.4	63	43	69	57
Dc10	AEP Air (A)	18-Jun-12	47.3	59	42	63	55
Dc11	AEP Water	18-Jun-12	45.6	62	42	66	58
Dc12	AEP Water	18-Jun-12	44.3	61	41	66	56
Dc13	AEP Water	19-Jun-12	47	60	43	63	57
Dc14	AEP Water	19-Jun-12	42.7	59	41	63	53
Dc15	AEP Water	19-Jun-12	44.5	59	40	62	55
Dc16	AEP Water	19-Jun-12	44.3	60	42	64	54
Dc17	AEP Water	19-Jun-12	45.6	59	42	66	53
Dc18	AEP Water	20-Jun-12	43.2	56	39	61	55
Dc19	AEP Water	20-Jun-12	42	60	40	63	52
Dc20	AEP Water	20-Jun-12	47.2	62	42	64	54
Dc21	AEP Water	20-Jun-12	47	61	42	66	55
Dc22	AEP Air (A)	21-Jun-12	47	59	43	63	56
Dc23	AEP Air (A)	21-Jun-12	48.3	62	43	65	55
Dc24	AEP Air (A)	21-Jun-12	45.5	59	43	63	55
Dc26	Blood Collection	23-Jun-12	39.3	53	38	57	50
Dc28	Blood Collection	24-Jun-12	49.4	60	45	66	57
Dc30	Blood Collection	24-Jun-12	43.6	60	41	63	53
Dc32	Blood Collection	24-Jun-12	44.2	59	42	64	52
Dc33	Blood Collection	24-Jun-12	45.8	59	41	62	52
Dc35	Blood Collection	24-Jun-12	45.6	59	41	61	53

Aerial experimental setup

Before testing, we lightly restrained the turtles by wrapping them in elastic veterinary wrap to reduce excessive muscle movement and placed them on top of several pieces of vibration-reducing foam. An amplified speaker (speaker: DI 6.5R Definitive Technology,

Owings Mills, Maryland 21117, USA; sensitivity: 90 dB; 0.26-30 kHz; amplifier: Servo 120A, Samson Technologies, Inc. Hauppauge, NY 11788) was placed 40 cm directly in front of the turtle, and level with the turtle's ear. To reduce the possibility of the speaker causing a vibratory response during in the air trials, we suspended the speaker with string from a PVC pipe stand, which we also placed on vibration-reducing foam. Air temperatures averaged 27.6 ºC (range: 26.4-29.8 ºC) during data collection.

Sedation and anesthesia

Preliminary research showed collection of AEPs in leatherback hatchlings to be impossible due to the presence of myogenic electrical signal artifacts, which masked AEP signal recordings. To reduce these signal artifacts, we lightly sedated hatchlings in both air and water using midazolam at 2 or 3 mg/kg intravenous (IV) in the dorsal cervical sinus.

Anesthesia is reported to have effects on the collection of AEPs and hearing sensitivity measurements in some taxa (Yost 2007). Because we anticipated that the collection of underwater AEPs in leatherback hatchlings would require anesthesia (although ultimately they were achievable with only sedation), we first evaluated the effects of using anesthesia as a restraint for the collection of AEPs in hatchlings in air. We recorded aerial AEPs in five turtles with anesthesia using protocols established by Harms et al. (2007, 2009). Anesthesia was induced with dexmedetomidine (30 μg/kg) and ketamine (6 mg/kg) combined IV in the dorsal cervical sinus. Anesthesia was reversed with atimpamezole (300 μg/kg) half IV and half intramuscular (IM).

We defined sedation time to effect as the time at which the turtle could be prepared for AEP recordings. Time to full recovery was subjectively determined as the time at which the turtle was fully responsive and active. We recorded respiratory rates periodically by visual observation throughout the procedures and determined heart rate using a fetal heart rate Doppler flow probe (Pocket-Dop 3, CareFusion, Middleton, Wisconsin USA). Heart rate was recorded before administering sedation, after applying elastic wrap just before starting AEP recordings (midazolam groups only, not needed for ketamine-dexmedetomidine anesthetized group), at the conclusion of the AEP recordings, and after atipamezole administration for the anesthetized group. In the unsedated control group we recorded heart rate before and after venipuncture.

The number of movements (struggling bouts) in resistance to manual (elastic wrap) restraint that can potentially disrupt AEP recordings, were counted for midazolam-sedated and ketamine-dexmedetomidine-anesthetized turtles in air. These movements were not specifically recorded for midazolam-sedated turtles in water, because any such movement was taken as a signal to raise the turtle to the surface for a breath. We scored the turtles subjectively on the quality of their release beach crawl into the ocean, with a good release characterized by strong purposeful crawling and entry into the water, a fair release involving somewhat weaker crawling and greater difficulty negotiating the water entry, and a poor release distinguished by weak crawling and considerable difficulty with water entry and doubtful post-release survival.

Blood collection

Within five minutes of the conclusion of AEP recordings, we collected 0.1 ml of blood from the dorsal cervical sinus using a heparinized (heparin sodium USP, 1000 units/ml, APP Pharmaceuticals, LLC, Schaumburg, Illinois 60173, USA) 0.5 ml insulin syringe with integral 28 ga 1.27 cm needle with minimal dead space. For comparison with sedated and anesthetized

turtles, we collected blood from an additional six leatherback hatchlings shortly after emergence from the nest. Venous blood gas and lactate analysis was performed immediately after blood collection using the iStat Portable Clinical Analyzer (Heska Corporation, Loveland, Colorado 80538, USA) with CG4+ cartridges (Abaxis, Union City, California 94587, USA). Analytes measured were pH, pCO_2, pO_2 and lactate. Bicarbonate concentration is calculated from directly-measured values by the Henderson-Hasselbalch equation. The iStat instrument performs analysis of samples at 37 ºC and corrects pH, pCO_2, and pO_2 for patient temperature by human-based algorithms. We manually performed temperature corrections for sea turtles as previously described (loggerheads: Chittick et al. 2002, Harms et al. 2003; leatherbacks: Harms et al. 2007; ridleys: Innis et al. 2007). Packed cell volume (PCV) was determined by centrifugation (IMA Microhematocrit Mini-Centrifuge, Model MHMC206, International Medical Assistance, Inc., Indianapolis, Indiana 46240, USA) of heparinized 32 × 0.8 mm capillary hematocrit tubes (Drummond Scientific Co., Broomali, Pennsylvania 19008, USA).

Signal generation and AEP recording

To collect AEPs, we inserted needle electrodes (27 ga, 6 mm in length, Rochester Electro-Medical, Inc., Lutz, Florida 33559, USA) subdermally beneath scales on the top of the head (recording electrode); in the deltoid muscle of the shoulder (reference electrode); and either beneath the skin beneath the rear of the carapace (air: ground electrode) or water (water: ground electrode). An Evoked Potential Workstation (Tucker-Davis Technologies, Inc. Alachua, Florida 32615 USA) and laptop computer with SigGenRP and BioSigRP software (Tucker-Davis Technologies, Inc. Alachua, Florida 32615 USA) generated tonal stimuli and recorded AEP responses from the electrodes. We presented pulsed sinusoidal tonal stimuli, 50 ms in length, shaped with a Hanning window, with a 5 ms gate time, at a rate of 11 s^{-1}. We recorded responses to frequencies between 50 and 1600 Hz, and attenuated tones in 6 dB steps beginning at the loudest level that could be generated at each frequency and attenuating until no further AEP signal could be identified (after up to 1000 AEP signal averages). In order to increase the number of recordings for each individual, if an AEP response was detected before 1000 averages were completed, we advanced to the next SPL step down. We paused recordings whenever the turtles lifted their heads to breathe (air) or moved in any way to ensure we made all measurements with the head in the same position on the acoustic field.

Calibration

We calibrated the sound field and measured background noise using a hydrophone (HTI96-min, High Tech, Inc. Gulfport, Mississippi 39501, USA; sensitivity: -164 dBV/µPa; 0.02-30 kHz) in water and a microphone (M31, LinearX Systems, Inc. Tualatin, Oregon 97062, USA; sensitivity: -117dBV/20 µPa; 0.1-10 kHz) in air placed at the location of the center of the turtle's head with the turtle absent. Calibrations were made 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 24414 Hz. Underwater, this procedure accounts for reverberation in the tank, as opposed to calibrating with long duration tones. We collected background noise recordings using FieldLog (custom software, David Mann, University of Southern Florida) at 24414 Hz using the RP2.1 module and analyzed background noise frequency spectra using Matlab (version 7.14, MathWorks, Inc. Natick, Massachusetts 01760, USA). To ensure no recorded AEP signals were the result of

electrical artifacts, we collected AEPs from a deceased hatchling (found freshly deceased in the nest after all other hatchlings had emerged) at all frequencies tested using the same experimental setup after the turtle had been deceased for 12 hrs. No AEP signals were recorded from the deceased hatchling.

DATA ANALYSES

Because turtle movement and the presence of low-frequency background and electrical noise did not allow for the use of automated threshold detectors, we performed threshold analyses manually, a method commonly used in hearing investigations using AEPs (e.g. Casper and Mann 2006, Dow Piniak et al. 2012, Egner and Mann 2005, Martin et al. 2012, Mooney et al. 2010). We made visual inspections for presence or absence AEP signals in the time and frequency domains in BioSigRP and Matlab (Fig. 1). We used a 2048-point fast Fourier transform (FFT) to analyze the AEP signals in the frequency domain. An AEP was determined to be present if the signal showed a peak twice that of the stimulus frequency (eg 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 peak in the frequency domain. We defined threshold as the lowest sound level at which a peak in the FFT was recorded. To generate audiograms, we plotted the threshold (dB) for each frequency tested using Excel (Microsoft Corporation Redmond, Washington 98052, USA).

Minimum and maximum respiratory rate and temperature during the AEP recordings, recovery times, and post-procedure venous blood pH, pCO_2, pO_2, bicarbonate, lactate, PCV, and heart rate were compared among groups by the Kruskal-Wallis test followed by Dunn all pairs post hoc analysis, using a commercial software package (JMP 0.0, SAS Institute, Inc, Cary, North Carolina, USA). We compared the number of movements potentially or actually disrupting AEP measurements between the midazolam-sedated and ketamine-dexmedetomidine-anesthetized turtles in air by the Wilcoxon rank sums test (JMP). We compared heart rates before midazolam administration and after midazolam effect in the sedated groups, and before and after venipuncture in controls using the Wilcoxon matched pairs signed ranks test (JMP). We considered a p-value less than 0.05 statistically significant. To evaluate the effectiveness of using anesthesia as a restraint, we compared threshold levels in resulting audiograms and venous blood gas values before and after the procedures on five anesthetized (ketamine-dexmedetomidine) hatchlings and seven sedated (midazolam) hatchlings sedated using midazolam.

In order to determine the marine anthropogenic sources of sound leatherback sea turtles are capable of detecting, we compared the resulting audiograms to published frequency ranges and sound pressure levels (SPLs) produced by several common underwater low-frequency anthropogenic sources (seismic airgun arrays, offshore drilling, mid and low-frequency sonar, shipping, pile driving, operating wind turbines, helicopters, and planes) and analyzed the overlap of signals produced by these sources and signals detectable by leatherback sea turtles.

RESULTS

AUDITORY EVOKED POTENTIAL WAVEFORM CHARACTERISTICS

Recorded leatherback sea turtle AEP waveforms obtained from averaged responses to pulsed tonal signals increased in latency and decreased in amplitude as we attenuated the stimuli (Fig. 1a). Recorded AEP waveforms were twice the frequency of the presented stimuli (Fig. 1b).

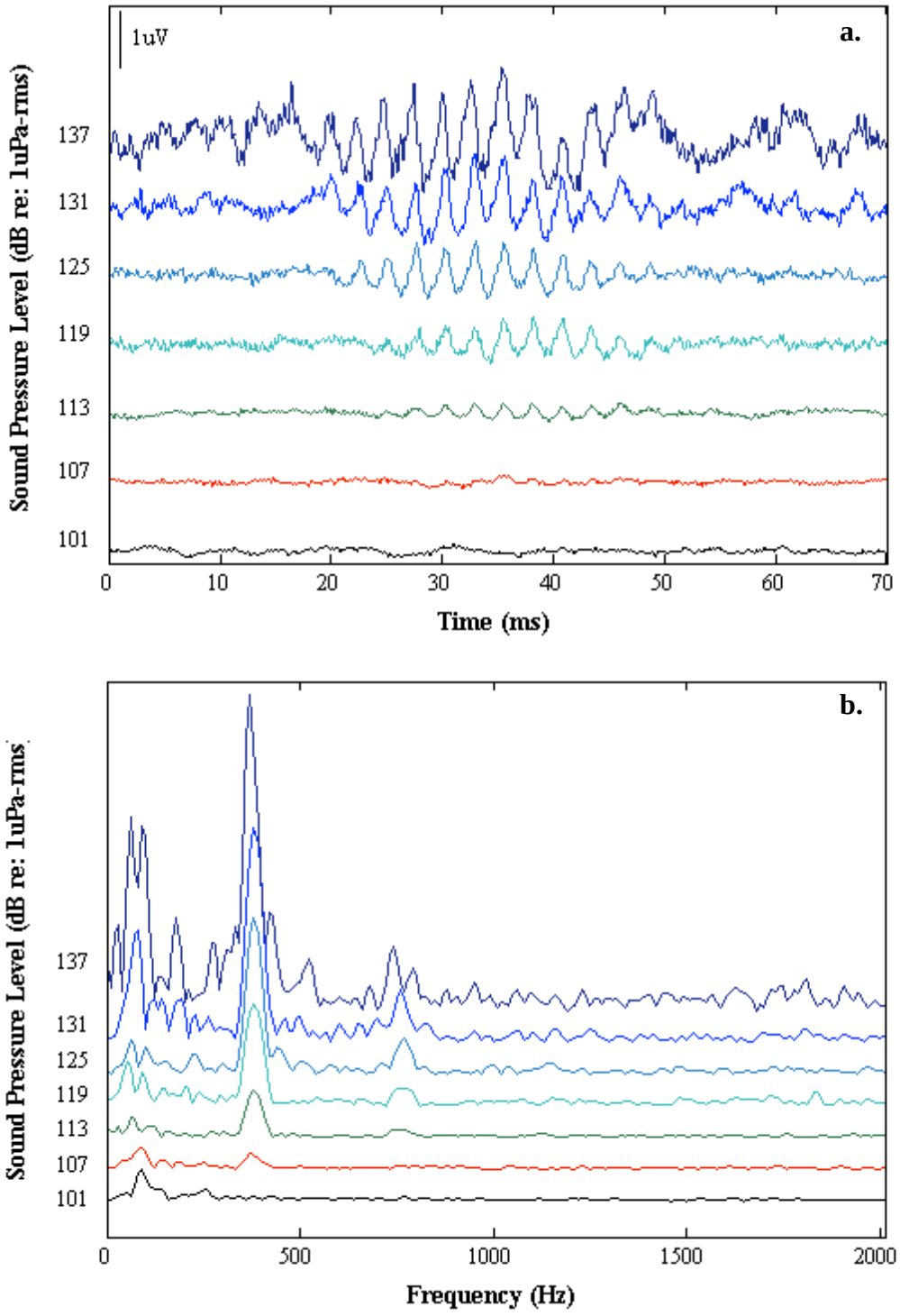

Figure 1. a. Underwater auditory evoked potential waveforms recorded from a leatherback sea turtle hatchling (*Dermochelys coriacea*, ID Dc18) and corresponding stimuli levels in response to an underwater signal of 200 Hz. b. 2048-point FFTs of recorded AEPs (presented in a.) showing peak at twice the frequency presented (400 Hz). Threshold level is presented in red (107dB re: 1 µPa-rms).

UNDERWATER HEARING

AEP waveforms recorded from averaged responses to underwater tonal stimuli appeared 17-20 ms after onset of the signal. Due to the duration of underwater experiments, full audiograms could not be collected for each individual. We recorded responses to three to five frequencies per hatchling and averaged the threshold levels to create one mean audiogram. Leatherback sea turtles responded to signals between 50 and 1200 Hz in water with maximum sensitivity between 100 and 400 Hz (Table 2; Fig 2). Sensitivity decreased sharply after 400 Hz. 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 continued to decrease with increasing frequency. Frequency threshold level differences among individuals ranged from <1 to 18 dB, however up to 6 dB of this variability could be due to the 6 dB step size used during the AEP measurements. While all individuals tested responded to 600 Hz, only four of the six tested responded to 800 Hz, just two of the five individuals tested responded 1200 Hz. No hatchlings responded to 1600 Hz at a level of 128-129 dB re: 1 µPa.

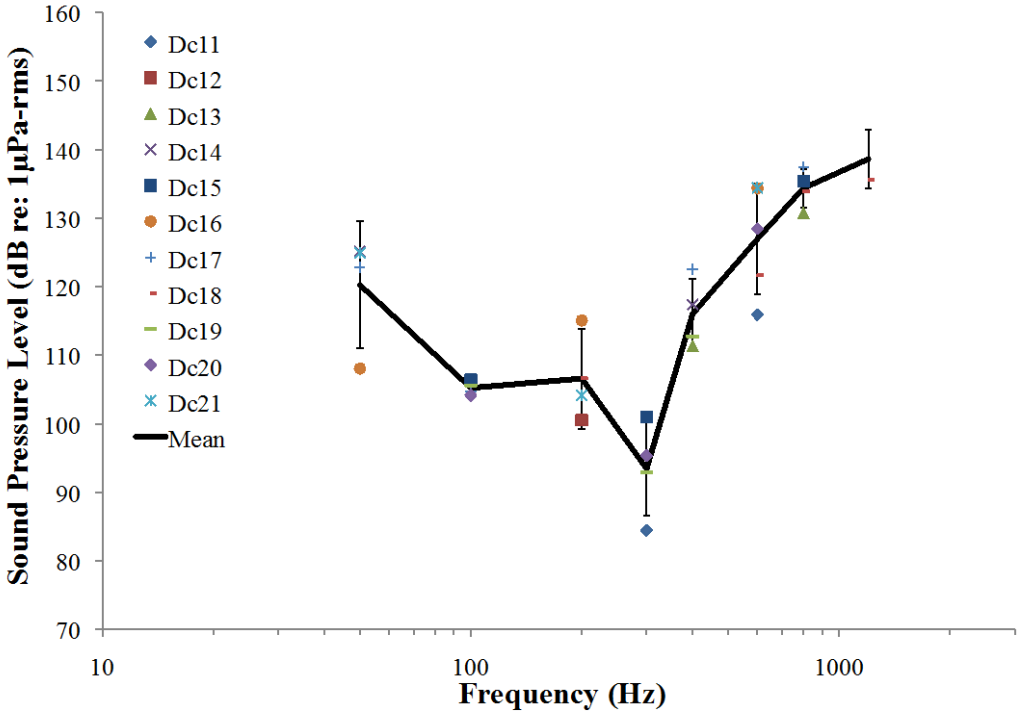

Figure 2. Underwater audiogram for leatherback sea turtle hatchlings (*Dermochelys coriacea*) (n=11). Because we recorded auditory evoked potentials in response to three to five frequencies per individual (and not the entire frequency range detectable by leatherbacks) one audiogram is presented, representing the mean threshold (± 1 SD) for all individuals for which each frequency was tested.

Table 2.

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). - Denotes a frequency not tested.

Turtle ID	Frequency (Hz)								
	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

AERIAL HEARING

AEP waveforms appeared 13-16 ms after tone presentation. Leatherback sea turtles responded to signals between 50 and 1600 Hz in air, with maximum sensitivity between 50 and 400 Hz (Table 3; Figs. 3 and 4). Sensitivity decreased sharply after 400 Hz. The lowest sensitivity recorded was 62 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. We found variability between individuals both in frequency threshold levels and highest frequency of response. Within the midazolam sedated group, frequency threshold level differences among individuals ranged <1 to 12 dB, and within the anesthesia sedated group frequency threshold level differences among individuals ranged from <1 to 13 dB, however up to 6 dB of this variability could be due to the 6 dB step size used during the AEP measurements. Similar to the underwater recordings, all individuals tested responded to 600 Hz, however in air nine of the 12 tested responded to 800 Hz, and eight of the 11 tested responded to frequencies >1000 Hz. We chose not to test above 1600 Hz as it seemed to be the upper frequency limit for the turtles (those that responded only heard the highest stimulus level presented), and we did not want to present turtles with potentially damaging levels of sound. On average, anesthetized turtles had lower threshold levels than sedated turtles, particularly at lower frequencies, however the difference between the two techniques was small, with mean thresholds for anesthetized hatchlings <7 dB lower (more sensitive) than those not anesthetized (Fig. 4).

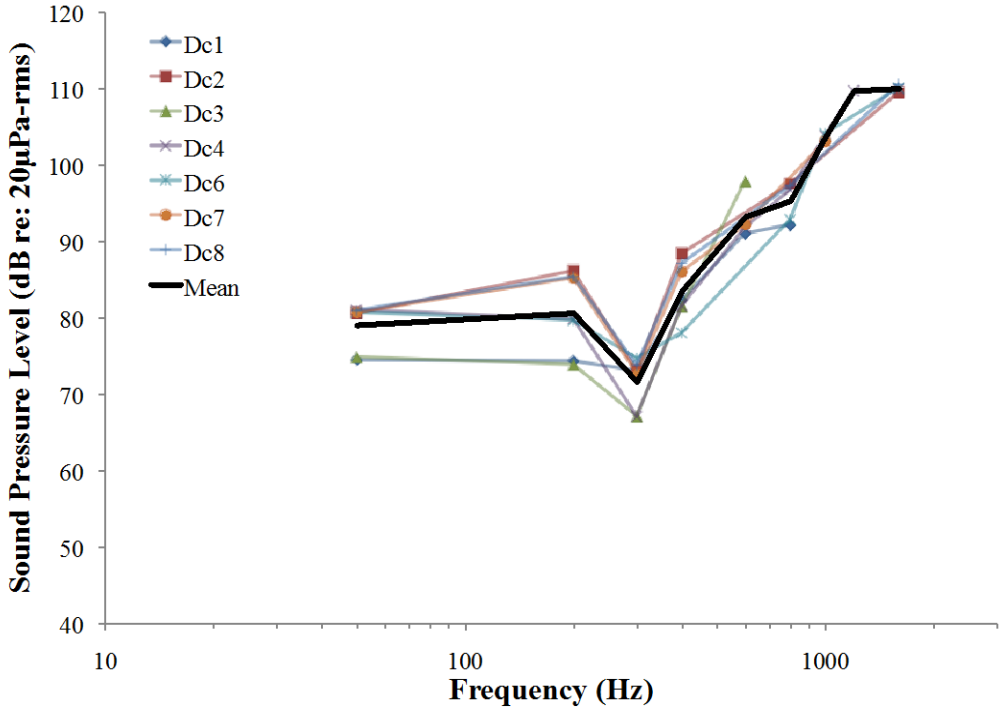

Figure 3. Aerial audiograms (n=7) for individual leatherback sea turtle hatchlings (*Dermochelys coriacea*) and mean audiogram for all sedated turtles.

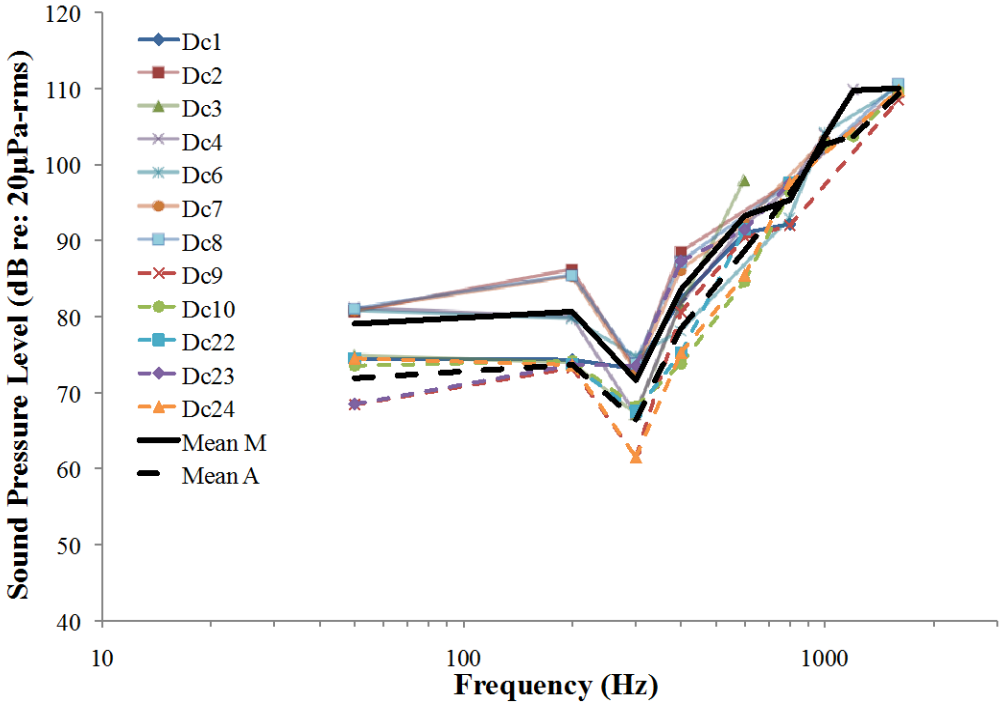

Figure 4. Aerial audiograms for individual leatherback sea turtles hatchlings (*Dermochelys coriacea*) and mean audiograms for sedated turtles (n=7, represented by solid lines) and anesthetized turtles (n=5, represented by dashed lines).

Table 3.

Aerial thresholds (dB re: 20 µPa-rms) for individual leatherback sea turtle hatchlings (*Dermochelys coriacea*), and mean thresholds for those sedated with: midazolam (M); anesthesia (A); and midazolam and anesthesia combined. Frequencies tested for which no auditory evoked potential was recorded are presented with > "highest sound pressure level presented" (dB re: 20 µPa-rms). - Denotes a frequency not tested.

Turtle ID	Sedation	Frequency (Hz)								
		50	**200**	**300**	**400**	**600**	**800**	**1000**	**1200**	**1600**
Dc1	M	74	74	73	82	91	92	>105	>110	>110
Dc2	M	81	86	73	88	-	98	-	-	110
Dc3	M	75	74	67	82	98	>97	-	-	-
Dc4	M	81	80	67	82	92	97	104	110	110
Dc6	M	81	80	75	78	-	93	104	-	110
Dc7	M	81	85	73	86	92	>97	103	-	>109
Dc8	M	81	85	74	87	-	97	-	-	111
Dc9	A	68	73	62	81	90	92	-	-	109
Dc10	A	74	74	68	74	85	96	103	104	110
Dc22	A	75	74	68	75	91	98	>103	-	-
Dc23	A	69	74	74	87	91	98	-	-	-
Dc24	A	75	74	62	75	85	98	-	-	110
Mean M	M	79	81	72	84	93	95	104	110	110
Mean A	A	72	74	66	78	89	96	103	104	109
Mean All	A & M	76	78	69	81	91	95	103	107	110

SEDATION, ANESTHESIA, AND VENOUS BLOOD GAS COMPARISONS

Weight, length, venous pH and blood gases, lactate, post-procedure heart rate, and minimum and maximum respiratory rate are summarized in Table 4. Temperatures varied slightly among groups ($p = 0.048$), with median (minimum – maximum) submerged temperatures (26.9 [25.9- 28.5] ºC) less than temperatures at the nest (27.9 [27.3- 28.2] ºC) but neither differing significantly from the sedation (27.4 [27.0- 29.8] ºC) or anesthesia (27.1 [26.4- 28.3] ºC) in air groups.

Time to sedative or anesthetic effect (median [minimum – maximum]) was significantly shorter for the ketamine-dexmedetomidine anesthetized turtles (0.5, [0.5, 1.0] min) than for midazolam sedated turtles (11 [4-16] min). Heart rate was significantly greater for hatchlings recently emerged from the nest than for post-procedure heart rate of sedated submerged hatchlings and for anesthetized hatchlings, while post-procedure heart rate of sedated hatchlings in air was intermediate (Table 2). Heart rate of sedated turtles decreased significantly from baseline values (80 [70-100] min^{-1}) following the initial effect of midazolam (75 [60- 90] min^{-1}; $p < 0.0001$). Heart rates of recently emerged hatchlings did not differ before and after venipuncture.

Table 4.

Median (minimum - maximum) weight, straight carapace length, venous pH and blood gases (at 37 ºC and temperature corrected [TC]), lactate, post-procedure heart rate, and minimum and maximum respiratory rates (excluding initial apneic period for ketamine-dexmedetomidine group) for leatherback sea turtle hatchlings (*Dermochelys coriacea*) sedated with midazolam in air, sedated with midazolam and submerged (brought to the surface to breathe every 45-60 seconds), anesthetized with ketamine-dexmedetomidine (reversed with atipamezole), or just emerged from the nest (no sedation or collection of auditory evoked potentials). Cells denoted by * are significantly greater than those denoted by †.

Analyte	Midazolam - Air (n=7)	Midazolam - Water (submerged) (n=11)	Ketamine-dexmedetomidine - Air (n=5)	No Sedation or Anesthesia (n=6)
Weight (g)	43.8 (41.1 - 45.7)†	44.5 (42.0-47.2)	47.3 (45.5-48.4)*	44.9 (39.3-49.4)
SCL (mm)	60 (58-63)	60 (56-62)	59 (59-63)	59 (53-60)
$pH_{37\,°C}$	7.309 (7.189-7.359)	7.216 (7.106-7.300)	7.27 (6.995-7.308)	7.245 (7.141–7.372)
pH_{TC}	7.471 (7.329-7.489)	7.357 (7.257-7.446)	7.407 (7.117-7.447)	7.376 (7.264-7.499)
$pCO_{2\ 37\,°C}$ (mm Hg)	64.0 (51.9-72.3)†	77.7 (62.7-101.5)*	67.8 (64.8-85.9)	75.2 (59.6-88.6)
$pCO_{2\ TC}$ (mm Hg)	42.1 (34.6-47.5)	49.9 (38.6-63.3)	44.0 (41.9-58.7)	49.8 (40.0-60.3)
$pO_{2\ 37\,°C}$ (mm Hg)	45 (31-48)	38 (11-65)	38 (35-44)	43 (26-50)
$pO_{2\ TC}$ (mm Hg)	40 (27-43)	34 (10-57)	34 (31-38)	38 (23-44)
HCO_3^- (mmol L^{-1})	30.8 (26.3-32.1)	32.0 (29.8-38.2)	31.7 (21.0-34.6)	32.6 (26.3-34.6)
Lactate (mmol L^{-1})	0.98 (0.40-5.78)†	1.58 (0.44-4.9)	1.81 (0.69-14.71)	4.99 (3.65-10.6)*
Heart rate (min^{-1})	80 (80-95)	80 (20-90)†	70 (60-80)†	97.5 (90-110)*
Minimum respiratory rate (min^{-1})	1.0 (0.5-1.0)	1.0 (1.0-1.0)	1.0 (0.5-0.4)	ND
Maximum respiratory rate (min^{-1})	2.6 (2.0-3.0)†	4.0 (2.0-6.0)	9.0 (3.6-11.0)*	ND

The median (minimum-maximum) apneic interval following ketamine-dexmedetomidine administration was six (5-12) minutes. Midazolam sedated turtles did not have an apneic interval apart from their normal inter-breath respiratory pause. Minimum respiratory rate (excluding the initial apneic interval of anesthetized turtles) did not differ among sedated and anesthetized groups. Maximum respiratory rate was significantly greater for anesthetized hatchlings than for midazolam sedated hatchlings in air, while the rate for midazolam sedated submerged hatchlings was intermediate.

Median (minimum, maximum) number of movements potentially or actually disrupting AEP measurements was more numerous for midazolam-sedated (23 [18, 45]) than for ketamine-dexmedetomidine-anesthetized (6 [1, 22]) turtles in air.

Venous blood pH did not differ among groups, although the ketamine-dexmedetomidine group included one turtle considered clinically acidotic (temperature-corrected pH = 7.117). Venous blood pCO_2 differed significantly among groups ($p = 0.0329$) at instrument temperature (37 ºC), with the midazolam sedation in air group having significantly lower values than the midazolam in water group, and the ketamine-dexmedetomidine in-air group and the unanesthetized recently emerged group falling intermediate between but not significantly different from the two midazolam groups. Because of slight temperature differences in water, however, the difference was not significant for temperature corrected values of venous pCO_2 ($p = 0.0676$). Venous pO_2 did not differ among groups, but values for the midazolam sedation in water group varied the greatest and included both the lowest and highest values (temperature corrected pO_2 = 10 and 57 mm Hg, respectively). Venous blood bicarbonate did not differ significantly among groups. Venous blood lactate differed significantly among groups ($p = 0.0415$), and was greater for hatchlings recently emerged from the nest than for turtles sedated with midazolam in air, with the other two groups falling intermediate between but not differing significantly from the high and low lactate groups. The ketamine-dexmedetomidine group did include a single hatchling with clinically relevant lactic acidemia, at 14.71 mmol L^{-1}, which coincided with the lowest pH value mentioned above (7.117).

Arousal effects of atipamezole were evident in the anesthetized group within one minute in all cases. Heart rate after atipamezole effect was recorded in only three instances, but heart rate increased by 30-50 min^{-1} in those cases. Median (minimum – maximum) time to full recovery following the procedure was significantly greater for turtles sedated and submerged (30 [15 – 347] min) than for turtles sedated in air (14 [6-33] min), with anesthetized turtles intermediate and not significantly different from the sedated groups (17 [12-49] min). The sedated and submerged group included one extreme outlier (347 min) that went on to have a good release. The second longest time to full recovery in that group reached full recovery in 48 minutes. Time from the end of the procedure until release did not vary among groups, with an overall median (minimum – maximum) of 7.1 (1.3-24.6) hours, determined primarily on optimizing release times for after sundown (19:00 – 23:30). All releases of hatchlings to the ocean were scored as good, except for one fair and one poor release out of 11 in the midazolam sedation in water group.

DISCUSSION

Results showed leatherback sea turtle hatchlings are able to detect sounds underwater and in air, responding to stimuli between 50 and 1200 Hz in water and 50 and 1600 Hz in air with maximum sensitivity between 100 and 400 Hz in water (84 dB re: 1 μPa-rms at 300 Hz) and 50

and 400 Hz in air (62 dB re: 20 μPa-rms at 300 Hz). Hearing sensitivity in both media declined considerably above 400 Hz. These represent the first measurements of leatherback sea turtle hearing sensitivity.

AUDITORY EVOKED POTENTIAL MEASUREMENTS

We found a wider frequency bandwidth of hearing in leatherback hatchlings (in both the upper and lower bounds) when compared to studies using different AEP methodologies (greens - cochlear potentials: Ridgway 1969; greens - partially submerged with an aerial sound source: Bartol and Ketten, 2006; loggerheads - direct tympanum stimulation via mechanical vibrator: Bartol et al. 1999; Kemp's ridley - partially submerged with an aerial sound source: Bartol and Ketten 2006) and similar frequency bandwidth of hearing in leatherback hatchlings when compared to studies using similar methodologies (greens: Dow Piniak et al. 2012; loggerheads: Martin et al. 2012). It is possible that higher stimuli SPLs may have elicited detectable AEP responses at higher frequencies (≥1200 Hz in water and ≥1600 Hz in air) or lower frequencies (<50 Hz in water and air). It is difficult to directly compare threshold levels between our study and many previous studies due the methodological differences described above, however, we recorded responses to much lower stimuli SPLs than Martin et al. (2012), who used similar methodologies to measure underwater AEPs for one adult loggerhead sea turtle (loggerhead adult: 110 dB re: 1 μPa, Martin et al. 2012); leatherback hatchling: 84 dB re: 1 μPa-rms, this study). Unfortunately, the small sample size in the Martin et al. (2012) study, and differences in species and age class limit the value of such a comparison. While it remains to be examined, it is possible that as sea turtles age, hearing sensitivity changes in either frequency bandwidth or thresholds of detection, or both.

Leatherback sea turtle AEP waveforms exhibit a frequency-doubling response at all frequencies tested, which has also observed in other species of sea turtle, fish, and squid AEPs (Casper and Mann 2006, Dow Piniak et al. 2012, Egner and Mann, 2005, Martin et al. 2012, Mooney et al. 2010). In fish it is hypothesized that this 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 double-firing and doubled response. Inner ear sound detection in sea turtles is thought to occur via the cochleae, rather than otoliths, but a differing orientation of limbic and basilar membrane hair cells could cause a similar double firing and doubled response.

One of the primary challenges of measuring hearing sensitivity using AEPs is the determination of thresholds. Low frequency AEP responses are particularly challenging to evaluate using AEP techniques. Because peak background and electrical noise levels occur at very low frequencies (<200 Hz), it can be difficult to distinguish low frequency peaks in the FFT caused by AEP presence from those caused by background noise, likely causing the determined thresholds at low frequencies to be conservative. While we were able to detect aerial AEP responses to 50 Hz in air, because of low signal-to-noise ratio or electrical interference, we were unable to record responses at100 Hz. Although we were unable to test below 50 Hz with our experimental setup, the relatively flat shape of the aerial audiogram below 200 Hz and the low threshold level of 50 Hz in water compared to the threshold of the upper frequency of detection, suggest that leatherbacks may be able to detect frequencies below 50 Hz in air and underwater.

Although 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, background noise in this study was very low in both media, >60 dB below

thresholds in air and >50 dB below thresholds underwater, making it unlikely that background noise masked measured thresholds.

USE OF SEDATION AND ANESTHESIA TO COLLECT AUDITORY EVOKED POTENTIALS

Sedation with midazolam at 2-3 mg/kg and anesthesia with ketamine (6 mg/kg) and dexmedetomidine (30 µg/kg) proved effective in facilitating AEP recordings in leatherback hatchlings. Unlike hardshell hatchlings for which hearing has been measured, AEP recordings of leatherback hatchlings without sedation had previously been impossible to collect in air or submerged. Full anesthesia for submerged AEP recordings has been accomplished in larger juvenile green turtles using specially designed double-cuffed endotracheal tubes to protect the airway and deliver positive pressure ventilation from the surface (Harms et al. 2009), but the small size of the glottis and trachea of leatherback hatchlings (<2 mm) precluded the use of cuffed endotracheal tubes that would have adequately protected the airway in a submerged anesthetized animal. Therefore, sedation was employed, allowing the turtle to control its airway voluntarily, while reducing motion artifact. The effects of sedatives on measured hearing sensitivity have not been explored, however resulting leatherback frequency bandwidth of sensitivity and thresholds are comparable to, if not more sensitive than, other sea turtle hearing studies for which sedation was not used, a negative effect seems unlikely.

Full anesthesia for in-air AEP measurements further reduced movement, likely reducing internal body noise and increasing the signal-to-noise ratio, allowing for the detection of AEPs at lower stimulus levels. Anesthesia appeared to have little effect on measured hearing sensitivity, with average thresholds for anesthetized hatchlings <7 dB lower (more sensitive) than those not anesthetized. Although the pattern of lower thresholds held for all individuals sedated with anesthesia, up to 6 dB of this difference could be attributed to the step size used in AEP measurements.

Acid-base balance and blood gases were not markedly affected by sedation (with or without submergence) or anesthesia. In fact, lactic acidemia was greater for the unsedated, unanesthetized, recently emerged group. The midazolam sedated submerged group tended towards hypercapnea, indicative of hypoventilation, despite a higher observed respiratory rate in the submerged group than in the sedated in-air group. There may have been subtle respiratory movements not detected in the aerial group that could breathe on demand. Lower heart rate in the submerged group may have been partially due to a forced dive reflex response (Lutcavage and Lutz 1997), while bradycardia in the anesthetized group is an expected response to dexmedetomidine (Plumb 2011). The higher maximum respiratory rate in ketamine-dexmedetomidine anesthetized turtles may have been a compensatory response to the initial apneic period.

Although as groups, the turtles did well across all treatments, there were individuals with values or clinical responses of concern. One turtle in the ketamine-dexmedetomidine anesthesia group exhibited a lactic acidosis (lactate 14.71 mmol/L, pH 7.117), but recovered well and had a good release. One turtle in the midazolam sedated submerged group experienced a profound bradycardia (20 min^{-1}) and prolonged recovery (347 min), but recovered well and had a good release. Another in this group appeared to recover rapidly from sedation but then became less active, experienced bradycardia as low as 20 min^{-1}, and had a poor release. Both of these turtles were treated with dexamethasone sodium phosphate at 2 mg/kg IM and a period of time in an improvised oxygen chamber. These two turtles had come from a nest that had been excavated

following the emergence of the main body of hatchlings, rather than from the emergence itself, and thus represented less vigorous turtles that would otherwise have perished in the beach. After the response observed with these two turtles, no further animals from this batch were utilized.

POTENTIAL IMPACTS OF MARINE ANTHROPOGENIC SOUND ON SEA TURTLES

Sea turtles may be affected by marine sound both physiologically and behaviorally. Effects of noise on sea turtles are largely unknown, because of a lack of information on hearing capabilities and behavioral responses to 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 area to reproduce and forage, but also those simply "passing through". As sea turtles can be found in nearly all temperate, tropical, coastal and offshore habitats, there is vast potential for temporal and spatial overlap between sea turtle habitat and marine anthropogenic sound is vast.

Physiological effects of marine sound

High-intensity sounds can cause physiological effects and even death in some species (Richardson et al. 1995). No data exist on the impacts on marine anthropogenic sound on the physiology of sea turtles. In general, animals may experience a temporary or permanent auditory sensitivity threshold shift (TTS or PTS), or loss of hearing. TTSs or PTSs are temporary or permanent increases in the threshold level of audibility for the ear at a particular frequency or frequencies (Yost 2007). For example, sonic booms from jet aircraft have been shown to cause temporary threshold shifts in desert tortoises (Bowles et al. 1999) and noise generated by air guns during seismic surveys has been found to permanently damage the ears of the pink snapper (McCauley et al. 2003). Increased noise in the ocean can also mask important acoustic cues, however no information exists on critical ratios and masking in sea turtles. A decrease in hearing sensitivity reduces an animal's ability to monitor its acoustic environment. Repeated exposures to sound sources can cause habituation or sensitization (decreases or increases in behavioral response) increasing long-term physiological effects. Cumulative effects of repeated exposures on physiology are not well understood.

Leatherback sea turtles and anthropogenic noise

Leatherback hearing sensitivity overlaps with the frequencies and source levels produced by low-frequency anthropogenic sources such as: seismic airgun arrays, offshore drilling, low-frequency sonar, pile driving, operating wind turbines, and traveling vessels. While the SPL of these sound sources varies depending on the source configuration and environmental variables, some examples of anthropogenic sound sources and SPLs at peak frequencies compared to the leatherback hearing range can be seen in Figure 5.

Leatherback sea turtles are largely pelagic, and inhabit near-shore waters only during reproduction and occasionally during foraging. The temporal and spatial overlap of leatherback sea turtle habitat and anthropogenic sound varies depending on the environment and the anthropogenic sound source. Within categories of sound source types, SPLs 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 the receiver's location in relationship to the source.

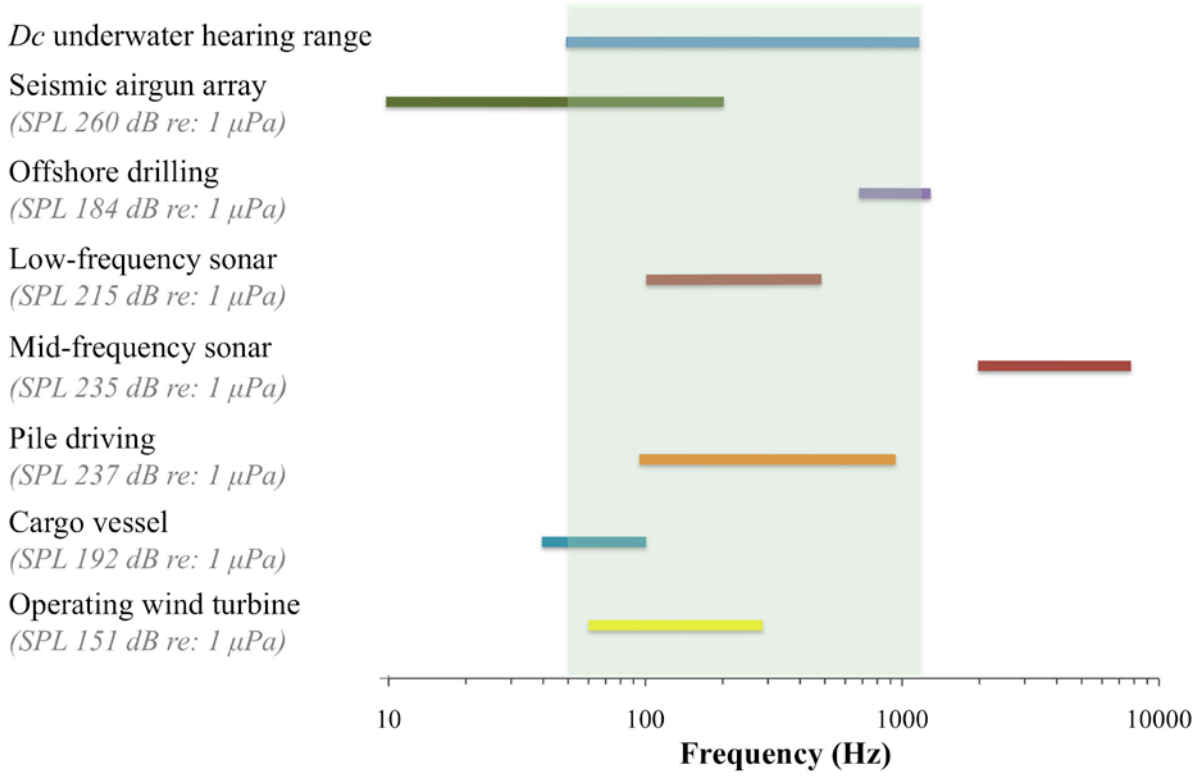

Figure 5. Comparison of the leatherback sea turtle (*Dermochelys coriacea*) underwater auditory frequency range to the frequency bandwidth of peak sound pressure levels of sounds produced by marine anthropogenic sources (seismic airgun array: Turner et al. 2006; offshore drilling: Blackwell et al. 2004; low-frequency sonar (SURTASS LFA sonar): Anonymous 2007; mid-frequency sonar (US Navy 53C ASW sonar): Evans and England 2001); pile driving (1000 kJ hammer): Hildebrand 2009; Cargo vessel (173 m in length, 16 knots): NRC 2003; operating wind turbine (wind speed 13 m^{-s}, 180 Hz: Wahlberg and Westerberg, 2005). Figure adapted from anthropogenic sources presented in Hildebrand 2009.

Oil and gas exploration and extraction presently occurs in many important sea turtle nesting and foraging habitats and generates high-intensity, low-frequency, impulsive sounds within the leatherback hearing range. In a report prepared for the International Association of Oil and Gas Producers Exploration and Production Sound and Marine Life Joint Industry Project, leatherback sea turtles are shown to be present in 11 of the 13 oil and gas industry offshore interest areas (Thorson et al. 2005). While drilling from stationary platforms occurs over long time scales, oil and gas exploration is an intermittent activity, and in some locations there is the potential to avoid leatherback high habitat use times.

Naval sonar also occurs intermittently (although while active, the sound is continuous), and often in designated areas or ranges. However many of these areas/ranges provide habitat for leatherback sea turtles. While leatherback hatchlings appear to be capable of detecting low-frequency sonar (<1000 Hz), frequencies for the peak SPL for mid-frequency sonar (2000-8000

Hz) appear out the range of sea turtle hearing sensitivity. It is important to note, however, that while making these hearing measurements, we did not expose leatherbacks to the high SPLs of mid-frequency sonar, and is possible that turtles are able to detect these higher frequencies at increased SPLs.

Pile driving occurs over small spatial and temporal scales and produces high-intensity, low-frequency, impulsive sounds with high peak pressures that can be detected by leatherback sea turtles. Like oil and gas exploration, due to the short time duration of many pile driving projects, it may be possible to avoid leatherback high habitat use times.

The construction of offshore wind farms generates high-intensity sounds (pile driving). In this case the spatial and temporal scales of pile driving could be quite large, as offshore wind farms can span tens of kilometers and require many months to install. However once in place, turbines generate continuous, moderate levels of low frequency sound that can be detected by leatherback sea turtles. Depending on environmental variables (wind speed) and turbine type, leatherbacks are unlikely to be able to detect these sounds at large distances away from the farm, however farms could disrupt leatherback 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 SPL 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 leatherback sea turtles. Areas of high use by commercial vessels (e.g. inshore ports and shipping lanes), and those used by recreational vessels (e.g. nearshore waters and inshore ports) overlap with leatherback sea turtle reproductive and foraging habitat at many locations. While no direct measurements of noise levels have been made in leatherback sea turtle habitat, Samuel et al. (2005) recorded levels of up to 113 dB re: 1 μPa (200-700 Hz) for small, recreational boats during high-use seasons 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.

CONCLUSIONS & RECOMMENDATIONS

In this study, we made the first measurements of underwater and aerial hearing sensitivity of leatherback sea turtles. Leatherback sea turtle hatchlings are able to detect sounds underwater and in air, responding to stimuli between 50 and 1200 Hz in water and 50 and 1600 Hz in air with maximum sensitivity between 100 and 400 Hz in water (84 dB re: 1 μPa-rms at 300 Hz) and 50 and 400 Hz in air (62 dB re: 20 μPa-rms at 300 Hz). When the hearing sensitivity of leatherback sea turtles and are compared with the source level and frequency range many of the high intensity, low frequency marine anthropogenic sources of sound commonly considered when evaluating about effects of noise on marine life, it is clear that leatherbacks (and all other sea turtle species for which hearing has been tested) are able to detect many of these sources. Now that we have evidence that leatherback sea turtles can detect sources of low-frequency anthropogenic sound, we recommend future studies investigate the potential physiological (critical ratios and temporary and permanent threshold shifts) and behavioral effects of exposure to these sound sources.

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