APPENDIX I

SEA TURTLE HEARING AND SENSITIVITY TO ACOUSTIC IMPACTS

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1. INTRODUCTION

There is growing concern over anthropogenic sound in the world's oceans and its potentially harmful effects on protected marine organisms, including sea turtles. Similar to other migratory marine species, sea turtles occupy different ecological niches throughout ontogeny, each characterized by unique acoustic conditions. Sea turtles spend the majority of their lives in the ocean; their only land-linked behaviors are egg deposition and hatching. Like many marine fishes and mammals, sea turtles use a range of habitats for each developmental stage (see review by Bolton, 2003). Once hatchlings reach the sea, they are pelagic, moving primarily with ocean currents. After a period of years, which varies both among species and populations, a critical ontogenetic habitat shift occurs whereby most sea turtles actively recruit to a demersal, neritic habitat and are considered juveniles. Finally, upon reaching maturity, all sea turtles maintain a discrete foraging area (this region frequently overlaps with the juveniles), migrating only to return to their natal nesting beach. The exception to this life history model in North Atlantic populations is the leatherback turtle (*Dermochelys coriacea*). Leatherbacks remain pelagic as both juveniles and adults and return to the neritic zone only for reproduction (Bolton, 2003).

Few studies have examined the role acoustic cues play in the ecology of sea turtles (Mrosovsky, 1972; Samuel et al., 2005; Nunny et al., 2008). There is evidence that sea turtles may use sound to communicate; the few vocalizations described for sea turtles are restricted to the "grunts" of nesting females (Mrosovsky, 1972). These sounds are low frequency and relatively loud, thus leading to speculation that nesting females use sounds to communicate with conspecifics (Mrosovsky, 1972). We know very little about the extent to which sea turtles use their auditory environment ("soundscape"). However, the passive acoustic environment for sea turtles changes with each ontogenetic habitat shift. In the inshore environment where juvenile and adult sea turtles generally reside, the ambient environment is noisier than the open ocean environment of the hatchlings; this inshore environment is dominated by low frequency sound (Hawkins and Myrberg, 1983), and, in highly trafficked areas, virtually constant low frequency noises from shipping, recreational boating, and seismic surveys compound the potential for acoustic impact (Hildebrand, 2005).

2. MORPHOLOGY

Much of the research on the hearing capacity of sea turtles is limited to gross morphological dissections (Wever, 1978; Lenhardt et al., 1985). The tympanum is a continuation of the facial tissue and is distinguishable only by palpitation of the area. Beneath the tympanum is a thick layer of subtympanal fat (**Figure I-1**), a feature that distinguishes sea turtles from both terrestrial and semi-aquatic turtles. Recent imaging data suggests that this layer of fat is similar to the fats found in the jaws of odontocete whales and functions as a low-impedance channel for sounds to the ear (Ketten et al., 1999). The middle ear cavity lies posterior to the tympanum; the Eustachian tube connects the middle ear with the throat (Wever, 1978; Lenhardt et al., 1985). As with most turtles, the middle ear is small and encased by bone. The ossicular mechanism consists of two elements: the extracolumella and the columella (stapes). The extracolumella is a cartilaginous disk under the tympanic membrane attached to the columella by ligaments. The columella, a long rod with the majority of its mass concentrated at each end, extends medially from the middle ear cavity through a narrow bony channel and expands within the oval window to form a funnel shaped end. The columella is free to move only longitudinally within this channel so when the tympanum is depressed directly above the middle of the extracolumella, the columella moves readily in and out of the oval window, without any flexion of the columella. The stapes and oval window are connected to the saccular wall by fibrous strands. It is thought that these stapedo-saccular strands relay vibrational energy of the stapes to the saccule (Wever and Vernon, 1956; Wever, 1978; Lenhardt et al., 1985). For semi-aquatic turtles, the columella is the main pathway for sound input to the inner ear; when the columella is clipped while leaving the tympanum intact, the animal displayed an extreme decrease of sensitivity of hearing (Wever and Vernon, 1956).

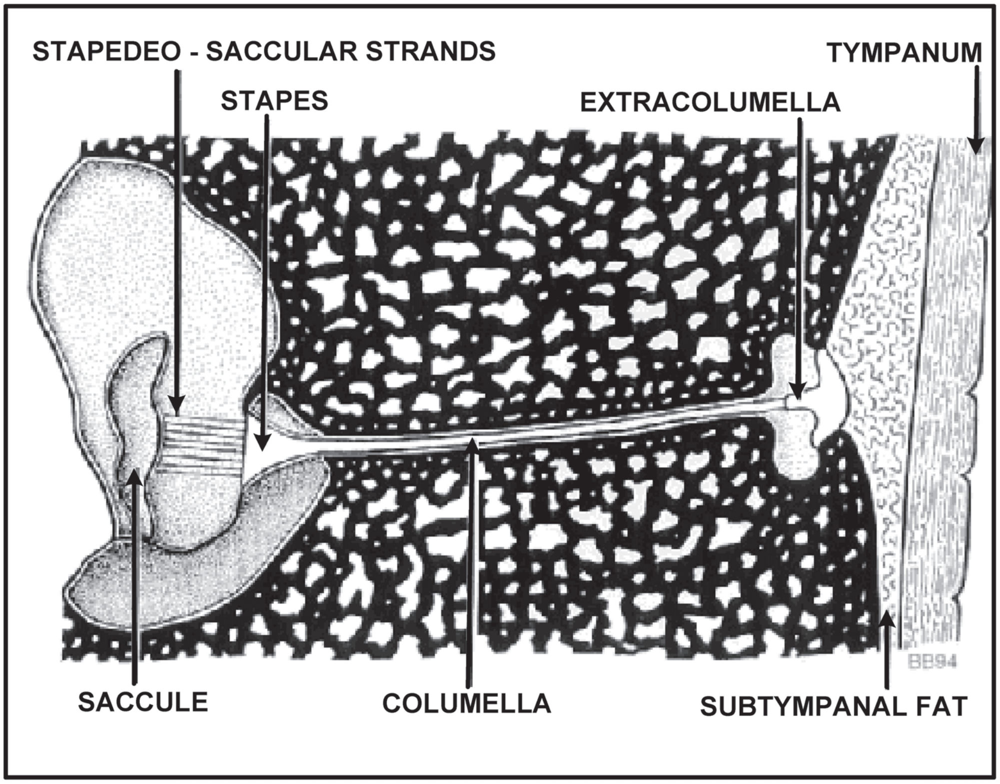

Figure I-1. Middle Ear Anatomy of the Juvenile Loggerhead Sea Turtle (Moein, 1994).

The auditory sense organ within the inner ear of the sea turtle cochlea is the basilar papilla (basilar membrane). This membrane is large and composed of dense connective tissue in sea turtles (rather than a thin basilar membrane found in terrestrial turtles) (Wever, 1978; Hetherington, 2008). This basilar papilla is positioned opposite the round window and lies within the pathway of fluid displacement due to columella motion. In most reptiles, and presumably in sea turtles as well, the tectorial membrane lays over the hair cells of the basilar papilla. For sea turtles, the innervations of the hair cells may be accomplished through the movement of the overlying tectorial membrane rather than the movement of the papillae (Hetherington, 2008).

Based on the functional morphology of the ear, it appears that sea turtles receive sound through the standard vertebrate tympanic middle ear path. This ear, however, is adapted to underwater sound, not aerial. For the terrestrial vertebrate, the middle ear is an impedance transformer between sound in air (environment) and sound in fluid (inner ear). This impedance mismatch can be overcome by having a high convergence ratio between the tympanic membrane and oval window (thus amplifying the force acting on the inner ear) and by having a multiple bone ossicular mechanism that acts as a lever system to amplify force. The convergence ratio of the tympanic membrane to oval window in sea turtles is reported to be lower than other semi-aquatic turtles (Lenhardt et al., 1985), and sea turtles lack an osscicular mechanism that acts as a lever (having only a single straight columella). Thus, the sea turtle ear appears to be a poor receptor for aerial sounds. However, this ear is well adapted to water conduction sound. The dense layer of fat under the tympanum acts as a low-impedance channel for underwater sound (similar to that pathway found in odontocetes [Ketten et al., 1999]). Furthermore, the retention of air in the middle ear of these sea turtles suggests that they are able to detect sound pressures.

3. ELECTROPHYSIOLOGICAL RESPONSE TO SOUND

Electrophysiological studies on hearing have been conducted on juvenile green turtles (*Chelonia mydas*) (Ridgway et al., 1969; Bartol and Ketten, 2006), juvenile Kemp's ridley turtles (*Lepidochelys kempii*) (Bartol and Ketten, 2006), and juvenile loggerhead turtles (*Caretta caretta*) (Bartol et al., 1999;

Lavender et al., 2010, 2011). Electrophysiological responses, specifically auditory evoked potentials (AEPs), are the most widely accepted technique for measuring hearing in situations in which normal behavioral testing is impractical. The AEPs reflect the synchronous discharge of large populations of neurons within the auditory pathway and, thus, are useful monitors of the functioning of the throughput of the auditory system. Most AEP research has concentrated on the use of responses occurring within the first 10 ms following presentation of click or brief tone burst stimuli. This response has been termed the auditory brainstem response (ABR) and consists of a series of 5-7 patterned and identifiable waves. Corwin et al. (1982) recorded AEPs from five classes of non-mammalian vertebrates (including the red eared turtle, *Pseudemys scripta elegans*) and found the response, recorded outside the brain, to be congruous with the criteria for "conventional" ABRs. Furthermore, these techniques are noninvasive and can be performed on conscious subject animals (Bullock, 1981; Corwin et al., 1982).

Ridgway et al. (1969) measured auditory cochlear potentials of green turtles using both aerial and vibrational stimuli. Thresholds were not measured; instead, cochlear response curves of 0.1 µV potential were plotted for frequencies ranging from 50 to 2,000 Hz. Green turtles detect a limited frequency range (200-700 Hz) with best sensitivity at the low tone region of about 400 Hz. Though this investigation examined two separate modes of sound reception (i.e., air and bone conduction), sensitivity curves were relatively similar, suggesting that the inner ear is the main structure for determining frequency sensitivity. To measure electrophysiological responses to sound stimuli, Bartol et al. (1999) collected ABRs from juvenile loggerhead turtles. Vibratory stimuli were delivered directly to the dermal plates over the loggerhead turtle's tympanum. Thresholds were recorded for both tonal and click stimuli. Best sensitivity was found in the low frequency region of 250-1000 Hz. The decline in sensitivity was rapid after 1,000 Hz, and the most sensitive threshold tested was at 250 Hz. More recently, Bartol and Ketten (2006) collected underwater ABRs from hatchling and juvenile loggerhead and juvenile green turtles. For these experiments, the speaker was suspended in air while the turtle's tympanum remained submerged underwater. All turtles tested responded to sounds in the low frequency range, from at least 100 Hz (lowest frequency tested) to no greater than 900 Hz. Interestingly, the smallest turtles tested, hatchling loggerheads, had the greatest range of hearing (100-900 Hz) while the larger juveniles responded to a much narrower range (100-400 Hz). Hearing sensitivity of green turtles also varied with size; smaller greens had a broader range of hearing (100-800 Hz) than that detected in larger subjects (100-500 Hz). Lavender et al. (2010, 2011) have recorded underwater AEPs using a Navy J9 underwater speaker from loggerhead turtles, their ages ranging from yearlings to subadults. Under these conditions, loggerheads were found to respond to frequencies between 50-1000 Hz.

4. BEHAVIORAL RESPONSES TO SOUND

Multiple studies have attempted to examine the behavioral responses of juvenile loggerheads to sound in their natural environment, both in controlled settings (O'Hara and Wilcox, 1990; Moein et al., 1995; McCauley et al., 2000; Lavender et al., 2011) and as observed *in situ* (Holst et al., 2007; Weir, 2007; DeRuitter and Doukara, 2010). Behavioral audiograms have been collected from multiple size classes of loggerhead turtles (Lavender et al., 2011). Behavioral audiograms require the animal to perform a task in the presence of auditory stimuli; though time consuming (it can take months to train a turtle to sound), behavioral audiograms are a more sensitive measure of hearing threshold than electrophysiological responses and ascribe a critical behavioral component to hearing trials. Lavender et al. (2011) recorded audiograms using a two-response, forced-choice approach, whereby the turtles were required to vary behavior according to presence or absence of sound, permitting a behavioral measure of acoustic sensitivity. Lavender et al. (2011) have found that while loggerheads respond to similar frequencies as previous studies (50-1,000 Hz), their threshold levels are actually more sensitive than reported using electrophysiological methods.

Several sea turtle behavioral studies have been initiated to assist in the development of an acoustic repelling device for sea turtles. O'Hara and Wilcox (1990) attempted to create a sound barrier for loggerhead turtles at the end of a canal using seismic airguns. The test results indicated that airguns were effective as a deterrent for a distance of about 30 m when the sound output of this system was approximately 220 dB re 1 µPa at 1 m in the 25-1,000 Hz range. However, this study did not account for the reflection of sound by the canal walls, and the stimulus frequency and intensity levels are ambiguous. Moein et al. (1995) investigated the use of airguns to repel juvenile loggerhead turtles from hopper dredges. A net enclosure was erected in the York River, Virginia to contain the turtles, and an airgun was stationed at each end of the net. Sound frequencies of the airguns ranged from 100 to 1,000 Hz at three decibel levels (175, 177, and 179 dB re 1 μPa at 1 m). Avoidance of the airguns was observed upon first exposure. However, after three separate exposures to the airguns, the turtles habituated to the stimuli. McCauley et al. (2000) examined the response of sea turtles (one green and one loggerhead turtle) to an airgun signal. For these trials, the turtles were placed in cages, and behavior was monitored as a single airgun approached and departed. During these trials, the turtles showed a noticeable increase in swimming behavior when the airgun level was above 166 dB re 1 μPa at 1 m and became erratic and increasingly agitated above 175 dB. Because these animals were caged, avoidance behavior could not be monitored. However, the researchers speculated that avoidance would occur at 175 dB re 1 μPa at 1 m, the point at which the animals were acutely agitated (McCauley et al., 2000).

Researchers have also attempted to monitor sea turtle avoidance to sound during an active seismic survey (Weir, 2007; DeRuiter and Doukara, 2010). Weir (2007) observed 240 animals during a 10-month seismic survey off the coast of Angola. Behaviors were recorded at time of first sighting and as the vessel and towed equipment moved in relation to the turtle. Fewer turtles were observed near the airguns as they were firing (as opposed to the "gun-off" state). However, the source of agitation for the turtle could not be identified; the turtle could have reacted to the ship and towed equipment rather than specifically to the airgun (Weir, 2007). DeRuiter and Doukara (2010) observed turtles during active operation of an airgun array as well and found a startle response (rapid dive) to the airgun. However, again, these authors could not distinguish the stimulus source of the startle response as they did not perform a control with the airguns off (DeRuiter and Doukara, 2010).

5. EFFECTS OF ANTHROPOGENIC NOISE

There is growing concern over anthropogenic sound in the world's oceans and the potentially harmful effect it has on protected marine organisms. Anthropogenic noises can originate from a multitude of sources, including (but not limited to) shipping traffic, seismic surveys for petroleum exploration, military sonar operations, pile driving, etc. These sounds have the potential to impact an animal in several ways: trauma to hearing (temporary or permanent), trauma to non-hearing tissue (barotraumas), alteration of behavior, and masking of biologically significant sounds (McCarthy, 2004).

Hearing damage is usually categorized as either a temporary or permanent injury. Temporary threshold shifts (TTS) are recoverable injuries to the hearing structure and can vary in intensity and duration. Normal hearing abilities return over time; however, animals often lack the ability to detect prey and predators and assess their environment during the recovery period. In contrast, permanent threshold shifts (PTS) are permanent loss of hearing through loss of sensory hair cells (Clark, 1991). Few studies have looked at hair cell damage in reptiles, and it is still unknown if sea turtles are able to regenerate hair cells (Warchol, 2011). There are almost no data on the effects of intense sounds on marine turtles and, thus, it is difficult to predict the level of damage to hearing structures. Clear avoidance reactions to seismic signals at levels between 166-179 dB re 1μPa have been observed (Moein et al., 1995; McCauley et al., 2000); however, both of these studies were done in a caged environment, so the extent of avoidance could not be monitored. Moein et al. (1995) did observe a habituation effect to the airguns; the animals stopped responding to the signal after three presentations. This lack of behavioral response could be a result of TTS or PTS.

The BOEM concludes that there is incomplete or unavailable information (40 CFR 1502.22) about sea turtles that use the AOI with respect to their physiology and behavioral response to intense sounds. The available data and information about sea turtles using the AOI is reported to the best of our ability in this document. The BOEM has used what scientifically credible information is available and applied it using accepted scientific methodologies. What is known about representative species, however, in combination with observation and interpretation of behavioral response to stimuli does allow some inferences to be drawn that allow reasonably foreseeable significant adverse impacts on sea turtles to be understood well enough so that mitigations can be designed to avoid or reduce them.

The BOEM has determined that incomplete or unavailable data or information on sea turtle physiology and behavioral response to intense sounds is adequate to understand reasonably foreseeable adverse impacts and is not essential to a reasoned choice among the alternatives, including the No Action alternative.

Anthropogenic noises below injury level have the potential to mask relevant sounds in the animals' environment. Masking sounds can interfere with the acquisition of prey or mate, the avoidance of predators, and, particularly in the case of sea turtles, identification of an appropriate nesting site (Nunny et al., 2008). Sea turtles appear to be low frequency specialists and, thus, the potential masking noises would fall within at least 50-1,000 Hz. These maskers could have diverse origins, ranging from natural to anthropogenic sounds (Hildebrand, 2005). The overall behavioral changes that can occur due to obscuration of sound scenery can have major ecological consequences for sea turtles. However, there are no quantitative data demonstrating masking effects for sea turtles.

Clearly, more research on the behavioral and physiological responses to sounds needs to be conducted on sea turtles before appropriate noise exposure criteria can be developed for reduced fitness, injury, and death. While the research community is making progress in the frequency range of hearing for sea turtles, there are few data on hearing loss/damage, hair cell regeneration, masking, and behavioral responses. Inner ear research on hair cell population needs to be conducted on multiple species and multiple age classes by using histology/imaging techniques to analyze variations in auditory anatomy among stages and species. The critical point that noise disrupts scene analysis and masks signals should be explored and quantitative data on masking needs to be collected for sea turtles. When looking at behavioral responses, research beyond the "startle response" must be conducted. Controlled experiments in the natural environment need to be conducted to document and classify reactions to sound as either nuisance (i.e., causing the animal to move away, changing the animals' behavior to another acceptable consequence) or injurious (i.e., preventing the animal from completing essential behavior). The results of these research studies could provide new data on the hearing ability and response to sound for sea turtles and a quantitative base for assessing potential impact of man-made sound sources on multiple species of sea turtles across habitats and developmental stages.

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