LIVING MARINE RESOURCES PROJECT 40

# Temporary Threshold Shifts in Underwater Hearing Sensitivity in Freshwater and Marine Turtles

## NEED

The Navy, National Marine Fisheries Service (NMFS) and other federal agencies require quantitative thresholds to examine the potential impacts of underwater sound on protected species. Basic audiometric information is available for some sea turtle species, however, data on the susceptibility of sea turtles to noise induced hearing loss (threshold shifts) is lacking. There is a need to obtain auditory temporary threshold shift (TTS) information for sea turtles. Due to their protected status under the Endangered Species Act, it is necessary to first determine the feasibility of generating TTS in a closely related surrogate—a non-ESA listed turtle species (e.g. red-eared slider, eastern painted turtle, pond slider, etc.). If feasible, steps to obtain TTS information for an ESA-listed sea turtle may be undertaken in follow-on research efforts.

Loggerhead sea turtle.
*NOAA*

## SOLUTION

This project will examine TTS in two species of freshwater aquatic turtles and potentially provide the cumulative sound exposure levels and durations that induce these TTS. The work also will include examining the turtles' ear anatomy to support physiological comparisons between freshwater and marine turtle hearing apparatus. This will help to identify potential TTS susceptibility of sea turtles based on freshwater turtle data. Results will provide researchers, managers and stakeholders critical data to improve estimates of acoustic effects to both freshwater and sea turtles and to inform the development of appropriate mitigation measures to reduce potential effects to sea turtles from low-frequency anthropogenic sound. This project is co-funded by the Navy LMR program and the National Oceanic and Atmospheric Administration (NOAA).

## METHODOLOGY

Initial underwater hearing measurements and TTS assessments will be conducted with two freshwater turtle species—the eastern painted turtle (*Chrysemys picta picta*) and red-eared slider (*Trachemys scripta elegans*). Physiological auditory evoked potential (AEP) methodology will be used. Testing two species will increase sample sizes, which will support both developing robust TTS measurement methods and

identifying if there are methodological challenges/differences between the species. Additionally, comparing TTS onset in the two surrogate taxa will contribute to understanding potential TTS variability between turtle species. The AEPs will be followed by sound exposure trials and anatomical imaging as summarized below.

- **AEP testing**

  Baseline hearing sensitivity will be measured by recording AEPs, a rapid, non-invasive technique that can be used to measure hearing in a diverse array of taxa including fishes, squid, seabirds, odontocetes, manatees, pinnipeds, sharks and sea turtles.

  Initial hearing thresholds to determine a baseline audiogram will be measured at a variety of frequencies between 50 and 5,000 Hz (with additional frequencies added as needed). This method is well-established and encompasses the full anticipated range of turtle hearing. At each frequency, sound levels will be decreased until AEP responses can no longer be detected (threshold).

- **Sound exposure trials**

  Sound exposure trials will explore duration and sound pressure levels required to induce TTS onset and develop a TTS onset predictive curve. The trials will expose turtles to broadband white noise that spans their auditory frequency range and is likely to cause TTS. Eastern painted turtles and red-eared sliders can detect acoustic signals between 30 and 5,000 Hz with maximum sensitivity <1,000 Hz. Fatiguing noise sound pressure levels will start at 140 dB and increase up to 160-170 dB sound pressure level (SPL), after which durations will be increased to increase overall sound exposure level (SEL).

- **Anatomy**

  The project will also examine the similarities and potential differences of the auditory anatomy of control animals and those exposed to sound to identify potential short and long-term anatomical effects of TTS. Auditory hair-cell damage and loss in some marine taxa have served as indicators of sound exposure and these indicators could apply to turtles. Defining methods by which to assess damage would support examining other turtle species in the future.

  Three methods are being considered. The first involves using x-ray computed microtomography (μCT) to examine morphology on the micro-scale. Although μCT on turtle ears has not been previously conducted, and using these methods to gauge hair cell health and status may be challenging, it offers a good, non-invasive first step. Second, researchers may seek to image auditory hair cells using fluorescent immunohistochemical procedures (which provide high resolution imaging at a cellular level) and other readily available methods used in an array of animals from fish, to invertebrates and mammals. The third imaging option is scanning electron microscopy (SEM).

Based on the results of these efforts, the team will explore dose-dependent effects to begin to create a noise-based, dose-dependent model of TTS. This will allow regulators and data users to predict the sound levels and durations that may produce TTS onset in turtle species. Project products also will outline the methods likely needed to induce and measure TTS in sea turtles, if feasible.

Green sea turtle.

## SCHEDULE

All permit approvals and arrangements for animal acquisition are expected to be completed by the end of calendar year 2019. During 2020 the team will complete the initial AEP recordings and TTS onset evaluation, attempt to induce TTS at additional hearing frequencies and complete initial TTS analyses. Additional efforts during 2020 will include developing hair cell imaging methods, analyzing data and writing reports. During 2021, the team will develop a TTS matrix that presents TTS onset relative to sound exposure and duration, quantify hair cell condition relative to noise exposures and complete TTS matrix analyses. Final reports, conference presentations and manuscripts for submission to peer-reviewed journals are expected by the close of 2021.

## PROJECT BENEFITS

Because no TTS data currently exist for turtles, the audiograms and TTS data produced by this research will provide experts with appropriate data when developing the next phase of TTS criteria. The data also will inform analyses of the effects of sound-producing activities on sea turtles.

## TRANSITION

Results will be provided to the project funders and the sea turtle and marine bioacoustics research and management communities through regular reports, conference presentations and publications in peer-reviewed scientific journals. The major products of this proposed research will be underwater audiograms and underwater TTS onset data for two species of freshwater turtles. The project will also provide protocols that will contribute to investigations of noise-induced hearing loss in other turtle species, including sea turtles.

## ABOUT THE PRINCIPAL INVESTIGATORS

Aran Mooney is an Associate Scientist in the Biology Department at the Woods Hole Oceanographic Institution, where he leads the Sensory Ecology and Bioacoustics Laboratory. His research addresses how marine animals detect and use sound and how animals may be affected by anthropogenic noise. Dr. Mooney holds a Ph.D. in Zoology (marine biology emphasis) from the University of Hawaii.

Wendy Dow Piniak is a Visiting Assistant Professor in the Marine Science and Conservation Division of the Nicholas School of the Environment at the Duke University Marine Lab. Dr. Piniak's research focuses on sea turtle acoustic ecology. She has experience measuring turtle hearing and conducting field studies examining sea turtle behavioral responses to sound. Dr. Piniak holds a Ph.D. in Marine Science and Conservation from Duke University.

## About the LMR Program

The Living Marine Resources (LMR) program seeks to develop, demonstrate, and assess data and technology solutions to protect living marine resources by minimizing the environmental risks of Navy at-sea training and testing activities while preserving core Navy readiness capabilities. For more information, contact the LMR program manager at exwc_lmr_program@navy.mil or visit www.navfac.navy.mil/lmr.