OCS Study
BOEM 2023-079

Workshop Report: Methods to Examine Behavioral and Physiological Responses of Sea Turtles to Sound

U.S. Department of the Interior
Bureau of Ocean Energy Management
Sterling, VA

OCS Study
BOEM 2023-079

Workshop Report: Methods to Examine Behavioral and Physiological Responses of Sea Turtles to Sound

December 2023

Authors:
Craig A. Harms
Douglas P. Nowacek
Wendy E. D. Piniak

Prepared under Agreement No. M20AC10008-02
By
North Carolina State University
College of Veterinary Medicine and
Center for Marine Sciences and Technology
303 College Circle
Morehead City, NC 28557

U.S. Department of the Interior
Bureau of Ocean Energy Management
Sterling, VA

REPORT AVAILABILITY

To download a PDF file of this report, go to the U.S. Department of the Interior, Bureau of Ocean Energy Management Data and Information Systems webpage (http://www.boem.gov/Environmental-Studies-EnvData/), click on the link for the Environmental Studies Program Information System (ESPIS), and search on 2023-079. The report is also available at the National Technical Reports Library at https://ntrl.ntis.gov/NTRL/.

CITATION

Harms CA, Nowacek, DP, Piniak WED (North Carolina State University, Morehead City, NC). 2022. Workshop report: methods to examine behavioral and physiological responses of sea turtles to sound. Sterling (VA): U.S. Department of the Interior, Bureau of Ocean Energy Management. 59 p. Report No.: OCS Study BOEM 2023-079.

ABOUT THE COVER

Computed tomography (CT) of the skull of a juvenile Kemp's ridley (*Lepidochelys kempii*), cross section through the ears, where behavioral and physiological responses to sound begin. Image captures the middle ear spaces (straight arrow) and columellas (curved arrow). Image credit: Craig Harms.

ACKNOWLEDGMENTS

The authors thank the participants (see Appendix A) for their valuable time and informed perspectives that will provide important guidance on how best to address data gaps in sea turtle behavioral and physiological responses to ocean noise. Carrie Kappel and her team of top-notch facilitators set the workshop up for success with a well-conceived pre-meeting framework, kept the discussions on point, and provided detailed and well-organized notes that greatly eased the compilation of this report. The U.S. Department of the Interior, Bureau of Ocean Energy Management (BOEM) funded this workshop, and the authors particularly thank project manager Jacob Levenson, BOEM Division of Environmental Studies, for his efforts integrating the science and policy of ocean noise and sea turtle conservation.

Contents

List of Figures ii

List of Tables ii

List of Abbreviations and Acronyms iii

1 Introduction 1

1.1 Sea Turtles and Anthropogenic Sound 1
1.2 Sea Turtle Hearing, Physiology, and Behavior 2
1.2.1 Sea Turtle Hearing 2
1.2.2 Sea Turtle Physiological Responses to Sound 3
1.2.3 Sea Turtle Behavioral Responses to Sound 3
1.3 Policy Context 4
1.3.1 Endangered Species Act (ESA) 4
1.3.2 National Environmental Policy Act 5
1.3.3 Acoustic Criteria 5
1.4 Workshop Purpose and Design 6

2 Workshop Questions and Summarized Discussion 8

2.1 Overarching Study Design 8
2.1.1 Sea Turtles Examined 8
2.1.2 Exposure Sound Source 9
2.1.3 Exposure Protocol 11
2.1.4 Data Analysis 13
2.2 Behavior Study Design Questions 13
2.2.1 Experimental Protocol—Behavior 13
2.2.2 Geography 15
2.2.3 Biologging Tag Employed 16
2.2.4 Measured Behavioral Response Parameters 17
2.3 Physiology Study Design Questions 18
2.3.1 Experimental Protocol—Physiology 18
2.3.2 Measured Physiological Response Parameters 19
2.4 Workshop Takeaways and Recommendations 23
2.4.1 State of the Science and Research Priorities 23
2.4.2 General Recommendations and Approaches 23

3 References 27

Appendix A: Workshop Agenda and Attendees 35

Appendix B: Summary of Available Literature 38

List of Figures

Figure 1. General approach for planning exposure experiments to investigate responses to sound 11

Figure 2. Summary of the suggested experimental design exposure experiments to investigate responses to sound. 12

List of Tables

Table 1. Identified pros and cons of conducting sound exposure experiments with wild turtles in their natural habitat and captive sea turtles in tanks or an open-water enclosed pen 9

Table 2. Identified pros, or advantages, and cons, or disadvantages of using real sources and simulated sources in sound exposure experiments 10

Table 3. Behavioral response study sound exposure approaches 12

Table 4. Strengths, weaknesses, and data that can be acquired from sound exposure experiments conducted in captive tank-based, field-based pen, and field-based controlled exposure experiments 14

Table 5. Summary of biologging tools for sea turtle behavioral response studies 16

Table 6. Behavioral response parameters appropriate for investigation sound exposure experiments conducted in captive tank-based, field-based pen, and field-based controlled exposure experiments 18

Table 7. Response variables of potential interest in evaluating physiological effects of exposure to sound 21

Table B-1. Summary of representative studies examining sea turtle (and related aquatic species) physiological response to stressors 38

Table B-2a. Summary of published in-water sea turtle behavioral response to sound studies 47

Table B-2b. Summary of published in-water sea turtle behavioral response to sound studies: exposure context variables 49

Table B-2c. Summary of published in-water sea turtle behavioral response to sound studies: noise exposure metrics 51

List of Abbreviations and Acronyms

BOEM	Bureau of Ocean Energy Management
cm	centimeter
dB	decibel
DHEA	dihydroepiandrosterone
DPS	distinct population segment
DTH	delayed type hypersensitivity
ECG	electrocardiogram
ESA	Endangered Species Act
HR	heart rate
Hz	hertz
H/L	heterophil/lymphocyte ratio
HPA	hypothalmic-pituitary-adrenal
kg	kilogram
m	meter
NMR	nuclear magnetic resonance
µPa	micropascal
NOAA	National Oceanic and Atmospheric Administration
NEPA	National Environmental Policy Act
NMFS	National Marine Fisheries Service
PTS	permanent threshold shift
RMS	root mean square
RR	respiratory rate
s	second
SEL	sound exposure level
SPL	sound pressure level
TTS	temporary threshold shifts
U.S.	United States
USFWS	United States Fish and Wildlife Service
WBC	white blood cells

1 Introduction

1.1 Sea Turtles and Anthropogenic Sound

As anthropogenic sound inputs in the ocean continue to increase globally with the expansion of shipping, construction, and energy exploration, a growing number of research efforts focus on potential impacts of sound on marine organisms (National Research Council 2000, 2003, 2005, Nowacek et al. 2007, Popper and Hastings 2009, Richardson et al. 1995). Documented impacts of anthropogenic sound include stress, which may repress growth, reproduction, and immune system functions (Rolland et al. 2012, Romano et al. 2004, Wright et al. 2007), displacement (Miller et al. 2005, Pirotta et al. 2014, Slotte et al. 2004), behavioral change (Nowacek et al. 2007, Popper and Hastings 2009, Richardson et al. 1995, Southall et al. 2016), hearing impairment (Finneran 2015), and soundscape masking (Erbe et al. 2016, Halpern et al. 2008, Richardson et al. 1995). Most studies conducted thus far have focused on marine mammals and fishes (Nowacek et al. 2007, Slabbekoorn et al. 2010), while current information regarding noise impacts on sea turtles is limited (Nelms et al. 2016, Popper et al. 2014).

Sea turtles can be found in nearly all temperate, tropical, coastal, and offshore habitats, and these habitats overlap spatially and temporally with anthropogenic sound sources and sound-producing activities, including seismic airguns used for oil and gas prospecting and scientific purposes, pile driving used for construction activities, drilling used for resource extraction, explosives, military and non-military sonar, and vessel movement. Most populations are highly migratory, traveling great distances between developmental, foraging, and reproductive habitats. Current literature indicates that sea turtles can detect low-frequency sounds produced by anthropogenic sources such as pile drivers, active sonars, and seismic airguns (Bartol et al. 1999, Bartol and Ketten 2006, Dow Piniak 2012, Dow Piniak et al. 2012, Hildebrand 2009, Lavender et al. 2014, Martin et al. 2012, Piniak et al. 2016, Ridgway et al. 1969). For the sea turtle species and age classes studied thus far, underwater hearing was shown to be most sensitive at frequencies below 1,000 Hertz (Hz) (Dow Piniak 2012, Dow Piniak et al. 2012, Lavender et al. 2014, Martin et al. 2012, Piniak et al. 2016), where anthropogenic noise is most prominent.

Additional research is needed to determine the frequencies, sound pressure levels, and exposure durations that may impact the physiology (stress) and behavior of sea turtles (Nelms et al. 2016, Popper et al. 2014). While several studies (Harms et al. 2003, Hoopes et al. 2000, Hunt et al. 2016a, 2016b, 2019, Innis et al. 2007) have examined physiological responses of sea turtles to stressful events (*e.g.*, incidental or directed capture in fishing nets, cold stunning, handling, transport), to our knowledge, no studies have examined physiological (stress) responses of sea turtles to sound. To date, few studies have examined the behavioral responses of sea turtles to sound; however, several of these studies indicate that sea turtles respond behaviorally (diving or changing swim direction/speed) and/or physiologically (behaviors indicating stress or temporary hearing loss) to low-frequency acoustic stimuli (DeRuiter and Larbi Doukara 2012, McCauley et al. 2000, Moein et al. 1994). Most existing studies were conducted in enclosed or semi-enclosed environments and have focused on examining responses to high-intensity seismic airguns, and most studies also lack key information necessary to determine the exposure sound levels or durations at which responses occurred to accurately assess the potential impacts of sound-producing activities. Due to the paucity of knowledge pertaining to noise impacts on sea turtles, mitigation measures originally developed for marine mammals currently are relied upon with little understanding of their efficacy for sea turtle species (Elliott et al. 2019, Nelms et al. 2016).

To accurately assess the potential impacts of anthropogenic activities on sea turtles, we must better understand if and how sea turtles are impacted by the sounds produced by anthropogenic activities. Well-planned studies are needed to address existing uncertainties and information gaps in our understanding of behavioral and physiological responses of sea turtles to sound. Behavioral response studies (especially those conducted in open water on cryptic species) are inherently complex and difficult to implement, and physiological studies require careful and robust controls to interpret measurements of stress response because conducting the study itself (*e.g.*, capture, handling, and transport of animals) can impact data collected. Interdisciplinary expertise is needed to conceptualize, plan, and implement such studies with particular attention to balancing experimental design, feasibility, cost, and scope.

1.2 Sea Turtle Hearing, Physiology, and Behavior

1.2.1 Sea Turtle Hearing

Although the biological significance of hearing remains largely uninvestigated, research has shown that sea turtles are able to detect and respond to underwater and aerial sounds, and may use sound in their environment to aid in navigation, prey identification, predator avoidance, and environmental awareness (Piniak et al. 2016). Sea turtles have also been shown to produce sounds in air and underwater; however, the potential role of these sounds in communication is not understood (Charrier et al. 2022, Cook and Forrest 2005). The functional morphology of the sea turtle ear remains poorly understood, but their ears are thought to be adapted for the reception of underwater low-frequency auditory and vibratory acoustic stimuli (Lenhardt 1982, Ketten 2008). Electrophysiological and behavioral studies of sea turtle hearing have demonstrated that loggerhead, green, Kemp’s ridley, leatherback, and hawksbill sea turtles detect low-frequency acoustic and/or vibratory stimuli underwater and in air (Bartol and Ketten 2006, Bartol et al. 1999, Piniak et al. 2016, Dow Piniak 2012, Dow Piniak et al. 2012, Lavender et al. 2014, Martin et al. 2012, Ridgway et al. 1969). Sea turtles generally appear to be most sensitive to underwater acoustic stimuli below 1,000 Hz, with best sensitivity below 400 Hz, though variation in threshold levels and frequencies of maximum sensitivity exist between species and age classes (see Dow Piniak 2012 for species comparisons), and several data gaps in species and life stages still exist.

Long-duration and/or high-intensity sounds can impact hearing sensitivity of marine animals. At high cumulative sound exposure levels (SEL, measured in decibels referenced to 1 micropascal squared per second [dB re: 1 $\mu Pa^2/s$]), animals may experience temporary threshold shifts (TTSs) or permanent threshold shifts (PTSs) in auditory sensitivity, or loss of hearing (Popper et al. 2014, Southall et al. 2019). TTSs or PTSs are temporary or permanent increases in the threshold level of audibility. TTS has been observed in several marine taxa; however, TTS and PTS have not been examined in sea turtles (though ongoing studies are examining TTS in freshwater turtles, *e.g.*, Salas et al. 2022). For example, mid-frequency tones, sonar signals, and seismic watergun sounds have been observed to cause TTS in dolphins and beluga whales (Finneran et al. 2002, 2005, Mooney et al. 2009), octave-band noise has been observed to cause TTS in pinnipeds (Kastak et al. 2005), and noise generated by seismic airguns has been found to cause TTS in fish (Popper et al. 2005). Decreases in hearing sensitivity may reduce an animal's ability to receive and behaviorally respond to acoustic environmental cues. Repeated exposures can cause behavioral habituation and/or sensitization, depending on species and source, thus potentially increasing long-term physiological (hearing and stress) effects.

1.2.2 Sea Turtle Physiological Responses to Sound

Studies evaluating physiological responses of sea turtles to sound are generally lacking. In an extreme case study, the physiological impacts of explosives were evaluated, though this study did not examine stress response (via standard endocrine and blood gas measurements), but rather observed external indicators of physiological harm. Kilma et al. (1988) examined the impacts of explosives on sea turtles by placing turtles in cages near sites where explosives were used to remove offshore petroleum drilling platforms. To determine impact zones for sea turtles they placed juvenile Kemp's ridley and loggerhead sea turtles in underwater steel cages at 229, 366, 549, and 915 meters (m) away from the detonation of four 23-kilogram (kg) charges. Received sound pressure levels at each cage were estimated via propagation models to be 221, 217, 213, and 209 dB re 1 μPa, respectively. These received levels should be evaluated carefully; Vaida et al. (2008) reviewed the utilized models and determined the resulting received levels were inaccurate as the charges were buried in sediment rather than in the water column as modeled. Two Kemp's ridleys and two loggerheads at 366 m and one loggerhead at 915 m were found unconscious after charge explosions. One Kemp's ridley at 229 m had a prolapsed cloaca, and all loggerheads had an abnormal pink coloring of the skin at the base of the throat and flippers. Kilma et al. (1988) also noted an increase in the number of sea turtle strandings after explosive removal of platforms.

Several studies exist on sea turtle physiological responses to various non-auditory stressors, including fishery interactions, disease, environmental conditions, capture, transport, and handling (see studies summarized in Appendix B, Table B1); the variables investigated in these studies could be applied to evaluate responses to sound. Physiological responses to capture, transport, and handling are critical to bear in mind as potential confounding factors to control for in experimental design of sea turtle sound response studies. Physiological differences associated with gigantothermy in leatherbacks may affect responses and create logistical challenges of obtaining biological samples from adult leatherbacks without substantial impacts of capture and handling. Although stress response studies inevitably gravitate towards the adrenocortical system (corticosterone for turtles), there is a wide range of additional response variables to stressors that could be evaluated.

1.2.3 Sea Turtle Behavioral Responses to Sound

Our understanding of behavioral responses of sea turtles to sound lags behind other marine taxa (Nelms et al. 2016). Few studies have examined the behavioral responses of sea turtles to sound; however, of those conducted, several have observed that sea turtles respond behaviorally (diving, changing swim direction and/or speed) to low-frequency acoustic stimuli (see studies summarized in Appendix B, Table B2). Most studies have been conducted in enclosed or semi-enclosed environments and have focused on examining responses to high-intensity seismic airguns. Several studies also lack key information (*i.e.*, exposure level and duration, contextual metrics) necessary to derive behavioral disturbance thresholds needed to accurately assess the potential impacts of sound-producing activities (see Southall et al. (2021) for an example).

McCauley et al. (2000) exposed one green and one loggerhead sea turtle in an open-water cage to an approaching-departing single airgun (Bolt 600B, 20-in^3 chamber); the turtles increased their swimming activity when received levels reached 166 dB re 1 μPa root mean square (RMS) sound pressure level (SPL) and demonstrated more erratic behavior at received levels greater than 175 dB re 1 μPa RMS. O'hara and Wilcox (1990) observed that loggerhead sea turtles avoided airguns (Bolt 600B with 165-cm^3 capacity and Bolt pneumatic popper with 13-cm^3 capacity, presented at 140 kg/cm^2 airgun pressure) in a 300 x 45 m enclosure in a 10 m deep canal; behaviors were not consistent (some turtles swam toward the airguns), and the study did not report received sound levels. Moein et al. (1994) repeatedly exposed loggerhead turtles to airguns presented at three source sound levels (175, 177, and 179 dB re 1 μPa) in an 18 x 61 m enclosure in a 3.6 m deep river. They reported that loggerheads exhibited avoidance behavior during the first airgun exposure, but that repeated exposure did not elicit significant behavioral responses, suggesting that the turtles had habituated to the sound or had experienced a temporary shift in hearing capabilities (received sound levels were not reported). Physiological measurements (blood chemistry) showed increases in stress levels (as measured by increases in glucose and white blood cell count), and pre- and post-exposure hearing measurements showed a change in hearing physiology (phase shifts or non-repeatability of response recordings) and temporary decrease in hearing sensitivity in some turtles. Although the results of these studies provide valuable data points and are often referenced when analyzing and determining the level of sound anticipated to cause behavioral change in sea turtles, these studies are limited in that they do not examine the responses of freely swimming turtles.

Contrasting results have been found among observational studies conducted in open water, with loggerhead sea turtles diving immediately after exposure airgun shots from an airgun array (array source level 252 dB re 1 μPa [peak]) at a modeled received level of 191 dB re 1 μPa (peak) at 130 m from the array (the median distance at which turtles dove; DeRuiter and Doukara 2012), and olive ridley, green, and hawksbill sea turtles exhibiting no response when exposed to airguns (no received levels measured/modeled; Gurjão et al. 2005, Weir 2007). Differences in results of these studies may be attributed to variations in airgun source levels, frequencies, propagation distances from sources, focal species, or other uncontrolled and unmeasured biological or environmental parameters. It can be difficult to interpret or compare results because the existing studies are primarily observational, use different methodologies, and often do not include received sound level and frequency characteristics, distance to source, and/or behavioral context.

1.3 Policy Context

1.3.1 Endangered Species Act (ESA)

All species of sea turtles in the United States (U.S.) are listed as threatened or endangered under the ESA. An endangered species is a species in danger of extinction throughout all or a significant portion of its range, and a threatened species is one that is likely to become endangered within the near future throughout all or in a significant portion of its range. Kemp's ridley (*Lepidochelys kempii*), leatherback (*Dermochelys coriacea*), loggerhead (*Caretta caretta*; North Pacific distinct population segment [DPS]), green (*Chelonia mydas*; Central West Pacific and Central South Pacific DPSs), and hawksbill (*Eretmochelys imbricata*) sea turtles are listed as endangered. Loggerhead (Northwest Atlantic Ocean DPS), green (North Atlantic, South Atlantic, Central North Pacific, and East Pacific DPSs), and olive ridley (*Lepidochelys olivacea*) sea turtles are listed as threatened, except for the breeding colony populations of olive ridleys on the Pacific coast of Mexico, which are listed as endangered. In the United States, the National Oceanic and Atmospheric Administration's (NOAA's) National Marine Fisheries Service (NMFS) and the U.S. Fish and Wildlife Service (USFWS) jointly administer the ESA for sea turtles. NMFS has jurisdiction for sea turtles when they are in the marine environment, and for activities that have the potential to affect sea turtles and their habitats in the marine environment. USFWS has jurisdiction for sea turtles when they are in the terrestrial environment, and for activities that have the potential to affect sea turtles and their habitats in the marine environment. NMFS and USFWS work jointly (with NMFS as the lead) to attend to stranded sea turtles in the marine environment or when washed ashore from the marine environment.

Section 9 of the ESA prohibits the take (defined to include harassing, harming, pursuing, hunting, shooting, wounding, killing, trapping, capturing, or collecting or attempting to engage in any such conduct), including take that occurs incidental to (not the purpose of) an otherwise lawful activity, of listed species and DPSs. Pursuant to ESA section 4(d), NMFS has issued regulations extending the prohibition of take, with exceptions, to threatened sea turtles (50 CFR 223.205 and 223.206). For Federal actions, such as those authorized by Bureau of Ocean Energy Management (BOEM), NMFS may grant exceptions to the take prohibitions with an incidental take statement pursuant to ESA section 7. To do so, NMFS must determine the activity that will result in incidental take is not likely to jeopardize the continued existence of the affected listed species.

1.3.2 National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires all Federal agencies to consider all environmental impacts when planning Federal actions that are proposed within the United States and its territories. NEPA mandates that Federal agencies prepare a concise public document that provides an assessment of the potential effects any major Federal actions may have on the human environment. Major Federal actions include activities that Federal agencies fully or partially fund, regulate, conduct, or approve.

BOEM manages offshore energy and marine minerals exploration and development in accordance with the ESA and NEPA. To accurately assess impact and calculate takes of sea turtles as required under NEPA and ESA, there is a need to address the information gaps in anthropogenic noise impacts to potentially impacted endangered and threatened species and their habitats. Current leases for energy development and marine minerals extraction along the US East Coast and in the Gulf of Mexico directly overlap sea turtle habitats (Hart et al. 2010 2013, Iverson et al. 2020), including critical habitat designated by the ESA (*e.g.*, loggerhead critical habitat: 79 FR 39855).

1.3.3 Acoustic Criteria

To quantify the effects of various sounds on protected species, including sea turtles, acoustic criteria are needed. Acoustic criteria typically represent received levels at the animal and that are likely to result in impacts to hearing, non-auditory physiological impacts, or behavioral disturbance. No data exist on noise-induced threshold shifts in sea turtles. Thus, current criteria use data from fish TTS studies as a surrogate, which is not ideal given the differences in auditory anatomy between these taxa (DoN 2017). Furthermore, there are limited studies addressing the behavioral disturbance from noise on sea turtles (discussed previously). Many of these studies were done in a laboratory or working with caged individuals, which creates challenges in interpreting how these behavioral responses would correlate to wild individuals. Thus, currently, NMFS relies on a single RMS received threshold (175 dB re 1 μPa RMS SPL) to represent behavioral disturbance of all sound sources to sea turtles.

For sea turtles, there are several key data gaps associated with the development of acoustic criteria. For example, having more data on free-ranging individuals would aid in making behavioral criteria more representative of real-world exposure scenarios. However, there first needs to be a better understanding of baseline sea turtle behavior, so behaviors associated with noise exposure can be put in the appropriate context. Additionally, having more data on various species and sources is needed to distinguish which factors are important to consider in acoustic criteria (*e.g.*, impulsive/non-impulsive, continuous/intermittent, stationary/mobile sources). Ultimately, having more data is helpful, but standards are needed to facilitate comparing disparate datasets appropriately and offer guidance for future studies.

There have been many lessons learned from the development of marine mammal acoustic criteria (Southall et al. 2019; Southall et al. 2021) and that are helpful in informing updated sea turtle acoustic criteria. Namely, it is always challenging to develop implementable thresholds capable of capturing all the possible variability expected with responses and that can be easily applied by managers and other user groups. Also, it is important to consider if there are particularly sensitive species, life stages, or contexts that should be represented. Furthermore, similar to marine mammals (Southall et al. 2021), after behavioral response data have been collected, it will be important to examine the appropriateness of a severity scale to help quantify different types of responses related to their likelihood to result in fitness consequences to sea turtles. It will also be necessary to identify the most appropriate metric for assessing various sea turtle responses (*e.g.*, RMS SPL, cumulative sound exposure level) and which other factors beyond received level (such as source proximity or behavioral context) should be incorporated into the criteria. Finally, as more data become available, there needs to be a plan for the criteria to evolve via a timely, transparent process.

1.4 Workshop Purpose and Design

Behavioral and physiological response studies are inherently challenging as they require examination of a variety of response parameters, can be logistically complex, and are often expensive. Designing these studies is particularly challenging for protected species such as sea turtles, as few similar studies have been conducted. Results from behavioral and physiological response studies can provide researchers, managers, and stakeholders critical data to improve estimates of noise impacts to sea turtles and guide the development of appropriate mitigation measures to reduce potential impacts.

To assist in the development of sea turtle behavioral and physiological response studies that will address research needs and questions (based on regulatory needs and data gaps), we created a working group of experts and convened a workshop. The goal of the workshop was to determine the appropriate methodologies to investigate the behavioral and physiological responses in sea turtles anthropogenic noise, thereby filling information gaps and allowing for accurate impact assessments and the development of effective mitigation measures.

The working group was composed of scientists active in the fields of marine taxa behavioral and physiological responses to sound, and policy makers/managers who would use the resulting data to assess impacts (take under the ESA, conduct analyses under NEPA, and/or the creation of acoustic criteria for sea turtles) (see Appendix A, Attendees). The working group met virtually for a facilitated two-day workshop from October 28–29, 2021. To prepare the group for discussion, pre-meeting materials were provided to the participants prior to the workshop, including the agenda and summaries of previous behavioral and physiological response to sound (or other appropriate stressors) studies (see Appendices A and B), and an introductory presentation to frame the issue and the goals of the workshop.

The workshop was designed to lead the participants through a series of facilitated sessions to answer questions to identify research priorities and discuss the strengths and weaknesses of methodological approaches to identify recommended approaches. Questions were discussed in breakout groups, and breakout groups were formed to draw on expertise in the group and introduce new ideas from experts with experience related to, but outside of, the focus of the question. After the breakout sessions, each breakout group reported back to all participants, allowing for broader discussion and additional feedback. In addition to taking detailed notes, the facilitators used a virtual whiteboard to frame overall goals and discussion topics for participants and record key takeaways from discussion.

This workshop report is framed by three discussion topics: 1) overarching study design (covering topics related to studies of both behavior and physiology), 2) behavioral response studies, and 3) physiological response studies. The summarized discussions and recommendations in this report will provide researchers and managers with the information needed to determine the most suitable methodology to examine sea turtle behavioral and physiological responses to sound to meet their data needs. As there are several possible experimental approaches depending on the data need (research question) and funding available, the report also provides discussion of the strengths and weaknesses of, and important considerations for, different approaches and design parameters so users can better understand the trade-offs associated with particular methodological approaches.

2 Workshop Questions and Summarized Discussion

2.1 Overarching Study Design

2.1.1 Sea Turtles Examined

What species, size, or age class should be prioritized for the behavioral/physiological response studies?

Participants identified loggerhead, Kemp's ridley, and leatherback sea turtles as the most important species (followed by green sea turtles) to prioritize for behavioral and physiological response studies. Participants focused on examining potential impacts to sea turtles in the US and North America, and prioritized these species based on their prevalence in U.S. waters, their status under the ESA, and the amount of overlap between the species' habitat use and potential sources of anthropogenic sound (for example, areas currently planned for offshore wind development and oil and gas exploration).

Among the age classes (e.g., hatchlings, juveniles, subadults, adults), participants identified juveniles of both sexes and reproductive females as the most important to prioritize for behavioral and physiological response studies. Reproductive females were prioritized because impacts to this life stage are most likely to have immediate consequences to the population. Adult males can be more difficult to access, but participants noted that adult males should be opportunistically examined if available. Although hatchlings are more readily available and abundant, juveniles were prioritized due to their high reproductive value (large juveniles or subadults) to the population and because they inhabit both neritic and pelagic habitats, where they may encounter different anthropogenic sources of sound and consequently the potential impacts of those sounds. Participants noted that understanding potential differences in responses between age classes is important and highlighted studies to examine different age classes.

What are the pros and cons of using wild-caught versus captive turtles?

Workshop participants agreed that experiments with both wild-caught and captive turtles could be appropriate, but study subjects depend on the research question. Participants identified several pros and cons to working with each group (**Table 1**). The discussion also focused on some limitations that researchers should consider when designing experiments with turtles in captivity. Captive studies are most appropriate for looking at startle response, orientation to sound source, change in behavioral state, and physiological responses, while other behavioral responses (*e.g.*, displacement, dive behavior) require open-water field studies with freely swimming turtles. Participants also discussed the importance of distinguishing the differences between sea turtles that have been in captivity for either the short or long durations, as long-term residents may not exhibit behaviors that are representative of wild individuals and may have diminished hearing compared to short-term residents (*e.g.*, turtles in rehabilitation for short periods of time).

Table 1. Identified pros and cons of conducting sound exposure experiments with wild turtles in their natural habitat and captive sea turtles in tanks or an open-water enclosed pen

Type of Study/Source of Sea Turtle	Pros	Cons
Studies conducted with wild sea turtles in their natural environment	• Best reflects real-world impacts and natural context • Allows for study of leatherback sea turtles (which cannot be held in captivity) • May allow examination of full suite of responses and natural behavior • Experience stimuli in natural setting without tank (enclosed environment) biases • Potential for greater sample sizes, diverse age classes, etc. • Allows for collection of some long-term response data (depending on data collection tool)	• Expensive and logistically challenging • Measurements of received level are more challenging and may require use of technology (calibrated tags) • Context and confounding variables harder to control for and understand • Sample size may be limited depending on sea turtle density • If capturing sea turtles to tag, turtles may have a response to the capture event that confounds response to planned stimuli • Turtles may leave area if disturbed without allowing recovery of tag and/or recording device
Studies conducted with captive sea turtles in tank or open-water pen	• Easier sampling of a variety of response parameters • Allows for control of more variables • Captive turtles generally more manageable for physiological experiments • Repeated sampling over time of same individual (useful for habituation/ sensitization studies) • Allows for monitoring of long-term health impacts • Sea turtles may be available in high numbers if stranding event (*e.g.*, cold-stun event) • Useful for particular behaviors (*e.g.*, startle response, orientation to sound source, change in behavioral state)	• Experimental environment may not be reflective of real-world exposure conditions • Captive turtles may already be "stressed"; may not be at normal baseline; responses may not be reflective of wild turtles' responses • If from rehabilitation: turtle may still be recovering from prior stranding event or affected by a disease state and may have received medications that impact response • Extremely challenging acoustic/particle velocity environments in most enclosures for impulsive/low-frequency noise sources • Background noise exposure of life support system (pumps, etc.) • Limitations on which behavioral responses you can assess—difficult or impossible to assess changes in diving or displacement (distance) • Limited to species, age and sex classes in captivity • Not ideal or feasible for leatherbacks • Non-natural diet

2.1.2 Exposure Sound Source

What are the most appropriate and highest priority sound sources to evaluate for sea turtles (e.g., airguns, sparkers, pile driving, explosives)?

With respect to which anthropogenic sources of sound are most important to examine for potential impacts to sea turtles, participants ranked acute sources of sound (*e.g.*, pile driving, airguns, explosives, sparkers, sonar) higher than chronic sources of sound (*e.g.*, vessels). Of highest priority for studies are sources that: 1) overlap with the sea turtle hearing range, 2) overlap spatially and temporally with sea turtle habitat use, and 3) are frequent and loud enough to present a potential risk to sea turtles. High-priority sources thus include small and coastal pile driving (vibratory, impact, or down-the-hole) used for coastal construction, large pile driving (largely impact) used for construction of offshore wind farms, airguns used for seismic surveys, and explosives (confined and unconfined). Participants from regulatory agencies noted the particular interest in large pile driving activities due to the increase in offshore wind energy development in U.S. waters. Medium, or second tier priority sources include boomers, sparkers, and bubble guns used for other geological and geophysical exploration and/or characterization of the bottom for other activities (*e.g.*, cable laying). These sources can produce sounds near the upper end of frequencies detectable by sea turtles, though they can include lower frequencies as well. Participants noted the importance of examining potential impacts of chronic sources of sound such as small and large vessels. Vessel noise levels do vary by location and time, though are present in most tropical and temperate waters inhabited by turtles. However, these sources contribute more to elevating background noise levels (which can have impacts such as behavioral disruptions and masking), and they are lower priority for exposure studies. Other low-frequency sources such as Navy low-frequency active sonar are also a concern, though to a lesser extent, based on their relatively low prevalence.

What are the pros and cons of using real sources (e.g., airgun) and alternate transducers that simulate sounds (e.g., J-9)?

Participants identified several pros and cons to working with real sound sources versus transducers to simulate sounds (**Table 2**).

Table 2. Identified pros, or advantages, and cons, or disadvantages of using real sources and simulated sources in sound exposure experiments

Source Type	Pros	Cons
Real Source	• Realistic exposure (*e.g.*, signal characteristics, presentation) • Includes other factors related to events that we may not be aware of and are not able to capture with simulation	• Typically difficult to access or coordinate exposures • Many of the real sources cannot be used in a captive situation (though, previously single airguns have been used in large tanks) • Can be challenging to control • Can be dangerous due to high source level
Simulated Source	• Allows for reproducibility • Can decouple distance and sound level • Does not have to be as loud as the real source • Can be deployed closer to the animal • Large Navy speakers can reasonably replicate ship noise and likely an airgun pulse with a multi speaker setup	• Unable to actually produce the full signal of a noise event from a source like pile driving and therefore may not observe the real response • Rise time and full frequency band are difficult to reproduce • Source proximity and source level are related

2.1.3 Exposure Protocol

What is the most appropriate exposure protocol (e.g., control period followed by single stimulus vs. multiple presentations of stimulus)? How many stimuli should be presented?

The exposure protocol and number of stimuli will depend on the sound source, the question(s) of interest, and what is feasible/realistic for the source. An overall recommendation is that the exposure presentation should be as close to the real-world situation as possible (*e.g.*, a single pile driving hammer strike or single airgun is unrealistic). The participants created a suggested general approach for planning exposure experiments when investigating responses (physiological or behavioral), which is summarized in **Figure 1**.

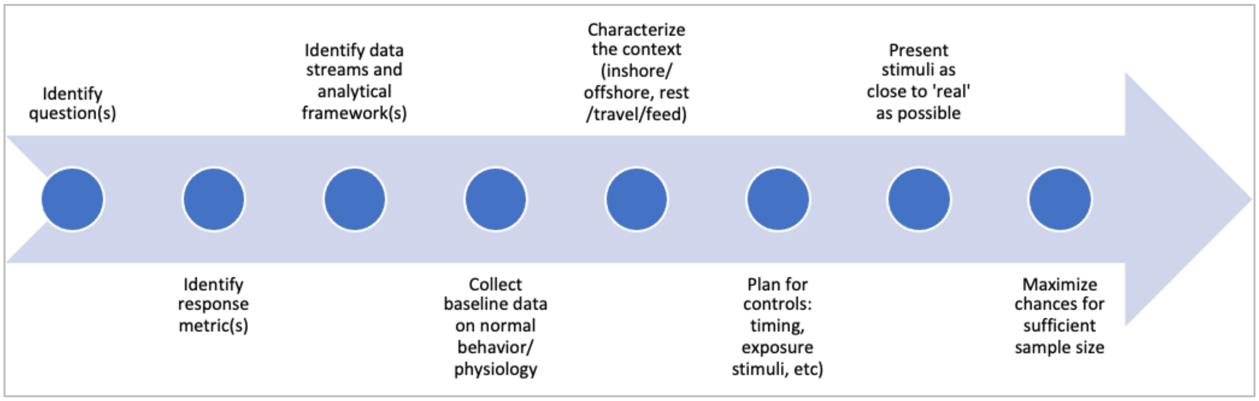

Figure 1. General approach for planning exposure experiments to investigate responses to sound

The next topic addressed was whether it is better to use a single sound stimuli versus multiple sound stimuli for an exposure paradigm. The participants worked to delineate pros and cons of three different regimes: control-single stimulus, control-multiple stimulus, and control-stimulus-post stimulus (also referred to as before, during, and after stimulus). Table 3 describes the pros and cons of the different approaches, with the favored paradigm being the "control-stimulus-post stimulus."

Participants highlighted two approaches that were not favored and felt it was important to note that these situations should be avoided. The first was "at surface" exposures to sound. Participants felt this approach should be avoided because 1) sound propagation changes at the air-water interface, 2) waves and wind-generated noises are greater at the surface, 3) turtles spend the majority of their time away from the surface (divers rather than surfacers), and 4) the response of surfacing may be a variable of interest in behavioral studies. The second was testing in the presence of other animals, specifically with captive (permanent or temporary) turtles. Participants felt this should be avoided because sea turtles spend most of their time alone, and being in the presence of other sea turtles may influence their behavioral responses.

Table 3. Behavioral response study sound exposure approaches
Pros (advantages to each approach) appear in black text.
Cons (disadvantages to each approach) appear in red text.

Control: Single Stimulus	Control: Multiple Stimuli	Control: Stimulus – Post Stimulus
Simplest to implement [pro]	Most similar to actual exposures [pro]	Allows for time to acclimate (control), measurements during stimulus, and then recovery time (with potential for measurements of variables post exposure) [pro]
Allows direct comparison to baseline behavior [pro]	Harder to implement with free-swimming turtles but could be implemented in a large net pen or mesocosm if turtles are allowed time to acclimate and the pen is large and deep enough to allow realistic responses [pro]	May need repeat sampling in post-stimulus phase [pro]
Most useful for startle response [pro]	Easier to implement inshore than offshore [pro]	Longer to conduct, may lead to smaller sample sizes [con]
Experimental time period sufficient to capture the multiple-hour time course of changes in adrenal hormones in sea turtles [pro]	Most useful for habituation and sensitization studies; extinguishing of habituation should be considered [pro]	-
Novel stimulus may result in a bigger behavioral and heart rate response [con]	Timing of presentations of stimuli should be as close to real world as possible [pro]	-
Does not allow assessment of habituation/sensitization [con]	Difficult to determine which signal has caused an observed response [con]	-

The next identified task was to look holistically at a behavioral study approach design, in other words, what an "ideal" design would look like given the limited information currently available. The participants' suggested approach is summarized in **Figure 2**, which provides a timeline view of how to execute the study.

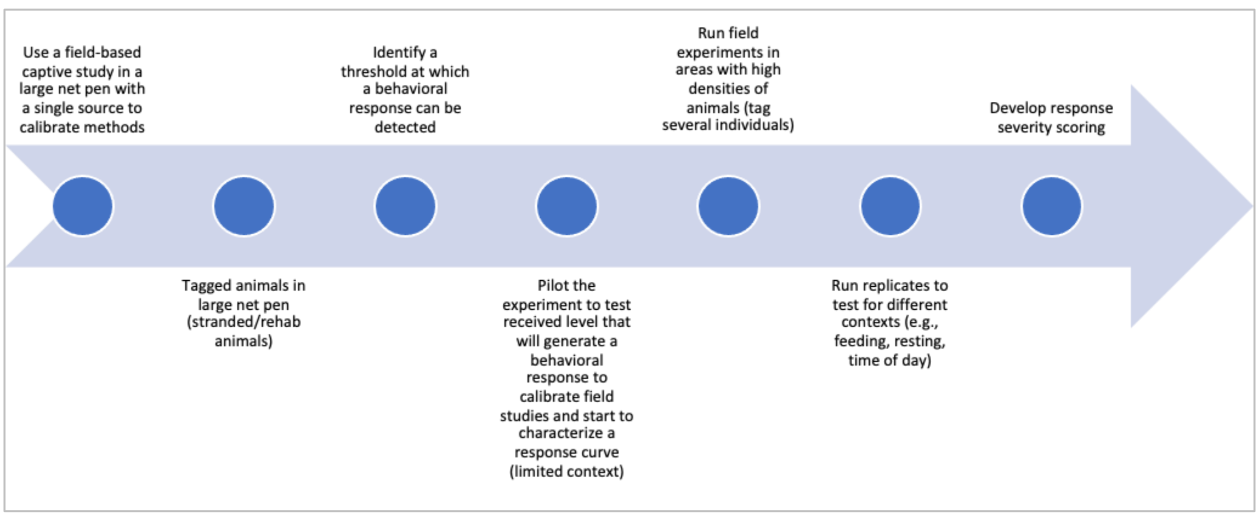

Figure 2. Summary of the suggested experimental design exposure experiments to investigate responses to sound.

Though all participants agreed that ultimately these behavioral response questions should be addressed with wild turtles, captive turtles can be useful in answering some specific questions we have regarding certain aspects of behavioral response and/or mitigation effectiveness. For example, captive turtles can be helpful in assessing questions of amplitude ramp-up of various sources. These ramp-up studies may suffer from some contextual complications, but they can be useful to measure how quickly (or not) their responses change with respect to ramp-up. Next, captive turtles can be useful in helping us understand potential impact of other, non-noise stressors as they may or may not be exacerbated by noise exposure (*e.g.*, assess the response to handling in turtles exposed to noise vs. those not exposed to noise).

Finally, the participants identified several open questions related to exposure protocol(s). Consider, for example, the issue of multiple samples from the same individual or individuals within a group and effects of the sampling protocol on subsequent responses (*i.e.*, whether the same animals can be used or separate groups will be required for different time points). Next, what is the appropriate temporal scale over which to present a stimulus and look for a response? What stimulates a response? What response(s) result in an impact at the individual level? At the population level? And, lastly, the participants identified potential limitations on these experiments, including permit restrictions, costs of tags and equipment, boat, and staff time.

2.1.4 Data Analysis

Given anticipated small sample size, what are the most appropriate statistical analyses to use?

Small sample sizes are the norm for behavioral studies of virtually every large marine vertebrate, though, luckily for sea turtle research, the marine mammal field has benefitted from considerable resources being put towards behavioral response studies; specifically, for this case, the analysis methods can be applied to these small and often variable samples. This marine mammal research is highly relevant for sea turtles and is helpful in both experimental design as well as statistical analyses, *e.g.*, change-point analyses, response modeling, use of each animal as its own control (*e.g.*, Harris et al. 2018). When questions of necessary sample sizes arise, as they perennially do, some sort of power analysis is usually recommended to inform experimental design. Conducting a power analysis, though, does require some knowledge of what the measured quantities will be (*i.e.*, responses) and some expectation or presumption of detectability. Also, sample size must be taken into account when the number of samples or animals is limited by permits. Given what is known about sea turtle behavior and plasticity, a general recommendation was made to target a sample size of 20 individuals for behavior studies.

2.2 Behavior Study Design Questions

2.2.1 Experimental Protocol—Behavior

What are the strengths and weaknesses of captive tank-based studies, field-based pen studies, and field-based controlled exposure experiments with freely swimming turtles? What types of data can be acquired with each of these experiments?

Participants identified and discussed several strengths and weaknesses of various experimental designs and identified the types of data that could be (or would be most appropriate) for each.

These are summarized in **Table 4**. Note that some strengths and weaknesses highlighted by the group also have implications for studies of physiological responses (hearing and stress).

Table 4. Strengths, weaknesses, and data that can be acquired from sound exposure experiments conducted in captive tank-based, field-based pen, and field-based controlled exposure experiments.

Study Location	Strengths	Weaknesses	Data Acquired

Captive tank-based studies	• Easier than experiments with free-swimming turtles
	• Opportunity to examine responses in same individual multiple times
	• Easier to recapture turtles for resampling and tag recovery
	• Life history of individuals better known
	• Easier access for physiological measures
	• Longer observation time frame (before/after)
	• Predictable / controlled exposure levels
	• Stereotyped behaviors can be documented
	• Easier instrumentation
	• Power, ruggedness
	• Pre-exposure hearing tests possible
	• Animals are habituated to manipulation
		• Acoustic limitations
		  ○ Highly reverberant, shallow, noise cancellation issues from standing waves
		  ○ The larger the tank the better
		• Animals have been living in a noisy tank environment (water pumps, filtration, terrestrial sounds, etc.)
		• Confined space prevents some behaviors (lateral distance, dive depth)
		• Rehab condition may lead to non- representative results
		• Only certain species/sizes of turtles can live in a tank and stay healthy
		• Not suitable for leatherbacks
			• Startle response
			• TTS onset, recovery time
			• First cuts at repeated physiologic measurements
			• Effects on several physiologic parameters
			• Effects of ramp-up protocols
			• Effects of repeated exposures (*e.g.*, sensitization, habituation)
			• If turtle is later released: long-term follow up via tags?
			• Effects on growth rate

Field-based pen studies	• Same strengths as captive-based studies above, plus the following: • Real source can be used if pen can be proximal • Generally less expensive? • Higher sample sizes • Longer acclimation after tagging • More realistic environment	• Limited spatial scale • Potentially capturing unnatural behaviors • Not suitable for leatherbacks • Suite of responses might be driven by size of pen • Permits and permissions may take longer to acquire • Potentially limited pens/sites available near enough turtles • Allows for adequate acclimation • Potential entanglement	• Startle response • TTS onset, recovery time, habituation • Beginnings of behavioral responses • Habituation/ sensitization • If large enough could look at heading, direction • Effects of ramp-up protocols • Long-term health effects of stress responses
Field-based controlled exposure experiments	• Realistic behavioral repertoire • Opportunity to capture full suite of responses via long-term tags • Realistic sound sources • Opportunity to examine habitat or seasonally dependent impacts	• Complicated and logistically challenging • Small sample sizes • Expensive • Data points might be lost with turtles leaving the study site • Cannot control for other stimuli • Difficulty accessing *cheloniid* species in certain areas (*e.g.*, New England)	• Natural dive behavior • Realistic exposures and responses • Longer term responses • Behavioral responses • Recovery periods • Exposure-response relationship • Context dependence

2.2.1.1 Summary of Other Considerations

Participants identified and discussed many of the pros and cons of conducting these studies with wild vs. captive turtles (Section 2.1.1). There are, though, some other points made during the workshop that are worth summarizing. Captive and wild studies can be complementary, with captive studies being useful for establishing protocols to be used with wild turtles. However, any synergy would extend only so far, as the situations can be so different behaviorally and acoustically (*e.g.*, a captive turtle may sink to the bottom and stay there, while a wild turtle may actively swim away from the source, both in pursuit of lower received levels).

We have discussed several different scenarios that utilize captive vs. wild turtles, and there is also the intermediate option of conducting studies in a large field-based pen. These pens should be as large as possible, *e.g.*, aquaculture pens are often 8,000 m^2, and a pen could be located adjacent to or at predetermined distances from a sound source (*e.g.*, drilling, pile driving) to test multiple animals before and after an exposure to such a realistic sound source. These open-water pens are not without their challenges but can provide a mesocosm approach to answering these difficult questions. Finally, other options can be explored, *e.g.*, using captive animals in large settings (or enclosures) and conducting studies in canals, saltwater ponds, blue holes or cenotes, or even isolated nesting beaches using a shark-exclusion net that leaves the turtles access to land, where they might go to avoid the sound.

2.2.2 Geography

What are the considerations for offshore versus inshore studies?

For behavioral studies conducted in the field, the participants agreed that the location of the study should be driven by the sound source and species/age class planned for investigation. If feasible, the group favored exposures to be as realistic as possible. As examples, studies of behavioral responses of nesting females in response to near-shore anthropogenic sound should be conducted in inshore habitats, and studies of the behavioral responses of pelagic juveniles to deep-water sources of sound should occur in offshore habitats. With that said, the participants identified several strengths and weaknesses of conducting studies in inshore and offshore habitats to consider during study design.

Inshore habitats are easier and less expensive to access and often have higher densities of sea turtles, making capture and measurement of sea turtle behavior more feasible, less expensive, and less technically challenging. Inshore habitats co-occur with many important breeding and feeding areas, making inshore environments advantageous for examining behavioral responses to sound during foraging and breeding activities. Inshore areas also co-occur with activities BOEM regulates (*e.g.*, coastal pile driving). Participants noted the importance of effects of shallow and shoal environments on sound propagation when designing experiments. Finally, inshore studies on nesting females may be advantageous as tags can be deployed and retrieved on nesting beaches; however, one disadvantage to working with nesting females is that females may rest between subsequent nesting events and may not be expressing a full range of normal behaviors. Permits from NMFS, USFWS, and state agencies may be required for these types of studies.

Offshore habitats are much more challenging and costly to access, and often present harder working conditions (due to weather, sea state, etc.). Sea turtle densities are often lower in offshore habitats, and turtles may be more dispersed in the study area. However, offshore habitats co-occur with both important foraging habitats and activities BOEM regulates (*e.g.*, offshore pile driving, seismic surveys, etc.), making offshore habitats more realistic for measuring responses to these types of anthropogenic activities.

2.2.3 Biologging Tag Employed

What biologging tools are available and what type of data can they provide?

Participants identified several biologging tools that could be used for behavioral response to sound studies (**Table 5)**. Methods for measuring behavioral variables in freely swimming turtles depend on the question and timescale of response, and different types of biologging tags have different strengths and weaknesses. For example, to measure fine-scale, short-term (*i.e.*, seconds to hours) responses, high-resolution acoustic recording tags are well suited to collect data to address these questions. For longer (*i.e.*, days to weeks) responses, satellite linked tags are the only viable option. There is emerging tag technology (single-molecule-real-time, or SMRT, tag) that samples animal behavior and the acoustic environment, and are satellite linked.

Table 5. Summary of biologging tools for sea turtle behavioral response studies
A relatively recent paper discussed the advantages and challenges of several tag/sensor types (Johnson et al. 2009), and while new sensors have been incorporated (*e.g.*, cameras), this table serves to frame the comparison(s) and set up the discussion of the appropriate technology for a particular question.

Biologging Tool	Data Collected	Advantages/Limitations
Accelerometer	Swim/movement speed, sudden changes in behavior (*e.g.*, startle)	Can be difficult to interpret behavior
Magnetometer	Heading	Can track horizontal displacement
Animal-borne cameras, including stereoscopic cameras	Visual data of what the turtle sees, its swimming behavior, or both	Can see if turtle is on the bottom; Small visual ranges
Heart rate monitors	Heart rate	Electrocardiograms (ECGs) difficult in hard shelled turtles but recent techniques in low-amplitude ECG signal processing show promise (Sakamoto et al. 2021, Kinoshita et al. 2022)
Time-depth recorders	Depth and dive time	Can track vertical displacement or changes in foraging behavior
Acoustic recording tags (dTag, Loggerhead Instruments, Inc., AMX tag, CATcams, FaunaTag, etc.)	Movement (speed, depth, pitch, roll, etc.) and received SPL	Pair movement with received acoustics; only allows for short durations of measurement
Acoustic telemetry tags	Geolocation with respect to an array of acoustic receivers	Requires extensive acoustic receiver array. If turtles move out of the array, no location information is collected
Satellite Fastloc tags	Geolocation	Locations useful and allows for longer-term data collection; behavior data available can be limited
IMU (inertial measurement unit)	Combination of accelerometer, gyroscopes, and sometimes magnetometers	Good measurements of body movements and orientations Corrections for tag placement/orientation can be challenging
CTD/environmental loggers	Conductivity (salinity), temperature, and depth	Can be used to explore water masses/depths used by turtles; gives no information about actual movements

What are their strengths and weaknesses when deployed on sea turtles?

Biologging tags will be useful in virtually all situations, but especially 1) when response variable(s) include movement, including changes in depth; 2) when response variable(s) involve metabolic quantities (*e.g.*, heart rate, respiration rate); 3) tags attached to captive or pen turtles can generate data (*e.g.*, movement parameters) that can be used to develop analyses of similar tag data in wild studies, including 'starting points' for source levels or other experimental parameters; 4) in turbid water conditions where video is ineffective.

Johnson et al. (2009) provide an excellent review of the use of high-resolution biologging tags, and while there has been significant advances in the sensors deployed on these tags since their review, their discussion of pros and cons is still valuable. There are some important considerations when using biologging tags. To attach these tags, the turtles must be handled for some period of time, and thought needs to be put toward addressing the 'acclimation period' after which the animal has returned to baseline condition, and its behavior is no longer influenced by the tagging operations. Captive and/or pen animals tagged but not exposed could be utilized to address this issue, and behavioral and physiological measurements should be collected. Currently, the research community operates on the assumption that 1-day post-tagging and handling is acceptable. Finally, the future availability of new satellite transmission systems may facilitate the development and use of new tags that could bridge data gaps (*e.g.*, the coarse nature of dive behavior measured by Argos-based tags).

2.2.3.1 Other Instrumentation

Hydrophones separate from those on any tags attached to the animal(s) can be useful for documenting received level(s) and/or sound mapping of a tank or pen. These received level measurements can be integral to the overall plan by, for example, documenting the actual received level at turtle location(s) and/or verifying propagation modeling done in concert with the experiments. Cameras or drones located in or above a tank or net (and potentially used in concert with animal-bourne cameras) may provide additional imagery to document response(s).

2.2.4 Measured Behavioral Response Parameters

What are the most appropriate and/or important behavioral parameters to monitor for sea turtles and on what time scales?

Participants agreed that the final suite of behavioral response parameters chosen will be dictated by the research question and sound source of interest. They identified several behavioral response parameters that could be examined in both freely swimming, wild turtles, and captive turtles (in tanks or open-water pens) (**Table 6**). Participants highlighted the importance of investigating responses that have the potential to lead to fitness consequences, including changes in behavioral state (implications for energetics), changes in dive duration and swim speed (implications for foraging and energetics), and avoidance (indicator of displacement away from important habitats).

Participants noted that different parameters may need to be observed or measured at different time periods after exposure. For example, a startle response must be examined immediately after the onset of the sound, while other parameters (like changes in behavioral state, dive behavior, swim speed, and avoidance) should be investigated on longer time scales and only after sea turtles have had the opportunity to return to baseline behavior after any handling or capture. The participants also discussed the need to examine responses to several combinations of time and a range of sound levels, including the same timeframe sea turtles would experience in a real-world exposure and varying sound levels to mimic exposures at different distances from the source. Finally, the participants discussed the need for repeated exposures to determine if sea turtles habituate or become sensitized to sounds over time.

Table 6. Behavioral response parameters appropriate for investigation sound exposure experiments conducted in captive tank-based, field-based pen, and field-based controlled exposure experiments

Type of Study/ Source of Sea Turtle	Response Parameter
Studies conducted with wild sea turtles in their natural environment	• Startle response—still unclear whether turtles have the startle reflex response (see Götz and Janik 2011), but do show rapid onset responses • Avoidance (horizontal and vertical)—displacement • Diving behavior changes • Swim speed • Changes in behavioral state: time spent foraging, swimming, and resting • Internal temperature to get at change in feeding regime • Resource use and selectivity
Studies conducted with captive sea turtles in tank (T) or open-water pen (P)	• Startle response (P, T) • Avoidance, change in heading (P, T depending on size) • Heart rate and stress biomarkers (P, T) • Change in behavioral state (resting, swimming) • Swim speed (P, depending on size)

How can context and response variable scoring be applied to sea turtles?

The context of exposure, as we have learned with marine mammals, can significantly influence an animal's response to an acoustic stimulus. For example, is the animal feeding vs. traveling? Has it been exposed to the sound source before or recently? Some of the important contextual variables to measure include the quality and availability of foraging habitat or prey, ambient noise conditions, the effects of ramp-up or exposure to many different received levels, etc. The importance of context is discussed in some marine mammal focused papers, including Southall et al. (2021) and Ellison et al. (2012). The severity of a response can also be affected by context. Scoring the severity of responses is also very important, and the sea turtle research community should consider adopting an approach similar to that taken by Southall et al. (2021).

2.3 Physiology Study Design Questions

2.3.1 Experimental Protocol—Physiology

What is the appropriate sampling time?

Physiological responses are dynamic and time dependent, so the sampling time can be critical in determining whether a response has occurred. Further, stress of handling animals for sampling can have marked effects on subsequent samples. Serial sampling beginning immediately after exposure provides information about the effects related to prior sampling events or handling, as well as the effects of the exposure of interest. For that reason, sample size generally needs to be increased and sampled by groups at a particular post-exposure time rather than sampling individuals several times through an entire time series, unless samples can be obtained without otherwise impacting the study animals. Metrics to assess are not specific to any particular stressor, requiring robust controls for reliable interpretation. For acute glucocorticoid responses, the output that received the majority of discussion time in the workshop, it is important to collect baseline samples quickly, before or within 10 minutes of stimuli, and then again during (for one cohort) and after (for another cohort) exposure across about a 6-hr time course. Other outputs may have different timelines. For longer-term effects, comparisons of different populations with and without exposure over time would likely be more informative than pre-exposure baselines vs. post-exposure changes.

Examples of qualitative timescales for measuring different variables include the following:

- Hours: hormones, blood gases, lactate
- Days: hormones in fecal samples
- Months: endocrine assays from scute clips
- Years: reproduction, growth and morphometrics

2.3.2 Measured Physiological Response Parameters

Several physiological parameters could be informative for assessing sea turtle reaction to auditory stressors. The breakout group participants focused on physiology gravitated towards endocrine responses, and more specifically towards corticosterone, the primary glucocorticoid of sea turtles, as one essential output to evaluate in the context of the hypothalmic-pituitary-adrenal (HPA) nonspecific response to a wide range of stressors. Additional endocrine responses of interest would be thyroid hormones (thyroxine, triiodothyronine) for metabolic impacts, reproductive hormones (estradiol, testosterone, progesterone) for potential reproductive effects, aldosterone (mineralocorticoid product of the adrenal cortex) for potential impacts on electrolyte balance, and catecholamines (epinephrine, norepinephrine, and their metabolites) for sympathetic (fight-or-flight) response to acute stressors. Non-endocrine responses discussed included hematology (complete blood count, heterophil/lymphocyte ratio, packed cell volume, total solids), blood-based immunology assays, morphometrics (weight, body condition, carapace measurements, subcutaneous fat depth, growth over time), heart rate and rhythm, and gross pathology and histopathology. These factors would be applied to assess general health status to confirm healthy subjects are being evaluated, as well as to determine possible responses to sound exposures.

The techniques considered most promising to be informative and feasible included blood sampling for short-term responses of endocrine, hematological, and immunological variables; fecal samples for mid-term (days) endocrine responses; scute clips for long-term (months) endocrine responses; morphometrics (carapace length and width, weight); and subcutaneous fat depth for long-term effects on growth and body condition. Body condition can be assessed by subjective scoring or by calculation based on weight and length as derived from Fulton’s K in fisheries science (Harris et al. 2017, Tristan and Norton 2017). Ultrasound for subcutaneous fat thickness has been described for leatherbacks (Harris et al. 2016) but is less well investigated for use in hard shell turtles.

Fecal steroid methods are not yet developed for sea turtles but could be adapted from other species and validated. Measuring steroids in feces is more expensive and time consuming than in blood or plasma. Different methods of feces collection may be needed depending on species; cloacal irrigation is often nonproductive, and colonic irrigation is challenging in green turtles without endoscopic guidance. A fresh sample of spontaneously produced feces when the turtle is out of water is simplest, but timing the sample depends on the turtle's gastrointestinal schedule.

The participants briefly discussed several additional considerations for measuring physiological response variables. The choice of response variables will depend on whether the focus is acute, ephemeral noise exposure or chronic, persistent noise exposure. The stress response has not been as well characterized in sea turtles as it has been in mammals. As a starting point, non-marine turtles may be a useful surrogate model, with greater accessibility of research subjects and potentially fewer permitting constraints. Many response variables are easy to measure within an acute time frame, but longer-term effects are of greater interest. It will be important to choose variables that can be linked to longer-term impacts on sea turtle fitness, but many of the longer-term effects of parameters of interest (*e.g.*, corticosterone and suppression of reproductive hormones) have not been well studied in sea turtles. Blood gases and lactate exhibit more transient effects than other variables considered, but if those effects are strong and lead to tissue damage, they may still have lasting effects that will be important to understand. Physical trauma, impacts to hearing, and other histopathology may occur, depending on the severity of exposure. To identify effects, experimenters will need to assess animals before, during, and after acoustic exposure. This need will either require methods that do not affect the measured variables (*e.g.*, simple handling may increase plasma lactate or corticosterone concentrations) or separate cohorts sampled at different stages. It is important to recognize that both free-ranging and rehabilitating turtles may have been exposed to other stressors or external factors (*e.g.*, pre-existing injuries or noise exposure) prior to the investigations, which could confound the outcomes. Samples for many of the variables of interest are relatively simple to collect (*e.g.*, blood samples), whereas the cost of analyses is likely to be a greater constraint. Long-term follow up and tracking of physiological responses is particularly challenging in such long-lived and wide-ranging species.

A tabular summary of response variables of potential interest in evaluating physiological effects of sound exposure are described in **Table 7**. Though corticosteroid responses received the most attention during the workshop discussions—and would be a primary variable to consider in evaluating physiological effects of noise exposure—it is recognized that for a more comprehensive assessment, a wider range of variables should be considered; however, it would be both cost- and time-prohibitive to evaluate all of the variables discussed. The workshop participants did not resolve which other variables should be prioritized and left that decision to future investigators to choose and justify. Further, the difficult question of how or whether to combine physiological and behavioral experiments was left unresolved. It could be more efficient and reduce the number of animals subjected to experimental conditions to combine protocols. However, sampling for many of the physiological variables of interest would reasonably be expected to alter future behaviors and physiologic variables. Therefore, any proposals that combine behavioral and physiologic protocols must address this concern either by employing sampling methods that do not affect or minimally affect subsequent assessments, or designating separate cohorts to evaluate different effects or timing of effects.

Participants agreed that the minimum sample size should be 8–10 individuals for hormone studies, but data are lacking for sea turtles so larger sample sizes may be needed, depending on the variability in the results. Power analyses should be performed to determine the appropriate sample size.

Table 7. Response variables of potential interest in evaluating physiological effects of exposure to sound

*References cited below are offered by way of example and are not intended to be exhaustive.

Response Variable	Category	Notes/Considerations
Corticosterone	Endocrine	Easy to collect; standard component of any stress response (HPA) study (Gregory et al. 1996, Hunt et al. 2012, Hunt et al. 2019) Timescale: hours
Epinephrine	Endocrine	Easy to collect but unstable sample requiring rapid processing (Hamann et al. 2003) Timescale: hours
Norepinephrine	Endocrine	Easy to collect but unstable sample requiring rapid processing (Hamann et al. 2003) Timescale: hours
Metanephrines	Endocrine	May be somewhat more stable option for evaluating epinephrine/norepinephrine, but not applied to sea turtles yet to our knowledge Timescale: hours
Testosterone; estradiol/ progesterone	Endocrine	Easy to collect; primarily applicable to adults (Allen et al. 2015). Timescale: hours
Thyroxine; tri-iodothyronine if detectable	Endocrine	Easy to collect, indicates metabolic status (Hunt et al. 2012)
Aldosterone	Endocrine	Easy to collect; currently under study by New England Aquarium group: https://www.morrisanimalfoundation.org/article/stress-hormone-study-endangered-sea-turtles
DHEA (dihydroepiandrosterone)	Endocrine	Correlates positively to some immune responses in other vertebrates, not sure if data exist for turtles (Whitham et al. 2020)
WBC	Hematology, Immunology	Standard component of assessing health status (Stacy and Innis 2017); could be done to confirm study population is healthy regardless of response to acoustic stressor
H/L (heterophil/lymphocyte ratio)	Hematology, Immunology	Readily calculated from WBC data; loosely correlates with glucocorticoid and glucose responses in many taxa (Muñoz et al. 2013)
Hematocrit	Hematology	Standard component of assessing health status (Stacy and Innis 2017); could be done to confirm study population is healthy regardless of response to acoustic stressor
Blood gases, lactate, acid-base	Physiology	Can change rapidly with handling (Mones et al. 2021) Timescale: hours
Plasma chemistry panels (glucose, electrolytes)	Physiology	Glucose and electrolytes supportive of corticosterone response; somewhat different time scales (Stacy and Innis 2017)
Weight	Morphometrics	Longer-term response (Tristan and Norton 2017)
Body condition index	Morphometrics	Longer-term response (Harris et al. 2017, Stamper et al. 2005, Tristan and Norton 2017)
Fat depth via ultrasound	Morphometrics	Longer-term response (Harris et al. 2016)
Percent body fat	Morphometrics	Perhaps measured by electrical impedance (Kophamel et al. 2023 and in prep)

Response Variable	Category	Notes/Considerations
Growth	Morphometrics	Long-term, follow-up challenge, but probably important, especially for juveniles Timescale: months to years
Oxidative burst	Immunology	Innate immune response, adaptable across taxa; assays must be done on fresh samples (Rossi et al. 2016, Rousselet et al. 2013)
Leukocyte coping capacity (LCC)	Immunology	Variation on oxygen radical production by leukocytes (Huber et al. 2019); nonspecific immune response, adaptable across taxa; assays must be done on fresh samples
Phagocytosis	Immunology	Innate immune response; adaptable across taxa; assays must be done on fresh samples (Rossi et al. 2016, Rousselet et al. 2013)
Delayed type hypersensitivity (DTH)	Immunology	In vivo immune function assay (Muñoz et al. 2013)
Lymphocyte proliferation	Immunology	Cell mediated immune response capacity; adaptable across taxa with various mitogens; assays must be done on fresh samples (Keller et al. 2006, Rousselet et al. 2013)
NK activity	Immunology	Innate immune response; assays must be done on fresh samples (Rousselet et al. 2013)
Lysozyme	Immunology	Innate immune response (Keller et al. 2006)
Heart rate (HR)	Other	ECGs difficult in hard shelled turtles, but recent techniques in low-amplitude ECG signal processing show promise (Sakamoto et al. 2021, Kinoshita et al. 2022); unclear whether LED HR sensor (like in FaunaTag) could be adapted to work in turtles
Respiratory rate (RR)	Other	Sometimes not useful because of breath-holding, but easy to collect (Harris et al. 2017, Tristan and Norton 2017)
Reproductive success	Other	Long-term, follow-up challenge Timescale: years
Epibiota coverage	Other	Nonspecific, insensitive to minor impacts (Stamper et al. 2005)
Unconsciousness/reduced responsiveness	Physical	Acute effect (Harms et al. 2017, Tristan and Norton 2017)
Gross pathology	Physical	Comprehensive indications of health status at time of death (Stacy and Innis 2017)
Histopathology	Physical	Comprehensive indications of health status at time of death (Stacy and Innis 2017)
NMR metabolomics	Metabolics	Early phases of development and interpretation (Niemuth et al. 2015, 2019)
Ghrelin and leptin	Physiology	Could provide insights on foraging impacts of the sound stressor (Goldberg et al. 2013)
Behavior collected via datalogger	Other	As correlate of health in rehabilitation (Arkwright et al. 2020) or after sound exposure (vs. primary behavior evaluation discussed in that section)
Microbiome	Other	Sample collection simple; analysis complex; associations with disease or physiological states not yet established, but active area of investigation (McNally et al. 2021)

Response Variable	Category	Notes/Considerations
Scute clips	Endocrine	For retrospective/accumulative hormone signature (*e.g.*, compare a noise-exposed population to non-exposed or less exposed populations) Timescale: months? (Day et al. (2010), but for Hg rather than hormones; Baxter-Gilbert et al. (2014) for corticosterone in nail clippings of painted turtles)
Fecal hormones	Endocrine	For retrospective analysis (past several days?) (Umapathy et al. 2015, Wasser et al. 2000)

2.4 Workshop Takeaways and Recommendations

2.4.1 State of the Science and Research Priorities

Many important data and knowledge gaps exist in our understanding of the impact of anthropogenic noise on sea turtles, particularly with respect to physiological responses and long-term fitness and population consequences of behavioral disturbance in sea turtles. There is a pressing need for increased investment in research (both dollars and effort) to fill these gaps, particularly given the ongoing and recent increases in offshore energy development in areas that overlap the habitat of vulnerable populations of sea turtles in U.S. waters. These data are critical to accurately assessing the impacts of anthropogenic sound sources on sea turtles.

Participants prioritized focusing research efforts on the most vulnerable sea turtle species (or populations), both in conservation status and in the amount of overlap between species' habitat use with potential sources of anthropogenic sound. Participants prioritized studies focused on the potential impacts of acute sources of sound (*e.g.*, pile driving, airguns, explosives, sparkers, sonar) higher than chronic sources of sound (*e.g.*, vessels). The highest priority for studies are sources that overlap with the sea turtle hearing range, overlap spatially and temporally with sea turtle habitat use, and are frequent and loud enough to present a potential risk to sea turtles, including coastal and offshore pile driving airguns used for seismic surveys, and explosives (confined and unconfined). Making the link between observed behavioral and physiological shifts and long-term fitness consequences is a high priority, and studies should focus on a variety of response parameters, including those that can be most directly tied to reproduction, foraging success, and survival.

2.4.2 General Recommendations and Approaches

A key theme often revisited during the workshop was that the most appropriate methodological approach for conducting studies of physiological and behavioral responses of sea turtles to sound should be driven by the species and sound source of interest, context (*e.g.*, inshore vs. offshore, migrating vs foraging individuals, etc.), and the question of interest (*e.g.*, threshold to startle, habituation, displacement, changes in foraging or reproduction, or other biologically meaningful response). Multiple experimental approaches could and should be pursued in parallel. Though experiments with freely swimming wild turtles in their natural habitat are preferred, insights/lessons learned from captive studies can inform studies with freely swimming wild turtles. Coordination and communication among those efforts and across the interested research and regulatory community will help speed progress and increase efficiency.

It is important to focus on the questions and carefully select response variables that align with the question, categorize and quantify contextual variables, and integrate analytical techniques in the experimental design. These types of experiments take significant time to plan and require permits to conduct (either from NMFS, USFWS, or both), which can take up to one year to procure. Lessons learned and techniques from studies of sound impacts on other marine species can be applied to sea turtles, including employing multiple controls (non-exposed individuals and populations) to collect baseline data; sampling before, during, and after sound exposure; incorporating/recording context and response variable scoring; improved tagging techniques, and experimental and statistical design (given often small sample sizes); and improved physiological methods. Finally, it is critical that study subject, sound exposure, and context parameters are carefully identified and characterized during experiments and included in reports/publications (see Southall et al. (2021) for a list of parameters).

Both physiological and behavioral studies are needed, and there will be benefits to pursuing those studies separately, and, when feasible, in combination; however, simultaneous physiological and behavioral studies present challenges, depending on the response variables being assessed. Wildlife studies are increasingly integrating measures of physiology and behavior, as movement is relevant to both and informs energetics and rates; for example, accelerometers and movement tags can generate data on energetic expenditure. A key challenge to conducting behavioral and physiological studies concurrently is that the disturbance that results from each capture and handling event will affect subsequent physiological measurements (*e.g.*, Mones et al. (2021)). Additionally, when studying freely swimming wild turtles, it may be difficult to recapture and collect physiological measurements from turtles after exposure. After an animal has been tagged, baseline physiological data have been collected, and the animal has re-acclimated, the stimulus can be presented and behavioral responses observed, yielding multiple behavior data points. The animal would then need to be recaptured to collect physiological samples and measurements representing its exposed condition. Given that this yields only a single time point, one would need multiple cohorts to increase sample size.

Recommended experimental approaches, to be pursued in series or parallel include are listed from least to most challenging to implement:

- Tank experiments with single sound stimulus
    - Turtles: Short-term rehabilitation animals or wild-caught animals preferable over long-term captives
    - Response parameters: Limited behavior (startle response, orientation to source, change in behavioral state), basic physiology
    - Output: Initial sound response thresholds
- Net pen experiments with single and multiple sound stimuli
    - Turtles: Rehabilitation animals ready for release or wild
    - Response parameters: More comprehensive range of behavioral responses, complex interactions of more physiology metrics
    - Outputs: Thresholds for broader suite of behavioral and physiological responses; insights to inform field-based studies with free-swimming turtles
- Semi-enclosed net pen experiments at nesting beaches with controlled sound source (real or simulated)
    - Turtles: Wild

- Measure: More comprehensive range of behavioral responses, longer tagging sequences, larger sample sizes
- Output: Thresholds for broader suite of behavioral responses; insights to inform field-based studies with freely swimming turtles
- Caveat: Nesting sea turtles range more widely than any net pen, and confinement during reproductive cycles may have unpredictable unintended consequences

- Field-based experiments with controlled sound source (real or simulated)
  - Turtles: Wild
  - Response parameters: Full range of behavioral responses
  - Output: Thresholds for broader suite of behavioral responses; insights to inform field-based studies with freely swimming turtles
- Natural/field-based experiments with planned development activities (real sound source)
  - Turtles: Wild
  - Response parameters: Full range of behavioral responses
  - Outputs: Thresholds for broader suite of behavioral responses under realistic conditions
  - Requirements: Cooperation of industry
- Natural comparative experiments
  - Turtles: Wild populations with different levels of baseline chronic noise exposure and noise risk
  - Response parameters: Full range of behavioral and physiological responses as feasible
  - Outputs: Better characterization of baseline; insights into the effects of chronic noise; insights into links between noise disturbance and long-term fitness

2.4.2.1 Behavioral Studies Considerations

The methodological approach that provides the most realistic exposure scenarios and breadth of behavioral response data are those conducted with freely swimming wild turtles and with real sources using the "control-stimulus-post stimulus" experimental paradigm. However, given that these studies are logistically challenging and expensive, and considering the paucity of data available for sea turtle behavioral responses to sound for use in informing mitigation and management, all agreed that other experimental paradigms would assist in filling data gaps (including those conducted with captive turtles, simulated sound sources, etc. as outlined above).

If these alternate experimental paradigms are pursued, they will require careful study design and a firm understanding of the types of data that can be collected with these approaches and their strengths and weaknesses (**Table 4**). The participants suggested a step-wise approach of conducting studies with sea turtles in large open-water pens prior to conducting studies with freely swimming sea turtles, particularly to refine techniques for deploying and retrieving biologging tools, and examining behavioral baselines and initial behavioral responses to different sources of sound (**Figure 2**). When feasible, researchers and managers should take advantage of opportunities for natural (in situ) experiments (*e.g.*, measurements of behavior during planned development activity), as ongoing and planned activities may assist in generating more quickly relevant data based on real-world conditions.

2.4.2.2 Physiological Studies Considerations

Investigations of physiological effects of sound are expected to include an assessment of endocrine responses, in particular, but not limited to, corticosterone as an indicator of the HPA axis. To acquire a more comprehensive assessment of physiological effects of sound, investigations should include multiple endocrine effects (*i.e.*, response variables in addition to corticosterone) and non-endocrine effects.

The workshop participants did not make recommendations on which specific response variables to prioritize. Many options are suggested in **Table 7**. Selection of response variables will depend on investigative team capabilities, practical considerations for working with protected species (which have distinct handling and husbandry requirements over a wide range of sizes), and the case presented by prospective investigators for their proposed array. A range of time points should be evaluated, from acute (short term) to chronic (long term).

It is anticipated that in-depth physiological response studies would be better suited to controlled captive settings than free-ranging settings, although some physiological response variables may be more adaptable to a variety of settings; net pen experiments offer an intermediate option. Additionally, natural or opportunistic experiments may arise, based on differing sound exposures to otherwise similar free-ranging sea turtle populations. Allowing for some acclimation time, turtles recently collected from the wild or short-term rehabilitation animals would be preferable to turtles that have been in managed care for long durations. Turtles participating in noise response studies should be judged as healthy based on physical examination and hematology findings, and, if available, known history. The physiological effects of capture and handling for sampling must be considered with appropriate controls and judicious timing.

3 References

Allen CD, Robbins MN, Eguchi T, Owens DW, Meylan AB, Meylan PA, Kellar NM, Schwenter JA, Nollens HH, LeRoux RA, Dutton PH. 2015. First assessment of the sex ratio for an East Pacific green sea turtle foraging aggregation: validation and application of a testosterone ELISA. PLoS One. 10(10): e0138861. doi:10.1371/journal.pone.0138861.

Arkwright AC, Archibald E, Fahlman A, Holton MD, Crespo-Picazo JL, Cabedo VM, Duarte CM, Scott R, Webb S, Gunner RM, Wilson RP. 2020. Behavioral biomarkers for animal health: a case study using animal-attached technology on loggerhead turtles. Frontiers in Ecology and Evolution. 7:504.

Bartol SM, Musick JA, Lenhardt M. 1999. Auditory evoked potentials of the loggerhead sea turtle (*Caretta caretta*). Copeia. 3:836-840.

Bartol SM, Ketten DR. 2006. Turtle and tuna hearing. In: Swimmer Y, Brill R (eds) Sea turtle and pelagic fish sensory biology: developing techniques to reduce sea turtle bycatch in longline fisheries. Pp. 98–105. Report No.: NOAA Technical Memo NMFS-PIFSC-7.

Baxter-Gilbert JH, Riley JL, Mastromonaco GF, Litzgus JD, Lesbarreres D. 2014. A novel technique to measure chronic levels of corticosterone in turtles living around a major roadway. Conservation Physiology. 2(1). doi:10.1093/conphys/cou036.

Charrier I, Jeantet L, Maucourt L, Régis S, Lecerf N, Benhalilou A, Chevallier D. 2022. First evidence of underwater vocalizations in green sea turtles *Chelonia mydas*. Endangered Species Research 48:31–41.

Cook SL, Forrest TG. 2005. Sounds produced by nesting leatherback sea turtles (*Dermochelys coriacea*). Herpetological Review. 36(4):387–9.

Day RD, Keller JM, Harms CA, Segars AL, Cluse WM, Godfrey MH, Lee AM, Peden-Adams M, Thorvalson K, Dodd M, Norton T. 2010. Comparison of mercury burdens in chronically debilitated and healthy loggerhead sea turtles (*Caretta caretta*). Journal of Wildlife Diseases 46:111–117.

DeRuiter SL, Doukara KL. 2012. Loggerhead turtles dive in response to airgun sound exposure. Endangered Species Research. 16:55–63.

Dow Piniak WE. 2012. Acoustic ecology of sea turtles: implications for conservation. Doctoral Thesis. Duke University. 136 p.

Dow Piniak WE, Eckert SA, Harms CA, Stringer EM. 2012. Underwater hearing sensitivity of the leatherback sea turtle (*Dermochelys coriacea*): assessing the potential effect of anthropogenic noise. Herndon (VA): US Dept. of the Interior, Bureau of Ocean Energy Management. 35 p. Report No.: OCS Study BOEM 2012-01156.

Elliott BW, Read AJ, Godley BJ, Nelms SE, Nowacek DP. 2019. Critical information gaps remain in understanding impacts of industrial seismic surveys on marine vertebrates. Endangered Species Research. 39:247–254.

Ellison WT, Southall BL, Clark CW, Frankel AS. 2012. A new context-based approach to assess marine mammal behavioral responses to anthropogenic sounds. Conservation Biology. 26(1):21–28.

Erbe C, Reichmuth C, Cunningham K, Lucke K, Dooling R. 2016. Communication masking in marine mammals: a review and research strategy. Marine Pollution Bulletin. 103(1-2):15–38.

Finneran JJ, Schlundt CE, Dear R, Carder DA, Ridgway SH. 2002. Temporary shift in masked hearing thresholds in odontocetes after exposure to single underwater impulses from a seismic watergun. The Journal of the Acoustical Society of America. 111:2929–2940.

Finneran JJ, Carder DA, Schlundt CE, Ridgeway SH. 2005. Temporary threshold shift in bottlenose dolphins (*Tursiops truncatus*) exposed to mid-frequency tones. The Journal of the Acoustical Society of America. 118:2696–2705.

Finneran JJ. 2015. Noise-induced hearing loss in marine mammals: a review of temporary threshold shift studies from 1996 to 2015. Journal of the Acoustical Society of America. 138:1702–1726.

Goldberg DW, Leitao SAT, Godfrey MH, Lopez GG, Santos AJ, Neves FA, de Souza ÉP, Moura AS, Bastos JD, Bastos VL. 2013. Ghrelin and leptin modulate the feeding behaviour of the hawksbill turtle *Eretmochelys imbricata* during nesting season. Conservation Physiology. 1:10.1093/conphys/cot016.

Götz T, Janik VM. 2011. Repeated elicitation of the acoustic startle reflex leads to sensitisation in subsequent avoidance behaviour and induces fear conditioning. BMC neuroscience. 12(1):1–3.

Gregory LF, Gross TS, Bolten AB, Bjorndal KA, Guillette Jr LJ. 1996. Plasma corticosterone concentrations associated with acute captivity stress in wild loggerhead sea turtles. General and comparative endocrinology. 104:312–320.

Gurjão L de, Freitas J de, Araújo D. 2005. Observations of marine turtles during seismic surveys off Bahia, Northeastern Brazil. Marine Turtle Newsletter. 1–3.

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, d'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R. 2008. A global map of human impact on marine ecosystems. Science. 319:948–952.

Hamann M, LImpus CJ, Whittier JM. 2003. Seasonal variation in plasma catecholamines and adipose tissue lipolysis in adult female green sea turtles (*Chelonia mydas*). General and comparative endocrinology. 130:308–316.

Harms CA, Mallo KM, Ross PM, Segars AL. 2003. Venous blood gases and lactates of wild loggerhead sea turtles (*Caretta caretta*) following two capture techniques. Journal of Wildlife Diseases. 39(2):366–374.

Harms CA, Valenta ALS, Stacy BA, Wyneken J. 2017. Nervous system. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea turtle health & rehabilitation. Plantation (FL): JRoss Publishing, pp. 417–448.

Harris HS, Benson SR, James MC, Martin KJ, Stacy BA, Daoust PY, Rist PM, Work TM, Balazs GH, Seminoff JA. 2016. Validation of ultrasound as a noninvasive tool to measure subcutaneous fat depth in leatherback sea turtles (*Dermochelys coriacea*). Journal of Zoo and Wildlife Medicine. 275–279.

Harris HS, Flint M, Stewart KM, Harms CA. 2017. Field techniques. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea turtle health & rehabilitation. Plantation (FL): JRoss Publishing, pp. 819–857.

Harris CM, Thomas L, Falcone EA, Hildebrand J, Houser D, Kvadsheim PH, Lam FP, Miller PJ, Moretti DJ, Read AJ, Slabbekoorn H. 2018. Marine mammals and sonar: dose-response studies, the risk-disturbance hypothesis and the role of exposure context. Journal of Applied Ecology. 55(1):396–404.

Hart KM, Zawada DG, Fujisaki I, Lidz BH. 2010. Inter-nesting habitat-use patterns of loggerhead sea turtles: enhancing satellite tracking with benthic mapping. Aquatic Biology. 11(1):77–90.

Hart KM, Lamont MM, Sartain AR, Fujisaki I, Stephens BS. 2013. Movements and habitat-use of loggerhead sea turtles in the northern Gulf of Mexico during the reproductive period. PLoS One. 8(7).

Hildebrand JA. 2009. Anthropogenic and natural sources of ambient noise in the ocean. Marine Ecology Progress Series. 395:5–20.

Hoopes LA, Landry Jr AM, Stabenau EK. 2000. Physiological effects of capturing Kemp's ridley sea turtles, *Lepidochelys kempii*, in entanglement nets. Canadian Journal of Zoology. 78(11):1941–1947.

Hunt KE, Innis C, Rolland RM. 2012. Corticosterone and thyroxine in cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*). Journal of Zoo and Wildlife Medicine. 43:479–493.

Hunt KE, Innis CJ, Kennedy AE, McNally KL, Davis DG, Burgess EA, Merigo C. 2016a. Assessment of ground transportation stress in juvenile Kemp’s ridley sea turtles (*Lepidochelys kempii*). Conservation Physiology. 4(1).

Hunt KE, Innis CJ, Merigo C, Rolland RM. 2016b. Endocrine responses to diverse stressors of capture, entanglement and stranding in leatherback turtles (*Dermochelys coriacea*). Conservation Physiology. 4(1).

Hunt KE, Innis C, Merigo C, Burgess EA, Norton T, Davis D, Kennedy AE, Buck CL. 2019. Ameliorating transport-related stress in endangered Kemp's ridley sea turtles (*Lepidochelys kempii*) with a recovery period in saltwater pools. Conservation Physiology. 7(1).

Innis CJ, Tlusty M, Merigo C, Weber ES. 2007. Metabolic and respiratory status of cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*). Journal of Comparative Physiology B. 177(6):623–630.

Iverson A, Benscoter A, Fujisaki I, Lamont M, Hart KM. 2020. Migration corridors and threats in the Gulf of Mexico and Florida Straits for loggerhead sea turtles. Frontiers in Marine Science. 7(208).

Johnson M, de Soto NA, Madsen PT. 2009. Studying the behaviour and sensory ecology of marine mammals using acoustic recording tags: a review. Marine Ecology Progress Series. 3(395)55–73.

Kastak D, Southall BL, Schusterman RJ, Kastak CR. 2005. Underwater temporary threshold shift in pinnipeds: effects of noise level and duration. The Journal of the Acoustical Society of America. 118:154–3163.

Keller JM, McClellan-Green PD, Kucklick JR, Keil DE, Peden-Adams MM. 2006. Effects of organochlorine contaminants on loggerhead sea turtle immunity: comparison of a correlative field study and in vitro exposure experiments. Environmental Health Perspectives. 114:70–76.

Ketten DR. 2008. Underwater ears and the physiology of impacts: Comparative liability for hearing loss in sea turtles, birds, and mammals. Bioacoustics. 17(1-3)312–315.

Kilma EF, Gitschlag GR, Renaud ML. 1988. Impacts of the explosive removal of offshore petroleum platforms on sea turtles and dolphins. Marine Fisheries Review. 50(3):33–42.

Kinoshita C, Saito A, Kawai M, Sato K, Sakamoto KQ. 2022. A non-invasive heart rate measurement method is improved by placing the electrodes on the ventral side rather than the dorsal in loggerhead turtles. Frontiers in physiology 13:811947. doi: 10.3389/fphys.2022.811947.

Kophamel S, Ward LC, Ariel E, Mendez D, O'Brien LM, Burchell L, Munns SL. 2023. A standardised protocol for measuring bioelectrical impedance in green turtles (*Chelonia mydas*). Physiological and Biochemical Zoology. 96(2).

Lavender AL, Bartol SM, Bartol IK. 2014. Ontogenetic investigation of underwater hearing capabilities in loggerhead sea turtles (*Caretta caretta*) using a dual testing approach. Journal of Experimental Biology. 217:2580–2589.

Lenhardt ML. 1982. Bone conduction hearing in turtles. Journal of Auditory Research. 22:153–160.

Martin KJ, Alessi SC, Gaspard JC, Tucker AD, Bauer GB, Mann DA. 2012. Underwater hearing in the loggerhead turtle (*Caretta caretta*): a comparison of behavioral and auditory evoked potential audiograms. Journal of Experimental Biology. 215(17):3001–3009.

McCauley RD, Fewtrell J, Duncan AJ, Jenner C, Jenner M-N, Penrose JD, et al. 2000. Marine seismic surveys – a study of environmental implications. APPEA Journal. 40(8):692–708.

McNally KL, Mott CR, Guertin JR, Bowen JL. 2021. Microbial communities of wild-captured Kemp's ridley (*Lepidochelys kempii*) and green sea turtles (*Chelonia mydas*). Endangered Species Research. 45:21–36.

Miller GW. 2005. Monitoring seismic effects on marine mammals–southeastern Beaufort Sea, 2001-2002. In: Armsworthy SL, Cranford PJ, Lee K. (Eds). Offshore oil and gas environmental effects: monitoring, approaches and technologies. Columbus (OH): Battelle Press.

Moein S, Musick J, Keinath J, Barnard D, Lenhardt M, George R. 1994. Evaluation of seismic sources for repelling sea turtles from hopper dredges. Final report submitted to the US Army Corps of Engineers, Waterways Experiment Station. Gloucester Point (VA): Virginia Institute of Marine Science (VIMS), College of William and Mary. 42p.

Mones AB, Gruber EJ, Harms CA, Lohmann CMF, Lohmann KJ, Lewbart GA. 2021. Lactic acidosis induced by manual restraint for health evaluation and comparison of two point-of-care analyzers in healthy loggerhead sea turtles (*Caretta caretta*). Journal of Zoo and Wildlife Medicine. 52(4):1195–1204.

Mooney TA, Nachtigall PE, Vlachos S. 2009. Sonar-induced temporary hearing loss in dolphins. Biology Letters (UK). 5:565–567.

Muñoz FA, Estrada-Parra S, Romero-Rojas A, Gonzalez-Ballesteros E, Work TM, Villaseñor-Gaona H, Estrada-Garcia I. 2013. Immunological evaluation of captive green sea turtle (*Chelonia mydas*) with ulcerative dermatitis. Journal of Zoo and Wildlife Medicine. 44:837–844.

National Research Council. 2000. Marine Mammals and low-frequency sound: progress since 1994. Washington (DC): National Academies Press.

National Research Council. 2003. Ocean noise and marine mammals. Washington (DC): National Academies Press.

National Research Council. 2005. Marine mammal populations and ocean noise: determining when noise causes biologically significant effects. Washington (DC): National Academies Press.

Nelms SE, Piniak WE, Weir CR, Godley BJ. 2016. Seismic surveys and marine turtles: an underestimated global threat? Biological Conservation. 193:49–65.

Nowacek DP, Thorne LH, Johnston DW, Tyack PL. 2007. Responses of cetaceans to anthropogenic noise. Mammal Review. 37:81–115.

O'hara J, Wilcox JR. 1990. Avoidance responses of loggerhead turtles, *Caretta caretta*, to low frequency sound. Copeia. 2:564–567.

Piniak WE, Mann DA, Harms CA, Jones TT, Eckert SA. 2016. Hearing in the juvenile green sea turtle (*Chelonia mydas*): a comparison of underwater and aerial hearing using auditory evoked potentials, PloS One. 11(10).

Pirotta E, Brookes KL, Graham IM, Thompson PM. 2014. Variation in harbour porpoise activity in response to seismic survey noise. Biology Letters. 10:20131090.

Popper A, Hawkins A, Fay R, Mann D, Bartol S, Carlson TJ, Coombs S, Ellison WT, Gentry RL, Halvorsen MB, Lokkeborg S, Rogers PH, Southall BL, Zeddies DG, Tavolga WN. 2014. Sound exposure guidelines for fishes and sea turtles: a technical report prepared by ANSI-accredited standards committee S3/SC1 and registered with ANSI. ASA S3/SC1 4.

Popper AN, Hastings M. 2009. The effects of anthropogenic sources of sound on fishes. Journal of Fish Biology. 75:455–489.

Popper AN, Smith ME, Cott PA, Hanna BW, MacGillivray AO, Austin ME, Mann DA. 2005. Effect of exposure to seismic airgun use on hearing of three fish species. Journal of the Acoustical Society of America. 117:3958–3971.

Richardson WJ, Greene CR Jr, Malme CI, Thomson DH. 1995. Marine mammals and noise. San Diego (CA): Academic Press.

Ridgway SH, Wever EG, McCormick JG, Palin J, Anderson JH. 1969. Hearing in the giant sea turtle, *Chelonia mydas*. Proceedings of the National Academy of Sciences of the United States of America. 64:884–890.

Rolland RM, Parks SE, Hunt KE, Castellote M, Corkeron PJ, Nowacek DP, Wasser SK, Kraus SD. 2012. Evidence that ship noise increases stress in right whales. Proceedings of the Royal Society B: Biological Sciences. 279:2363–2368.

Romano TA, Keogh MJ, Kelly C, Feng P, Berk L, Schlundt CE, Carder DA, Finneran JJ. 2004. Anthropogenic sound and marine mammal health: measures of the nervous and immune systems before and after intense sound exposure. Journal of Fisheries and Aquatic Sciences. 61:1124–1113.

Rossi S, de Queiroz Hazarbassanov NG, Sánchez-Sarmiento AM, Prioste FE, Matushima ER. 2016. Immune response of green sea turtles with and without fibropapillomatosis: evaluating oxidative burst and phagocytosis via flow cytometry. Chelonian Conservation and Biology. 15:273–278.

Rousselet E, Levin M, Gebhard E, Higgins BM, DeGuise S, Godard-Codding CA. 2013. Evaluation of immune functions in captive immature loggerhead sea turtles (*Caretta caretta*). Veterinary Immunology and Immunopathology. 156:43–53.

Sakamoto KQ, Miyayama M, Kinoshita C, Fukuoka T, Ishihara T, Sato K. 2021. A non-invasive system to measure heart rate in hard-shelled sea turtles: potential for field applications. Philosophical Transactions of the Royal Society B. 2;376(1830):20200222.

Salas AK, Capuano A, Harms CA, Piniak WED, Mooney TA. 2022. Noise induced hearing loss in two freshwater turtles (*Trachemys scripta elegans* and *Chrysemys picta*). The Effects of Noise on Aquatic Life Conference, Berlin, Germany.

Slabbekoorn H, Bouton N, van Opzeeland I, Coers A, ten Cate C, Popper AN. 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends in Ecology and Evolution (Amst). 25:419–427.

Slotte A, Hansen K, Dalen J, Ona E. 2004. Acoustic mapping of pelagic fish distribution and abundance in relation to a seismic shooting area off the Norwegian west coast. Fisheries Research. 67:143–50.

Southall BL, Nowacek DP, Miller PJ, Tyack PL. 2016. Experimental field studies to measure behavioral responses of cetaceans to sonar. Endangered Species Research. 31:293–315.

Southall BL, Finneran JJ, Reichmuth C, Nachtigall PE, Ketten DR, Bowles AE, Ellison WT, Nowacek DP, Tyack PL. 2019. Marine mammal noise exposure criteria: updated scientific recommendations for residual hearing effects. Aquatic Mammals. 45(2):125–232.

Southall BL, Nowacek DP, Bowles AE, Senigaglia V, Bejder L, Tyack PL. 2021. Marine mammal noise exposure criteria: assessing the severity of marine mammal behavioral responses to human noise. Aquatic Mammals. 47(5):421–64.

Stacy NI, Innis CJ. 2017. Clinical pathology. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea turtle health & rehabilitation. Plantation (FL): JRoss Publishing. pp. 147–207.

Stamper MA, Harms C, Epperly SP, Braun-McNeill J, Avens L, Stoskopf MK. 2005. Relationship between barnacle epibiotic load and hematologic parameters in loggerhead sea turtles (*Caretta caretta*), a comparison between migratory and residential animals in Pamlico Sound, North Carolina. Journal of Zoo and Wildlife Medicine. 36:635–641.

Tristan TA, Norton TM. 2017. Physical examination. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea turtle health & rehabilitation. Plantation (FL): JRoss Publishing, pp. 99–121.

Tyson RB, Piniak WE, Domit C, Mann D, Hall M, Nowacek DP, Fuentes MMPB. 2017. Novel bio-logging tool for studying fine-scale behaviors of marine turtles in response to sound. Frontiers in Marine Science. 4:219.

Umapathy G, Deepak V, Kumar V, Chandrasekhar M, Vasudevan K. 2015. Endocrine profiline of endangered tropical chelonians using noninvasive fecal steroid analyses. Chelonian Conservation and Biology. 14:108–115.

U.S. Department of the Navy. 2017. Technical report: criteria and thresholds for U.S. Navy acoustic and explosive effects analysis (phase III). San Diego, California: SSC Pacific.

Vaida ST, Hammer RM, Racca R, Hannay D, Thompson MJ, Balcom BJ, Phillips NW. 2008. Review of the potential impacts to sea turtles from underwater explosive removal of offshore structures. Environmental Impact Assessment Review. 28(2008):267–285.

Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S, Monfort SL. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of non-domestic mammalian and avian species. General and comparative endocrinology. 120:260–275.

Weir CR. 2007. Observations of marine turtles in relation to seismic airgun sound off Angola. Marine Turtle Newsletter. 116:17–20.

Whitham JC, Bryant JL, Miller LJ. 2020. Beyond glucocorticoids: integrating dehydroepiandrosterone (DHEA) into animal welfare research. Animals. 10:1381; doi:10.3390/ani10081381.

Wright AJ, Soto NA, Baldwin AL, Bateson M, Beale CM, Clark C, Deak T, Edwards EF, Fernández A, Godinho A, Hatch LT. 2007. Do marine mammals experience stress related to anthropogenic noise? International Journal of Comparative Psychology. 20:274–316.

Appendix A: Workshop Agenda and Attendees

A.1 Workshop Agenda

BOEM-Duke University-North Carolina State University Workshop: Methods to examine behavioral and physiological responses of sea turtles to sound

Date/Time: October 28-29, 2021, 1:00 pm - 5:00 pm ET
Hosts: Wendy Piniak, Doug Nowacek, Craig Harms, Jake Levenson
Facilitator: Carrie Kappel (kappel@nceas.ucsb.edu) 831.869.1503
Zoom: Meeting ID: 814 9018 9488 Passcode: 010263

Workshop Purpose

Develop a methodological framework to examine behavioral and physiological responses of sea turtles to sound and analyze the strengths and weaknesses of specific methodological approaches.

ZOOM TIPS FOR OUR MEETING

- **Show up as fully as if you were in person.**
  - Avoid multitasking.
  - Give your full presence to the meeting and to the other participants.
- **Help us hear you and your fellow participants.**
  - Mute liberally and quickly when not speaking to limit background noise.
  - If you can mute notifications on your devices this also helps.
  - If your space tends to echo, consider using a headset.
- **Let us see you.**
  - If you are comfortable having your video on, turn on your camera and adjust your lighting so your face is well lit.
  - Turn on gallery view in the upper right to see everyone.
    - *Also: Settings > Video > Display up to 49 participants*

Thursday, October 28, 2021

1:00 - 1:20	Welcome and Opening Remarks from Jill Lewandowski, BOEM
1:20 - 1:35	Introductions
1:35 - 2:20	Project Overview and Q&A
2:20 - 3:00	Breakout Session One: Behavior and physiology, sound sources and focal turtles
3:00 - 3:10	*Break*
3:10 - 3:30	Report Outs and Discussion
3:30 - 4:10	Breakout Session Two: Stimulus selection, presentation, and exposure protocol for wild vs captive sea turtles
4:10 - 4:20	*Break*
4:20 - 4:45	Report Outs and Discussion
4:45 - 5:00	Day One Closing

Friday, October 29, 2021

1:00 - 1:15	Welcome and Day Two Objectives
1:15 - 1:35	Reflections on Day One
1:35 - 2:20	Breakout Session Three: Behavioral and physiological response variables, sampling design and statistical analysis
2:20 - 2:30	*Break*
2:30 - 3:00	Report Outs and Discussion
3:00 - 3:45	Breakout Session Four: Further details on methods and tools
3:45 - 3:55	*Break*
3:55 - 4:35	Report Outs and Discussion
4:35 - 4:50	Synthesis
4:45 - 5:00	Closing

A.2 Attendees

Workshop organizers

- Doug Nowacek - Duke University Marine Lab and Pratt School of Engineering
- Craig Harms - North Carolina State University, College of Veterinary Medicine, Center for Marine Sciences and Technology
- Wendy Piniak - NOAA NMFS, Office of Protected Resources
- Jacob Levenson - BOEM, Division of Environmental Studies

Participants

- Kyle Baker - BOEM Offshore Renewable Energy Projects (OREP)
- Elizabeth Burgess - New England Aquarium
- Alex Conrad - BOEM, Center for Marine Acoustics
- Alasdair Davies - Arribada Initiative
- Sam Denes - BOEM, Center for Marine Acoustics
- Stacy DeRuiter - Calvin University
- Kara Dodge - New England Aquarium
- Mariana Fuentes - Florida State University
- Catriona Harris - University of St. Andrews
- Kathleen Hunt - George Mason University / Smithsonian-Mason School of Conservation
- Jill Lewandowski - BOEM
- Charles Muirhead - Duke University Marine Lab, Nicholas School of the Environment
- Samir Patel - Coonamessett Farm Foundation
- Doug Piatkowski - BOEM, Marine Minerals Division
- Amy Scholik-Schlomer - NOAA NMFS, Office of Protected Resources
- Maria Serrano - North Carolina State University, College of Veterinary Medicine, Center for Marine Sciences and Technology
- Nick Sisson - NOAA NMFS, Greater Atlantic Regional Fisheries Office
- Erica Staaterman - BOEM, Center for Marine Acoustics
- Brandon Southall - Southall Environmental Associates, University of California Santa Cruz, Duke University Marine Lab
- Brian Stacy - NOAA NMFS, Office of Protected Resources
- Kathy Tuxbury - New England Aquarium

Facilitators

- *Lead:* Carrie Kappel - NCEAS/Independent consultant and facilitator
- Isabella Clark - LegacyWorks Group
- Jessica Gomez - LegacyWorks Group
- Stephanie Dashiell - Independent consultant and facilitator

Appendix B: Summary of Available Literature

Table B-1. Summary of representative studies examining sea turtle (and related aquatic species) physiological response to stressors

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
Allen et al. 2015	*Chelonia mydas*	East Pacific	survey	field	69	subadult, adult	NA	NA	NA	plasma testosterone	sex ratio	easy to collect, could be applied to adult reproductive evaluation
Arkwright et al. 2020	*Caretta caretta*	Spain	experimental	captive	33	-	NA	NA	FI +/- DCS	datalogger behavior comparison of healthy and unhealthy turtles in rehab	some degree of association between injuries/illness and movement patterns	qualitative
Caliani et al. 2019	*Caretta caretta*	Italy, Spain	experimental/ opportunistic	captive	88 rehab 11 free ranging	juveniles, subadults	NA	NA	rehab	WBC, H/L, respiratory burst total antioxidant status, lysozyme	most measures elevated in rehab turtles, highest values in first 2 mo, normalized after 1 yr, monocytes and eosinophils declined in hospitalized animals; considered lysozyme and eosinophils valid indicators of inflammation and physiologic stress	variable causes of stranding/rescue; variable sampling times; suggests some immune response variables to consider, and long time frames of recovery
Flower et al. 2018	*Caretta caretta*	USA	survey	field	37	adult females	NA	NA	Nesting	corticosterone, hematology, plasma chemistry, reproductive success	no correlation between corticosterone and repro success, some incidental correlations	shotgun approach, single time point
Goldberg et al. 2013	*Eretmochelys imbricata*	Brazil	experimental	field	41	nesting females	NA	NA	progression of nesting season	ghrelin and leptin	decreasing leptin, increasing ghrelin through nesting season, associated with suppression during and resumption of foraging following nesting season	two other response variables to consider if sound affects foraging
Gregory et al. 1996	*Caretta caretta*	USA	experimental	field/capture	-	subadult, adult	NA	NA	trawl or tangle net, confinement, serial blood samples	corticosterone	plasma corticosterone peaked at 3 hr, declined at 6 hr, higher for trawl captures, higher for subadults, higher in summer than winter	serial sampling introduces sequential stressor; demonstrates issues of sample timing, season/temperature, and size class

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
Hamann et al. 2003	*Chelonia mydas*	Australia	survey	field	134	adult females	NA	NA	seasonal change, reproductive status, restraint time	epinephrine, norepinephrine, lipolysis	no change in epi/norepi within 600 sec restraint time from capture; seasonal variation	easy to collect but unstable sample requiring rapid processing or stabilization, and complex laboratory processing
Harms et al. 2003	*Caretta caretta*	USA	experimental	field, capture	22	subadult	NA	NA	trawl or pound net, capture handling	blood gases and lactate	trawl capture had greater effects, pH recovered at 30 min, lactate increased at 30 min for pound net and did not recover for trawl	only two time points; indicates even the less stressful capture and handling still has effects
Harms et al. 2017	sea turtles	general	review	field, captive	NA	all	NA	NA	NA	neurological examination	review	techniques for examining neurologic function
Harris et al. 2016	*Dermochelys coriacea*	USA	survey	field	36	immature and adult	NA	NA	live capture vs dead stranded and nesting	SC fat depth by ultrasound	noninvasive measure of body condition	method development and validation; operator dependent
Harris et al. 2017	sea turtles	general	review	field	NA	all	NA	NA	NA	field techniques	review	field techniques applicable to many sorts of investigations
Hoopes et al. 2000	*Lepidochelys kempii*	USA	experimental	field, capture	58	-	NA	NA	entanglement nets, recovery times in holding tanks vs in-water cages	lactate, epinephrine, norepinephrine, electrolytes, glucose	lactate, epinephrine, norepinephrine elevated initially (at 1hr post capture) and took about 6 hr to decline to near baseline; in-water cages had quicker recoveries	highlights issues of sample timing
Hunt et al. 2012	*Lepidochelys kempii*	USA	survey	captive	87	immature and adult	NA	NA	cold stunning and convalescence	corticosterone and free thyroxine (fT4)	high corticosterone/low fT4 on admission, corticosterone negatively correlated with WBC; no difference at admission between survivors and nonsurvivors	easy to collect, indicates metabolic status; high/low to low/high marks recovery from cold-stun stressor
Hunt et al. 2016	*Lepidochelys kempii*	USA	experimental	captive	26	juveniles	NA	NA	ground transport 13 or 26 hr	glucose, corticosterone, blood gases, electrolytes, WBC, H/L, HR, RR	glucose elevated after both transport durations, corticosterone elevated only after 26 hr transport	highlights issues of sample timing

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
Hunt et al. 2016b	*Dermochelys coriacea*	USA	survey	field	32	adult	NA	NA	capture, entanglement, stranding	corticosterone, thyroxine	corticosterone and thyroxine higher in entangled and stranded than in healthy captures; corticosterone increased bewteen 25 and 50 min postcapture	indicates effects of capture and sample timing, but with markedly greater impacts from more severe stressors
Hunt et al. 2019	*Lepidochelys kempii*	USA	experimental, opportunistic	captive	18	juveniles	NA	NA	transport (~21h); pool recovery	corticosterone, glucose, WBC, H/L, blood gases, lactated, electrolytes, hematocrit	corticosterone, glucose, WBC, H/L elevated post-transport, corticosterone and glucose reduced after 6h pool recovery; minor K differences; WBC and H/L elevation persisted	integrates multiple response variables, different timing for resolution
Hunt et al. 2020	*Lepidochelys kempii, Caretta caretta*	USA	experimental	captive	8 per bin	juveniles	NA	NA	ground transport 6, 12, 18, 24h	corticosterone, glucose, WBC, H/L, blood gases, lactate, electrolytes	corticosterone and glucose elevated at all time points, WBC and H/L elevated at 12, 18, 24 hr for Cc, Cc indicated greater impact than Lk	good controls and binning; indicates sample timing and species (+/- size) effects
Injaian et al. 2020	birds, reptiles	multiple	meta-analysis	field	variable	variable	urbanization	variable	urbanization, noise	corticosterone	equivocal to no effects detected	sound levels unknown for most studies included; many variables, many uncontrolled variables; but one of few stabs at effects of noise on reptiles in the field
Innis et al. 2007	*Lepidochelys kempii*	USA	experimental	captive	26	juveniles	NA	NA	cold stunning, convalescence	plasma biochemicals, blood gas, lactate	metabolic and respiratory acidosis	multiple days to recovery/sampling
Keller et al. 2006	*Caretta caretta*	USA	experimental, survey	field and cell culture	27	juveniles	NA	NA	organochlorine exposure	mitogen-induced lymphocyte proliferation, lysozyme	lymphocyte proliferative response correlate with sum PCBs; lysozyme negatively corelated with some OCs	blood samples simple to collect, immune assays must be run shortly thereafter, lab analysis labor intensive; methods broadly applicable to a variety of stressors

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
Kinoshita et al. 2022	*Caretta caretta*	Japan	experimental	captive	3	subadult/ adult	NA	NA	baseline	noninvasive unrestrained heart rate measurement	methods development, refinement of Sakamoto et al. (2021) to improve signal quality	potential for field application, electronics and signal processing highly technical (not off-the-shelf instrumentation)
Klima et al. 1988	*Lepidochelys kempii, Caretta caretta*	USA	experimental/ opportunistic	field	8	juvenile	explosions	modeled at 221, 217, 213, 209 dB by distance	explosive removal of offshore petroleum platforms	exposure at distances ranging from 229–915 m, free-swimming in cages	unconsciousness, cloacal prolapse, hyperemia of ventral throat and flippers for up to 3 wk.	extreme exposure, but clear major effects
Kophamel et al. 2023 and in prep	*Chelonia mydas*	Australia	experimental and survey	field	?	?	NA	NA	NA	estimated adipose tissue by electrical impedance	methods development	noninvasive estimation of % adipose tissue, validated to CT determination of adipose content, applicable to field settings, may be better indicator of body condition that condition indices
Lara and Vasconcelos 2021	zebrafish	China	experimental	captive	50/group	larvae	speaker	130 and 150 dB re 1 µPa, variable vs continuous	-	development/growth/yolk sac, cortisol (whole body), mortality, HR, behavior (dark avoidance, turning)	HR, yolk sac consumption, cortisol increased with increased noise at 3 and 5 days post-fertilization temporal variation more important than total duration of noise, 5 dpf larvae at 150 dB increased dark avoidance and impaired spontaneous alternation behavior	lethal cortisol measurement, but good combination of response variables

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
McNally et al. 2021	*Lepidochelys kempii, Chelonia mydas*	USA	survey	field	50	immature	NA	NA	baseline	microbiome	methods development	sample collection simple, analysis complex, associations with disease or physiological states not yet established, but active area of investigation, including through rehabilitation, cold stunning, ontogenetic shifts
Mones et al. 2021	*Caretta caretta*	USA	experimental	captive	16	yearling	NA	NA	15 min PE	lactate, blood gases	median plasma lactate concentration increased 6.54 mmol/L	indicates constraints of handling effects on physiologic responses
Muñoz et al. 2013	*Chelonia mydas*	Mexico	experimental	captive	15	13 mo	NA	NA	ulcerative dermatitis	hematology, histopathology, IG levels, delayed type hypersensitivity (DTH; PHA injection at cloacal skin fold)	affected turtles had lower weight, reduced DTH, higher H/L ratios	another immunologic response variable
Niemuth et al. 2015	*Caretta caretta*	USA	experimental	captive	5	juveniles	NA	NA	NA	NMR metabolomics	methods development, effects of sample type (plasma vs whole blood) and processing time evaluated; <40–50 min recommended	many response variables, small sample sizes, baseline
Niemuth et al. 2019	*Lepidochelys kempii, Caretta caretta, Chelonia mydas*	USA	experimental	field, rescue	39 (various group sizes)	juveniles	NA	NA	cold stuning	tear NMR metabolomics	PCA five biomarkers differed between affected and unaffected (propylene glycol, glycerol, lactate, formate, and unidentified metabolite)	pooled samples, low sample sizes, overlapping results
Owens and Morris 1985	*Chelonia mydas,* others	-	review	-	variable	variable	NA	NA	-	many hormones (pituitary, adrenal, repro, thyroid); histo	multiple	wide array of response variables from which to choose, depending on focus; size, sex, and seasonal variation

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
Rossi et al. 2016	*Chelonia mydas*	Brazil	experimental	field	38	juveniles, subadults	NA	NA	Fibropapillomatosis (FP)	oxidative burst, phagocytosis via flow cytometry	no differences in leukocyte activity; differences in leukocyte populations	more specific immunological assessment that hematology, but labor intensive and not much if any separation
Rousselet et al. 2013	*Caretta caretta*	USA	experimental	captive	65	immature and adult	NA	NA	baseline	lymphocyte proliferation, NK activity, phagocytosis, respiratory burst	immunological methods development/validation	more immunologic response variables
Sakamoto et al. 2021	*Lepidochelys olivacea, Caretta caretta, Chelonia mydas, Eretmochelys imbricata*	Japan	experimental	captive	11	subadult, adult	NA	NA	baseline	noninvasive unrestrained heart rate measurement	methods development; resting mean HR 6.2/min, swimming at surface 14.0/min	worked for Cc, Lo, one Cma, not Cm or Ei. Potential for field application, electronics, and signal processing highly technical (not off-the-shelf instrumentation)
Schock et al. 2013	*Chelonia mydas*	USA	survey	field	20 (3–7)	juveniles, subadults	NA	NA	FP	NMR metabolomics	methods development, some differences	many response variables, small sample sizes, baseline
Shertzer et al. 2018	*Caretta caretta*	USA	experimental	field	1,401	juveniles	NA	NA	seasonal changes	testosterone	testosterone higher in juvenile males, higher in summer/warmer water in both sexes	seasonal and sex differences to bear in mind if evaluating reproductive hormones
Silvestre 2014	reptiles	-	review	-	-	-	-	-	several	behavior, autonomic nervous system, neuroendocrine, immune/hematology	reviews different response variables, sample timing, population effects; general	-
Stacy and Innis 2017	sea turtles	general	review	field and captive	NA	all	NA	NA	NA	clinical pathology	review	standard component of assessing health status
Stamper et al. 2005	*Caretta caretta*	USA	survey	field	57	juveniles, subadults	NA	NA	migration	epibiota coverage, clinical pathology, body condition	some significant clin path differences in migratory group, no differences in epibiota coverage or condition index	epibiota coverage insensitive indicator of health, until debilitation is more advanced

Source	Species	Location	Method	Setting	Sample Size	Life Stage	Sound Source	Sound Level	Stressor	Response Variables	Result	Limitations; Implications
Stewart et al. 2016	*Chelonia mydas*	Barbados	experimental/ opportunistic	field	29	juveniles	NA	NA	feed supplementation, tourist interaction	morphs, PE, BCI, epibiota, hematology, plasma biochems	multiple differences noted, consistent with more abundant less natural food sources	not particularly applicable to sound perhaps, but a field study, with multiple response variables related to health/physiology
Tristan and Norton 2017	sea turtles	general	review	field, captive	NA	all	NA	NA	NA	physical examination	review	standard component of assessing health status
Whitham et al. 2020	vertebrates	general	review	mixed	NA	na	NA	NA	several	dehydroepiandrosterone (DHEA)	review of DHEA employed in animal welfare research	more complete picture of HPA axis, correlates positively to some immune responses in other vertebrates, not sure if data exist for turtles

Notes: BCI = body condition index, CT = computed tomography, DCS = decompression sickness, DHEA = dehydroepiandrosterone, DTH = delayed type hypersensitivy (Type IV hypersensitivity), FI = fishery interaction, FP = fibropapillomatosis, H/L = heterophil/lymphocyte ratio, HR = heart rate, fT4 = free thyroxine (i.e., not protein-bound), K = potassium, NA = not applicable, NMR = nuclear magnetic resonance, OC = organochlorine, PCA = principal component analysis, PCB = polychlorinated biphenyl, PE = physical examination, PHA = phytohemagglutinin (a mitogen), WBC = white blood cell count

Sources:

Allen CD, Robbins MN, Eguchi T, Owens DW, Meylan AB, Meylan PA, Kellar NM, Schwenter JA, Nollens HH, LeRoux RA, Dutton PH. 2015. First assessment of the sex ratio for an East Pacific green sea turtle foraging aggregation: validation and application of a testosterone ELISA. PLoS One. 10(10): e0138861. doi:10.1371/journal.pone.0138861.

Arkwright AC, Archibald E, Fahlman A, Holton MD, Crespo-Picazo JL, Cabedo VM, Duarte CM, Scott R, Webb S, Gunner RM, Wilson RP. 2020. Behavioral biomarkers for animal health: a case study using animal-attached technology on loggerhead turtles. Frontiers in Ecology and Evolution 7:504.

Caliani I, Poggioni L, D'Agostino A, Fossi MC, Casini S. 2019. An immune response-based approach to evaluate physiological stress in rehabilitating loggerhead sea turtles. Veterinary immunology and immunopathology.. 207:18-24.

Flower JE, Norton TM, Andrews KM, Parker CE, Romero LM, Rockwell KE, Mitchell MA. 2018. Baseline corticosterone, hematology and biochemistry results and correlations to reproductive success in nesting loggerhead sea turtles (*Caretta caretta*). Journal of Zoo and Wildlife Medicine. 49(1):9-17

Goldberg DW, Leitao SAT, Godfrey MH, Lopez GG, Santos AJ, Neves FA, de Souza ÉP, Moura AS, Bastos JD, Bastos VL. 2013. Ghrelin and leptin modulate the feeding behaviour of the hawksbill turtle *Eretmochelys imbricata* during nesting season. Conservation Physiology. 1:10.1093/conphys/cot016.

Gregory LF, Gross TS, Bolten AB, Bjorndal KA, Guillette Jr LJ. 1996. Plasma corticosterone concentrations associated with acute captivity stress in wild loggerhead sea turtles. General and comparative endocrinology. 104:312-320.

Hamann M, LImpus CJ, Whittier JM. 2003. Seasonal variation in plasma catecholamines and adipose tissue lipolysis in adult female green sea turtles (*Chelonia mydas*). General and comparative endocrinology. 130:308-316.

Harms CA, Mallo KM, Ross PM, Segars A. 2003. Venous blood gases and lactates of wild loggerhead sea turtles (*Caretta caretta*) following two capture techniques. Journal of Wildlife Diseases. 39:366-374.

Harms CA, Valenta ALS, Stacy BA, Wyneken J. 2017. Nervous system. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea Turtle Health & Rehabilitation. Plantation, FL: JRoss Publishing, pp. 417-448.

Harris HS, Benson SR, James MC, Martin KJ, Stacy BA, Daoust PY, Rist PM, Work TM, Balazs GH, Seminoff JA. 2016. Validation of ultrasound as a noninvasive tool to measure subcutaneous fat depth in leatherback sea turtles (*Dermochelys coriacea*). Journal of Zoo and Wildlife Medicine. 275-279.

Harris HS, Flint M, Stewart KM, Harms CA. 2017. Field techniques. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea Turtle Health & Rehabilitation. Plantation, FL: JRoss Publishing, pp. 819-857.

Hoopes LA Landry AM, Stabenau EK. 2000. Physiologic effects of capturing Kemp's ridley sea turtles, *Lepidochelys kempii*, in entanglement nets. Canadian Journal of Zoology. 78(11):1941-1947.

Hunt KE, Innis C, Rolland RM. 2012. Corticosterone and thyroxine in cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*). Journal of Zoo and Wildlife Medicine. 43:479-493.

Hunt KE, Innis CJ, Kennedy AE, McNally KL, Davis DG, Burgess EA, Merigo C. 2016a. Assessment of ground transportation stress in juvenile Kemp's ridley sea turtles (*Lepidochelys kempii*). Conservation Physiology. 4(1).

Hunt KE, Innis CJ, Merigo C, Rolland RM. 2016b. Endocrine responses to diverse stressors of capture, entanglement and stranding in leatherback turtles (*Dermochelys coriacea*). Conservation Physiology. 4(1).

Hunt KE, Innis C, Merigo C, Burgess EA, Norton T, Davis D, Kennedy AE, Buck CL. 2019. Ameliorating transport-related stress in endangered Kemp's ridley sea turtles (*Lepidochelys kempii*) with a recovery period in saltwater pools. Conservation Physiology. 7(1).

Hunt KE, Merigo C, Burgess EA, Buck CL, Davis D, Kennedy A, Lory L, Wocial J, McNally K, Innis C. 2020. Effects of ground transport in Kemp's ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) turtles. Integrative Organismal Biology. 2020;2(1):obaa012, doi:10.1093/iob/obaa012.

Injaian AS, Francis CD, Ouyang JQ, Dominoni DM, Donald JW, Fuxjager MJ, Goymann W, Hau M, Husak JF, Johnson MA, Kircher BK. 2020. Baseline and stress-induced corticosterone levels across birds and reptiles do not reflect urbanization levels. Conservation Physiology. 8(1):coz110.

Innis CJ, Tlusty M, Merigo C, Weber ES. 2007. Metabolic and respiratory status of cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*). Journal of Comparative Physiology B. 177(6):623-630.

Keller JM, McClellan-Green PD, Kucklick JR, Keil DE, Peden-Adams MM. 2006. Effects of organochlorine contaminants on loggerhead sea turtle immunity: comparison of a correlative field study and in vitro exposure experiments. Environmental Health Perspectives. 114(1):70-76.

Kinoshita C, Saito A, Kawai M, Sato K, Sakamoto KQ. 2022. A non-invasive heart rate measurement method is improved by placing the electrodes on the ventral side rather than the dorsal in loggerhead turtles. Frontiers in Physiology. 13:811947, doi: 10.3389/fphys.2022.811947.

Klima EF, Gitschlag GR, Renaud ML. 1988. Impacts of the explosive removal of offshore petroleum platforms on sea turtles and dolphins. Marine Fisheries Review. 50:33-42.

Kophamel S, Ward LC, Ariel E, Mendez D, O'Brien LM, Burchell L, Munns SL. 2023. A standardised protocol for measuring bioelectrical impedance in green turtles (*Chelonia mydas*). Physiological and Biochemical Zoology. 96(2).

Lara RA, Vasconcelos RO. 2021. Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. Scientific Reports. 11:6615.

McNally KL, Mott CR, Guertin JR, Bowen JL. 2021. Microbial communities of wild-captured Kemp's ridley (*Lepidochelys kempii*) and green sea turtles (*Chelonia mydas*). Endangered Species Research. 45:21-36.

Mones AB, Gruber EJ, Harms CA, Lohmann CMF, Lohmann KJ, Lewbart GA. 2021. Lactic acidosis induced by manual restraint for health evaluation and comparison of two point-of-care analyzers in healthy loggerhead sea turtles (*Caretta caretta*). Journal of Zoo and Wildlife Medicine. 52(4):1195-1204.

Muñoz FA, Estrada-Parra S, Romero-Rojas A, Gonzalez-Ballesteros E, Work TM, Villaseñor-Gaona H, Estrada-Garcia I. 2013. Immunological evaluation of captive green sea turtle (*Chelonia mydas*) with ulcerative dermatitis. Journal of Zoo and Wildlife Medicine. 44:837-844.

Niemuth JN, Harms CA, Stoskopf MK. 2015. Effects of processing time on whole blood and plasma samples from loggerhead sea turtles (*Caretta caretta*) for 1H-HMR-based metabolomics. Herpetological Conservation and Biology. 10:149-160.

Niemuth JN, Harms CA, Stoskopf MK. 2019. Sea turtle tears: a novel, minimally invasive sampling method for 1H-NMR metabolomics investigations with cold stun syndrome as a case study. Journal of Wildlife Diseases. 868-873.

Owens DW, Morris JA. 1985.The comparative endocrinology of sea turtles. Copeia. 1985(3):723-735.

Rossi S, de Queiroz Hazarbassanov NG, Sánchez-Sarmiento AM, Prioste FE, Matushima ER. 2016. Immune response of green sea turtles with and without fibropapillomatosis: evaluating oxidative burst and phagocytosis via flow cytometry. Chelonian Conservation and Biology. 15:273-278.

Rousselet E, Levin M, Gebhard E, Higgins BM, DeGuise S, Godard-Codding CA. 2013. Evaluation of immune functions in captive immature loggerhead sea turtles (*Caretta caretta*). Veterinary Immunology and Immunopathology. 156:43-53.

Sakamoto KQ, Miyayama M, Kinoshita C, Fukuoka T, Ishihara T, Sato K. 2021. A non-invasive system to measure heart rate in hard-shelled sea turtles: potential for field applications. Philosophical Transactions of the Royal Society B. 2;376(1830):20200222.

Schock TB, Keller JM, Rice M, Balazs GH, Bearden DW. 2013. Metabotyping of a protected non-model organism, green sea turtle (*Chelonia mydas*), using 1H NMR spectroscopy and optimized plasma methods for metabolomics. Current Metabolomics. 1:279-290.

Shertzer KW, Avens L, McNeill JB, Hall AG, Harms CA. 2018. Characterizing sex ratios of sea turtle populations: A Bayesian mixture modeling approach applied to juvenile loggerheads (*Caretta caretta*). Journal of Experimental Marine Biology and Ecology. 504:10-9.

Silvestre AM. 2014. How to assess stress in reptiles. Journal of Exotic Pet Medicine. 23:240-243.

Stacy NI, Innis CJ. 2017. Clinical pathology. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea Turtle Health & Rehabilitation. Plantation, FL: JRoss Publishing, pp. 147-207.

Stamper MA, Harms C, Epperly SP, Braun-McNeill J, Avens L, Stoskopf MK. 2005. Relationship between barnacle epibiotic load and hematologic parameters in loggerhead sea turtles (*Caretta caretta*), a comparison between migratory and residential animals in Pamlico Sound, North Carolina. Journal of Zoo and Wildlife Medicine. 36:635-641.

Stewart K, Norton T, Mohammed H, Browne D, Clements K, Thomas K, Yaw T, Horrocks J. 2016. Effects of “swim with the turtles” tourist attractions on green sea turtle (*Chelonia mydas*) health in Barbados, West Indies. Journal of Wildlife Diseases. 52(2s):S104-17.

Tristan TA, Norton TM. 2017. Physical examination. In: Manire CA, Norton TM, Stacy BA, Innis CJ, Harms CA (eds.). Sea Turtle Health & Rehabilitation. Plantation, FL: JRoss Publishing, pp. 99-121.

Whitham JC, Bryant JL, Miller LJ. 2020. Beyond glucocorticoids: integrating dehydroepiandrosterone (DHEA) into animal welfare research. Animals 10:1381; doi:10.3390/ani10081381.

Table B-2a. Summary of published in-water sea turtle behavioral response to sound studies
Guided by Table 1 of Southall et al. (2021).

Source	Location	Method	Setting	Sample Size	Species	Age Class	Sex	Behavioral State	Result	Limitations
DeRuiter and Larbi Doukara 2012	Mediterranean Sea (off Algerian coast)	Observational (at-sea)	Wild	164	*Caretta caretta*	Unknown	Unknown	Basking, swimming at surface	Of the 86 turtles whose dive behavior was observed, 57% dove and 43% did not. Six turtles were observed to have startle responses before diving after an airgun shot. Median distances at which turtles dove: 191 dB re 1 µPa (peak) (modeled) at 130 m from the array.	No controls for effects of vessel presence. Modeled received levels.
Eckert et al. 1998	Trinidad	Observational (using biologging tools)	Wild	3	*Dermochelys coriacea*	Adult	Female	Not reported	Inconclusive.	Small sample size and technical difficulties.
Gurjão et al. 2005	Brazil	Observational (at-sea)	Wild	8	*Chelonia mydas and Eretmochelys imbricata*	Unknown	Unknown	Not reported	No avoidance behavior was observed (all observed >500m from source).	Limited sampling window and small sample size. No source or received sound levels reported.
Hazel et al. 2009	Australia	Experimental	Wild	1,890	*Chelonia mydas*	Adult	Unknown	Foraging or resting on benthos	Greater vessel speed increased the probability that a turtle would fail to flee from the approaching vessel. Authors suggest turtles rely on visual cues rather than acoustic cues in this scenario (or are habituated to vessel noise) as greater speed (higher sound levels) did not induce flee response.	No source or received sound levels reported.
Lavendar et al. 2014	Texas, USA	Experimental - behavioral hearing tests	Captive	8	*Caretta caretta*	Post-hatchling and juvenile	Unknown	NA – Behavioral audiogram	Turtles responded to sounds with SPL as low as 76 dB re: 1 uPa and tones 100–1,000 Hz.	Trained turtles detected and responded to low-frequency tonal signals; however, results cannot predict if/how turtles will respond to signals with these frequency components in wild settings.
Lenhardt et al. 1994	USA	Experimental	Wild (in pen)	5	*Caretta caretta*	Juvenile and adult	Unknown	Not reported	Turtles showed no significant approach or avoidance behavior in response to sound.	Confined setting – results cannot be applied to open-water situations. No source or received sound levels reported.
Lenhardt et al. 1994	USA	Experimental	Captive	5	*Caretta caretta*	Juvenile and adult	Unknown	Not reported	Startle responses recorded, but no specific stimuli produced the startle response.	Confined setting – results cannot be applied to open-water situations. No source or received levels reported.

Source	Location	Method	Setting	Sample Size	Species	Age Class	Sex	Behavioral State	Result	Limitations
Lenhardt 1994	USA	Experimental	Captive	2	*Caretta caretta*	Juvenile	Unknown	Resting	Both turtles always responded to low-frequency sound by swimming. No animal returned to the bottom or stopped swimming.	Confined setting – results cannot be applied to open-water situations. No source or received levels reported.
Martin et al. 2012	Florida, USA	Experimental - behavioral hearing tests	Captive	1	*Caretta caretta*	Adult	Female	NA - Behavioral audiogram	Turtle responded to sounds with SPL as low as 98 dB re: 1 uPa and tones 100–800 Hz.	Trained turtles detected and responded to low-frequency tonal signals; however, results cannot predict if/how turtles will respond to signals with these frequency components in wild settings.
McCauley et al. 2000	Australia	Experimental	Captive	2	*Caretta caretta and Chelonia mydas*	Not reported	Unknown	Not reported	Turtles displayed 'alarm' response at an estimated 2km from an operating seismic vessel and behavior indicative of avoidance estimated at 1 km.	Small sample size. Confined setting – results cannot be applied to open-water situations.
Moein et al. 1994	USA	Experimental	Wild (in pen)	10	*Caretta caretta*	Juvenile and adult	Unknown	Not reported	On first exposure, turtles occupied positions farther away from airguns than expected by chance, suggesting an avoidance response. No significant difference in seond exposure suggesting habituation. Hearing tests showed TTS in some turtles after exposure, potentially impacting behavioral responses.	Confined setting – results cannot be applied to open-water situations. No source or received levels reported. Potential impact of hearing loss.
O'Hara and Wilcox 1990	USA	Experimental	Captive (turtles of wild origin)	31	*Caretta caretta*	Juvenile	Unknown	Not reported	Turtles in higher air gun pressure exposure trials avoided areas near air guns (within ~30m), however turtles repeatedly approached airguns and spent time in areas close to air guns.	Confined setting – results cannot be applied to open-water situations. No source or received levels reported.
Tyson et al. 2017	Brazil	Experimental (at-sea, CEE)	Wild	1	*Chelonia mydas*	Juvenile	Unknown	Inferred resting or swimming (foraging state unknown)	Biologging tool proof of concept study.	Most received signals were below reported levels of hearing sensitivity.
Weir 2007	Offshore Angolan coast	Observational (at-sea)	Wild	240	*Lepidochelys olivacea, Dermochelys coriacea, Caretta caretta and unidentified*	Unknown	Unknown	Basking (94%), breathing at the surface, swimming at the surface	Inconclusive. Median distance to the array did not differ between full array (airguns on) and airguns off. Most startle responses observed were due to visual cues and vessel/towed equipment in very close proximity.	No controls for effects of vessel and towed equipment presence.

Table B-2b. Summary of published in-water sea turtle behavioral response to sound studies: exposure context variables
Guided by Table 1 of Southall et al. (2021).

Source	Sound Source	Exposure Type (start of exposure)	Source–Animal Range (start of exposure)	Source Depth (m)	Animal Depth (m)	General Source Movement (relative to subject)	Navigational Constraints (is subject confined in any way?)	Exposure Novelty (is source type common/ rare for area?)	Exposure Similar to Predator Sounds?	Other Species Present in the Area?	Predator Species Present in the area?	Other Anthropogenic Presence/ Noise in Area? (type and proximity)
DeRuiter and Larbi Doukara 2012	Seismic airgun array	Observations made during seismic survey	NA	11.5	Observations made at the surface	Approaching/ departing	None	Rare	No	Not reported	Not reported	Not reported
Eckert et al. 1998	Ongoing seismic survey (turtles tagged on nesting beach near seismic survey)	NA	NA	Not reported/ Unknown	NA (behavioral not linked to known seismic survey activity)	Unknown	None	Rare	No	Not reported	Not reported	Not reported
Gurjão et al. 2005	Seismic survey (# airguns and additional details not reported)	Not reported	NA	Not reported	Observations made at surface	Not reported	None	Rare	No	Not reported	Not reported	Not reported
Hazel et al. 2009	Vessel movement/noise - 40 horsepower outboard motor at three speeds	Vessel transects in known habitat	Unknown - source approached animal	Motor depth (<1m)	Benthic (on or near substrate) – 2–4	Source approached subject	Depth – turtles could not dive to avoid sound/vessel	Common	No	Not reported	Not reported	Not reported
Lavendar et al. 2014	J9 Speaker - tonal signals 50–1,200 Hz	NA - Behavioral audiogram	Not reported	0.3	Not reported	Stationary source	NA – Behavioral audiogram	Rare	No	No	No	Minimal
Lenhardt et al. 1994	J15 Speaker - tonal signals (250, 500, and 750 Hz)	Not reported	Not reported	Not reported	Not reported	Stationary source	Net pen in river	Rare	No	Not reported	Not reported	Not reported
Lenhardt et al. 1994	J15 Speaker – tone burst, noise burst and frequency sweeps (250 and 500 Hz and white noise)	Not reported	Not reported	Not reported	Not reported	Stationary source	Oval tank (6.9x4.6x1.3m)	Rare	No	No	No	Not reported
Lenhardt 1994	Water coupled speaker, 20–80 Hz tones and sweeps	Exposure started after 2 min of observed resting	Not reported	Speaker coupled to outside of tank	1	Stationary source	Circular tank, 1m depth (dimensions not reported)	Rare	No	No	No	Not reported
Martin et al. 2012	Speaker – tonal signals 100–1131 Hz	NA – Behavioral audiogram	1	0.5	~1	Stationary source	NA – Behavioral audiogram	Rare	No	No	No	Tank pumps

Source	Sound Source	Exposure Type (start of exposure)	Source–Animal Range (start of exposure)	Source Depth (m)	Animal Depth (m)	General Source Movement (relative to subject)	Navigational Constraints (is subject confined in any way?)	Exposure Novelty (is source type common/ rare for area?)	Exposure Similar to Predator Sounds?	Other Species Present in the Area?	Predator Species Present in the area?	Other Anthropogenic Presence/ Noise in Area? (type and proximity)
McCauley et al. 2000	Single airgun (20 in^3)	Not reported	NA	Not reported	Not reported	Approaching/departing	Cage in open water	Rare	No	Not reported	Not reported	Not reported
Moein et al. 1994	Seismic airgun (one at each end of net pen)	When turtles in center of net equidistant from airguns	Not reported	Not reported	Not reported	Stationary source	Net pen in river	Rare	No	Not reported	Not reported	Not reported
O'Hara and Wilcox 1990	Airgun with 165 cm^3 capacity (1) and pneumatic popper 13 cm^3 capacity (2) presented simultaneously	Not reported	Not reported	2	Unknown	Stationary source	Exposures too place in a canal (with net to prevent leaving canal)	Rare	No	Not reported	Not reported	Not reported
Tyson et al. 2017	Vessel noise	Opportunistic (freely swimming turtle encountering vessels in habitat)	Unknown	Motor depth (<1m)	Variable (data available/ reported in figures)	Variable	None	Common	No	Not reported	Not reported	Yes, other vessels present
Weir 2007	Two airgun arrays (24 airguns 30–290 cu. in. each) fired alternately	Observations made during seismic survey	NA	4–8	Observations made at surface	Not reported	None	Rare	No	Not reported	Not reported	Not reported

Table B-2c. Summary of published in-water sea turtle behavioral response to sound studies: noise exposure metrics
Guided by Table 1 of Southall et al. (2021).

Source	Continuous or Intermittent Exposure	Interval Between Exposures	Individual Duration (s)	Total Exposure Duration	Order of Multiple Exposures (identify seqency/order)	Harmonics Present? (none, few, many)	Sound Source Level (e.g., RMS SPL, SEL, SELcum, peak-to-peak)	Received Level @ Change Point of Max. if No Change (e.g., RMS SPL, SEL, SELcum, peak-to-peak)
DeRuiter and Larbi Doukara 2012	Intermittent	19.4 s	Not reported	NA	NA	Not reported	252 dB re: 1 uPa (peak)	Various received levels modeled and probability of diving response as a function of min range from airgun reported
Eckert et al. 1998	Intermittent	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Gurjão et al. 2005	Intermittent	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Hazel et al. 2009	Continuous during approach	NA	NA	Not reported	NA	Not reported	Not reported	Not reported
Lavendar et al. 2014	Intermittent	11–14 presentations per s	0.05	NA – Behavioral audiogram	To identify threshold tone intensity was reduced until no response was recorded	Not reported	Multiple – Behavioral audiogram	Threshold levels (lowest response recorded) at 76 dB re: 1uPa at 800 Hz (SPL)
Lenhardt et al. 1994	Intermittent	15 min	0.12	1.1 per s for 5 min	Not reported	Yes	Not reported	Not reported
Lenhardt et al. 1994	Intermittent	15 min	Not reported	Not reported	Random	Yes	Not reported	Not reported
Lenhardt 1994	Continuous	NA	60	1 min	NA	Not reported	Not reported	Reported as vibration "startles" ~12–16 dB (Intensity dB re: 1um RMS displacement)
Martin et al. 2012	Intermittent	NA	2	NA – Behavioral audiogram	To identify threshold tone intensity was reduced until no response was recorded	Not reported	Multiple–- Behavioral audiogram	Threshold levels (lowest response recorded) at 98 dB re: 1 uPa at 100 Hz (SPL)
McCauley et al. 2000	Intermittent	10 s	Not reported	1–2 hours	NA	Not reported	Not reported	166 dB re: 1uPa RMS increased swimming activity, 175 dB re: 1uPa RMS erratic behavior
Moein et al. 1994	Intermittent	10 min	Not reported	Discharged every 5–6s for 5 min	Quietest to loudest exposure, random determination of which airgun discharged	Not reported	175, 177, and 179 – units and distance measured not reported (equidistant from two airguns in pen)	Not reported
O'Hara and Wilcox 1990	Intermittent	7.5 or 15 s	Not reported	20–36 hours (duration of noise exposure)	NA	Not reported	Not reported	Not reported

Source	Continuous or Intermittent Exposure	Interval Between Exposures	Individual Duration (s)	Total Exposure Duration	Order of Multiple Exposures (identify seqency/order)	Harmonics Present? (none, few, many)	Sound Source Level (e.g., RMS SPL, SEL, SELcum, peak-to-peak)	Received Level @ Change Point of Max. if No Change (e.g., RMS SPL, SEL, SELcum, peak-to-peak)
Tyson et al. 2017	Intermittent	NA	Variable	Variable	NA	Yes	Unknown	Variable (approaching, departing vessels)
Weir 2007	Intermittent	18.75–25 (reported as m)	Not reported	1.5–12 hours	NA	Not reported	Minimum intensity within frequency bandwidth (<120 Hz) 203–208 dB re: 1 uPa per Hz @ 1m	Not reported

Sources for Tables B-2a, B-2b, and B-2c:

DeRuiter S, Larbi Doukara K. 2012. Loggerhead turtles dive in response to airgun sound exposure. Endangered Species Research. 16:55–63.

Eckert S, Bowles A, Berg E. 1998. The effect of seismic airgun surveys on leatherback sea turtles (*Dermochelys coriacea*) during the nesting season. Final report to BHP Petroleum (Trinidad) Ltd.

Gurjão L de, Freitas J de, Araújo D. 2005 Observations of marine turtles during seismic surveys off Bahia, Northeastern Brazil. Marine Turtle Newsletter. 1-3.

Hazel J, Lawler IR, Hamann M. 2009. Diving at the shallow end: Green turtle behaviour in near-shore foraging habitat. Journal of Experimental Marine Biology and Ecology. 371:84-92.

Lavender AL, Bartol SM, Bartol IK. 2014. Ontogenetic investigation of underwater hearing capabilities in loggerhead sea turtles (*Caretta caretta*) using a dual testing approach. The Journal of Experimental Biology. 217:2580-9.

Lenhardt M. 1994. Seismic and very low frequency sound induced behaviors in captive loggerhead marine turtles (*Caretta caretta*). In: Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-351, pp. 238–241.

Lenhardt M, Moein S, Musick J, Barnard D. 1994. Evaluation of the response of loggerhead sea turtles (*Caretta caretta*) to a fixed sound source. In: Prepared for the U.S. Army Corps of Engineers, Waterways Experiment Station Tech Report.

Martin KJ, Alessi SC, Gaspard JC, Tucker AD, Bauer GB, Mann DA. 2012. Underwater hearing in the loggerhead turtle (*Caretta caretta*): a comparison of behavioral and auditory evoked potential audiograms. The Journal of Experimental Biology. 215:3001-9

McCauley R, Production A, Association E. 2000. Marine seismic surveys: a study of environmental implications. APPEA. 692-708.

Moein SE, Musick JA, Keinath JA, Barnard DE, Lenhardt M, George R. 1994. Evaluation of seismic sources for repelling sea turtles from hopper dredges. Report from Virginia Institute of Marine Science, Gloucester Point, VA, to US Army Corps of Engineers.

O'Hara J, Wilcox J. 1990. Avoidance responses of loggerhead turtles, *Caretta caretta*, to low frequency sound. Copeia. 1990:564-567.

Southall BL, Nowacek DP, Bowles AE, Senigaglia V, Bejder L, Tyack PL. 2021. Marine Mammal Noise Exposure Criteria: Assessing the Severity of Marine Mammal Behavioral Responses to Human Noise. Aquatic Mammals. 47(5):421-64.

Tyson RB, Piniak WE, Domit C, Mann D, Hall M, Nowacek DP, Fuentes MM. 2017. Novel bio-logging tool for studying fine-scale behaviors of marine turtles in response to sound. Frontiers in Marine Science. 4:219.

Weir C. 2007. Observations of marine turtles in relation to seismic airgun sound off Angola. Marine Turtle Newsletter.

U.S. Department of the Interior (DOI)

DOI protects and manages the Nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors the Nation's trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities.

Bureau of Ocean Energy Management (BOEM)

BOEM's mission is to manage development of U.S. Outer Continental Shelf energy and mineral resources in an environmentally and economically responsible way.

BOEM Environmental Studies Program

The mission of the Environmental Studies Program is to provide the information needed to predict, assess, and manage impacts from offshore energy and marine mineral exploration, development, and production activities on human, marine, and coastal environments. The proposal, selection, research, review, collaboration, production, and dissemination of each of BOEM's Environmental Studies follows the DOI Code of Scientific and Scholarly Conduct, in support of a culture of scientific and professional integrity, as set out in the DOI Departmental Manual (305 DM 3).