OCS Study
BOEM 2022-004

Behavioral effects of sound sources from offshore renewable energy construction on the black sea bass (*Centropristis striata*) and longfin squid (*Doryteuthis pealeii*)

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

OCS Study
BOEM 2022-004

Behavioral effects of sound sources from offshore renewable energy construction on the black sea bass (*Centropristis striata*) and longfin squid (*Doryteuthis pealeii*)

April 2023

Authors:
Jenni A. Stanley
Paul E. Caiger
Ian T. Jones
Sofie M. Van Parijs
Beth Phelan
Katie Shelledy
T. Aran Mooney

Prepared under M17PG00029
By
Woods Hole Oceanographic Institution
Marine Research Facility
266 Woods Hole Road,
Falmouth, MA 02540

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

DISCLAIMER

This study was funded, in part, by the U.S. Department of the Interior, Bureau of Ocean Energy Management (BOEM), Environmental Studies Program, Washington, DC, through Interagency Agreement Number M17PG00029 with the National Oceanic and Atmospheric Administration National Marine Fisheries Service Northeast Fisheries Science Center. This report has been technically reviewed by BOEM, and it has been approved for publication. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of BOEM, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REPORT AVAILABILITY

CITATION

Stanley, J, Mooney, TA, Jones, IT, Phelan, B, Van Parijs, SM, Shelledy, K. (Woods Hole Oceanographic Institution, Woods Hole MA). 2023. Behavioral effects of sound sources from offshore renewable energy construction on the black sea bass (*Centropristis* striata) and longfin squid (*Doryteuthis pealeii*). Woods Hole, MA): U.S. Department of the Interior, Bureau of Ocean Energy Management. 140 p. Report No.: OCS Study BOEM 2022-004. Contract No.: M17PG00029.

ACKNOWLEDGMENTS

There are a large number of people who have assisted on this project through the three years that without their help this project would have not possible.
We would like to thank the staff at National Oceanic and Atmospheric Administration (NOAA) James J. Howard Marine Sciences Laboratory, NJ, for fish husbandry, experimental assistance and general helpful support. Specifically, Gina Badlowski, John Rosendale and Jeffrey Pessutti for conducting large tank experiments with the black sea bass. Abigail Keller and Julian Olden for statistical assistance. We would also like to thank the Passive Acoustics Group, Northeast Fisheries Science Center, NOAA, WH, MA, for general support and advice.

We would also like to thank Marie Renahan for assisting with experiments and husbandry at Woods Hole Oceanographic Institution, and James Michaelson for advice on computed tomography imaging and Zhongchang Song and Walid Naciri for opportunistic imaging. We also thank the staff at the Marine Biological Laboratory's Marine Resources Department, Woods Hole, MA, and the captain and crew of R/V Gemma for collecting and providing squid used in this study. Zhongchang Song, Hadley Clark, James Peyla, and Madison Schumm played important co-author roles in data collection, analysis, and manuscript revisions. Seth Cones, Jessica Perelman, Destiny Reese, Francesco Caruso, Paul Caiger, and additional volunteers assisted with squid transport and husbandry. We thank Casey Zakroff for helpful advice on squid husbandry and Rodger Hanlon for valuable input on analysis and interpretation of the behavioral results. Blake Hansen provided valuable statistical advice.

Arthur Newhall, Gopu Potty, Ying-Tsong Lin, James Miller, and their colleagues provided audio recordings and particle acceleration data from the Block Island Wind Farm. Rick Galat, Ed O'Herty, and the ESL Facilities support team at WHOI enabled much of this work. Finally, we gratefully acknowledge Brian Hooker, Erica Staaterman and team members at the Bureau of Ocean Energy Management for their support and advice throughout the project.

Jenni Stanley
Aran Mooney
Ian Jones
Beth Phelan

Table of Contents

List of Figures

List of Tables

Abbreviations and Acronyms

BOEM	Bureau of Ocean Energy Management
MBL	Marine Biological Laboratory
OWF	offshore wind farm
PRI	pulse repetition interval
PSD	power spectral density
SPL	sound pressure level (dB re 1 μPa)
$SPL_{z\text{-}pk}$	zero-to-peak sound pressure level
$SAL_{z\text{-}pk}$	zero-to-peak sound acceleration level
SE	Standard error
SEL_{cum}	Cumulative sound exposure level
SEL_{ss}	Single strike sound exposure level
TL	Total length
X_{z-pk}	Median zero-to-peak
WHOI	Woods Hole Oceanographic Institution

1 Introduction

1.1. Background on underwater sound

Sound travels efficiently in the ocean at about 1,500 m/s—five times faster than it does in air. Acoustic signals consist of two components: sound pressure and particle motion. The former is a compression and rarefaction wave. It is a scalar quantity that acts in all directions. It can be described in terms of its magnitude, as well as its temporal and frequency characteristics. In contrast, particle motion is a back-and forth motion and, as such, is a vector quantity. Accordingly, particle motion can be described not only by specifying its magnitude and temporal and frequency characteristics but also by its direction of motion. Sound pressure is expressed in SI units of pascals (Pa) or micropascals (µPa). Particle motion may be expressed in terms of the particle displacement (SI unit: meter) or its time-related derivatives: particle velocity (meters per second) or particle acceleration (meters per second squared). Sound intensity is the product of the sound pressure and the particle velocity, for which the SI units are watts per meter squared (W/m^2).

In addressing acoustic impacts, it is fundamentally important to understand sensitivity. All fishes (including elasmobranchs) and an increasingly identified number of invertebrates detect and use particle motion, particularly at frequencies below several hundred hertz. Detection of pressure in water requires a compressible cavity such as an air bubble or swim bladder. Some fishes have evolved with air bubbles located just under external hair cells or with a variety of swim bladder extensions to the inner ears, all adaptations that enhance their detection of pressure. When addressing the effects of sounds on fishes and invertebrates, it is vital to describe the sounds in terms of particle motion as well as sound pressure. This may be done by measuring the particle motion directly or by conducting experiments under free-field acoustic conditions, where particle motion can be predicted from measurements of sound pressure. However, near boundaries, such as the seafloor and sea surface, or water that does not act as the free and far field, particle motion cannot easily be predicted by pressure so direct measurements are ideal.

1.2. What is known about the effects of noise on commercially important marine organisms

This topic also is thoroughly discussed in the background section of each study.

There are several review papers discussing in great detail the potential effects of anthropogenic sounds on marine animals, written by the pioneers of the field; (Hawkins et al. 2015, Hawkins and Popper 2016, Popper and Hawkins 2019). There are also publications on several meetings on the Effects of Noise on Aquatic Life, which outline much of the research being conducted in this field (Popper 2012, Popper and Hawkins 2016).

1.2.1. Fishes

Over the past several decades, there has been increasing scientific interest and in turn mounting evidence in the potential effects of anthropogenic sounds on marine fishes (Reviews; Popper et al. 2003b, Popper and Hawkins 2019). Initially, much of the research focused on the effects on marine mammals (Southall et al. 2009, Popper and Hawkins 2019), however, through better understanding and research there is growing evidence of effects upon organisms which make up a much greater part of the marine biomass, including fishes (both commercially and ecologically significant), invertebrates and plankton (Popper 2012, Popper and Hawkins 2016).

The added sound humans are emitting into the marine environment could potentially have a wide range of effects on commercially important fish species, with exposure to very intense or loud sounds resulting in damage to hearing structures, body tissues or even death. However, more importantly are the associated issues with sound and the potential to affect an animal's behavior that could also result in effects on populations and ecosystems. These include movement away from breeding or feeding grounds, diversion from migration routes, and interference with acoustic communication which can subsequently affect reproductive behaviors and prevent correct detection of other biologically relevant sounds.

The range of structures and activities involved during offshore wind farm construction brings a variety of potential noise sources and levels. One of the most significant activities during the construction is the installation of foundations (International Organization for Standardization 2017). This is most often achieved using impact or vibrational/percussive hammers that can produce a wide range of peak source sound levels. Contact of the pile with the water and striking of the hammer on the pile create acoustic waves that radiate out from the pile through the water column and substrate via multiple paths, resulting in loud, high-energy, impulsive sounds with sharp rise times (for a review see Andersson et al. 2016). Underwater sound levels (both particle motion and sound pressure) and detection distances vary substantially by site and depend on many factors, including substrate characteristics, depth, pile diameter, size of impact hammer, and how they are measured. However, sound pressure levels measured from field examples are on the order of 220 dB re 1 μPa at a range of ~10 m and 200 dB re 1 μPa at a range of 300 m from 0.75 m and 5 m diameter piles, respectively (Reinhall and Dahl 2011b). The predominant energy is below 500 Hz, with some energy extending past 1 kHz, and with sharp rise times to maximum energy. The measured frequency range directly overlaps the auditory bandwidth of many fish species across multiple lifestyles (e.g., pelagic, epibenthic, demersal), including cod, salmon, black sea bass, flatfish, and squid, to name a few (Chapman and Hawkins 1973, Hawkins and Chapman 1975, Mooney et al. 2010, Popper et al. 2019, Stanley et al. 2020). Predicting effects can be complicated because an acoustic pulse changes as it propagates. Measured peak-to peak sound pressure levels may be 205 dB re 1 μPa at 100 m, but signals are still detectable out to 70 km (Bailey et al. 2010). At close range (1 km), the initial waveform peak is pronounced, lasting 10 ms; however, durations increase to 200 ms at 40 km, illustrating that signals become less impulsive at greater distances (Bailey et al. 2010). The particle motion component and substrate transmission have been far less monitored and reported. However, the particle motion component is likely far more relevant to many important fisheries species (fishes and invertebrates). Miller et al. (2018) measured and estimated the particle velocity and sound pressure levels from pile-driving activity during construction of the Block Island Wind Farm. They reported zero-to-peak total sound velocity levels of ~110 dB re 1 nm/s (vector sum) (tetrahedral hydrophone array) and ~124 dB re 1 nm s–1 (geophone), and peak-to-peak received sound pressure levels of ~185 dB re 1 μPa (tetrahedral hydrophone array) from one hammer strike 500 m from the activity (Miller et al. 2018).

A number of studies have investigated the effects of construction noise exposure on fishes. These studies used a range of methods and species. Consequently, results indicate a variety of impacts ranging from severe physical injury to no effect, making it difficult to extrapolate across taxa.

Studies investigating the lethal and permanent effects caused by pile driving noise have revealed a variety of results in multiple species, ranging from mortality to damage to hearing tissues and other organs (Popper and Hastings 2009b). For example, many injury types were observed in hybrid striped bass/white bass (*Morone chrysops/saxatilis*) in large and small size classes when exposed to simulated pile driving signal using a High Intensity Controlled Impedance Fluid Filled wave tube (Casper et al. 2013a). Injury number and severity increased with fish size. Similar results were also found in lake sturgeon (*Acipenser fulvescens*) and Nile tilapia (*Oreochromis niloticus*), with injury occurring at the lowest levels tested 204 SELcum (204 dB re 1 μPa^2*s) and 174 SELss (174 dB re 1 μPa^2*s), with more severe and greater number of injuries occurring at the loudest cumulative and single-strike sound exposure levels. Yet species differ; at these levels there were no injuries observed in ‘hogchoker’ flatfish (*Trinectes maculatus*) (Halvorsen et al. 2012). Using different methods, caged northern anchovy (*Engraulis mordax*) and common sole larvae (*Solea solea*) showed no increase in mortality or pathology compared to control groups when exposed to 4-min of pile driving (9.75 m from a 0.61 m diameter pile) and simulated pile driving sound levels (up to 210 dB re 1 μPa^2 zero-to-peak (z-p)) (Abbott et al. 2005, Bolle et al. 2012). Fishes with physoclistous swim bladders appear to be more susceptible to injury from impulsive noise sources, including pile driving, than fishes with physostomous swim bladders (Halvorsen et al. 2012, Casper et al. 2013a). Fishes without swim bladders, such as sole or hogchokers (as mentioned above) may be less susceptible to injury.

As the sound from pile driving can propagate large distances, there is a much larger area where sub-lethal sound levels are a concern, including changes to respiration rates, oxygen uptake, stress and stress markers, swimming and schooling behavior, alarm responses, and feeding or foraging behavior. European seabass (*Dicentrarchus labrax*), the most widely studied of the fishes, have been found to change their schooling structure and dynamics, becoming less cohesive, directionally ordered, and poorly correlated in speed and directional changes when exposed to playbacks of pile driving (SEL$_{cum}$ 154 dB re 1 μPa^2*s). Exposure significantly disrupted the organization of their shoals and abilities to coordinate their movements with one another, behaviors which are ecologically beneficial for information exchange and reducing predation risk (Herbert-Read et al., 2017). Additionally, when exposed to impulsive, low-frequency noise (200-1000 Hz, mean SPLz-p 180-192 dB re 1 µPa) seabass exhibit increased swimming speeds and depths, reduced inter-fish distances, increased startle responses, and increased movement away from the sound source (Neo et al. 2016). Black seabream (*Spondyliosoma cantharus*) and European seabass have been found to increase ventilation rates and/or oxygen uptake when exposed to replayed and *in situ* pile driving noise (184 SEL_{cum}, 184 dB re 1 µPa^2*s) whereas European plaice (*Pleuronectes platessa*) showed no significant differences (Bruintjes et al. 2016b, Poulton et al. 2016). Furthermore, intense, impulsive sounds have been documented to affect primary (cortisol) and secondary responses (adenylate, glucose, lactate) in European seabass at considerable distances from the source (2000 m). Concern arises if homeostasis is not recovered, or repeated effects arise, because tertiary responses may affect growth, disease resistance and fecundity, which in turn can have population-level effects by reducing reproductive capacity and abundance (Debusschere et al. 2016).

However, some studies show little to no responses to exposures and support the premise that certain species will be at more risk to noise impacts than others, and even individuals or populations within a species could show substantial variation in responses. Telemetry tagged sheepshead (*Archosargus probatocephalus*) showed no significant decrease in daytime residency or displacement during 35 days of pile driving at a wharf complex (Iafrate et al. 2016).

One of the most prevalent, yet poorly understood, sublethal effects of underwater noise is auditory masking. This is where a receiver experiences an increase in the threshold of acoustic detection or discrimination of a signal which could potentially lead to partial or complete loss of received signal, misinterpretation of the signal, and/or changes in a response, due to an unwanted masking noise containing sufficient energy inside the detectible frequency range (Hawkins and Chapman 1975, Dooling and Blumenrath 2016, Popper and Hawkins 2019). Masking is frequently examined with respect to continuous noise, however impulsive noise sources, such as percussive pile-driving, can also impair detection. During the construction of OWFs, pile driving can occur episodically at one location for days to weeks at a time. Similar to continuous sounds (Hawkins and Chapman 1975, Stanley et al. 2017), impulsive pile driving noise has the potential to decrease an animal's communication space and/or listening range, with subsequent effects to fitness, during this time (Pine et al. 2020). Because of the intermittent nature of the signal there are potential 'masking releases' that could occur, yet, as noted earlier, impulsive pile driving noise tends to "smear" toward more continuous noise through distance and propagation effects (Bailey et al. 2010). Beyond simple masking, lower-level intermittent sounds can also cause distraction, limiting detection of biologically relevant communication or predator sounds (Chan et al. 2010).

1.2.1.1. Black sea bass (*Centropristis striata*)

The black sea bass *Centropristis striata* (Linnaeus 1758), which is the study species for Studies 1 – 4, is a principal target species in the western North Atlantic. This is a warm temperate species that shows an attraction toward structurally complex habitats, including rocky reefs, cobble and rock fields, stone coral patches, exposed stiff clay, and mussel beds (Steimle et al. 1999). Black sea bass occur along the entire eastern seaboard of North America. However, the species exists as three populations or stocks: northern, southern, and Gulf of Mexico. For the northern stock, which is the focus of this project, Cape Cod is typically the northernmost endpoint, with this population undergoing a seasonal migration, moving north and inshore from southern and deeper waters respectively in late spring (Steimle et al. 1999). This stock also supports a valuable commercial and recreational fishery (South East Data 2018). There is some circumstantial evidence that *C. striata* communicate acoustically (Fish and Mowbray 1970), and potentially during spawning events, however these signals were made under duress and in a small tank environment (making precise measurements difficult), behavioral relevance is therefore unknown. Additionally, there is one study that elicited young of the year in this species to approach a predetermined feeding space when presented with a 280 Hz pure tone (Lindell et al. 2012). However, prior to the study there are no published records of either sound-production or the auditory thresholds/sensitivities of *C. striata*. Therefore, whether sounds from anthropogenic activities (e.g., pile driving) are within the communication and/or hearing range of this species has yet to be definitively confirmed. Similarly, there have been no previous studies investigating the effects of pile-driving on the behavior of black sea bass.

1.2.2. Cephalopods

Cephalopods are mollusks belonging to the class Cephalopoda, including squid, cuttlefishes, and octopuses (and nautiluses, which are not considered here). These animals, especially select species of squid such as *Doryteuthis* (formerly *Loligo*) *pealeii*, common cuttlefish *Sepia officinalis*, and octopuses such as *Octopus vulgarus*, have long been the subjects of neurobiological and behavioral research (Gilbert et al., 1990; Hanlon & Messenger, 2018). For certain cephalopod species, much is known about the behaviors of these enigmatic animals, including behaviors employed for defense, camouflage, feeding, and reproduction, and they are models for ethology and neurobiology (Hanlon & Messenger, 2018; Hanlon et al., 1999a; Shashar & Hanlon, 2013). Extensive investigations have been conducted on cephalopod sensory systems, including visual receptors, mechanoreceptors, chemoreceptors, and neural pathways between these sensory systems, the brain, and “effectors”, e.g., muscles that carry out behavioral responses to sensory stimuli (Gleadall & Shashar, 2004; Hanlon & Messenger, 1988; Hanlon & Shashar, 2003; Wells & Wells, 1956). Further, learning and cognitive abilities, many of which are considered “advanced” among invertebrate taxa, have been described in multiple cephalopod species, including habituation of *Lolliguncula brevis* to visual stimuli (Long et al., 1989), and associative learning, spatial learning, and memory of *S. officinalis*, *O. vulgarus*, and other species (Mather, 1991; Mather & Kuba, 2013; Scatà et al., 2016; Schnell et al., 2021). Several species such as the Hawaiian bobtail squid (*Euprymna scolopes*) have been cultured as model organisms for studying developmental biology, neurobiology, gene regulation, co-speciation, and various aspects of behavior and physiology (Hanlon et al., 1997; Kerwin et al., 2021; Montague et al., 2021; Zepeda et al., 2017).

1.2.2.1. Longfin squid (*Doryteuthis pealeii*)

The longfin squid *D. pealeii*, which is the study species for Studies 5 – 7, has been the study subject of decades of neurophysiological and behavioral research. It has long been a model species for biomedical and neurobiological work, and much of the basic knowledge of nerve fiber mechanisms has been obtained from the giant axon of this species (Gilbert et al., 1990). Behaviors of *D. pealeii* have been extensively observed and quantified, including anti-predator defense behaviors and strategies (Crook et al., 2014; Staudinger et al., 2011), and reproductive behaviors around egg spawning beds (Shashar & Hanlon, 2013). A detailed ethogram describing their behavioral repertoire has been published (Hanlon et al., 1999).

Compared to other marine invertebrate phyla, the morphology of the cephalopod sound detection sensory organ, the statocyst, is relatively well understood (Budelmann, 1979, 1990; Hanlon & Budelmann, 1987). However, *bioacoustic* research on cephalopods is in its infancy; little is known about their sensitivities to sound outside a handful of studies (e.g., Budelmann and Williamson 1994; Mooney et al. 2010; Packard et al. 1990; Samson et al. 2016). There is a clear avenue to leverage the broad wealth of foundational neurophysiological, behavioral, and ecological research on cephalopods toward examining these animals' acoustic sensitivities. Beyond interest among research groups, cephalopods are of considerable commercial value, making up a 6% share (USD) of global exports and about 4.5% of global capture production from 2012–2018 (FAO, 2021). Select species hold great commercial value in certain regions. For instance, the longfin squid (*D. pealeii*) fishery in New England has had annual landed values of about $30 million since 2010 (NMFS, 2021). Cephalopoda is further considered an ecological keystone taxon because many species occupy central positions in marine food webs (Boyle & Rodhouse, 2005). Cephalopods comprise large portions of the diets of many marine mammals, seabirds, and predatory fish. For example, squid can constitute over 50% of regional seabird diets and up to 95% of odontocete diets [ibid]. In turn, many cephalopods are opportunistic predators that feed on a wide variety of prey throughout their lifetime, such as copepods (consumed by pelagic pre-adults), benthic crustaceans and bivalves, and fishes (Boyle & Rodhouse, 2005; Hunsicker & Essington, 2006). The widespread ecological roles and the commercial values of cephalopods incentivize their use in research to better understand marine invertebrates' ecological uses of sound and how anthropogenic noise pollution may adversely impact individual fitness and populations.

Currently, cephalopods comprise ca. 15-20% of total global fisheries landings (Clarke 1977, Clarke 1996, Hunsicker et al. 2010). The market squid (*Doryteuthis opalescens*) of California has been the state's largest and most valuable fishery since 2009 as catches can exceed 66% (135,018 tons) of the total commercial landings (CA Dept of Fish & Wildlife 2016). Indirectly as prey, squid such as *Illex, Doryteuthis* and *Loligo sp.* support some of the most important fisheries in the Atlantic and Pacific (tuna, hake and swordfish) (Clarke 1996, Boyle and Rodhouse 2005).

D. pealeii are readily available in Woods Hole from May-December, allowing for a long experimental season. They are migratory, pelagic and a well-established model for sensory ecology and neurobiological investigations (Hanlon et al. 2013b). The species is broadly distributed on the Atlantic continental shelf and is the primary commercial cephalopod species of the western north Atlantic at approximately 16.6 mt and $41 million yr^{-1} since 2000 (NOAA 2010, 2011). They are consumed by a wide range of predators and are considered a key prey for a variety of marine mammals, seabirds and finfish (Clarke 1996, Overholtz et al. 2000, NOAA 2010).

2 General Methodology

2.1. Animals, collection and husbandry

2.1.1. Black sea bass (*Centropristis striata*)

While there were some small differences between studies, methods of collection and husbandry were generally similar and were as follows: *Centropristis striata* for studies 1 – 4 were collected under Scientific Commercial Permit 175150, administered by the Commonwealth of Massachusetts Department of Fish and Game and under Scientific Collecting Permit MFA-SCP No.1834, administered by the State of New Jersey Department of Environmental Protection.

Juvenile *C. striata* were collected in baited minnow traps in estuarine habitats at Salt Pond, Falmouth and Great Harbor, Woods Hole (WH), MA, USA, as well as hand captured on SCUBA in coastal habitats in Buzzards Bay, Woods Hole. Adult BSB were collected in Vineyard Sound, MA, and coastal New Jersey (NJ) by line fishing.

Depending on the site of the experiments (WH or NJ) fish were transported to the NOAA James J. Howard Marine Sciences Laboratory (NJ) or the Environmental Systems Laboratory (ESL) at Woods Hole Oceanographic Institution, Woods Hole MA, where husbandry and experiments were conducted. Fish were held in flow-through holding tanks of at least 1.2 m (3400 L) in diameter and were kept at low stocking levels so that water inflow to tanks could be kept at low levels to minimize chronic tank noise disturbance. Depending on the time of year, fish were held at constant temperatures (see individual studies). All tanks were subject to a natural light cycle. Fish were fed every 2 days to satiation, with squid (*Doryteuthis pealeii*) or green shore crab (*Carcinus maenas*). All experiments and animal care were undertaken in accordance with approval of the National Oceanic and Atmospheric Administration Animal Care Policy and the Woods Hole Oceanographic Institution's Institutional Animal Care and Use Committee under ID number BI24843.00. Any fish that was deemed not healthy, owing to capture or otherwise, was not used in the experiments.

2.1.2. Longfin squid (*Doryteuthis pealeii*)

While there were some small differences between studies, methods of collection and husbandry were generally similar and were as follows: Squid were collected in Vineyard Sound via trawl, by the Marine Biological Laboratory (Woods Hole, MA). As the collection vessel returned to dock, squid with minimal physical damage (e.g., few or no skin lesions or tears) were gently hand-transferred to coolers filled with ambient temperature Vineyard Sound seawater. The squid were immediately driven to the ESL at Woods Hole Oceanographic Institution, Woods Hole MA, where husbandry and experiments were conducted. Upon arrival at the ESL, squid were immediately and gently hand-transferred into circular holding tanks of at least 1.2 m diameter (3400 L), with ambient flowing seawater. All tanks were located in a facility subject to a natural light cycle. Sexes were kept separate, and densities were below one squid per 680 L. During experimental periods, means + SD of environmental measurements across holding tanks were as follows: temperature: 20.63 + 1.06 °C; salinity: 30.49 + 1.40 PSU; pH: 7.93 + 0.05; DO saturation: 100 + 2%. Squid were hand-fed daily with *Fundulus* spp. collected from local estuaries (WHOI IACUC approval to TAM). Squid were held in these lab conditions for a minimum of 24 hours prior to being tested, and experimenters took care to minimize sound in and near the holding tanks during this period.

2.2. Pile driving signals used in current studies

Audio files of pile driving noise used for experiments were recorded during construction of Block Island Wind Farm on October 25th, 2015 (between 16:00 and 20:00 UTC), from a hydrophone (High Tech Inc., model: HTI-94-SSQ, sensitivity: -203.8 dB re 1 V/µPa, gain: 6 dB, flat frequency response from 2 Hz to 30 kHz) on a benthic sled located 26 m deep and 0.5 km away from a pile driving site (Amaral et al., 2018). The hydrophone recordings were provided by Arthur Newhall and Ying-Tsong Lin, (WHOI) and James Miller (University of Rhode Island). The hydrophone was about 1 m above the seabed, and part of a tetrahedral array of hydrophones used to calculate particle acceleration via pressure differentials between hydrophones along three orthogonal axes. The hydrophones were spaced 0.5 m apart and were the same model and had the same sensitivity and frequency response noted above. These acceleration data, in m/s^2, were provided to the authors by Gopu Potty (University of Rhode Island). These files were recorded at a 9766 Hz sample rate. The hollow steel pile used in the construction of the offshore wind platform had a diameter of 127.0 cm, a wall thickness of 3.8 cm, a rake of 13.27° with respect to vertical, and was driven up to 76.2 m deep into the seabed.

3 Study Species 1: Black Sea Bass (*Centropristis striata*)

3.1. Study 1: Ontogenetic variation in the auditory sensitivity of *Centropristis striata*

Published as: Stanley, J. A., Caiger, P. E., Phelan, B., Shelledy, K., Mooney, T. A., & Van Parijs, S. M. (2020). Ontogenetic variation in the auditory sensitivity of black sea bass (*Centropristis striata*) and the implications of anthropogenic sound on behavior and communication. *The Journal of Experimental Biology, 223*(13), jeb.219683. doi:https://doi.org/10.1242/jeb.219683.

3.1.1. Background

There is mounting evidence that the increasing anthropogenic noise in the world's oceans can have a range of negative physiological and behavioral effects on marine animals (National Research Council 2003, Popper and Hastings 2009a, Kight and Swaddle 2011). Much of the focus has traditionally been aimed at marine mammals and protected species (Williams et al. 2015), and subsequently, the corresponding regulatory efforts typically address these same taxa (Markus and Sánchez 2018). Fishes are also exposed to the same anthropogenic disturbances, are of huge ecological and economic importance, and yet do not have the same degree of legal protection (Hawkins and Popper 2016). Acute, loud sound sources such as seismic airguns and sonars can cause temporary auditory threshold shifts in fishes (Scholik and Yan 2001, Smith et al. 2004b), severe swim-bladder trauma (Halvorsen et al. 2006), or permanent damage to fish inner ears (McCauley et al. 2003). Furthermore, lower level and/or chronic noise can also have negative impacts on fishes, masking acoustic signals, decreasing signal-to-noise ratios, and thus interfering with a wide range of important behaviors, including feeding (Voellmy et al. 2014a), predator avoidance (Simpson et al. 2016b), group cohesion (Sara et al. 2007, Bruintjes and Radford 2013), settlement behavior (Holles et al. 2013, Simpson et al. 2016a), and/or spawning success (Nedelec et al. 2017, Stanley et al. 2017). These impacts can have fundamental ecological and evolutionary implications for species, especially ones that rely on acoustics in key stages of their life, and ultimately can reduce both fish populations and ecosystem functioning. Renewable energy developments are expanding globally to meet the increasing demand for electricity.

The development on the eastern seaboard of North America (Musial and Butterfield 2004, Snyder and Kaiser 2009, Dvorak et al. 2013) marks the first major marine wind energy installations to be permitted within U.S. waters. With the development of these renewable energy regions there will be an increase in pile-driving during the construction process. There is evidence that marine pile-driving can cause negative effects in fishes, including barotrauma (Casper et al. 2017), anti-predator behavior (Spiga et al. 2017), elevated ventilation rates (Radford et al. 2016), oxygen uptake rates (Bruintjes et al. 2016b), and disruption to schooling dynamics (Herbert-Read et al. 2017). Furthermore, fishermen have recently expressed concern that the sound produced during pile driving – along with benthic surveys and operation of renewable energy facilities – may have negative effects on the behavior and/or distribution of target species (Thomsen et al. 2006). Concerns extend to changes in catch rates and potential long-term sub-lethal behavioral impacts, such as avoidance of essential feeding and spawning habitats, and/or disruption of essential intraspecific communication (Allison et al. 2019). Conversely, the structure created by wind turbine foundation structures below the surface of the water can change the local habitat by creating an artificial reef which, increases heterogeneity, and attracts marine organisms. These reefs have the potential to attract many marine organisms, especially fishes, and research suggests that artificial reefs generally hold greater densities and biomass of fishes, and provide higher catch rates compared to surrounding soft bottom areas (Langhamer 2012). Anecdotally, recreational fishermen are finding this to be true at the Block Island Wind Farm in Rhode Island. However, exactly what site-specific factors supports artificial reefs productivity at the higher trophic levels is unknown (Allison et al. 2019).

Again, there are no published records of either sound-production or the auditory thresholds/sensitivities of *C. striata*. Therefore, whether sounds from anthropogenic activities (e.g., pile driving) are within the communication and/or hearing range of this species is yet to be definitively confirmed.

A common physiological measure of fish hearing is the use of auditory evoked potentials (AEPs). This technique is a non-invasive electrophysiological approach that measures neural responses in a subject to a given sound stimulus, it permits rapid evaluation of the auditory detection bandwidth and repeated testing of animals. Since its utility for fishes was first suggested (Bullock and Corwin 1979, Corwin et al. 1982), and the technique subsequently refined (Kenyon et al. 1998), AEPs have provided baseline auditory sensitivities for over 100 species of fish (Ladich and Fay 2013). Auditory evoked potential measurements do have their limitations. For instance, the thresholds are widely considered to be not as sensitive as behavioral thresholds, because they considered to be a subset (sensory and neurally) of the complete sound perception. However, the estimation of the frequency range (bandwidth) of a species' sound detection capabilities using AEPs is not thought to be of concern (Ladich and Fay 2013). Secondly, sound projection in laboratory tanks is particularly complex (Akamatsu et al. 2002, Rogers et al. 2016a), and likely contain particle motion information beyond what is typically considered the near-field limit (Higgs and Radford 2016). Nevertheless, with these limitations and proper calibrations in mind, AEPs still provide meaningful baseline audiograms. Furthermore, they are particularly useful in a comparative context, such as testing between different species of interest (e.g., Corwin et al. 1982, Kenyon et al. 1998), or for testing sound detection ability through ontogeny (e.g., Higgs et al. 2002, Caiger et al. 2013).

Hearing sensitivity is wide-ranging between fish species and is related to morphology. The basal mechanism for hearing is the mechanical stimulation of the inner ear hair cells (Popper and Fay 1973). However, several species have adapted specializations, such as bones or ligaments to reduce the distance to or connect the swim bladder to the inner ears, enabling detection of the pressure component of the sound field (Webb and Smith 2000, Radford et al. 2013). The traditional terms "specialists" and "generalists" have recently been downgraded in favor of considering fish with and without specializations at either end of a continuum of pressure detection capabilities (Popper and Fay 2011), though these terms still provide some use when keeping this continuum in mind.

The present study sought to document the first records of the auditory detection bandwidth of *C. striata* using auditory evoked potential measurements, measuring levels in terms of both the sound pressure and the experienced particle acceleration components of the sound field. Whether the auditory detection bandwidth or thresholds vary with size or age were assessed by testing across three size groups, from juvenile to adult. Additionally, the mechanism responsible for the sound detection ability in this species was investigated via gross dissections and micro CT imaging of the internal morphology. The potential implications for the assessed auditory detection bandwidth were discussed in relation to the pervasive anthropogenic noises that share acoustic space with this species.

3.1.2. Methods

3.1.2.1. Fish acquisition and maintenance

Centropristis striata were collected under Scientific Commercial Permit 175150, administered by the Commonwealth of Massachusetts Department of Fish and Game. Juvenile *C. striata* were collected in baited minnow traps in estuarine habitats at Salt Pond, Falmouth and Great Harbor, Woods Hole, MA., as well as hand captured on SCUBA in coastal habitat in Buzzards Bay, Woods Hole, MA. Adult *C. striata* were collected in Vineyard Sound, MA, and coastal New Jersey, by line fishing. Any fish that was deemed not healthy, due to capture or otherwise were not used in the experiments. Fish were held in flow-through holding tanks which were kept at low stocking levels so water inflow to tanks could be kept at low levels to minimize chronic tank noise disturbance. Temperature was kept constant for the duration of the trials, in both the holding tanks and AEP setup (14°C ± 2°) to eliminate any potential temperature effects on auditory thresholds. Fish were fed every two days to satiation, with squid (*Doryteuthis paeleii*) or green crab (*Carcinus maenas*). All experiments and animal care were undertaken in accordance with WHOI's IACUC Ethics under ID number BI24843.00.

3.1.2.2. Auditory evoked potentials

Sound detection thresholds were determined for 20 *C. striata* across three size classes using auditory evoked potentials (AEPs), including three dead controls. Four goldfish (*Carassius auratus*) were also measured and served as calibrated audiograms for comparison with other AEP studies in the literature. All *C. striata* and *C. auratus* fully recovered from these procedures, with the exception of the three euthanized control fish. This method provides an instantaneous measure of sound detection ability by measuring an electrical response to sound stimuli in the eighth cranial nerve and brainstem auditory nuclei. Methods used in the present study follow standard AEP methodology, largely adapted from (Higgs et al. 2002, Wright et al. 2005, Strobel and Mooney 2012, Caiger et al. 2013).

Auditory evoked potential experiments were undertaken in two separate laboratories between October 2017 and March 2018: Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA, USA, and the James J. Howard Marine Sciences Laboratory (NOAA), Sandy Hook, NJ, USA (see table 1). Trials were performed in a PVC tank [0.6 m wide, 0.95 m long, 0.7 m deep] (WHOI), and a fiberglass tank [0.65 m wide, 1.25 m long, 0.6 m deep] (NOAA). Fish were initially anaesthetized with a dilute solution of 100 % clove oil (0.1-0.5 mL x L, dependent on fish size) before the trials to permit placement in the fish holder and to reduce large movement during experiments. The anaesthetized fish were positioned laterally upon a custom fish holder (consisting of a plastic board covered in moldable plasticine) and affixed at a perpendicular angle to a plastic rod with a piece of elastic cloth material firmly positioned around the fish's body as a restraint. The operculum was left free to allow respiration to occur normally. The fish holder was then completely submerged in the water. Effects of clove oil as an anesthetic and its concentration on auditory evoked potentials was tested during this study using juvenile individuals, both dosed and non-dosed. There were no effects on the auditory thresholds and also enabled identification of the lowest concentration possible. No muscle relaxants were used for these experiments. The fish were placed ~8 cm below the water surface at the opposite end to the speaker (65 cm away) which was positioned in the middle of the water column facing the fish. Three 27 gauge (0.36 mm diameter) subdermal stainless-steel electrodes (Rochester Electromedical Inc., FL) coated in nail varnish for insulation (except for the tip) were used to collect the AEP signals. The responses of each fish were recorded using the same laptop, program and data acquisition card. The recording electrode was placed dorsally, just posterior to the operculum, the reference electrode was placed dorsally in the nasal region, and a ground electrode was placed in the fish holder's plasticine. Fish were periodically checked during experiments, mostly by means of operculum and mouth movement. Electrodes were connected to a Grass CP-511 bio-amplifier (Astro-Med Inc.), which amplified (10,000-fold) and filtered (10–3000 Hz) the responses. The responses were further filtered (30–3000 Hz) with a Krohn-Hite 3362 filter (Krohn-Hite Corporation, MA, USA). Copper wire and a carbon-rod earth grounded the amplifiers. All equipment ran on batteries to reduce electrical noise and were fully charged daily.

Table 1. Size (mm TL) and location (see text for more details) of AEP testing of Centropristis striata (small, medium and large) and Carassius auratus tested in this study.

Size class (mm total length)	WHOI								NOAA			Mean	SE
Small	94	95	78	93	77	78	78	75				83.5	2.8
Medium	278	295	270	290					280	293	296	283.3	3.5
Large				379	346				440	398	470	362.5	16.5
Carassius auratus	63	77	69	87								74	4.5

Auditory stimuli were digitally generated using custom Labview software (National Instruments; www.ni.com) implemented on a laptop computer (S6520 LifeBook S, Fujitsu). Signal polarity was alternated by this program and sounds were then converted from digital to analog using a data acquisition card (6062E PCMCIA, National Instruments) in the laptop. This card was connected to a BNC connector box (National Instruments) and then to an attenuator (Hewlett–Packard 350D) that was used to control the sound pressure levels in 5 dB steps. Signals were relayed to a battery-powered amplifier (PLA-2210, PYLE Chopper Series, Pyle Audio) and then to an underwater speaker (UW-30, ElectroVoice, Michigan, USA.) to play the outgoing stimuli. All sounds were concurrently monitored on a digital oscilloscope (Tektronix TPS 2014; www.tek.com).

Stimuli consisted of amplitude modulated tone bursts of seven different frequencies presented from 80 to 2000 Hz (80, 100, 150, 200, 300, 400, 600, 1000, 2000 Hz) covering the expected range of fish hearing and considering tank limitations (Popper and Fay 1999). Stimulus duration varied from 10-30 ms, dependent on frequency, with a 3 ms rise-fall time which was found to create purest tone. The presentation order of the frequencies was conducted randomly and were increased in 5 dB increments until a stereotypical AEP response was seen (up to a maximum source level of 147 dB re 1 µPa @ 1 m due to speaker limitations), and then continued for at least another 10 dB to examine supra-threshold responses. At least two measurements (10-15 dB) were made below the apparent threshold to ensure weak responses were not overlooked. A minimum of 800 responses (alternating stimuli presented at 90° and 270° phases) were averaged together for each sound level at each frequency to cancel any stimulus artefacts. The auditory threshold was visually defined as the lowest level at which a definitive response could be detected (see Figure 1 for an example). Visual detection has been shown to produce comparable results to the use of statistical approaches (Kenyon et al. 1998, Mann et al. 2001). As controls, euthanized fish were tested in the apparatus and live fish were presented with no stimulus (Figure 1).

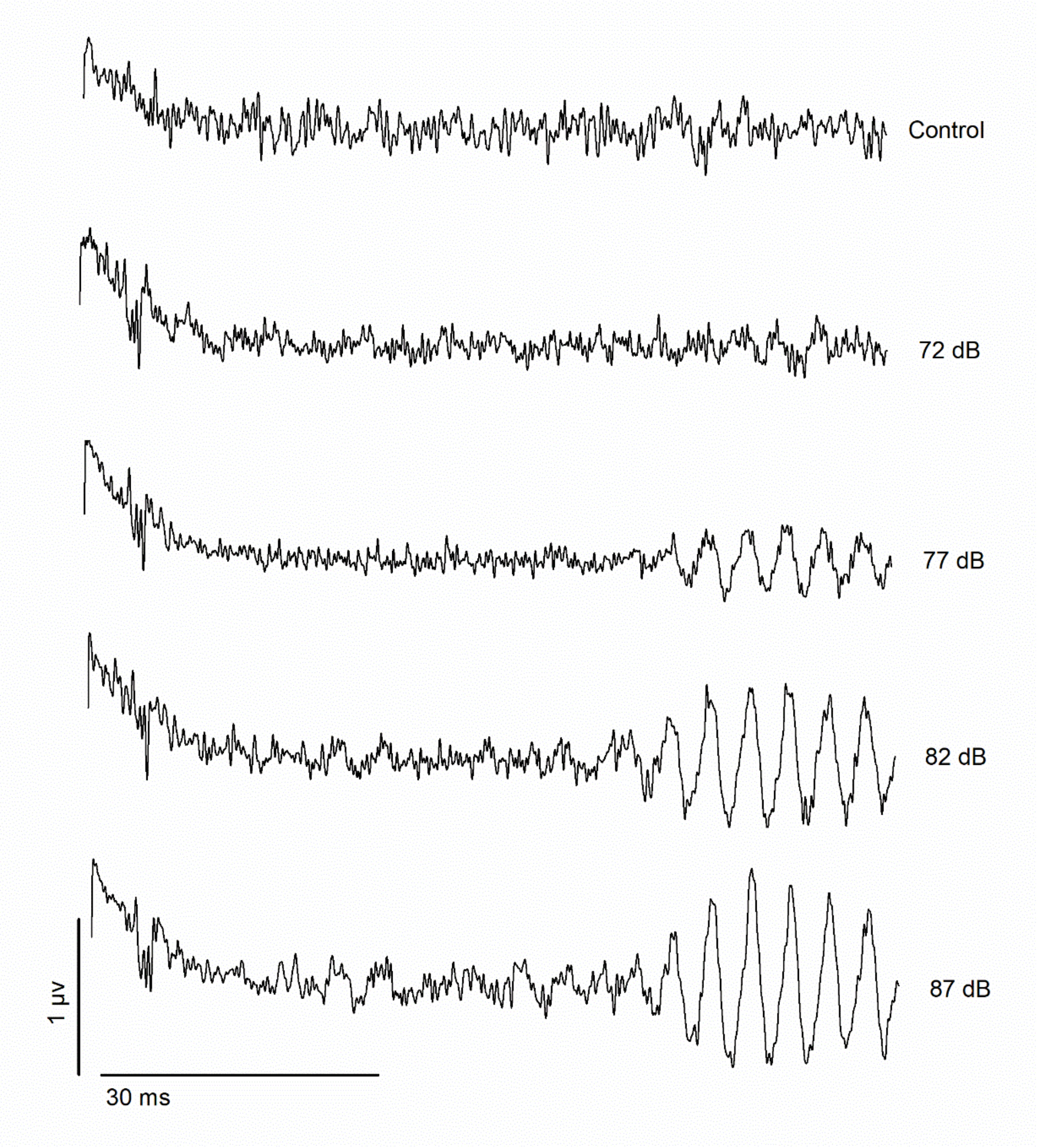


Figure 1. Example of auditory evoked potential waveforms from an individual Centropristis striata (93 mm TL) from the Small size class, in response to sound stimulus of 100 Hz pure tone bursts
The lowest sound pressure level to show a definitive response occurred at 77 dB in this example. Stimulus duration in this example was 30 ms, as indicated by the black bar in the lower left. Control AEPs (as shown here) were performed with a euthanized fish.

3.1.2.3. Acoustic calibration of experimental tanks

Sound pressure and particle motion in the tanks were calibrated four times during the experiments in the position the head is located for fish of all sizes, while the fish holder was in place. These were performed with a Reson TC4013 hydrophone (sensitivity -211 dB re 1 V/µPa)

(Teledyne Marine), an HTI-96 Min Series Hydrophone (High Tech Inc.) (sensitivity -165 dB re 1 V/ µPa) and a water proofed (Zeddies et al. 2012) triaxial ICP accelerometer (W356B11, PCB Piezotronics). The same test stimuli presented during the experiments were presented via the UW-30 loudspeaker during calibrations. The accelerometer was connected to a signal conditioner (Model 480B21, Piezotronics). From there the accelerometer and Reson hydrophone signal were directly input to two Krohn-Hite analog filters (3382, Krohn-Hite Corporation) which applied an anti-aliasing low-pass filter at 24 kHz. Filters were connected to a National Instruments DAQ board (USB 6251, National Instruments), which was connected to a laptop computer that run custom MATLAB (MathWorks, Natick, MA) scripts to allow recording and to ascertain the frequencies and absolute decibel levels using stimuli presentations.

3.1.2.4. Data analysis

To test for significant differences among auditory thresholds, two-tailed Kruskal-Wallis tests were used. Where significant differences were found, the Dunn's method was used to make pairwise comparisons. All significance levels were set at $\alpha = 0.05$. Non-parametric methods were used as data were not normally distributed and/or of uneven variance (Zar 1999).

3.1.2.5. Micro-computed tomography

Micro-computed tomography imagery was opportunistically conducted using an X-Tek HMXST 225 Micro-CT x-ray imaging system (Nikon Metrology), equipped with an open source x-ray tube with a maximum resolution of 3 – 5 µm in reflection mode and 2 µm in transmission mode. Imaging was performed at the Center for Nanoscale Systems (CNS) within the Laboratory for Integrated Science and Engineering (LISE), Harvard University. Euthanized individuals were prepared frozen and wrapped in layers of fine bubble wrap and inserted into a cardboard tube to stop any movement of body or body parts during the imaging process. These materials were used due to their low densities, whereby not affecting the imagery. Samples were transported to the imaging facilities in a cooler containing dry ice to keep them frozen. The tube was strapped vertically (nose down) onto the central circular imaging platform. Two individuals were imaged, 291 mm TL and 345 mm TL (focusing on inner ear and swimbladder region), however the latter's images were unusable due to computer malfunction, and re-imaging was not an option. Imagery of one individual took approximately 54 minutes under a 75 kV and 110 µA x-ray beam, which offered the least attenuation and best absolute contrast to noise ratio for the samples. 3D reconstruction was conducted using VG Studio MAX (v.2.2.6.80630 (Volume Graphics, NC, USA)) on a Dell PC running Windows 7, specialized for heavy workloads, which allowed visualization of different densities allowing segmentation of bone structures, soft tissues and air.

Six specimens, three from each of the Medium and Large size classes were euthanized after successful recovery from the AEP procedure. Specimens were dissected ventrally down the midline from anus to lower jaw, removing gills and other organs, to expose otic capsule and swimbladder. Extreme care was taken to observe any connective tissues or musculature leading to the inner ear region.

3.1.2.6. Pile driving recordings

During the late fall and winter of 2018/2019, Cashman Dredging & Marine Contracting Company were performing impact pile driving for the construction of a ferry berth in Woods Hole, MA, consisting of an 8 ft diameter pile using a hydraulic impact hammer. These activities were recorded to illustrate the potential frequency and intensity overlap between pile driving signals and sound detection abilities of *C. striata*. This activity was occurring in both the habitat and geographic region that *C. striata* are found and individuals in this study we caught within the same waters. Both the pressure and velocity components of the sound field during these activities were measured for approximately 30 min from an approximate distance of 200 m in 21 m water depth. Sound pressures and particle velocities were recorded at a 48 kHz sampling rate using both a SoundTrap hydrophone recorder (ST300 STD, Ocean Instruments Ltd) and a M20-PV sensor (Geospectrum Technologies, Nova Scotia) respectively.

$\mathrm{SPL_{z\text{-}pk}}$ and zero-to-peak sound acceleration levels ($\mathrm{SAL_{z\text{-}pk}}$) for individual pile pulses were calculated over a time window from 0.15 s before to 1 s after the time point of the detected pulse peak. $\mathrm{SPL_{z\text{-}pk}}$ and $\mathrm{SAL_{z\text{-}pk}}$ were calculated as:

$$\mathrm{SPL_{z-pk}} \text{ or } \mathrm{SAL_{z-pk}} = 20 * \log10\left(\frac{X_{peak}}{\mathrm{X0}}\right)$$

with X0 = 1 μPa or 1 m s^{-2} respectively

where X_{peak} was the maximum absolute μPa or m s^{-2} over a given measurement period, for pressure and acceleration respectively, with units of dB re 1 μPa and dB re 1 m s^{-2} for $\mathrm{SPL_{z\text{-}pk}}$ and $\mathrm{SAL_{z\text{-}pk}}$, respectively. To quantify sound energy distribution over frequencies from 20-20,000 Hz, power spectral density (PSD) curves were calculated in 1-Hz bins for both sound pressure and acceleration using Welch's method, with 80% overlap of time windows. Custom MATLAB scripts written specifically for this purpose were used to analyze both the pressure and particle velocities encountered.

3.1.3. Results

3.1.3.1. Sound detection measurements

The three size classes (Small, Medium and Large) of *C. striata* were used in this experiment. Size classes had a mean total length (TL) and range of 83.5 mm, 75 – 95 mm (n = 8), 284.4 mm, 270 – 296 (n = 8) and 408.8 mm, 346 – 470 (n = 4) respectively (Table 1). Responses to stimuli were observed from 80 to 1000 Hz, with only four of the 20 tested fish responding to the 1000 Hz signal, and no responses were elicited in any fish at 2000 Hz at the highest amplitudes possible before signal quality deteriorated (which was 147 dB re 1μPa for 1000 & 2000 Hz) (Figure 2). Responses were clear and consistent at 600 Hz and below. At no time did either of the two control types produce a result that resembled a response waveform, including when electrodes were placed in a euthanized fish, or when electrodes were placed in a live subject but presented with no stimulus. Response thresholds were at least 17 dB above ambient background sound in the experimental tanks, which remained below 62 dB re 1 µPa in all frequencies.

Major caution must be taken when comparing between our two trial locations, where tank dimensions slightly differed (all other AEP equipment and procedures were the same). However, to control for these differences, we tested fish in the same size class (Medium) at both locations to compare, which resulted in consistent results. The opportunity to test larger adults and the appropriately sized holding and test tanks to accommodate them at the second location was the rationale for using the two different setups.

The fish in the Small size/age class had the most sensitive low-frequency (<400 Hz) mean thresholds of the three size classes, ranging from 75–116 dB re 1µPa. This class was most sensitive at 150 Hz, followed closely by 200 and 100 Hz, and with three of eight fish responding to 1000 Hz stimuli with a mean threshold of 116 dB re 1 µPa (Figure 2). The fish in the Medium class overall had a very similar shaped audiogram to the Small class, which ranged from 77 – 123 dB re 1µPa, however, all frequencies were upward of three dB less sensitive. This class was also most sensitive at 150 Hz. Only one of eight fish responding to the 1000 Hz stimuli with a threshold of 122 dB re 1 µPa. Fish in the Large class were found to be the least sensitive, ranging from 90 – 108 dB re 1µPa and being as much as 25 dB less sensitive at 80 and 100 Hz compared to the Small and Medium classes. No fish tested in the Large class responded to 1000 Hz.

At the most sensitive frequencies within all size/age classes, 100, 150 and 200 Hz, there was a significant difference among classes ($H = 10.8$, $P = 0.005$, $H = 15$, $P < 0.001$, $H = 10.8$, $P = 0.004$ respectively). At 150 Hz the fish in the Small class were significantly more sensitive than the Large class ($Q = 3.2$, $P = 0.004$), but not significantly different from the Medium class. At 200 and 100 Hz the Small class was significantly more sensitive than both the Medium ($Q = 3.4$, $P = 0.002$ & $Q = 2.8$, $P = 0.014$ respectively) and Large classes ($Q = 2.9$, $P = 0.001$ & $Q = 2.5$, $P = 0.036$ respectively). Audiograms for particle accelerations encountered during the presentations (Fig. 2) were of a similar shape to the sound pressure audiograms with highest sensitivities at 150 Hz in all classes.

Micro computed tomography (MicroCT) showed the size and position of the saggital and lagenar otoliths, and the relative position of the swim bladder for a 291 mm *C. striata* (Figure 3). The distance between the closest point of sagittae and swim bladder was 35 mm. The fish that was imaged did not appear to have anterior projections of the swim bladder (Figure 3A, C, E), unlike the larger individuals dissected (Figure 3B).

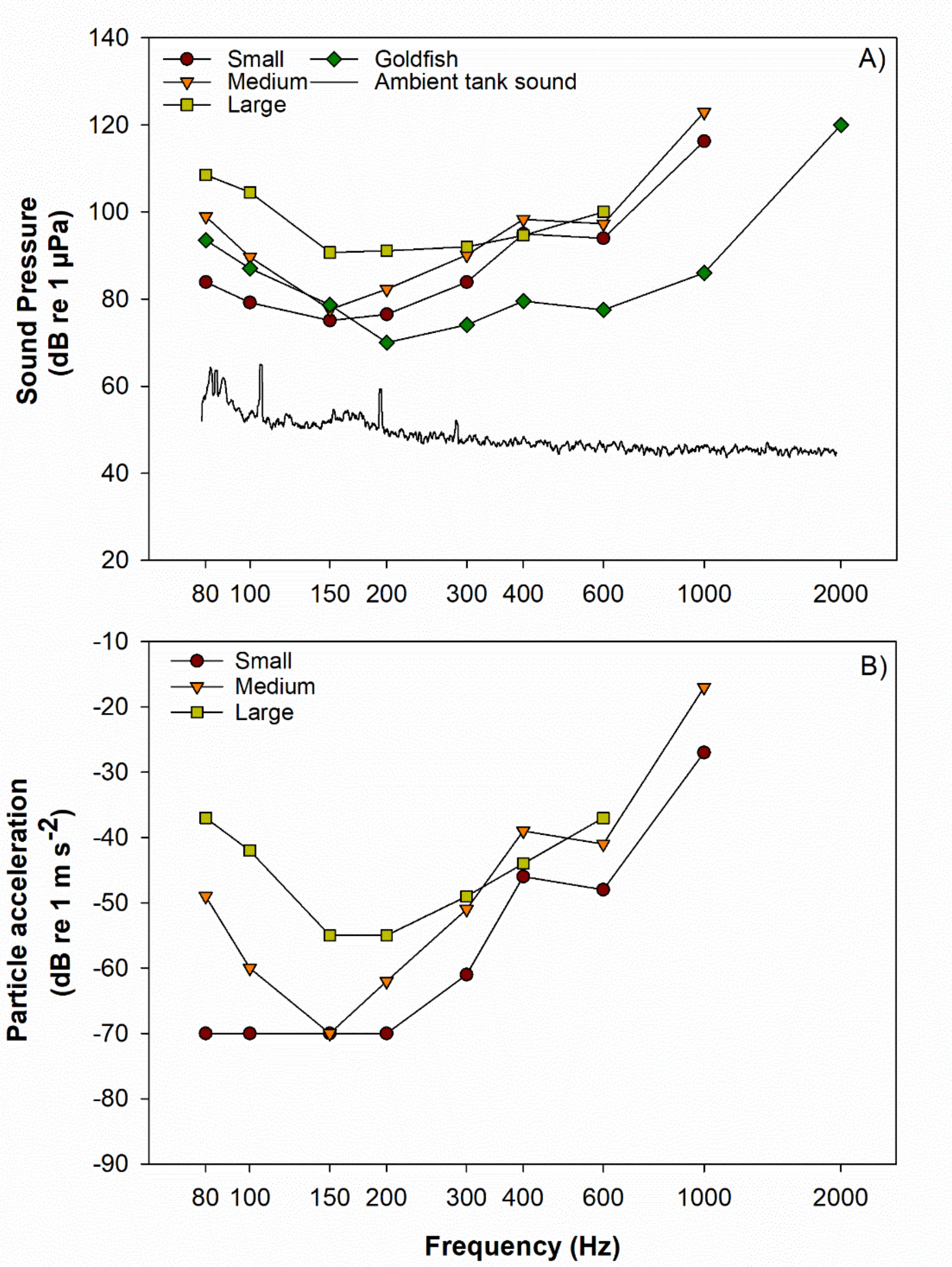


Figure 2. Sound pressure levels and measured particle acceleration at auditory thresholds of Centropristis striata (n=20) and Carassius auratus (n=4)

A). Mean (±SE) sound pressure levels at auditory thresholds for three size classes of *C. striata* and *C. auratus*, and ambient sound pressure levels of AEP tank, B) Particle acceleration levels measured at identified auditory thresholds. Flat response for the Small size class at frequencies 80 – 200 Hz due to noise floor of accelerometer. The ambient tank sound was also below the noise floor of the accelerometer.

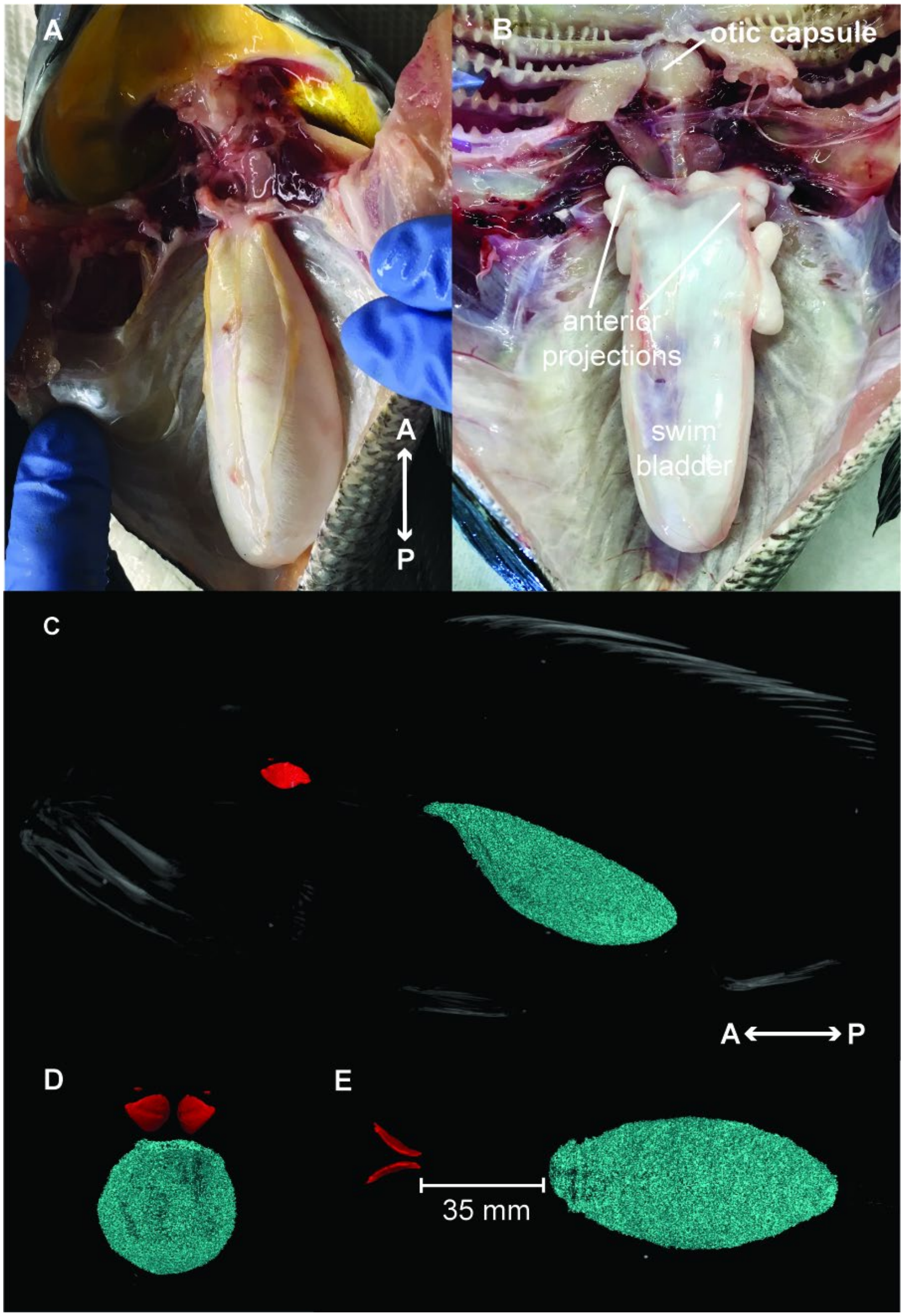


Figure 3. Dissection of Centropristis striata showing swim bladder and reconstruction of micro computed tomography (microCT) image

A) Dissection of a mature female *C. striata* (291 mm TL) showing otic capsules and swim bladder (75 mm in length) without anterior projections, B) Dissection of a mature female *C. striata* (345 mm TL) showing otic capsules and swim bladder (84 mm in length) with anterior asymmetric projections, C) lateral, D) anterior, and E) dorsal views of the reconstruction of microCT imaging slices demonstrating the spatial relationship between the swim bladder (blue) and otoliths (red: sagittae large, lapilli small) in mature female *C. striata* seen in A. (291 mm TL). A = anterior, P = posterior.

3.1.3.2. Pile driving recordings

For the 30 minutes of recorded pile driving in Woods Hole the highest received sound energy (pressure) was between 70 – 200 Hz (145 – 161.4 dB re 1µPa) with an inter-pulse interval of 1.46 s ± 0.092 SE (over 30 minutes of activity). This was within the range which *C. striata* had the greatest sensitivity to sound pressure (Figure 4). The recording had a median $X_{z\text{-}pk}$ of 187.8 dB re 1µPa and 14.1 dB re 1 m s^{-2}.

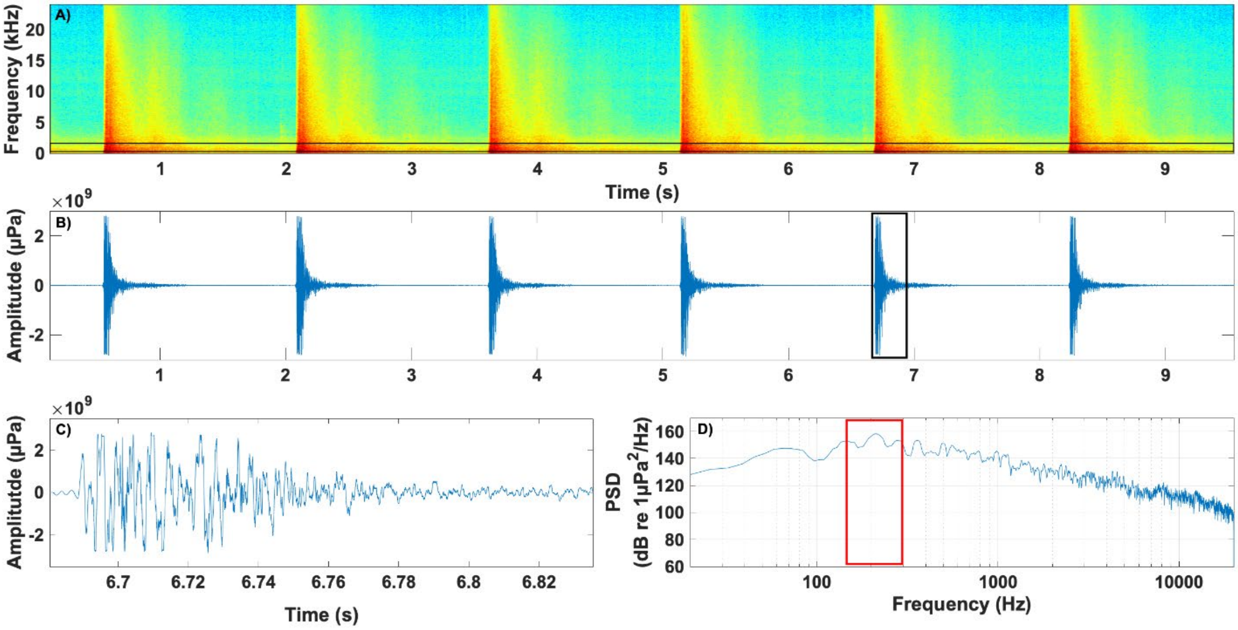


Figure 4. Acoustic characteristics (pressure) of 10 seconds of impact pile driving signal in Woods Hole, MA, at approximately 200 m distance in 21 m of water.
A) spectrogram of impact driving events illustrating frequency range, black outline indicates frequency range of sound detection in *Centropristis striata* (80 – 1000 Hz), B) wave form of events, C) reduced time resolution scale of the waveform to illustrate one pulse (black box from panel B), D) power spectral density (PSD) of impact driving event, red box indicates most sensitive detection range. 48 kHz sampling rate, spectrogram computed using a 1024-point fast Fourier transform (FFT), Hann-window, 80% overlap.

3.1.4. Discussion

3.1.4.1. Sound detection in *Centropristis striata*

This study represents the first published record of the auditory ability of *C. striata*. The audiogram of *C. striata* ranged from 80 to 1000 Hz, with the most sensitive thresholds at the lower frequencies. Eighty Hz was the lowest frequency tested (due to speaker limitations), so there is the possibility (and likelihood), given the hearing range of other fishes (Popper and Fay 1973) that this species could hear lower frequencies. However, peak sensitivity during this study was 150 Hz, and at 80 Hz *C. striata* was already significantly less sensitive. Thus, we can infer that at frequencies lower than 80 Hz, sound detection will most likely drop off rapidly, which is the case with most teleosts (Ladich and Fay 2013). Generally, the lowest frequency ranges of hearing in fishes is around 30-50 Hz (Ladich and Fay 2013), although there is some evidence certain species can detect infrasound (i.e. <20 Hz) (Sand et al. 2001). In other generally related perciform fishes, around 50-80 Hz is the lower frequency range observed (Ladich and Fay 2013). At the upper end of the bandwidth, 1000 Hz was the highest frequency detected by *C. striata*, and at no point did any fish detect 2000 Hz. In fact, the plotted value for 1000 Hz probably overestimates actual mean sensitivity, as less than half of the fish responded to this frequency. This is not surprising, as based on our dissections and the microCT imagery, this species does not appear to have any obvious ancillary structures (e.g., bones or ligaments) to transfer the pressure component detected in the swim bladder to the ears. However, the evidence of anterior projections of the swimbladder itself in a small number of mature adults should be further investigated, and whether these projections are consistent among the majority of individuals and/or they continue to develop for very large fish. Bony structures (e.g., Weberian ossicles, Fay and Popper 1974) and ligaments (e.g., otolaterophysic connection, Radford et al. 2013) – which would enhance the detection of higher frequencies – would be detectable in these images and dissections. However, even without ancillary structures, a small portion of pressure detection may be transduced through the soft tissue between the swim bladder and the otic capsule, particularly for smaller fishes, therefore improving the bandwidth of hearing (Popper et al. 2003a, Salas et al. 2019). This likely explains why the bandwidth extends to 1000 Hz and is not restricted to only 400 or 500 Hz, as is suggested to be the upper end of the purely particle motion component of hearing in fishes (Popper and Hawkins 2019).

In terms of sensitivity, relative to other fishes without specializations *C. striata* appears to have good sound detection capabilities. At the most sensitive frequency (150 Hz) the mean threshold was ~75-90 dB re 1 μPa, dependent on size class. The family Serranidae is not well represented in studies of hearing, predominantly limited to audiograms of larval stages. This is surprising for such a diverse and commercially and ecologically important family of fishes. The auditory detection bandwidths of larval serranids generally range from 100-1000 Hz, however two species could detect up to 2000 Hz (Wright et al. 2008, Wright et al. 2011) and the most sensitive levels were in the order of 110 dB re 1 μPa. Perhaps the small size of a larva and the relative closeness of the swim bladder to the otic capsule allows it to detect pressure more so than in adult fishes.

Many other perciform fishes have been tested using AEP methodology and share a similar audiogram shape with *C. striata* (most sensitive ~100-200 Hz and bandwidth ~50-2000 Hz). Typical maximum threshold levels of perciform fishes without ancillary organs vary widely from around 70 to 130 dB re 1 µPa, which is dependent not only on species, but also on age and the design of AEP setup (Popper and Fay 2011, Ladich and Fay 2013). Therefore, *C. striata* represents a fairly typical bandwidth of hearing for a perciform species without an identified otophysic connection and is at the more sensitive end of the spectrum, particularly at the low frequencies.

Lacking a definitive ancillary structure to transduce the pressure component of the sound field to the ears means purely sound pressure audiograms are not wholly representative for *C. striata*. In order to get an approximation for what the particle motion sensitivity was, we used an accelerometer in place of the fish's location in the tank, exposed to the same suite of sound stimuli. The particle acceleration audiogram for *C. striata* somewhat matched the pressure audiogram, being most sensitive at 100-200 Hz. This, along with the anatomical data, suggests that sound detection is predominantly particle motion derived at the lower frequencies, which is well documented (Popper and Hawkins 2019). However, the only way to remove a large amount of the pressure component is to perform the AEP trials with a pure motion stimulus device (e.g., shaker table). Further complicating the matter, is that it may not even be just the ears that are contributing to the detection of sound in fishes. Recent work has shown that the detection of sound stimuli in tanks is likely an integrative response from both the ear and the lateral line, at least at low frequencies (<400 Hz), and as such, it is recommended that AEPs should be acknowledged as acoustico-lateralis evoked potentials (Higgs and Radford 2016). However, the detailed distinction between the contribution of pressure vs particle motion or lateral line vs ears is not the major focus of this paper, but rather to present whether pile driving activities overlap the general bandwidth of this species, at levels that might interfere with life practices. The AEPs illustrate that they can indeed hear portions of the acoustic signal created when pile driving. Moreover, even if the detection thresholds were 50 dB less sensitive in the 150 – 300 Hz range, the acoustic signal from pile driving activity would still be detectible.

3.1.4.2. Ontogenetic variation in sound detection ability

Auditory sensitivity decreased with increasing size in *C. striata*. The negative correlation with *C. striata* size class and detection thresholds is possibly a function of the distance of the otoliths to the swim bladder, which will increase as the fish grows, or perhaps, distance from the AEP source to the sub-cutaneous electrodes. Many species have been found to improve hearing ontogenetically (Kenyon 1996, Schulz-Mirbach et al. 2012, Caiger et al. 2013), while much less common is a decline in hearing with development (Egner and Mann 2005) (although age-related hearing loss is observed in mammalian taxa). Therefore, the decreased bandwidth and sensitivity of *C. striata* is uncommon in fishes. Whether the decreases are simply a function of size, or is adaptive, is unknown. In our limited dissections, the 345 mm fish did appear to have some anterior projections of the swim bladder, compared to that of the 291 mm individual (Figure 3);

projections such as these are morphological adaptations that are well documented as enhancing hearing ability in fishes (e.g., Braun and Grande 2008). Further dissections of fish in both the Medium (n=3 291, 302, 309 mm) and Large (n=3, 345, 396, 400 mm) categories showed similar morphology, e.g., medium individuals showing no defined projections but with evidence of projections beginning to form, and large individuals with well-developed projections. Further study using a greater number, larger and individuals of both sexes is required to determine if this is both a consistent occurrence, and if these projections continue to develop. Moreover, if these projections are adapted to increase the pressure detection beyond that of juveniles, or more just to compensate the increasing gap between the swim bladder and ears as the fish grows is entirely unknown. The amplitude of the evoked potentials and consequently the increased thresholds with size class could potentially be a function of the relative placement of the electrodes. The electrodes we used were long enough to penetrate deep into the tissue of large fish, and extra care was taken to insert the electrodes proportionally close to the eighth cranial nerve with each fish, regardless of size, therefore we believe this is unlikely to be influencing results.

There are several potential ecological explanations for an ontogenetic increase in thresholds (i.e. decrease in hearing). In some fishes, hearing is most sensitive during the late larval and settlement stages, thought to be important for active habitat selection (Montgomery et al. 2001, Wright et al. 2010). Post-settlement, an enhanced sense of hearing is likely to be vital during the vulnerable juvenile stages of *C. striata*, where predation risk is highest. Then, as the juveniles become larger adults and their role in the food web changes from one of largely prey to predator, perhaps other sensory modalities become more utilized. There is also some anecdotal evidence of sound production in *C. striata*; however, to date there has been nothing published characterizing these sounds. From hundreds of hours of behavioral and acoustic observation in captivity, the authors have observed very little to no evidence that this species regularly uses acoustic communication outside of those observed during spawning events (Stanley *et al.,* unpublished data).

3.1.4.3. Utility of AEPs and tank caveats

It must be noted that while *C. striata* studied in our system was relatively sensitive, it is challenging to compare between fish AEP systems and subsequent detection thresholds, due to different acoustic conditions under which the experiments were conducted (e.g., different tanks, setups and procedures) (Popper et al. 2019). By testing *C. auratus*, we could directly compare our AEP results from this species with the wider literature (which also can show great variation). Our results show that *C. auratus* were well within the range reported in various literature (Ladich and Fay 2013), thereby qualifying the general detection bandwidth range and thresholds of *C. striata*.

A further limitation with AEP setups being used in restricted environments (e.g., tanks, both large and small) is the notoriously complex sound fields and the difficulties in quantifying them (Akamatsu et al. 2002, Ladich and Fay 2013). Moreover, in many tanks it is close to impossible to achieve a ratio between sound pressure and particle motion similar to that of a species natural habitat (Ladich and Fay 2013, Popper et al. 2019). Methods using sounds replayed through a loudspeaker do not separate the pressure and particle motion properties of the sound field, nor do they separate how the receiver is detecting the property. By measuring whole-field potentials across the brain and nerve roots, this method is likely detecting both the auditory and lateral line inputs, especially at the low frequencies (Higgs and Radford 2013, Garabon and Higgs 2017). Therefore, caution must be taken when treating AEPs as absolute hearing thresholds or relating threshold levels to detection of *in situ* sound sources. However, they undoubtedly serve as a useful starting point for assessing sound detection bandwidths in a species. Further work to better understand the species true sensitivities and/or their behavioral thresholds could include treatments that better control particle motion (i.e. using shaker tables), behavioral conditioning using very large tanks, or preferably, *in situ* fish cages (Popper et al. 2019).

3.2. Study 2: General behavioral changes in responses to replayed pile driving signal

Manuscript in Prep: Shelledy, K., Keller, A. G., Phelan, B., M., Stanley, J. A. Effects of replayed pile-driving on the general behavior of *Centropristis striata* in a controlled environment.

3.2.1. Background

Human activity has altered the worlds ocean soundscapes for decades, with commonly studied noise sources including motor vessels, geophysical surveys, and offshore construction (Hawkins et al. 2014). This increasing anthropogenic sound can have a range of physiological and behavioral effects on marine animals (Popper and Hastings 2009b). Although research and regulatory efforts associated with anthropogenic noise often focus on marine mammals and other protected species, fishes are also exposed to the same anthropogenic disturbances and altered ocean soundscapes. In salt water, sound waves travel 4.5 times faster than air, detectable as particle motion and pressure waves. Marine species rely on auditory cues to locate food, find mates, and avoid predators among other things. Thus, the effect of altered ocean soundscapes on marine organisms is a key concern as ocean development and use continues.

Acute, loud sound sources such as those produced by seismic surveying, sonar (low-frequency active), and pile driving activities have been found to produce physical, physiological, and behavioral effects on fish species. These range from temporary and permanent damage to hearing structures, swim bladder trauma (McCauley et al. 2003, Halvorsen et al. 2006, Casper et al. 2013a, Casper et al. 2013b), and temporary auditory threshold shifts in fishes close to the source (Scholik and Yan 2001, Smith et al. 2004a), and reduced observed abundances and reduced catch efforts at greater distances (Skalski et al. 1992, Carroll et al. 2017, Paxton et al. 2017). Furthermore, lower level and/or chronic noise can also have negative impacts on fishes, masking acoustic signals, decreasing signal-to-noise ratios, and thus interfering with a wide range of important behaviors, including feeding (Voellmy et al. 2014a), predator avoidance (Voellmy et al. 2014b, Simpson et al. 2016b), group cohesion and swimming dynamics (Sara et al. 2007, Bruintjes and Radford 2013, Herbert-Read et al. 2017), settlement behavior (Holles et al. 2013, Simpson et al. 2016a) and/or mating/spawning success (Nedelec et al. 2017, Stanley et al. 2017, Blom et al. 2019b). Previous studies have also examined the effect of elevated ambient sound and tones on different fish species. In response to playback of elevated ambient sound, studies have observed altered courtship (de Jong et al. 2018a, de Jong et al. 2018b), pronounced startle responses and milder “fear-related behaviors” (Purser and Radford 2011, Neo et al. 2015b, Purser et al. 2016), altered swimming and schooling patterns (Sabet 2016), cortisol level spikes (Smith et al. 2004b), and threshold shifts in hearing (Smith et al. 2004a). Tones of different frequency and intensity have yielded startle responses (Kastelein et al. 2008), altered swimming behavior (Kastelein et al. 2007), and noise source avoidance (Knudsen et al. 1992, 1994).

The foundations that support wind turbines in the ocean create structures that function as artificial reefs, changing the local marine habitat, increasing heterogeneity, and attracting high densities of marine organisms, including black sea bass. Previous research suggests that artificial reefs support greater density and biomass of fish, and compared to soft bottom areas, artificial reefs provide higher catch rates (Langhamer 2012). Anecdotal evidence from recreational fishermen suggest that the habitat-forming nature of wind turbine pilings produce higher catch rates at the Block Island Wind Farm in Rhode Island (Pers. Comm., 2019). Physiological examinations indicate that the frequencies at which black sea bass are most sensitive to sound directly overlap with frequencies of high-amplitude anthropogenic noise pollution, including noise from activities like shipping and the underwater construction required for offshore wind farms (Stanley et al. 2020) . However, the effects of pile-driving on the behavior of black sea bass have not yet been investigated.

Altered behavior not only has ecological and evolutionary consequences for fishes but can also induce cascading ecosystem effects and incur negative economic impacts for commercially important fish species. These impacts can have fundamental ecological and evolutionary implications for species, especially for those that rely on acoustics in key stages of their life, and ultimately can reduce both fish populations and ecosystem functioning.

In the present study, using a controlled tank environment, we examined how exposure to playbacks of impact pile driving signal influenced the general behavior of black sea bass in small groups. Time spent exhibiting seven recognized behaviors was recorded before exposure, during exposure, and after exposure for both control and experimental sound treatments. Since fish behavior is complex, multivariate statistical approaches were used to reveal behavioral patterns and determine how these behaviors change in complementary or non-complementary ways. Through principal component analyses and tests of discrimination among groups, this study aims to answer two general questions: 1) *Do black sea bass exposed to replayed pile-driving signal exhibit changes in behavioral patterns?* And 2) *Do changes in black sea bass behavioral patterns diminish during exposure to the signal?* By further understanding black sea bass behavioral responses to anthropogenic sounds and assessing their ability to habituate to sound exposure, we can gain a more comprehensive picture of offshore wind energy development's environmental impact.

3.2.2. Methods

3.2.2.1. Fish acquisition and maintenance

Adult *Centropristis striata* were wild-caught via line fishing off the New Jersey coast during July 2017 and July 2018 under Scientific Collecting Permit MFA-SCP No.1834, administered by the State of New Jersey Department of Environmental Protection. Any fish that was deemed not healthy, owing to capture or otherwise, were not used in experiments. Fish were held in 2.4 m diameter fiberglass tanks with flow through water supply at average temperatures of 16.4 ± 2.1 °C. All holding tanks were exposed to a 12:12 light cycle and fish were fed daily with blue crab and squid. Sound levels in holding tanks were approximately 100.7 – 104.3 dB re 1µPa in the 20 – 24,000 Hz range.

Pile-driving sound exposure experiments took place from May-September 2018. No fish was used in any experiment previously and was therefore naïve to the exposure signal and regime.

3.2.2.2. Experimental tank and audio set up

All experiments were conducted in a 183 cm diameter circular fiberglass tank filled to an 80 cm depth with ambient flowing seawater (Figure 5). The experimental tank was isolated from nearby vibrations with cinderblocks, plywood, and layers of rubber matting between the tank and the concrete floor of the laboratory.

A UW-30 underwater transducer (Electro-Voice, Fairport, NY) was suspended horizontally in the tank, 40 cm from the surface and 15 cm from the tank wall. To ensure fish did not swim behind the speaker, a mesh barrier was placed 15 cm in front of the speaker (Figure 5). The UW-30 was connected to a Pyle 2-channel stereo amplifier (2000 W Dual Channel – PLA2378), 12 V battery and Dell Latitude laptop with Adobe Audition used for playback of acoustic files. A cabled hydrophone (HTI-60-MIN/Low Noise, sensitivity: −203.8 dB re 1 V/µPa, gain: 6 dB, flat frequency response from 2 Hz to 30 kHz; High-Tech Inc., Long Beach, MS) was placed behind the mesh barrier 2 cm from the tank wall, 13 cm from the speaker and 40 cm from the surface to monitor ambient and sound playbacks within the tank. The hydrophone was attached to a laptop computer with data acquisition custom MATLAB scripts (Mathworks, Natick, MA). Two cameras, one positioned overhead (Sony HDR-AS50 ActionCam) and one underwater (GoPro 6), were used to record behavioral responses to the pile driving stimuli for quantification.

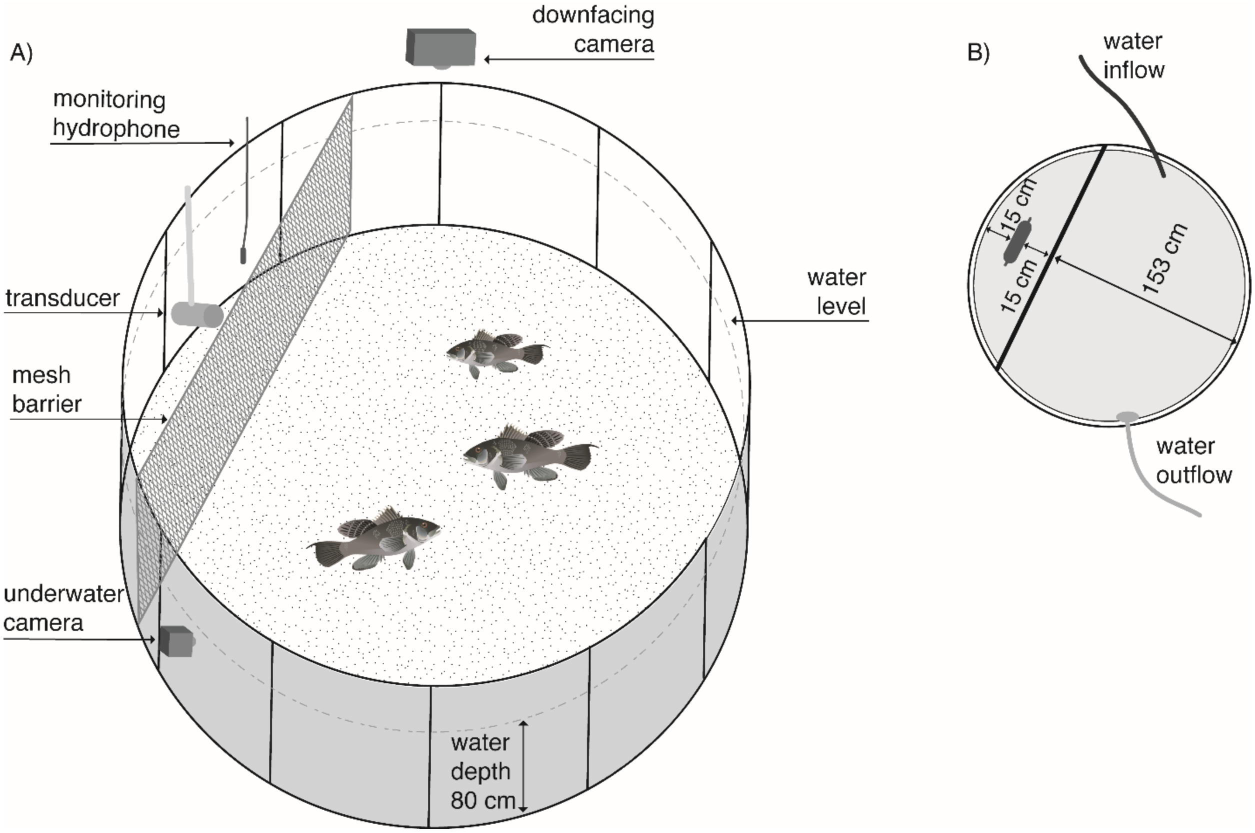


Figure 5. Schematic of experimental tank
Schematic of experimental tank set-up from top-down view. 1) downward facing camera, 2) locations of underwater cameras, 3) transducer (speaker), 4) hydrophone, 5) mesh barrier, 6) water inflow, 7) water outflow. Measurements, but not all objects shown, are to scale.

Audio files of pile driving signal used for experiments were recorded during construction of Block Island Wind Farm on October 25th, 2015 (between 16:00 and 20:00 UTC), from a hydrophone (HTI-94-SSQ, sensitivity: −203.8 dB re 1 V/μPa, gain: 6 dB, flat frequency response from 2 Hz to 30 kHz; High Tech Inc., Long Beach, MS) attached to benthic sled located 1 m from the seabed, 26 m deep and 0.5 km away from a pile driving site (Amaral et al. 2018, Amaral et al. 2020). These files were recorded at a 9766 Hz sample rate. The pile was a steel, hollow pile had a diameter of 127.0 cm, wall thickness of 3.8 cm, a rake of 13.27° with respect to vertical, and was driven up to 76.2 m deep into the seabed. These field data, along with particle acceleration calculated from hydrophones in the field, were provided to the authors.

To prevent pseudoreplication of playback stimuli, three distinct 15-minute pile driving playback files hereafter referred to as Pile1, Pile2, and Pile3, were extracted from two different pile driving bouts and prepared using Raven Pro 2.0 (The Cornell Lab of Ornithology, Ithaca, NY) prior to playback. A 3-s fade-in of the sound file (before the pile driving sound was emitted) was applied to each recording to prevent artifacts that could result from the playback beginning at a higher amplitude.

Each recording was amplified by a custom magnitude to obtain the highest playback sound levels possible without clipping, with the goal to match received zero-to-peak sound pressure levels in the tank with those present 0.5 km from the BIWF pile driving site, i.e., 190–194 dB re 1µPa. Median inter-pulse intervals were 1.53 s (IQR: 1.52–1.55 s), 1.81 s (IQR: 1.80–1.82 s) and 2.35 s (IQR: 2.32–2.42 s) for Pile 1, Pile 2, and Pile 3 respectively. Median zero-to-peak pulse amplitudes were 190.6 dB (IQR: 190.0–191.2 dB), 193.8 dB (IQR: 193.6–194.0 dB), and 194.2 dB (IQR: 193.6–194.5 dB) re 1 µPa, and median root-mean-square inter-pulse amplitudes were 140.7 dB (IQR: 140.5–141.0 dB), 138.4 dB (IQR: 138.2–138.7 dB), and 137.8 dB (IQR: 137.6–138.0 dB) re 1 µPa. Notably, pulse amplitude, inter-pulse time interval, and inter-pulse interval amplitude within a pile driving event will vary slightly from impact to impact and as the pile is driven into the sediment.

Here, playback amplitudes and inter-pulse interval were not manipulated to be identical within or across the three pile driving files because we were interested in studying behavioral impacts of pile driving noise at a simulated distance from the pile, rather than studying responses dependent on these specific metrics. The variability in these metrics across impulses reflects that which a wild fish may experience near an offshore pile driving site. For use in control trials, a 15- min-long silent file was played.

3.2.2.3. Acoustic calibration of experimental tanks

The experimental tank was calibrated in 20- cm increments in all 3 dimensions without animals present, creating a 3D array of received sound levels. At each position, the first minute of each of the three pile driving noise files was played and recorded at a 48 kHz sampling rate by a triaxial ICP accelerometer (Model W356B11, PCB Piezotronics) and Reson TC4013 hydrophone (Teledyne Marine) spaced 10 cm apart. The accelerometer was wired through a signal conditioner (Model 480B21, Piezotronics). The accelerometer signal and hydrophone were input to two analog filters (Model 3382, Krohn-Hite Corporation), which each applied an anti-aliasing low-pass filter at 24 kHz and a 20 dB gain. See Jones et al. 2020 and following sections for further details on data analyses for calibration.

3.2.2.4. Experimental procedures

In groups of three, black sea bass were transferred to the experimental tank and allowed to acclimate overnight. The morning of the following day, the hydrophone and cameras were set to record for the duration of the experiment. The sound exposure experiment consisted of three separate sound exposure periods at 9:00am, 11:30am, and 2:00pm EST. Each sound exposure period included 15 minutes of baseline pre-sound behavior, followed by 15 minutes of sound exposure to a silent track (control) or one of three randomly selected pile-driving tracks (Pile 1, Pile 2, or Pile 3), and then 15 minutes of recovery or post-sound behavior.

Following the 2 pm sound exposure treatment, fish were measured for total body length, weighed, and moved to a separate holding tank until they could be released. Individuals ranged in size from 24.5 – 47.0 cm (219-1314 g).

In total, eight control and thirteen experimental groups were run through the experimental treatment with three individuals in each group, and no individual used more than once. Temperatures in the test tank ranged from 14.3-17.3 °C with dissolved oxygen ranging from 3.07-8.15 mg/L.

3.2.2.5. Analysis of behavioral data

The first 15 seconds of video were analyzed for behavior (Table 2) at sound onset, corresponding to onset of pile-driving playback or control silence. Any changes in behavior were noted and then categorized (Figure 6).

Each 15-minute time segment was cut into one-minute intervals (00:00-01:00, 04:00-05:00, 09:00-10:00, and 14:00-15:00). Each 1-minute interval was then analyzed using Behavioral Observation Research Interactive Software (BORIS version 6.3.7). The number of seconds spent exhibiting each of seven behaviors (hereafter referred to as 'behavioral time budget') was recorded for each fish and each of the four 1-minute intervals. Recorded behaviors did not co-occur, and the dominant behavior was favored.

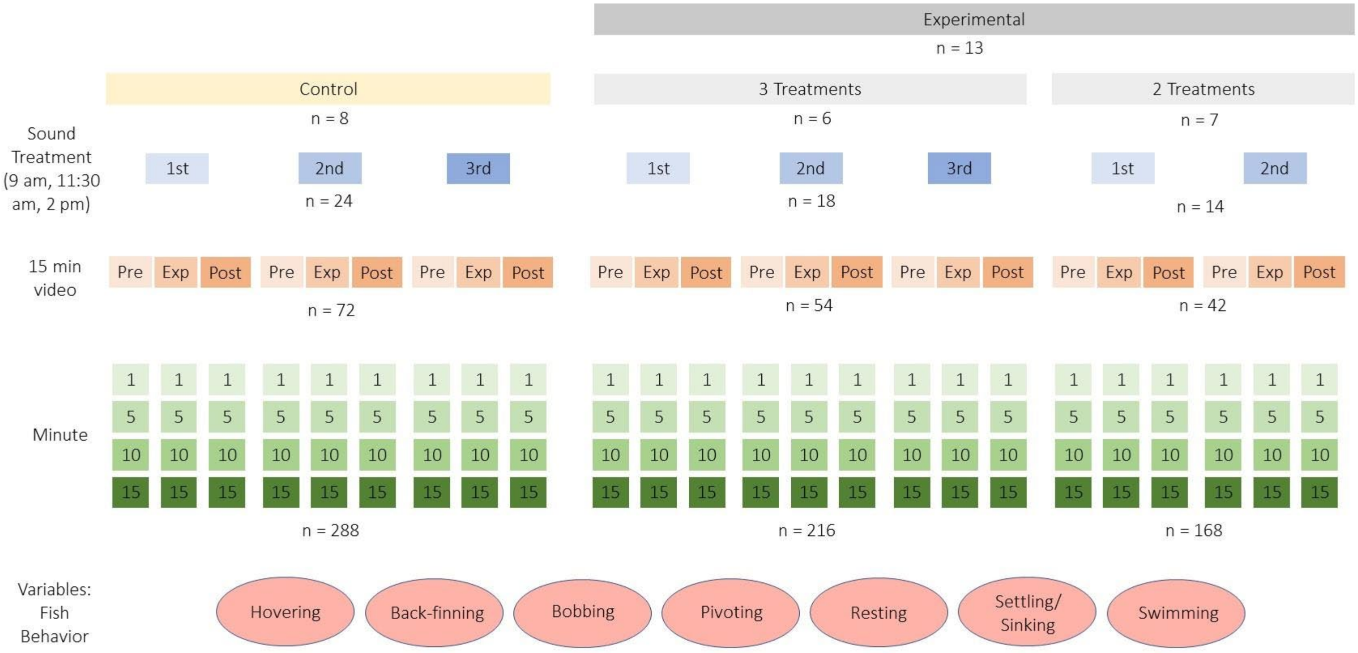


Figure 6. Schematic displaying the experimental and analysis procedure
Groups of three black sea bass individuals were separated into 21 small tanks. Thirteen tanks received sound treatment, with 6 receiving 3 treatments and 7 receiving 2 treatments. Within each session, the fish were recorded for 15 minutes before sound exposure, during sound exposure, and after sound exposure. During each 15-minute segment, fish behavior was recorded for the three fish at four one-minute time intervals. In each minute, the number of seconds spent exhibiting the above seven behaviors were recorded.

Table 2. Glossary of seven behaviors recorded for each fish.

Response Behavior Glossary	
Behavior	**Definition**
Resting	maintains position on tank floor
Swimming	movement along a plane in forward direction
Bobbing	movement across the air/water interface
Pivoting	change in body orientation around central point
Hovering	holding position while elevated in the water column
Back Finning	fish motions fins in backward circles
Sinking	lowering in the water column; pectoral fins are not being used to move body in forward direction

3.2.2.6. Statistical analysis

All data analyses were conducted in R version 3.6.3 using the packages ‘vegan’ (Oksanen 2018) and ‘Biostats’ (McGarigal 2016). A total of 2,007 black sea bass behavioral time budgets were included in the analysis. Twenty-six data points were missing due to video camera malfunction or lack of visibility, reducing the total number of observations to 1,981. Since the missing data were randomly distributed throughout the dataset, the missing observations were simply removed. The behavioral time budgets were averaged across the three black sea bass in each group.

Within each 15-minute video segment, the behavioral time budgets were converted into a proportion of the total recording time (4 minutes of behavior recording), resulting in 167 observations. The behavioral time budget proportions underwent an arcsine square-root transformation, suitable for proportional data, and the data were evaluated using a Mardia Kurtosis test of multivariate normality. The proportional behavioral time budgets were considered to be a member of one of six groups: Control: Pre-Exposure, Control: Exposure, Control: Post-Exposure, Treatment: Pre-Exposure, Treatment: Exposure, Treatment: Post-Exposure. A permutational multivariate analysis of variance (perMANOVA) was used to determine if the black sea bass behavioral patterns in multivariate space were significantly different among the six groups. A series of pair-wise perMANOVA tests were conducted to compare each of the six groups directly against each other. Since perMANOVA tests assume independence in observations, strata for sessions (9 am, 11:30 am, and 2 pm treatments) were used to retain the data structure during permutation and to satisfy the assumption of time independence between observations. A test of multivariate homogeneity of group dispersions was conducted pairwise to determine if the dispersions of the groups were significantly different (Anderson 2001).

To determine if there were any behavioral changes in response to exposure replayed pile driving a principal component analysis (PCA) was conducted to search for and summarize behavioral patterns associated with group differences. The PCA was performed using a variance-covariance approach and a Euclidean distance matrix. Statistical significance of each principal component (PC) axis was tested using a Monte Carlo randomization test, using 1000 permutations to compare the observed eigenvalues to the distribution of eigenvalues under the null hypothesis of no real correlation structure. Structure correlations between the original behavior variables and the principal component scores were calculated, and the significance of the variable loadings (behavior significance) was determined through permutation. On each PC axis, behavior loadings were included only if they were significantly correlated to the axes ($p < 0.01$ and $r^2 > 0.1$). The observations in multivariate space were visualized by separating observations by the six groups and by adding 95% confidence ellipses.

To assess whether any changes in behavior in response to exposure to replayed pile driving signal diminished during the exposure withing each 15-minute video segment, the behavioral time budgets were subsetted by minute (1st minute, 5th minute, 10th minute, and 15th minute). The behavioral time budgets were converted into a proportion of the total recording time (one minute), resulting in 167 observations for each of the four one-minute subsets. The behavioral time budget proportions underwent an arcsine square-root transformation. The four one-minute subsets were analyzed individually and were considered to be a member of one of six groups: Control: Pre-Exposure, Control: Exposure, Control: Post-Exposure, Treatment: Pre-Exposure, Treatment: Exposure, Treatment: Post-Exposure. Pairwise perMANOVA tests and pair-wise tests of multivariate dispersion were conducted for each one minute segment using the same methods previously described with the approach using the total recording time (combined four minute segments). A PCA was conducted on each one-minute subset separately to search for and summarize behavioral patterns associated with group differences in each minute. The PCA and tests of variable loading significance were conducted using the same methods previously described with the approach using the total recording time (combined four- minute segments).

Finally, to determine if previous exposures to replayed pile driving signal effect behavior, within each 15-minute video segment, the proportion of time spent resting, bobbing, and swimming was averaged for minutes 01, 05, 10, and 15. A Mann-Whitney U-test was used to determine if behavioral changes between control and sound treatment groups increased after subsequent exposures. The non-parametric Mann Whitney U-test tested if the control and sound treatment samples were likely to derive from the same distribution, and the test was conducted to compare the proportion of time spent exhibiting resting, bobbing, and swimming behaviors between sound and control behaviors for each 15-minute video segment (pre-exposure, exposure, post-exposure) and exposure treatment (first exposure, second exposure, and third exposure). P-values and effect sizes were calculated for all comparisons. Boxplots were created in R to compare subsequent exposures across video segments.

Further analyses are currently underway in preparation for the manuscript.

3.2.3. Results

3.2.3.1. Acoustic calibration of experimental tanks

Ambient sound levels (no playback) of the tank were low, starting at a PSD of ca. 81 dB re 1 uPa^2/Hz at 20–100 Hz and decreasing in a logarithmic fashion down to ca. 50 dB re 1 uPa^2/Hz at 600–1000 Hz. The acceleration levels of ambient sound in the tank and silent playback were below the self-noise floor of the accelerometer and were therefore unable to be quantified. This relatively quiet tank environment enabled us to isolate and assess potential effects of water-borne pile driving noise on fish while minimizing extraneous stimuli that are found in natural field environments.

The PSD curves of pile driving pulses in the tank indicated that noise playback created a substantially elevated sound field (by up to 51 dB) and this sound was generally similar among the three playback files, with some differences in spectral shape between pressure and acceleration metrics. The highest received pile driving pulse energy was between 100–300 Hz, within the most sensitive range *C. striata* sound detection abilities (Stanley et al. 2020; see Study 1). Across the frequency range of 20–1000 Hz, acceleration PSD of pile driving pulses was 20–30 dB higher than that of inter-pulse intervals. Spectral curves of sound pressure of pile driving pulses received in the tank had roughly similar shapes but lower amplitudes (by 20–40 dB) compared to those received in the field 0.5 km from the pile installation. These sound pressure levels in the tank were in the range of levels typically present in the water column 2–4 km away from the Block Island Wind Farm piles (Amaral et al., 2018). Acceleration PSD of pile driving pulses in the tank was about 20 dB higher than acceleration in the field at most frequencies from 20-1000 Hz, and about 40 dB higher near 300 Hz. Median peak particle velocity (Euclidian norm) across all tank recording positions and files was -40 (IQR: -43 to -37) dB re 1 m s^{-1}, compared with measured peak particle velocity of -70 dB re 1 m s^{-1} at about 1 m above the seabed and -60 dB re 1 m s^{-1} at the seabed, 0.5 km from the pile (Amaral et al., 2018). A prior study that modeled peak particle velocity propagation from simulated pile driving sound predicted -40 dB re 1 m s^{-1} at the seabed, 150 m away from the pile (Miller et al., 2016).

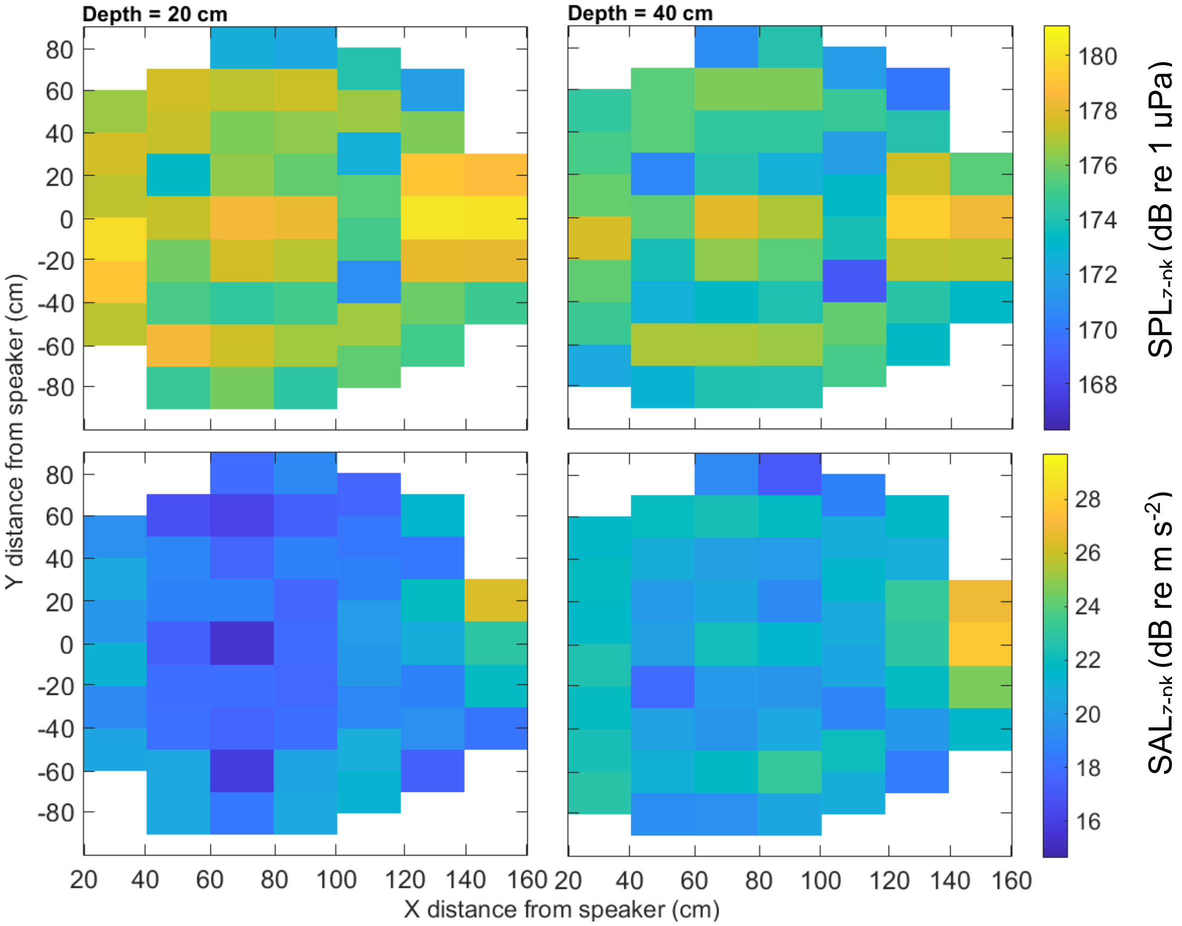


Figure 7. Schematic displaying the experimental and analysis procedure
Spatial maps of zero-to-peak acceleration (top) and pressure (bottom) for two water depths (columns), shown from top-down in the horizontal plane, with the front-center of the speaker set as the origin. Data were band-pass filtered to 20-1000 Hz and median zero-to-peak values of pile pulses (across 30 pulses, i.e., 1 min) are shown for each recording location.

3.2.3.2. Behavioral responses

Do C. striata exposed to pile-driving signal exhibit changes in behavioral patterns?

The series of pair-wise perMANOVA tests between the six groups (Control: Pre-Exposure, Control: Exposure, Control: Post-Exposure, Treatment: Pre-Exposure, Treatment: Exposure, Treatment: Post-Exposure) over the total recording time (combined four minute segments) showed significant (p-value < 0.05) or marginally significant (p-value < 0.10) differences in behavior between the Treatment: Exposure group and all three control groups and the Treatment: Pre-Exposure group. No significant difference in behavior was detected between the Treatment: Exposure and Treatment: Post-Exposure groups (Table 3).

Table 3. Results of pair-wise perMANOVA test between six groups for all combined recorded minutes in each 15-minute recording segment.
Each cell contains the R^2 and the associated p-value in parenthesis. Significant and marginally significant test statistics are bolded. (p-value < 0.05**; p-value < 0.10*)

	Control: Pre-Exposure	Control: Exposure	Control: Post-Exposure	Treatment: Pre-Exposure	Treatment: Exposure
Control: Pre-Exposure					
Control: Exposure	0.00085 (0.995)				
Control: Post-Exposure	0.0025 (0.939)	0.0031 (0.901)			
Treatment: Pre-Exposure	0.0046 (0.816)	0.0034 (0.863)	0.010 (0.520)		
Treatment: Exposure	**0.052 (0.062)***	**0.047 (0.073)***	**0.065 (0.024)****	**0.037 (0.072)***	
Treatment: Post-Exposure	0.026 (0.215)	0.023 (0.263)	0.039 (0.124)	0.018 (0.276)	0.012 (0.359)

The multivariate test of dispersion for the global comparison between all six groups indicated that there was no significant difference in dispersion between groups (5 df, 0.66 F-statistic, 0.665 p-value). The pair-wise multivariate tests of dispersion directly comparing each group indicated that there were no significant differences in dispersion between groups.

The principal component (PC) analysis ordinated the 167 black sea bass behavioral time budgets, and the first three PC axes explained significant variation in the behavioral time budgets. The first two PC axes explained 59.7% of the variation in the behavioral time budgets (PC1: 39.2%, PC2: 20.5%). All seven behavior variable loadings were statistically significant ($p\text{-value} < 0.001$). Behaviors including hovering ($r = 0.755$), back-finning ($r = 0.298$), bobbing ($r = 0.696$), pivoting ($r = 0.482$), sinking ($r = 0.327$), and swimming ($r = 0.627$) were positively correlated with PC1, while resting ($r = -0.931$) was negatively correlated with PC1 (Figure 8g). Behaviors bobbing and resting were negatively correlated with each other. Behaviors sinking and pivoting were positively correlated, and behaviors swimming and hovering were positively correlated. There was no correlation between sinking/pivoting and resting and sinking/pivoting and bobbing behaviors.

Differences between the six groups were visualized on the PC axes (Figure 8). The ordination of observations in the Treatment: Exposure group was consistent with the pair-wise perMANOVA test and displayed different behavioral patterns than the other five groups based on visual inspection. The Treatment-Exposure group was strongly associated with sinking, pivoting, and resting behaviors, and was not associated with bobbing behavior (Figure 8e). The other five groups were more strongly associated with bobbing behavior.

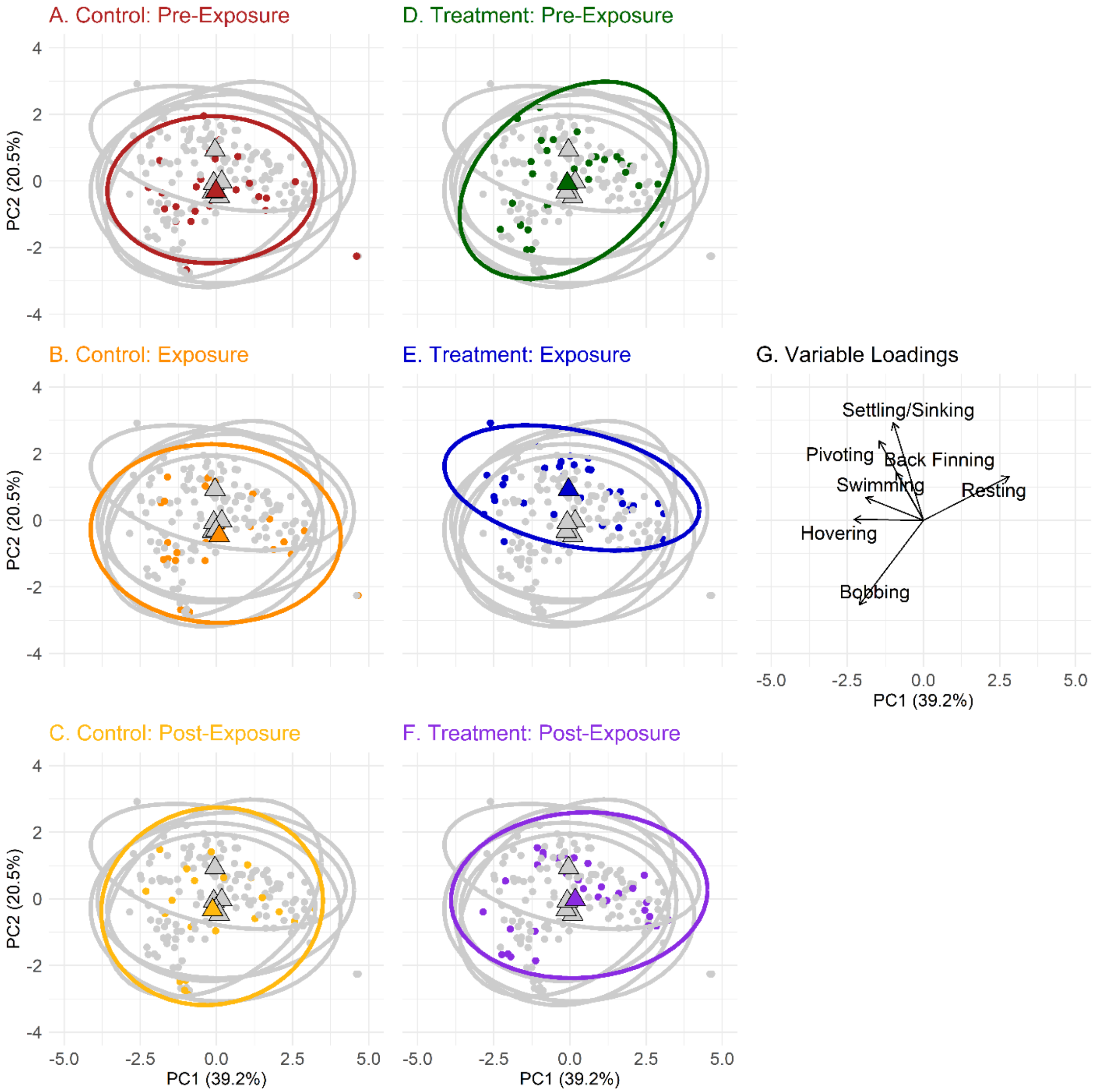


Figure 8. Multivariate ordination of black sea bass behavioral response before, during, and after exposure to wind turbine pile-driving sound

Each of the 167 points represents the behavior of a tank of black sea bass, ordinated in multivariate space based on principal component analysis. Each point belongs to one of six sound treatment or control groups, before, during, and after sound exposure. The triangles represent each group centroid. The observations are color-coded by group, and 95% confidence ellipses are included. ***A.*** Ordination of Control: Pre-Exposure group. ***B.*** Ordination of Control: Exposure group. ***C.*** Ordination of Control: Post-Exposure group. ***D.*** Ordination of Treatment: Pre-Exposure group. ***E.*** Ordination of Treatment: Exposure group. ***F.*** Ordination of Treatment: Post-Exposure group. ***G.*** Behavioral variable loadings on PC axes 1 and 2. The length of the vectors indicates the strength of the associated variable for describing the PCs. The direction of the vectors indicates the direction of the associated variable gradients in ordination space.

Do changes in C. striata behavioral patterns diminish during exposure to sound?

The minute 1 pair-wise series of perMANOVA tests indicated significant differences (p-value < 0.05) in fish behavior between the Treatment: Exposure group and all three control groups and the Treatment: Pre-Exposure group. The minute 5 pair-wise series of perMANOVA tests indicated significant (p-value < 0.05) or marginally significant (p-value < 0.10) differences between the Treatment: Exposure group and all three control groups and the Treatment: Pre-Exposure group. The Treatment: Post-Exposure group was marginally significantly (p-value < 0.10) different to the Control: Exposure and Control: Post-Exposure groups. In minutes 10 and 15, there were also significant (p-value < 0.05) and marginally significant (p-value < 0.10) differences between the Treatment: Exposure group and other groups.

In the minute 1 pair-wise tests of multivariate dispersion, the Treatment: Exposure group was significantly (p-value < 0.05) or marginally significantly (p-value < 0.10) different than all other groups. In the minute 10 pair-wise tests of dispersion, the Control: Exposure group was significantly (p-value < 0.05) or marginally significantly (p-value < 0.10) different than all other groups.

Separate principal component analyses ordinated 167 black sea bass behavioral time budgets during minute 1, 5, 10, and 15 of behavior recording. In minute 1, the first two PC axes explained significant variation (58.7% of total variation) in the behavioral time budgets. All seven behavior variable loadings were statistically significant (p-value < 0.001) (Figure 9a). In minute 5, the first two PC axes explained significant variation (54.9% of total variation) in the behavioral time budgets. All seven behavior variable loadings were statistically significant (p-value < 0.001) (Figure 9c). In minute 10, the first two PC axes explained significant variation (53.5% of total variation) in the behavioral time budgets. The back-finning behavior variable loading was no longer statistically significant (p-value = 0.431), but the remaining six behavior variable loadings were statistically significant (p-value < 0.001) (Figure 9e). In minute 15, the first two PC axes explained significant variation (53.0% of total variation) in the behavioral time budgets. The back-finning behavior variable loading was no longer statistically significant (p-value = 0.508), but the remaining six behavior variable loadings were statistically significant (p-value < 0.001) (Figure 9e).

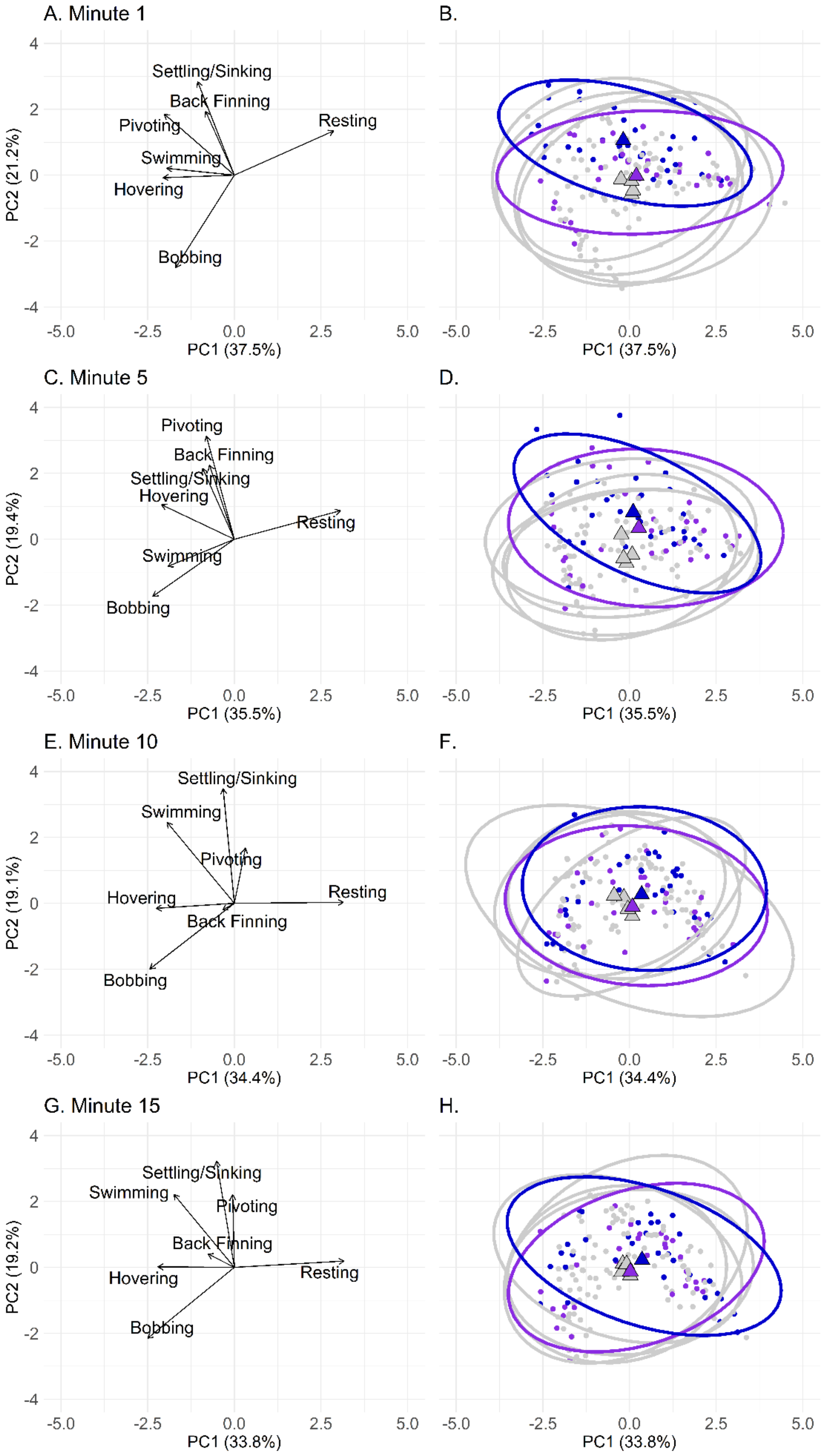

A. Minute 1
B.
Settling/Sinking
Back Finning
Pivoting
Resting
Swimming
Hovering
Bobbing
PC2 (21.2%)
PC1 (37.5%)
PC1 (37.5%)
C. Minute 5
D.
Pivoting
Back Finning
Settling/Sinking
Hovering
Resting
Swimming
Bobbing
PC2 (19.4%)
PC1 (35.5%)
PC1 (35.5%)
E. Minute 10
F.
Settling/Sinking
Swimming
Pivoting
Hovering
Resting
Back Finning
Bobbing
PC2 (19.1%)
PC1 (34.4%)
PC1 (34.4%)
G. Minute 15
H.
Settling/Sinking
Swimming
Pivoting
Back Finning
Hovering
Resting
Bobbing
PC2 (19.2%)
PC1 (33.8%)
PC1 (33.8%)
4
2
0
-2
-4
-5.0
-2.5
0.0
2.5
5.0

Figure 9. Behavioral time budget ordination based on principal component analysis for each one-minute segment within the 15-minute recording segment
For all PCA plots, blue points and ellipses correspond to the Treatment: Exposure group, purple points and ellipses correspond to the Treatment: Post-Exposure group, and 95% confidence ellipses are included. **A.** Behavioral variable loadings on PC axes 1 and 2 for minute 1. The length of the vectors indicates the strength of the associated variable for describing the PCs. The direction of the vectors indicates the direction of the associated variable gradients in ordination space. **B.** Ordination of the 167 behavioral time budgets in multivariate space for minute 1. **C.** Behavioral variable loadings on PC axes 1 and 2 for minute 5. **D.** Ordination of the 167 behavioral time budgets in multivariate space for minute 5. **E.** Behavioral variable loadings on PC axes 1 and 2 for minute 10. **E.** Ordination of the 167 behavioral time budgets in multivariate space for minute 10. **F.** Behavioral variable loadings on PC axes 1 and 2 for minute 15. **G.** Ordination of the 167 behavioral time budgets in multivariate space for minute 15.

During minutes 1 and 5, the variable loadings indicated that pivoting, back-finning, and sinking behaviors were positively correlated; swimming and hovering behaviors were positively correlated; and resting and bobbing behaviors were negatively correlated (Figure 9a, c). The variable loadings for minutes 10 and 15 were highly similar and indicated that sinking, swimming, and pivoting behaviors are positively correlated; resting and hovering behaviors were negatively correlated; and resting and bobbing behaviors were negatively correlated (Figure 9e, g).

Differences in behavioral time budgets for the six groups during each one-minute segment were visualized on the PC axes. During minute 1, the ordination of observations in the Treatment: Exposure group displayed different behavioral patterns than the other five groups based on visual inspection. The Treatment-Exposure group was strongly associated with sinking, pivoting, back-finning, and resting behaviors and was not associated with bobbing behavior. The other five groups were more strongly associated with bobbing behavior (Figure 9b). Minute 5 exhibited similar behavioral patterns to minute 1, although the Treatment: Exposure group displayed more variance than in minute 1 (Figure 9d). During minutes 10 and 15, the behavioral patterns in the six groups were visually indistinguishable (Figure 9f, h).

Do previous exposures to replayed pile driving signal affect C. striata behavior?

Boxplots comparing time budget distribution between exposures (1st, 2nd, 3rd) revealed elevated median time proportion spent resting and decreased median time proportions spent bobbing and swimming for experimental fish groups after subsequent exposures. Boxplots for control fish show no such trend (Figure 10).

When comparing sound and control groups using Mann Whitney U-tests, significant differences ($p<0.05$) occurred in trial three for bobbing (post-exposure) behavior, and marginally significant differences ($p<0.1$) occurred in trial three for resting (post-exposure) and swimming (post-exposure) behaviors.

A large effect size (>0.5) was found between control and sound groups during trial three for resting, bobbing, and swimming behaviors during the post-exposure period. A medium effect size (0.3-0.5) was found between control and sound groups during trial three for resting, bobbing, and swimming behaviors in the exposure period; during trial three for resting and bobbing in the pre-exposure period; and during trial two for bobbing behavior in the post-exposure period.

These results indicate a pattern of increased altered behavior in experimental groups after repeated sound exposures.

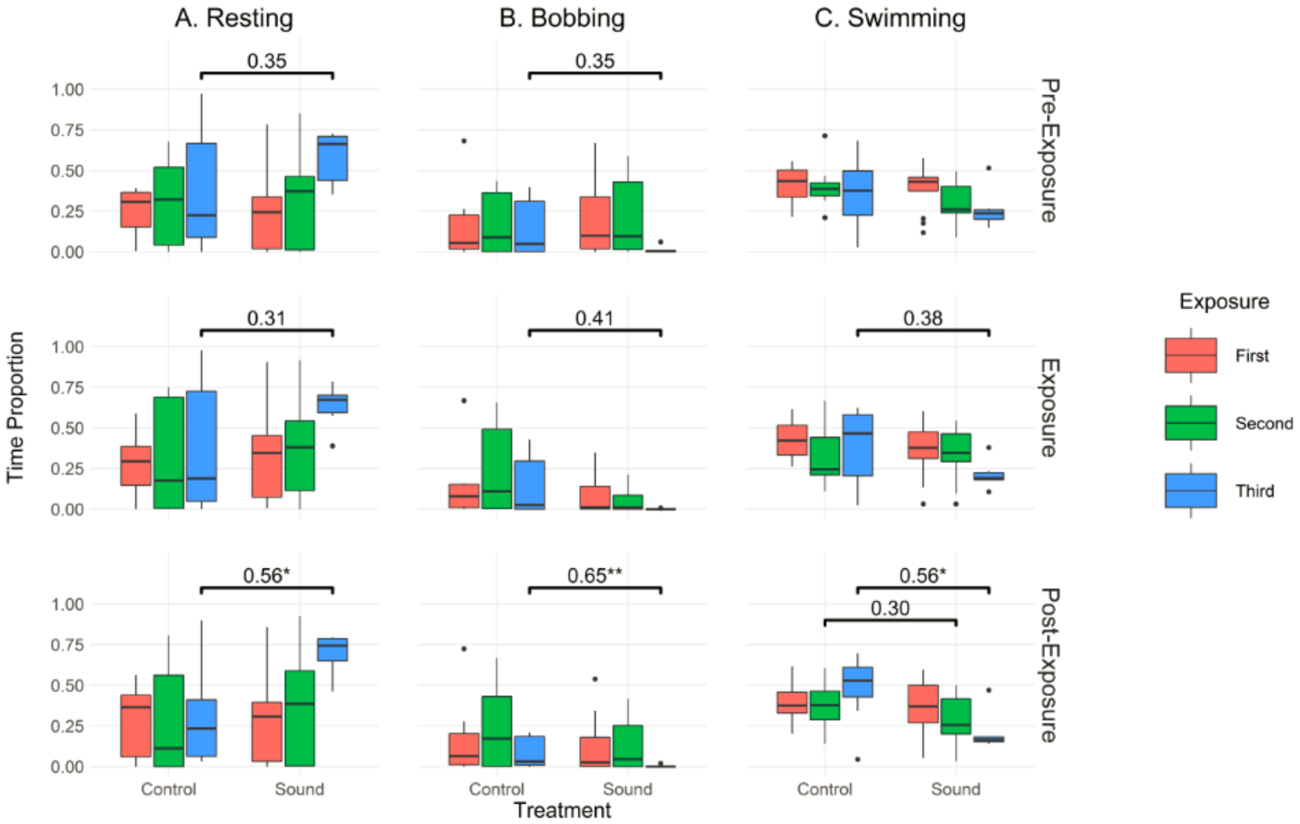


Figure 10. Boxplots of behavioral time budget for resting, bobbing, and swimming behaviors for averaged minute segments within each 15-minute recording segment
For all plots, red corresponds with control or experimental fish in their first sound 'exposure'; green corresponds with control or experimental fish in their second sound 'exposure'; and blue corresponds with control or experimental fish in their third sound 'exposure'. Plots are arrayed vertically by exposure phase (pre, during, and post-exposure) and horizontally by behavior (resting, bobbing, swimming). The x-axis corresponds with treatment group (control vs. sound) and the y-axis represents average proportion of time spent on a given behavior. A. Boxplots displaying proportion of time spent resting by fish in subsequent sound exposures (pre-exposure, during exposure, and post-exposure). B. Boxplots displaying proportion of time spent bobbing by fish in subsequent sound exposures (pre-exposure, during exposure, and post-exposure). C.

Boxplots displaying proportion of time spent swimming by fish in subsequent sound exposures (pre-exposure, during exposure, and post-exposure).

3.2.4. Discussion

The current study used a behavioral response design to examine the effect of pile-driving playback on black sea bass behavior in a controlled tank environment. A multivariate statistical approach was used to search for and summarize behavioral patterns and to examine interrelationships among behavioral variables. This study seeks to understand both black sea bass behavioral changes in response to pile-driving sounds, as well as black sea bass's ability to habituate to pile-driving sounds during exposure. The results of this investigation provide support for a measurable behavioral response to pile-driving sounds for black sea bass and that these changes in behavioral patterns are strongest at the onset of sound exposure.

Do black sea bass exposed to replayed pile-driving signal exhibit changes in behavioral patterns?
Through pair-wise tests of discrimination between groups, this study finds significant to marginally significant differences in black sea bass behavioral patterns during exposure to pile-driving signal, compared to control and pre-sound period behavior. During exposure periods (those exposed to one of three pile driving tracks) there were observed increases in certain behaviors such as sinking, pivoting or resting during signal exposure periods, compared to those in the Control groups (those exposed to a silent track during 15 min exposure period) and baseline pre-sound periods. These behaviors replaced higher amounts of time bobbing and swimming. This sinking and pivoting behavior is consistent with behavioral responses observed in other studies in other fish to anthropogenic sounds. For example, exposure to moderate sound levels affect swimming behavior of zebrafish by changing swimming speed and height (Neo et al. 2015a). Wild-caught Atlantic salmon have been reported to swim down in response to pure tones (Knudsen et al. 1992), and European seabass have been observed to swim faster, deeper, and away from pile-driving playback in large outdoor pens (Neo et al. 2016). These above observed behavioral patterns exhibit an opposite trend to certain Sebastes species. When exposed to air gun signals in an controlled tank environment, rockfish spend more time in the upper two-thirds of the enclosure (Pearson et al. 1992).

Historically, multivariate statistical approaches assessing animal behavior have been used in behavioral genetics to link rat behavioral patterns to genetic underpinnings (Berton et al. 1997). Similar approaches can be used to reveal behavioral patterns and relationships among behaviors in fish. Through the ordination procedures used in this study, a few behavioral patterns emerge. Time spent sinking and pivoting are positively correlated, and the time spent sinking-pivoting has no relationship to time spent resting. Swimming and hovering are positively correlated, while bobbing and resting are negatively correlated. Analyzing single behaviors concurrently in multivariate space can reveal aggregate behavioral patterns. Black sea bass's sinking-pivoting or resting response to pile-driving sound exposure creates a more detailed, mechanistic understanding of "reduced activity" and "freezing" which can inform more complex behaviors.

For example, the sinking-pivoting and resting behaviors in response to pile-driving sounds appeared to reduce activity and freeze behaviors, which could have ecological implications for black sea bass, including altered predator avoidance behavior, feeding behavior, and aggregation behavior necessary for spawning (Mercer 1989). Additionally, potential altered behavior could impact black sea bass fisheries directly by causing changes in distribution in the water column. However, the fishery utilized a variety of extraction methods including but not limited to; hook and line, long line, trap, and trawl, therefore, a potential change in catch position in the water column could be detected and methods altered accordingly.

There could also be potential indirect effects through the ecological consequences with the potential to affect population stability such as potential movements in and out of spawning areas during the time of sound exposure/construction. However, construction is usually limited in time in relation to a windfarms lifetime and potential movements could be reversed once construction is completed. There has been anecdotal evidence from fishermen that the added bottom structure provided by windfarms can increase animal abundance in an area, particularly if there was no structure there previously. However, to assertively answer these larger scale movement questions, further *in situ* experiments are required.

Do changes in black sea bass behavioral patterns diminish during exposure to the signal?
Black sea bass exhibited the strongest behavioral response to pile-driving sounds during the first minute of sound exposure, and these behavioral changes diminish throughout the 15-minute sound exposure segment. During minute 1, black sea bass exhibit a significantly distinct sinking-pivoting or resting behavioral response to sound exposure. Additionally, their behavior becomes significantly less variable during the first minute of sound exposure, compared to control (silent) and pre-exposure groups. These same behavioral patterns are apparent during minute 5 but diminish towards the end of the exposure by minutes 10 and 15. By minutes 10 and 15, the behavioral patterns of black sea bass during sound exposure were only marginally significantly different than some other groups based on perMANOVA tests. The diminished differences in behavioral patterns through the sound exposure suggest a degree of behavioral habituation to these signals. These findings are similar to previous studies showing evidence for intra-trial habituation. For example, after responding to intermittent sounds with altered behavior including increased swimming speed, swimming depth, and group cohesion, European seabass displayed reduced behavioral changes throughout exposure (Neo et al. 2016, Neo et al. 2018). However, a decrease in response does not necessarily denote habituation, where animals hear selectively while filtering out repeated or irrelevant sound signals (Rankin et al. 2009). The observed decrease in behavioral response can also be attributed to sensory adaptation, or reduced sensitivity of the hearing organs, as well as motor fatigue, or unresponsiveness due to exhaustion (Domjan 2015). Future work should determine the mechanism of behavioral recovery since different mechanisms vary in ecological implications.

Most studies investigating behavioral responses of fish to anthropogenic sound stimuli investigate intra-exposure behavioral habituation, but it is uncertain how repeated and extended exposure will affect fish behavior. The current studies observations suggest increased altered black sea bass behavior with subsequent exposures to sound (periods one – three) (Figure 10). Black sea bass exposed to pile driving signal tended to rest more and bob and swim less by the third sound exposure period (contrary to decreasing behavioral pattern within a period – see section above). Increased "resting" may be a "hunker down" behavioral response in response to the sound exposures. This trend did not emerge in control groups, negating the possibility that increased resting is normal for black sea bass throughout a normal day in the experimental tanks. However, the statistical power of these analyses is limited by the small sample size in the study control (n=8) and experimental groups (n=13). Some behavioral investigations have identified decreased behavioral changes in fish after weeks of repeated sound exposures to motor boat noise (Nedelec et al. 2016). However, there is broadly little evidence as to whether repeated exposure sessions over long time periods cause behavioral responses to accumulate, leading to either stronger responses through sensitization or diminished responses through de-sensitization or habituation. Future black sea bass behavioral analyses should assess these potential long-term behavioral responses.

3.2.5. Conclusions

As the industry for offshore wind energy grows in the United States, it is critical to investigate the full scope of offshore energy's environmental impact. Development along the eastern seaboard will overlap with the range of black sea bass, a fish species of ecological and commercial importance. This study investigates the behavioral response of black sea bass to replayed pile-driving signal patterns and frequencies, finding altered behavioral patterns during sound exposure. Multivariate analyses indicate these changes in behavioral patterns underly reduced activity, including greater time spent sinking, pivoting or resting. Additionally, black sea bass display diminished behavioral response withing an exposure period, which could indicate habituation, however, with increased responses among exposure periods (one - three), which could indicate re-sensitization. Future work should investigate how repeated and extended exposure will affect fish, and how behavioral response patterns are associated with changes in more complex behaviors, including changes in migration, feeding and breeding grounds, or stress-induced reduction in reproductive output.

3.3. Study 3: Feeding changes in response to replayed pile driving signal

Manuscript in Prep: Stanley, J. A., Renahan, M., Jones, I. T., Schumm, M., Phelan, B., M., Mooney, T. A. Changes in feeding and general behavior in *Centropristis striata* in response to replayed pile driving exposure in a controlled environment.

3.3.1. Background

As renewable energy development expands globally to meet demand for electricity, the world's oceans are becoming increasingly urbanized. Particularly, development on the eastern seaboard of North America has led to the first major marine wind energy installations to be permitted within U.S. waters, with the Block Island Wind Farm off the coast of Rhode Island as the U.S.'s first offshore wind farm. Development of these renewable energy regions will lead to an increase in pile-driving during the construction process. Pile-driving produces highly intensive and impulsive strike sounds as a metal pile is hammered into the ocean floor. Previous research suggests that marine pile-driving can cause negative behavioral effects in fishes, including anti-predator behavior (Spiga et al. 2017), disruption to schooling dynamics (Herbert-Read et al. 2017), avoidance of essential feeding and spawning habitats, and disruption of essential intraspecific communication (Allison et al. 2019). Additionally, fishermen have expressed concern that pile-driving sounds will have negative effects on the behavior and distribution of target species (Thomsen et al. 2006).

Relatively few studies have investigated changes in aquatic animals' feeding and foraging behavior during noise. Sustained reductions in animals' feeding behaviors due to anthropogenic stressors could lead to reduced survival, especially in regions with patchy prey distribution or limited prey abundance. The current study focused on the black sea bass, a species of commercial and recreational importance along the eastern coast of the United States. Black sea bass occur along the entire eastern seaboard of North America, and the northern-most population undergoes a seasonal cross-shelf migration, moving north and inshore from southern and deeper waters (Steimle et al., 1999). Black sea bass are largely carnivorous demersal feeders, consuming crustaceans (primarily crabs and shrimp), smaller fishes, molluscs, and echinoderms, with adults mainly eating crabs and fishes (Mercer 1989). Physiological examinations indicate that the frequencies at which black sea bass are most sensitive to sound directly overlap with frequencies of high-amplitude anthropogenic noise pollution, including noise from activities like shipping and the underwater construction required for offshore wind farms (Stanley et al. 2020; Study 1). To date there has been no clear evidence of a definitive ancillary structure to transduce the pressure component of the sound field to the ears, therefore it is expected that this species is largely detecting the particle motion component of the sound field.

In the present study, using a controlled tank environment, we examined how playbacks of impact pile driving signals influenced feeding success and behavior of *Centropristis striata* (black sea bass). In daytime experimental trials, non-living food pieces were added to the experimental tank to quantify the fish's' feeding behavior and time taken to successfully feed.

3.3.2. Methods

3.3.2.1. Experimental tank and audio set up

Experiments occurred in a cylindrical, 1.8-m-diameter tank in the ESL during daylight hours. Water was maintained at a depth of 0.9 m and temperature of 18.0 + 1.2 °C (mean + SD). A slow water inflow-maintained tank temperature and high dissolved oxygen levels. The bottom of the tank had a 3 cm layer of sand. An Aqua-30 speaker (DNH, Netherlands; frequency response: 0.08–20 kHz) connected to a Pyle 2-channel stereo amplifier (2000 W Dual Channel – PLA2378), 12-V battery and Dell Latitude laptop with Adobe Audition was used for playback of acoustic files. A hydrophone (High Tech Inc., USA; sensitivity: -165 dB re 1 V/µPa; frequency response: 0.002–30 kHz) connected to a SoundTrap ST4300 acoustic recorder (Ocean Instruments, NZ; 4 dB gain) was suspended in the tank to monitor sound respectively during experiments, with the ST4300 located outside of the tank. Trials were recorded with an overhead camera (Sony Handycam HDR-XR550), and two underwater cameras (GoPro Hero 6) facing each other from opposing sides of the tank at mid-depth, so that video of the entire inside of the tank was captured during experiments (Figure 11).

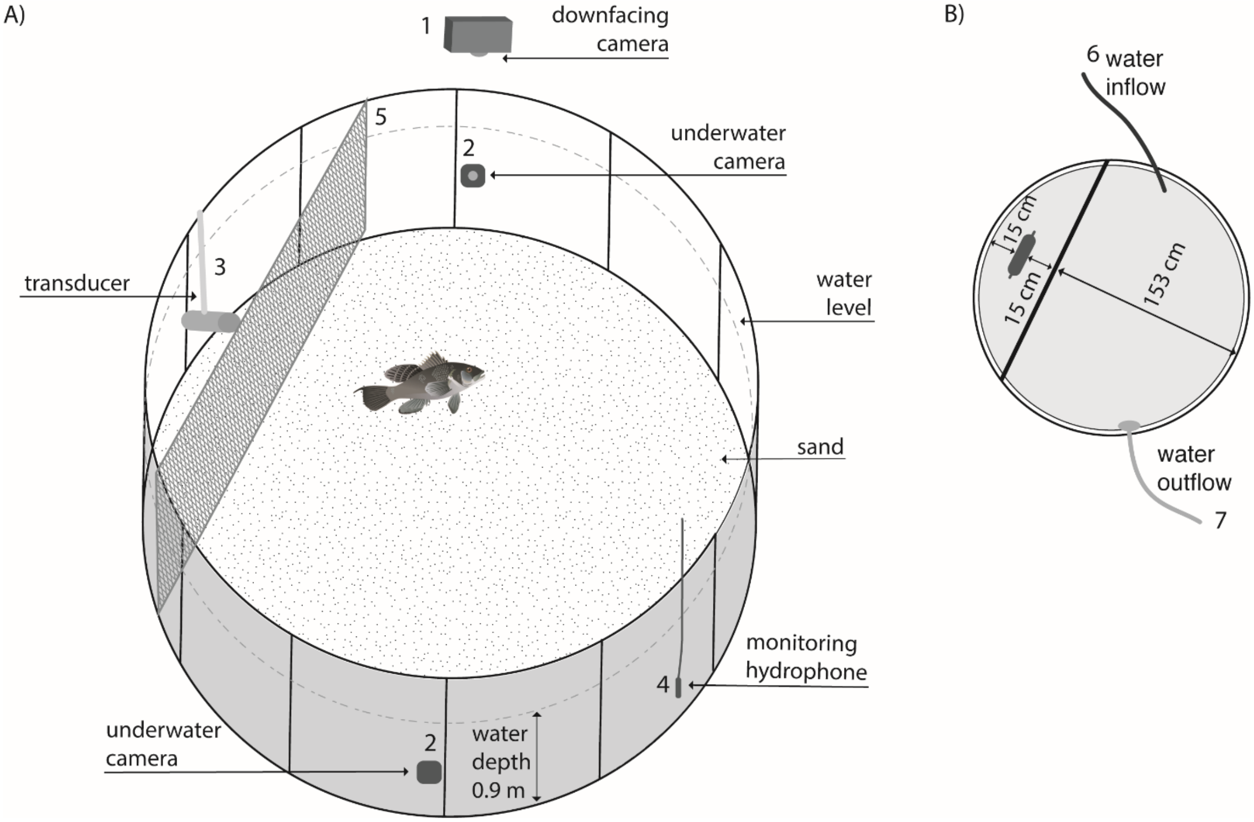


Figure 11. Schematic of experimental tank
Schematic of experimental tank set-up from top-down view. 1) downward facing camera, 2) locations of underwater cameras, 3) transducer (speaker), 4) hydrophone, 5) mesh barrier, 6) water inflow, 7) water outflow. Measurements, but not all objects shown, are to scale.

3.3.2.2. Experimental procedures

Experiments were conducted between June 27 and August 1, 2018, and June 21 and July 2, 2019 (n=18) and took place from 0800 and 1300 local time. All fish used in experiment were placed in an isolation tank and withheld food for 48 hours before experiment was to commence.

During exposure periods, fish were exposed to either 15-min long pile driving playbacks or 15-min long no sound control playbacks (hereafter referred to as “Treatment” and “Control” trials, respectively). Pile driving files had an inter-pulse interval of ~1.8 s, and were recorded from a hydrophone array 500 m away from a pile driven for the Block Island Wind Farm, 1 m above the seabed, in water 26 m deep (Amaral et al. 2018, Amaral et al. 2020). These field data, along with particle acceleration calculated from hydrophones in the field, were provided to the authors (see Acknowledgements). Three distinct 15-minute pile driving playback files were prepared using Raven Pro 2.0.

Fish were tested in isolation (one fish per experiment) during each experiment. A single fish was placed within the experimental tank between 1300 – 1400 local time the day before an experimental trial was to take place to enable acclimation it to its surroundings. During the trial, two underwater cameras and one overhead camera recorded video for scoring behavior after the trial had ended. Additionally, a four-channel cabled SoundTrap hydrophone recorded all sounds within the tank including any sounds made by the fish, ambient sounds, and the recording of the pile driving. One hour before the experiment was due to start underwater cameras were put in place in the experimental tank and set to record. The downward facing camera as well as the SoundTrap was also set to record at the start of the Acclimation period.

For all trials a strict exposure regime was followed (Figure 12), in which both the Treatment and Control trials began with a 15-minute Acclimation period. This was followed by either a 15-minute pile driving, or ‘Silent’ period and food was immediately released onto the surface of the water in the middle of the tank with minimal surface disruption (feeding event). The fish was then exposed to two further 15-minute pile driving recordings (average SPL 0-peak 174 dB re 1µPa, taken from field recordings at 500 m from source) or ‘Silent’ recordings, punctuated by 15 minutes of ambient sound (Figure 12). The three food pieces consisted of 10 g ± 1 g of fresh (deceased) longfin squid.

Two trained observers (50% overlap of analyzed trials) watched videos and recorded whether at each feeding even the fish was successful at feeding and if so, the amount of time it took for successful feeding. Further analysis of video is currently underway to quantify other behaviors in preparation for manuscript.

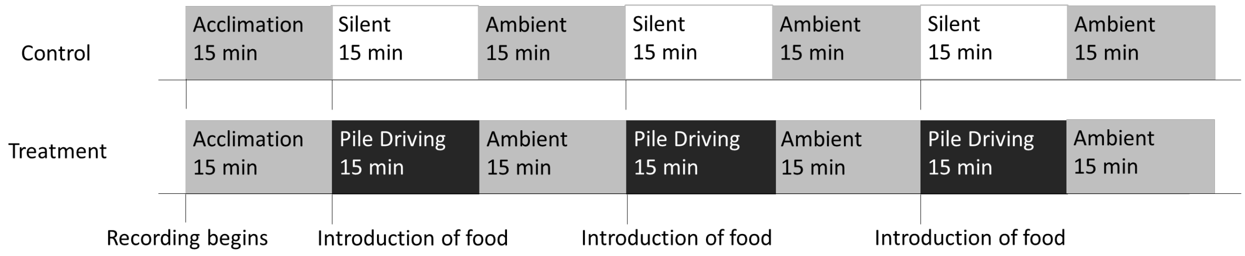


Figure 12. Schematic of exposure regimes in both Control and Treatment trials

3.3.2.3. Acoustic calibration of the experimental tank

Briefly, 1 min of a pile driving file (same as used in experiments) was played through the speaker and recorded in 20 cm increments in all three dimensions (240 recording positions total). It is assumed that the primary method for acoustic detection is particle motion as opposed to pressure, therefore, particle acceleration was recorded, as well as sound pressure for comparison with other studies. Recording instruments were affixed at the end of a PVC probe in the following configuration: a PCB triaxial accelerometer (model W356B11, frequency response: 0.5 Hz–5 kHz, sensitivity of each axis: 1.04 mV / m s-2) was centered at the recording position, and a Reson hydrophone (model TC4013, frequency response: 1 Hz–170 kHz, sensitivity: -211 dB re 1 V/µPa) was spaced 1.5 cm to the left of the accelerometer (facing the speaker).

Acoustic data analyses were performed following previous methods (Jones et al. 2020), and are briefly summarized here. Zero-to-peak levels of individual pile pulses, in decibels (dB), were calculated for particle acceleration ($\text{SAL}_{\text{z-pk}}$) and sound pressure ($\text{SPL}_{\text{z-pk}}$) as follows:

$$SAL_{z-pk} \text{ or } SPL_{z-pk} = 20 * \text{Log}_{10}(X_{pk})$$

where X_{pk} is the maximum absolute acceleration (µm s-2) or pressure (µPa). For simplicity, the 3D vector (Euclidean) norm of particle acceleration was calculated, and its magnitude is reported. Power spectral density (PSD) was calculated to visualize these magnitudes across frequencies. All acoustic metrics were limited to 20–1000 Hz, encompassing the hearing range of *C striata*.

3.3.2.4. Statistical analysis

To test for significant differences in feeding success between the two treatments Z-Tests were used on the data independently for each of the three feeding events. To test for a significant different in time to successful feeding between the two treatment the non-parametric Mann-Whitney Rank Sum Test was used. Non-parametric methods were used where the data were not normally distributed and/or of uneven variance (Zar 1999).

3.3.3. Results

3.3.3.1. Acoustic calibration of the experimental tanks

The sound field of pile driving playback in the experiment tank was spatially variable, with highest zero-to-peak levels (up to 145 dB re 1 μm s^{-2}) nearest the speaker, and a 20–40 dB drop-off horizontally toward the center of the tank (Figure 15). Vertically, sound levels were greater near the center of the tank water column, compared to near the surface and bottom. Acceleration levels were up to 20 dB higher in the tank than in the original field recording between 20 and – 100 Hz, and more similar in the between 400–1000 Hz range. Conversely, PSD of sound pressure was lower in the tank than the field recording, across frequencies. Ambient (no playback, speaker unpowered) and silent playback (silent file played through the powered speaker) spectra were similar to each other and were as much as 50–60 dB lower than the pile driving playback (Figure 15). The accelerometer was not sensitive enough to detect particle acceleration in these two conditions, therefore these recordings resulted in a flat PSD curve at the accelerometer's self-noise floor (55 dB re 1 μm s^{-2}).

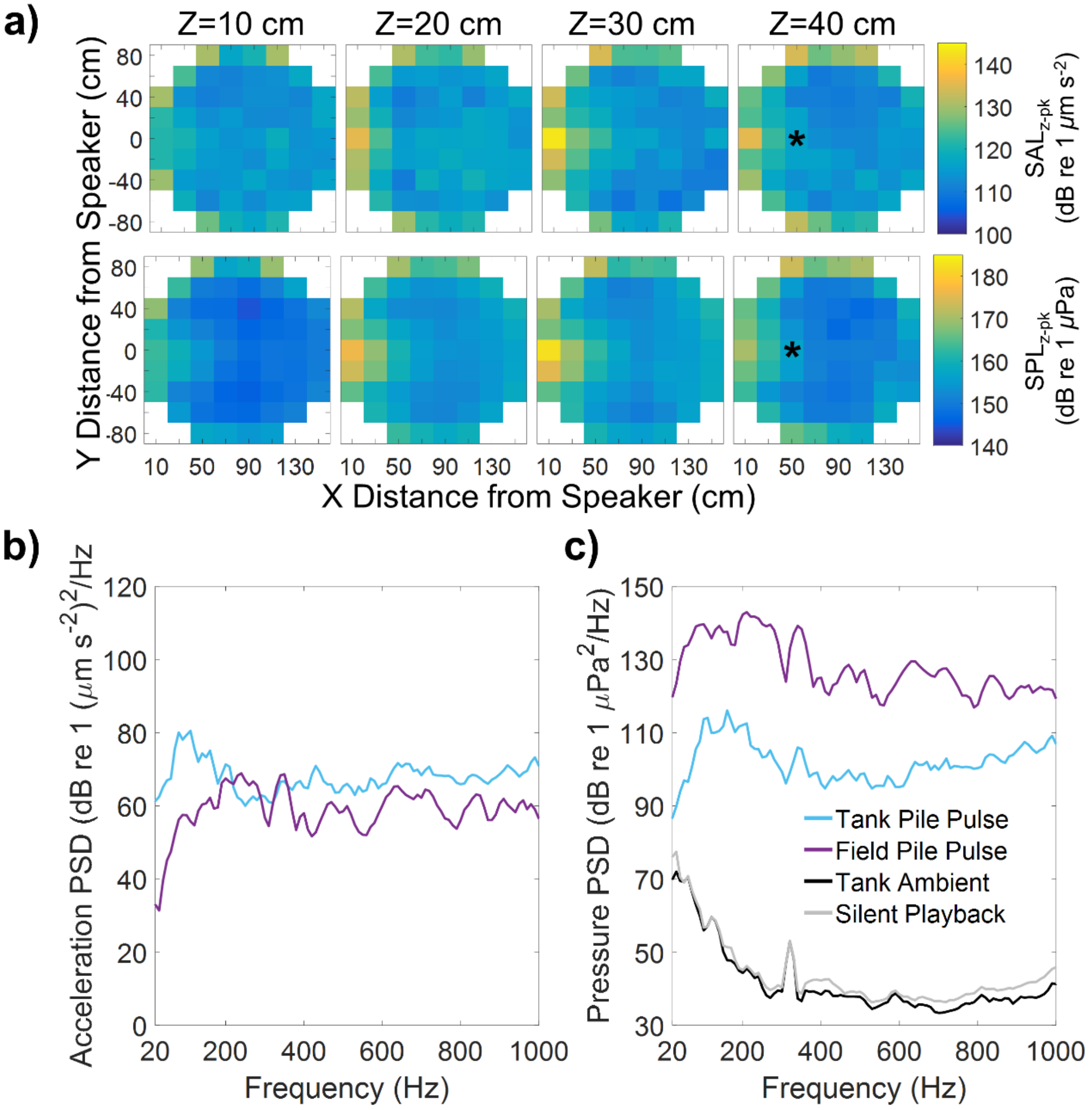


Figure 13. Spatial maps of pile driving playback recorded in the experiment tank
a) Spatial maps of zero-to-peak acceleration (top) and pressure (bottom) for four water depths (columns), shown from top-down in the horizontal plane, with the front-center of the speaker set as the origin. Data were band-pass filtered to 20-1000 Hz and median zero-to-peak values of pile pulses (across 30 pulses, i.e., 1 min) are shown for each recording location. b) Power spectra of pile driving playbacks in the tank compared with original field recordings, ambient tank sounds, and the silent audio file in the tank. Asterisks indicate the location of data shown on the power spectra plots. The accelerometer was not sensitive enough to detect ambient and silent playback conditions, hence these spectra are only presented in pressure.

3.3.3.2. Feeding success

Successful feeding occurred significantly less in Treatment trials exposed to pile driving signal compared to those in Control trials (Figure 13). During feeding event one, Control trials had 100 % of fish successfully feeding compared with 30 % in the Treatment trials (z test; z = 2.305, P = 0.02). Feeding event two had 66 % of fish successfully feeding compared to 10 % (z = 1.924, P = 0.05), and feeding event three had 50 % of fish successfully feeding compared to 10 % (z = 1.3, P = 0.19) in the Control and Treatment trials respectively. Although a significant difference in successful feeding between Treatment and Control trials was observed, it was also observed that some fish in both trial types reduced feeding as the experiment (number of feeding events) progressed. All individuals in the Control trials successfully fed during the first feeding period, whereas only 50 % of the same individuals successfully fed during the third feeding period. However, 30 % of individuals in the Treatment trials successfully fed during the first feeding period, and 10 % during the third feeding period (Figure 13). No individual used in the Treatment or Control trials fed in subsequent feeding trials after an unsuccessful feed, i.e., if a fish did not feed in the second feeding period, it did not go on to feed in the third feeding trial.

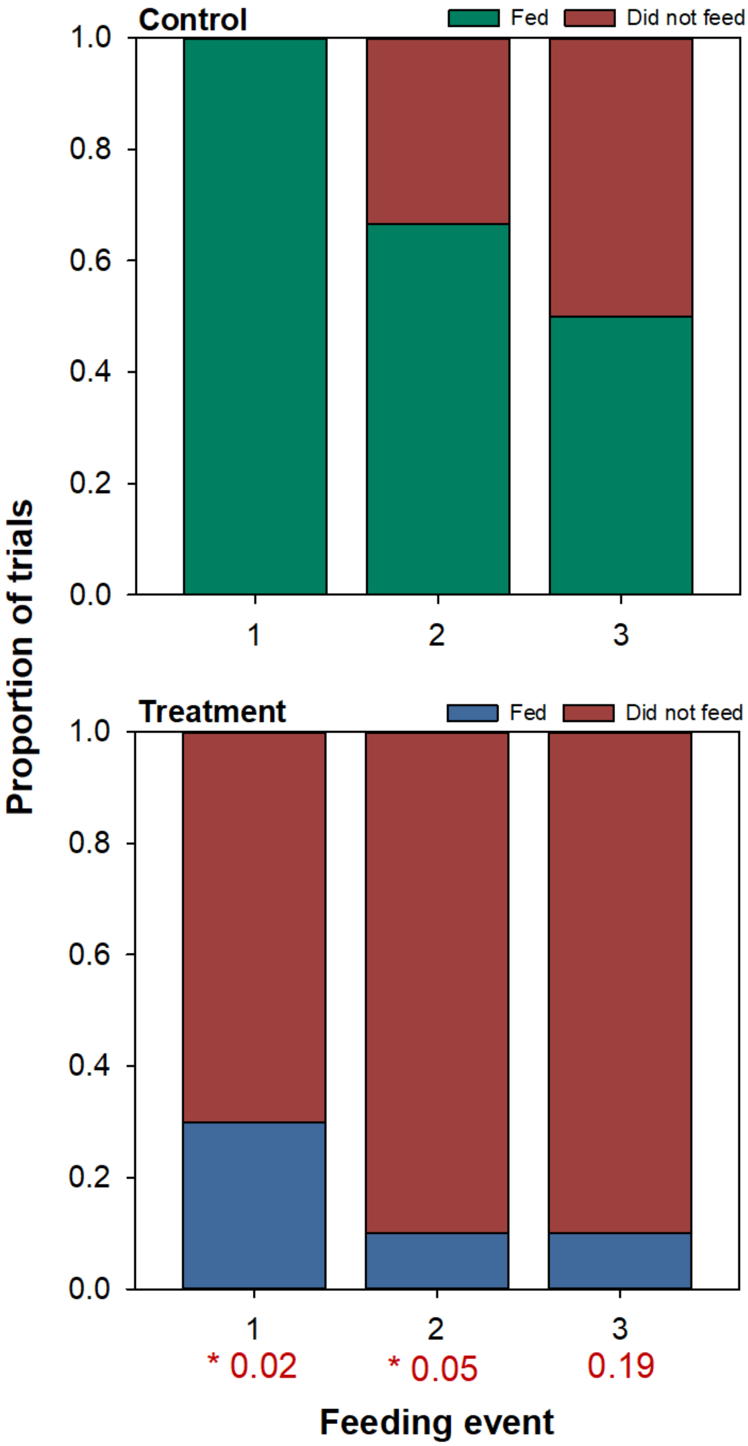


Figure 14. Proportion of trials with successfully feeding
Stacked histogram plot showing the proportion of trials which had individuals which successfully fed. Red asterisk indicates significant difference with respective p-values.

3.3.3.3. Time to feeding

In individuals which successfully fed, there was a significant difference between the time it took to feed between the Treatment and Control groups (Mann-Whitney Test; U=10.5, *P*=0.019) (Figure 14). In those individuals that successfully fed, the fish in the Treatment group, exposed to pile driving sound, took significantly longer (between 3 and 840 seconds, with a median of 281 s) to feed compared to the Control fish (between 2 and 10 seconds, with a median of 7.5 s).

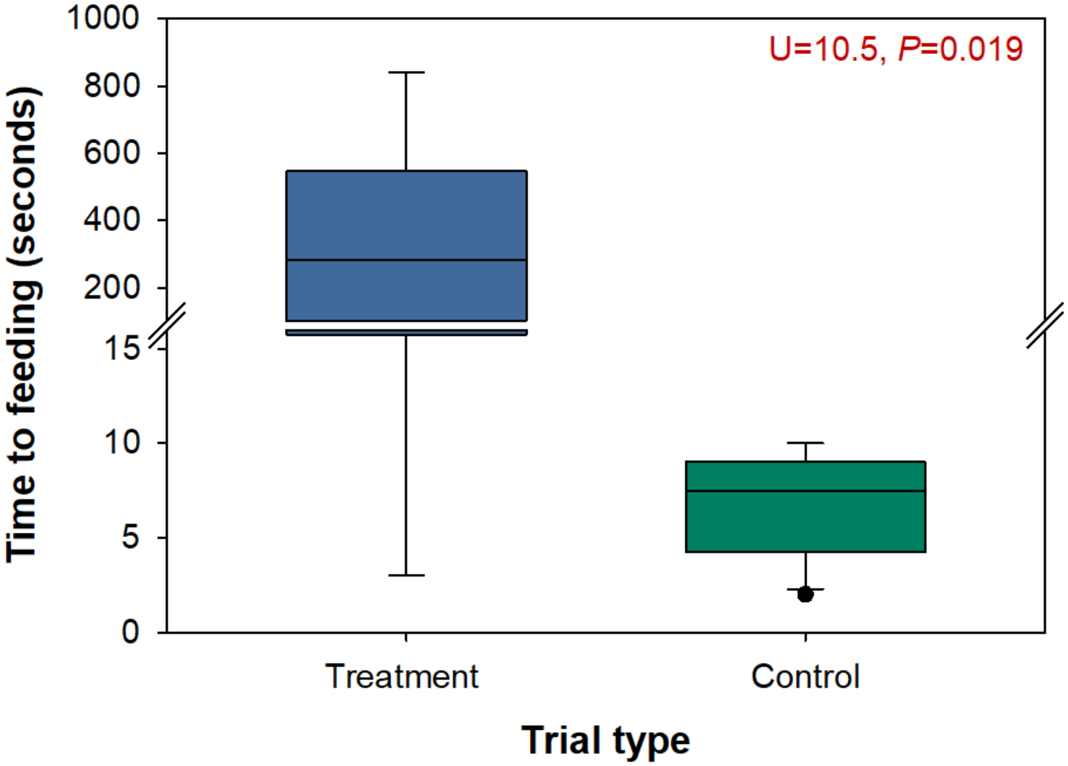


Figure 15. Time to successful feeding
Box plot showing the median and quartiles of time to feeding in seconds when individuals successfully fed during experimental trials and Mann-Whitney Test Statistic for the difference between the treatment and control trials.

3.3.4. Discussion

The fish exposed to the replayed pile driving signal showed lower rates of successful feeding and in the fish that did feed in the experimental group, they took significantly longer compared to that of the Control group. General behaviors have not yet been quantified for all experimental trials, but those so far showed similar results to those in Study 2, whereby activity levels were reduced from the onset of sound exposure. Clear behavioral changes were observed in fish exposed to pile driving, including freezing, startling, and seeking out of shelter near any structures such as the mesh barrier, the underwater speaker, and cameras. Behavioural/Physiological changes in the experimental group also included stress banding and overall lightening in color of the fish. These behavioral/physiological changes indicate a level of stress in these animals.

All fish, whether in the control or treatment trials, were less likely to feed in the second and third feeding, than the first, suggesting that some of the fish may have become satiated and were unwilling to feed due to lack of hunger or trial conditions were not conducive to multiple feedings, although this should not be the case due to very similar feeding methods in holding tanks.

Together, these results suggest that exposure to replayed pile driving signal in a controlled environment appears to alter the feeding activity of individual black sea bass, decreasing feeding success and increasing the time it took to feed, potentially reducing the fish's capacity to forage effectively. Although the extent of this has yet to be directly investigated, anthropogenic noise, during an acoustic exposure, may transfer attention from these essential tasks and toward the stressor, if observed as a likely danger.

3.4. Study 4: General behavioral changes in responses to replayed pile driving in a large tank environment

3.4.1. Background

There are many sources of sound in nature including wind, water and other animals. However anthropogenic activity has become pervasive expanding in scope and intensity as humans have expanded urban development. Anthropogenic noise has existed in the marine environment for many decades. More recently, the development of offshore wind energy sites has increased attention to the ecological consequences of anthropogenic noise underwater (Hildebrand 2009). While much of the research has focused on marine mammals and protected species, fish and invertebrates constitute a much larger environmental biomass and have a greater economic effect on society (Hawkins and Popper 2016). These added sounds may have a wide range of effects on fishes depending on their intensity and detection by the fish. Intense sound may result in mortal injury, or sound may result in a physiological effect that reduces their fitness, or sounds may affect their behavior, causing them to move away from the sound. Detecting the sound and moving in response to it could result in a range of effects even if there are no physiological impacts. Perhaps the movement causes them to leave essential fish habitat for feeding or breeding, or alters their migration routes, or interferes with communication, or detection of biologically important sounds. While many behavioral effects are likely to be minimal and have little or no effect on fish fitness and survival some behavioral responses may have substantial short- and long-term effects upon them. As a consequence, the addition of anthropogenic sounds to the aquatic environment has the potential to do significant harm to fishes (Popper and Hawkins 2019).

The black sea bass is a warm temperate fish in the family Serranidae; the geographic range of this species extends from Nova Scotia to southern Florida (Bowen and Avise 1990). Black sea bass (*Centropristis striata)* (BSB) support a valuable commercial and recreational fishery in the North, Mid and South Atlantic renewable energy planning areas. They show an attraction towards certain structurally complex habitats including rocky reefs, cobble and rock fields, stone coral patches, exposed stiff clay, and mussel beds, that occur within the current lease and planning areas (Steimle et al. 1999). Some of these habitats occur within the current renewable energy lease and planning areas either seasonally or year-round depending on water temperature. Black sea bass show affinity for certain habitats within the wind energy lease areas and are thus not a temporary resident of these lease areas (Guida et al. 2017). In the mid-Atlantic bight adult black sea bass undertake annual migrations across the continental shelf; individuals spend the winter offshore in the middle to outer continental shelf and migrate inshore in the spring as water temperatures increase (Musick and Mercer 1977, Fabrizio et al. 2005, Moser and Shepherd 2009). Black sea bass spawn in nearshore waters from April to October at depth between 18-45 m (Musick and Mercer 1977). In the fall, as inshore waters begin to cool adult and young-of-the-year black sea bass migrate offshore to the continental shelf. Mature males exhibit a bright blue hump on the nape during spawning season; however, differentiation of females from smaller mature males is not possible by external morphological characters alone (Steimle et al. 1999). The mid-Atlantic Bight stock is subject to recreational and commercial harvesting, with fisheries targeting individuals associated with reefs and other hard-bottom structure in coastal waters (Shepherd and Terceiro 1994). Fabrizio et. al. 2005 examined temporal patterns in habitat use by black sea bass at a former dredged material dump site off the coast of New Jersey with variable habitat characteristics. Black sea bass used the used the area for variable and prolonged periods of time (up to 6 months) with males (positively identified as male as opposed to unknown sex (female, subordinate males, and transitional males)) dispersing away earlier, presumably related to sex-specific pre-spawning behavior of the species. The distribution of black sea bass at the New Jersey site was not random and fish were detected most frequently at relatively shallow (<27.5 m) sites of varying bathymetry containing previously placed, coarse-grain material creating habitat preferred by black sea bass.

Commercial and recreational fishermen have expressed concern that sound produced during benthic surveys, pile driving, and operation of renewable energy facilities may be having negative effects of the behavior of the black sea bass, causing changes in catchabilities. There could be potential long-term sub-lethal behavioral impacts such as avoidance of essential feeding and spawning habitats and disruption of essential intraspecific communication (Bailey et al. 2010, Bailey et al. 2014). Effects of acute and chronic sound exposure may also affect necessary life functions for fish and invertebrates, including health and fitness, foraging efficiency, avoidance of predation, swimming energetics, migration, and reproductive behavior. Behavioral impacts can include startle responses or if capable, fish may leave the area of elevated noise levels (Popper and Hawkins 2019, Hawkins et al. 2020). The ability of a fish to detect and respond to biologically relevant sound is critical to maintain survival and fitness of individuals and populations (Popper and Hawkins 2019). Behavioral impacts to fish and invertebrate from anthropogenic noise remains a concern, as noise generated through pile driving may affect a much larger area for behavioral impacts than the area corresponding to potential mortality and injury (Hawkins and Popper 2016, Popper and Hawkins 2019). This species is known to detect sounds in the range of (80–1000 Hz) which may be used as environmental indicators but their sensitivities to anthropogenic sounds such as pile driving noise, and their behavioral responses to them, is not understood (Stanley et al. 2020).

In the present study, using a large (121,133 L) controlled tank environment, we examined how exposure to playbacks of impact pile driving signals influenced the general behavior of small groups of non-spawning black sea bass. Time spent exhibiting recognized behaviors was recorded the day before sound exposure, during a first day of exposure, the day after exposure, during a second day of exposure, and after the second exposure. By further understanding black sea bass behavioral responses to anthropogenic sounds and assessing their ability to habituate to sound exposure, we can gain a more comprehensive picture of offshore wind energy development’s environmental impact.

3.4.2. Methods

3.4.2.1. Fish acquisition and maintenance

Adult *Centropristis striata* were wild-caught via line fishing off the New Jersey coast during 2018 under Scientific Collecting Permit MFA-SCP No.1834, administered by the State of New Jersey Department of Environmental Protection. Any fish that was deemed not healthy, owing to capture or otherwise, was not used in experiments. Fish were held in 2.4 m diameter fiberglass tanks with flow through seawater. Temperature ranged from 12.4 – 20.9°C. All holding tanks were exposed to a 12:12 light cycle and fish were fed daily with Atlantic silverside (*Menidia Menidia*) and long finned squid (*Doryteuthis pealeii*). Sound levels in holding tanks were approximately 100.7 – 104.3 dB re 1 uPa in the 20 – 24,000 Hz range.

Pile-driving sound exposure experiments to measure the general behavior of non-spawning black sea bass adults took place from March 5, 2019, to May 21, 2019. All fish were measured for total length and weight and tagged in January prior to the start of the experiments. Black sea bass ranged in size from 252mm to 450mm (222.9 – 1150.4g). No fish was used in any experiment previously and was therefore naïve to the exposure signal and regime.

3.4.2.2. Experimental tank and audio set up

All experiments were conducted in an oval shaped 121,133 L fiberglass tank (Figure 16) at the James J. Howard marine Sciences laboratory at Sandy Hook, New Jersey. The tank (was 10.6 m long, 4.5 m wide, and 3 m deep, with eight rectangular observation windows (0.7 m wide and 1.2 m high) one in each end and three along each side. The tank was in its own room with concrete block walls and had independently controlled programmable lighting. Each session of the experiment was conducted using static water with no flow through to reduce noise and vibration in the tank. After an experimental session, the tank was emptied and refilled. Seawater upwells through coarse sand which covers the bottom of the aquarium (46 m^2) to a depth of 40cm then exits through drains at the top of the tank. For this experiment, photoperiod in the aquarium room was maintained at 12 h day:12 h night which was controlled by a computer driven bank of fluorescent lamps above the tank. Temperature in the tank ranged from 13.0 to 15.7 °C and averaged 14.1°C; salinity ranged from 22.1 to 22.3; avg 22.1; dissolved oxygen ranged from 7.41 to 8.45; Avg 7.86. cabled recording hydrophones and a remote recorder (SoundTrap 300) were deployed in the tank. Two shell bags with locally collected and washed oyster shell were placed on the bottom of the tank along the midline and approximately equidistant apart to encourage fish to display natural behaviors within the tank environment.

A J-9 underwater transducer (underwater speaker) (U.S. Underwater Defense Center, NY) was suspended horizontally in the tank, ~1.5 m from the substrate and surface and ~1 m from the tank wall and mesh barrier. To ensure fish did not swim behind the speaker, a mesh barrier was placed 1 m in front of the speaker (Figure 16). The J-9 transducer was connected to a Pyle 2-channel stereo amplifier (4000 W Dual Channel – PLA2678), truck battery and Dell Latitude laptop with Adobe Audition used for playback of acoustic files.

A cabled hydrophone (HTI-60-MIN/Low Noise, sensitivity: −203.8 dB re 1 V/µPa, gain: 6 dB, flat frequency response from 2 Hz to 30 kHz; High-Tech Inc., Long Beach, MS) was placed behind the mesh barrier 20 cm from the tank wall, 2 m from the speaker and 1.5 m from the surface, and the remote recorder at the other end of the tank to monitor ambient, bioacoustics and sound playbacks within the tank (not used for calibration). The hydrophone was attached to a Dell desktop computer with data acquisition custom MATLAB scripts (Mathworks, Natick, MA). Two cabled underwater cameras (Seaviewer 6000 HD) each positioned from each end of the experimental tank to get the entire field of view, were used to record behavioral responses to the pile driving stimuli for quantification. These cameras were also connected to the Dell Desktop Computer and audio and video were recorded with Noldus Media Recorder.

Audio files of pile driving signals used for experiments were recorded during construction of Block Island Wind Farm on October 25th, 2015 (between 16:00 and 20:00 UTC), from a hydrophone (HTI-94-SSQ, sensitivity: −203.8 dB re 1 V/µPa, gain: 6 dB, flat frequency response from 2 Hz to 30 kHz; High Tech Inc., Long Beach, MS) attached to benthic sled located 1 m from the seabed, 26 m deep and 0.5 km away from a pile driving site (Amaral et al. 2018, Amaral et al. 2020). These files were recorded at a 9766 Hz sample rate. The pile was a steel, hollow pile had a diameter of 127.0 cm, wall thickness of 3.8 cm, a rake of 13.27° with respect to vertical, and was driven up to 76.2 m deep into the seabed. These field data, along with particle acceleration calculated from hydrophones in the field, were provided to the authors.

To prevent pseudoreplication of playback stimuli, three distinct 30-minute pile driving playback files hereafter referred to as Pile 1, Pile 2, and Pile 3, were extracted from two different pile driving bouts and prepared using Raven Pro 2.0 (The Cornell Lab of Ornithology, Ithaca, NY) prior to playback. A 3-s fade-in of the sound file (before the pile driving sound was emitted) was applied to each recording to prevent artifacts that could result from the playback beginning at a higher amplitude.

Each recording was amplified by a custom magnitude to obtain the highest playback sound levels possible without clipping, with the goal to match as close as possible the received zero-to-peak sound pressure levels in the tank with those present 0.5 km from the BIWF pile driving site, i.e*.,* 190–194 dB re 1µPa. Median inter-pulse intervals were 1.56 s (IQR: 1.51–1.56 s), 1.82 s (IQR: 1.80–1.83 s) and 2.33 s (IQR: 2.31–2.42 s) for Pile 1, Pile 2, and Pile 3 respectively. Median zero-to-peak pulse amplitudes were 190.7 dB (IQR: 190.0–191.4 dB), 193.6 dB (IQR: 193.4–194.1 dB), and 194.3 dB (IQR: 193.6–194.6 dB) re 1 µPa, and median root-mean-square inter-pulse amplitudes were 140.9 dB (IQR: 140.5–141.6 dB), 138.0 dB (IQR: 138.0–138.6 dB), and 138 dB (IQR: 137.6–138.7 dB) re 1 µPa. Notably, pulse amplitude, inter-pulse time interval, and inter-pulse interval amplitude within a pile driving event will vary slightly from impact to impact and as the pile is driven into the sediment.

Here, playback amplitudes and inter-pulse intervals were not manipulated to be identical within or across the three pile driving files because we were interested in studying behavioral impacts of pile driving noise at a simulated distance from the pile, rather than studying responses dependent on these specific metrics. The variability in these metrics across impulses reflects that which a wild fish may experience near an offshore pile driving site. For use in control trials, a 15- min-long silent file was played.

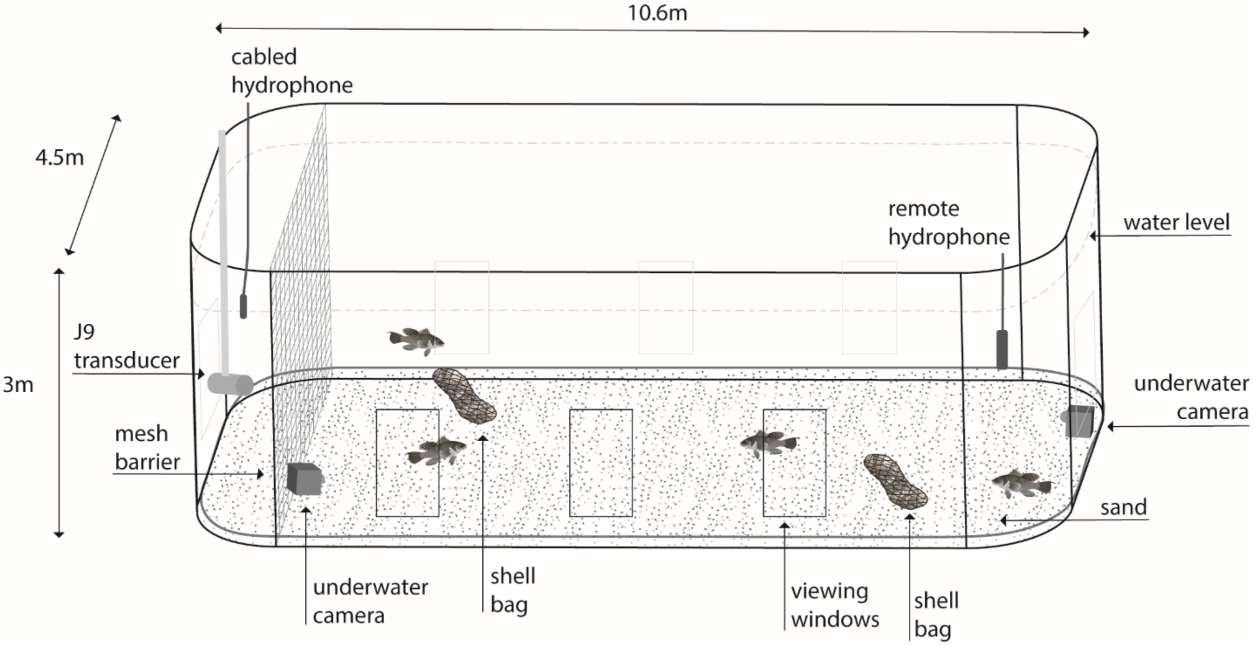


Figure 16. Schematic of large experimental tank
Schematic of experimental tank set-up from side view. Measurements, but not all objects shown, are to scale. An underwater speaker was positioned at one end of the tank and separated from the fish by a mesh barrier to prevent fish from getting too close to the speaker or behind the speaker. Two underwater cameras were positioned at opposite ends of the space the fish occupied in the tank.

3.4.2.3. Acoustic calibration of the experimental tank

The large experimental tank was calibrated at 54 locations, and at each location at four depths (216 total points of recording) within the tank using the playback set-up described in the above section. At each position, the first minute of each of the three pile driving noise files was played and recorded by four suspended cabled hydrophones (High Tech Inc., USA; sensitivity: -165 dB re 1 V/µPa; frequency response: 0.002–30 kHz) connected to a SoundTrap ST4300 acoustic recorder (Ocean Instruments, NZ; 4 dB gain, 48 kHz fs).

Acoustic data analyses were performed following previous methods (Jones et al. 2020), and are briefly summarized here. Zero-to-peak levels of individual pile pulses, in decibels (dB), were calculated sound pressure ($SPL_{z\text{-}pk}$) as follows:

$$SPL_{z-pk} = 20 * \mathrm{Log}_{10}(X_{pk})$$

where X_{pk} is the maximum absolute pressure (µPa). Power spectral density (PSD) was calculated to visualize these magnitudes across frequencies. All acoustic metrics were limited to 20–1000 Hz, encompassing the hearing range of *C striata*.

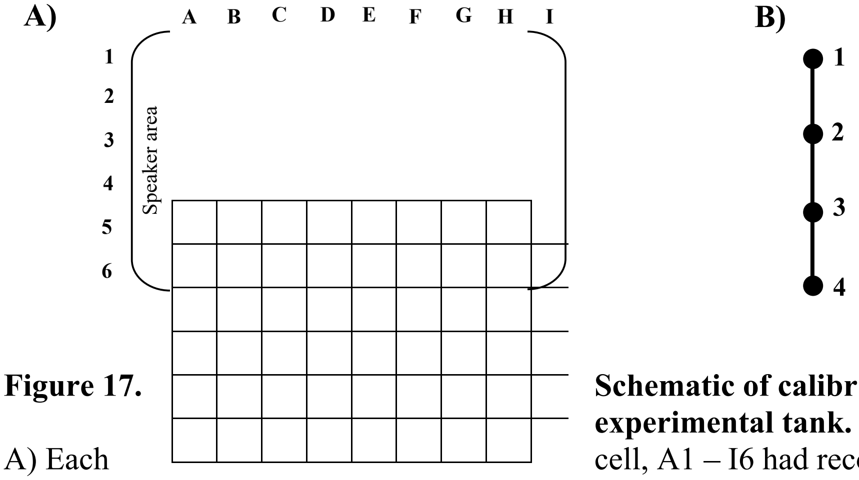


Figure 17. Schematic of calibration grid of the large experimental tank. A) Each cell, A1 – I6 had recordings taken at the center and at four vertical depths, B) Line of vertical hydrophone positions used during calibration 1 – 4, with closest to surface at 1 and closest to substrate at 4).

3.4.2.4. Experimental procedures

General behavior trials in the research aquarium at Sandy Hook were completed March 5-May 21, 2019. A total of six trials were run, each trial used five naïve adult black sea bass that were not in spawning condition, and which had overwintered in the laboratory before the trials. Each trial lasted five days. For a trial, five black sea bass were transferred to the experimental tank and allowed to acclimate overnight (Day 1 - Acclimation). The following morning (Day 2 – Pre-exposure), the five pre-exposure periods commenced at 9:00 am, 11:00 am, 1:00 pm, 3:00pm and 5:00pm EST with filming and power supplied to the underwater transducer (speaker), however, with no audio playback. On Day 3, (Exposure 1) occurred using the same 5 times of day with filming and an a random selection of pile driving recordings (Pile 1-3; see section above) from the Block Island Wind Farm construction were broadcast through the transducer for 30 minutes at each time period. On Day 4 – (Post-exposure), again the same five periods were used, with filming but no sound playback. Finally, on Day 5 (Exposure 2), filming and sound exposure occurred at 9:00AM, 11:00AM, 1:00PM with a random selection of Pile 1 – 3 recordings. The black sea bass were removed on Day 6 and the tank drained.

Table 4. Overview of six experimental treatment days, March 5 – May 21, 2019.

Day	Treatment	Sound
1	Acclimation	none
2	Pre-exposure	none
3	Exposure 1	Pile driving 5 times per day
4	Post-exposure	No pile driving
5	Exposure 2	Pile driving 3 times per day Followed by no pile driving
6	Post exposure 2	no pile driving

Black sea bass were of varying sizes within each group.

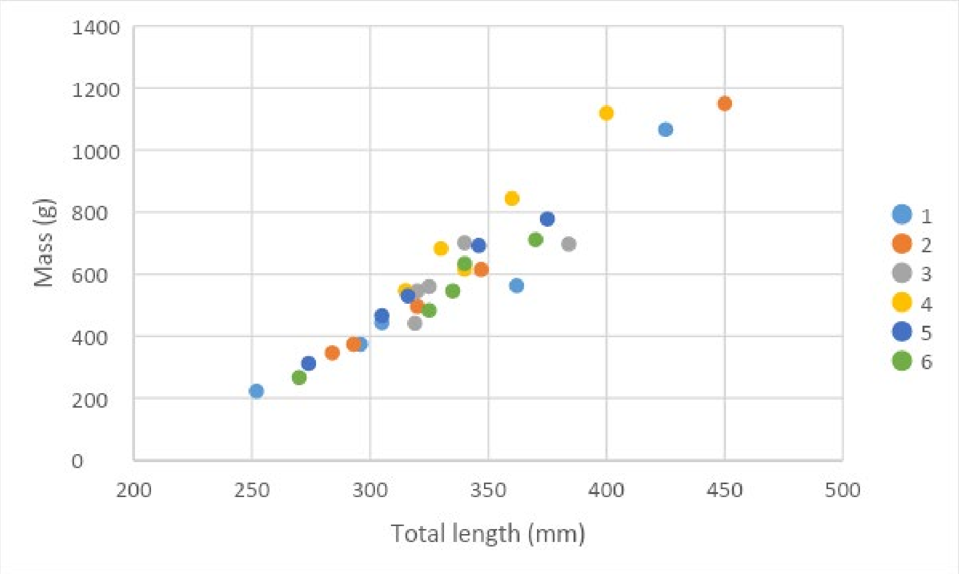


Figure 18. Scatterplot of black sea bass size distribution of groups from general behavior experiments in the large experimental tank.

3.4.2.5. Analysis of behavioral data

Underwater video and audio were set to automatically record for 30 minutes during each selected hour. There were five types of behaviors documented: Swim, Rest, Hover, Sink, Aggression. Noldus Observer XT software was used to review the video and record the frequency and duration of behaviors seen during video review. The "Other" category was added to account for the full amount of time recorded. During video review of the recorded times, the number of seconds spent exhibiting each of the five behaviors was recoded for each fish for 5 minutes each (5-minute observation period). While each fish was observed for 5 minutes, individual fish were not identifiable in subsequent hours and so the behaviors were averaged over the five fish during each hour.

3.4.2.6. Statistical analysis

All data analyses were conducted in R version 3.63 using the packages "vegan" (Oksanen et al 2011) and Biostats (McGarigal, 2009). The time spent on each behavior during the 5-minute observation period were converted into a proportion of the total 5 minutes observed. The proportions underwent an arcsine square-root transformation and the data were evaluated using a Mardia Kurtotis test of multivariate normality. Based on the results, the data cannot be considered as coming from a normal distribution and so a non-parametric MANOVA was used to determine if the behavioral time budgets were significantly different between the different hours of the day and between the different days of the trial. A series of pair-wise non-parametric MANOVA tests were also conducted to compare each of the six groups directly against each other.

Using packages "FactoMineR" and "factoextra (Kassambara, 2020), a principal component analysis (PCA). Statistical significance of each principal component (PC) axis was tested using a Monte Carlo randomization test, using 1000 permutations to compare the observed eigenvalues to the distribution of eigenvalues under the null hypothesis of no real correlation structure. Structure correlations between the original behavior variable and the principal component scores were calculated and the significance of the variable loading (behavior significance) was determined through permutation. On each PC axis, behavior loading were included only if significantly correlated to the axes (p,0.01). The observations in multivariate space were visualized by separating observation by the six groups and by adding 95% confidence ellipses.

3.4.3. Results

3.4.3.1. Acoustic calibration

Ambient sound levels (no playback) of the tank were low, starting at a PSD of ca. 85.6 dB re 1 uPa^2/Hz at 20–2000 Hz and decreasing in a logarithmic fashion down to ca. 78.2 dB re 1 uPa^2/Hz at 100–600 Hz. This relatively quiet tank environment enabled us to isolate and assess potential effects of water-borne pile driving noise on fish while minimizing extraneous stimuli that are found in natural field environments.

The sound field of pile driving playback in the experiment tank was spatially variable, with highest zero-to-peak levels (up to 162.1 dB re 1 uPa^2) at the calibration station furthest from the speaker, along the centerline of the tank, at the deepest sampling location (I3, 4), and lowest at the calibration station a few stations from the speaker, along the wall of the tank, at the shallowest sampling location (151, 1) (See figure 17). Sound levels were greatest towards the center and dropped off by as much as 4 dB at the sides of the tank at hydrophone 1 (surface) and by as much as 1.3 dB at hydrophone 4 (bottom). There was a slight gradient in the tank, with measurements along the midline (position 3) having the lowest levels closer to the speaker end (E3, 3; 150.9 dB) and highest at the location furthest from the speaker (I3, 3; 162.1 dB).

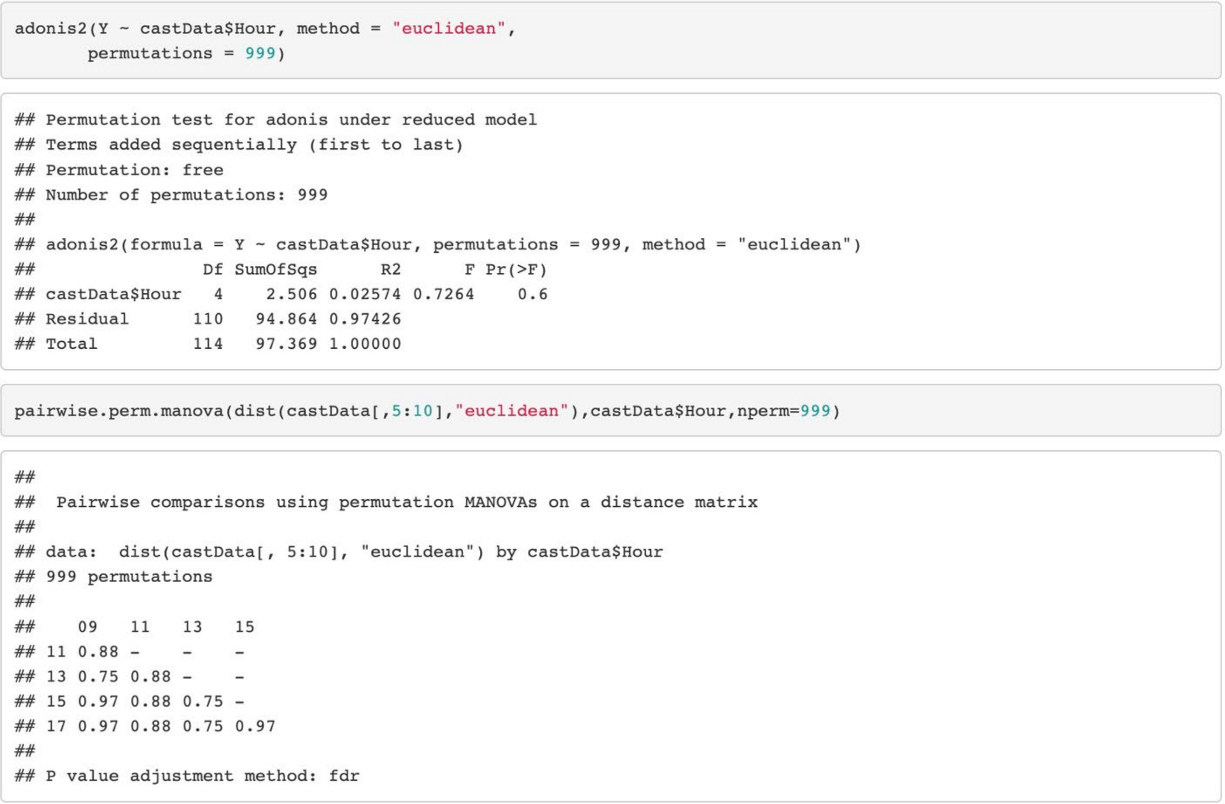
The recordings of the pile driving pulses in the tank indicated that noise playback created a substantially elevated sound field (by up to 77 dB) and this sound was generally similar among the three playback files, with some slight differences in spectral shape between pressure metrics that would also been seen in *in situ* activities.

The highest received pile driving pulse energy was between 20–300 Hz, within the most sensitive range *C. striata* sound detection abilities (Stanley et al. 2020; see Study 1). Spectral curves of sound pressure of pile driving pulses received in the tank had roughly similar shapes but lower amplitudes (by 40-50 dB) compared to those received in the field 0.5 km from the pile installation. These sound pressure levels in the tank were in the range of levels typically present in the water column 3–4 km away from the Block Island Wind Farm piles (Amaral et al., 2018).

3.4.3.2. Behavioral responses

The nonparametric MANOVA comparing times of day for differences in behavior pooled indicated there were no significant differences among the different hours of the day (*P*=0.602) (Table 5). Therefore, the data collected over the hours of a specific day (treatment) was pooled for further analysis.

Table 5. Results from One-Way MANOVA testing for a difference in behaviors with time of day.



```
adonis2(Y ~ castData$Hour, method = "euclidean",
       permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ castData$Hour, permutations = 999, method = "euclidean")
##                Df SumOfSqs      R2      F Pr(>F)
## castData$Hour   4    2.506 0.02574 0.7264    0.6
## Residual      110   94.864 0.97426
## Total         114   97.369 1.00000
```

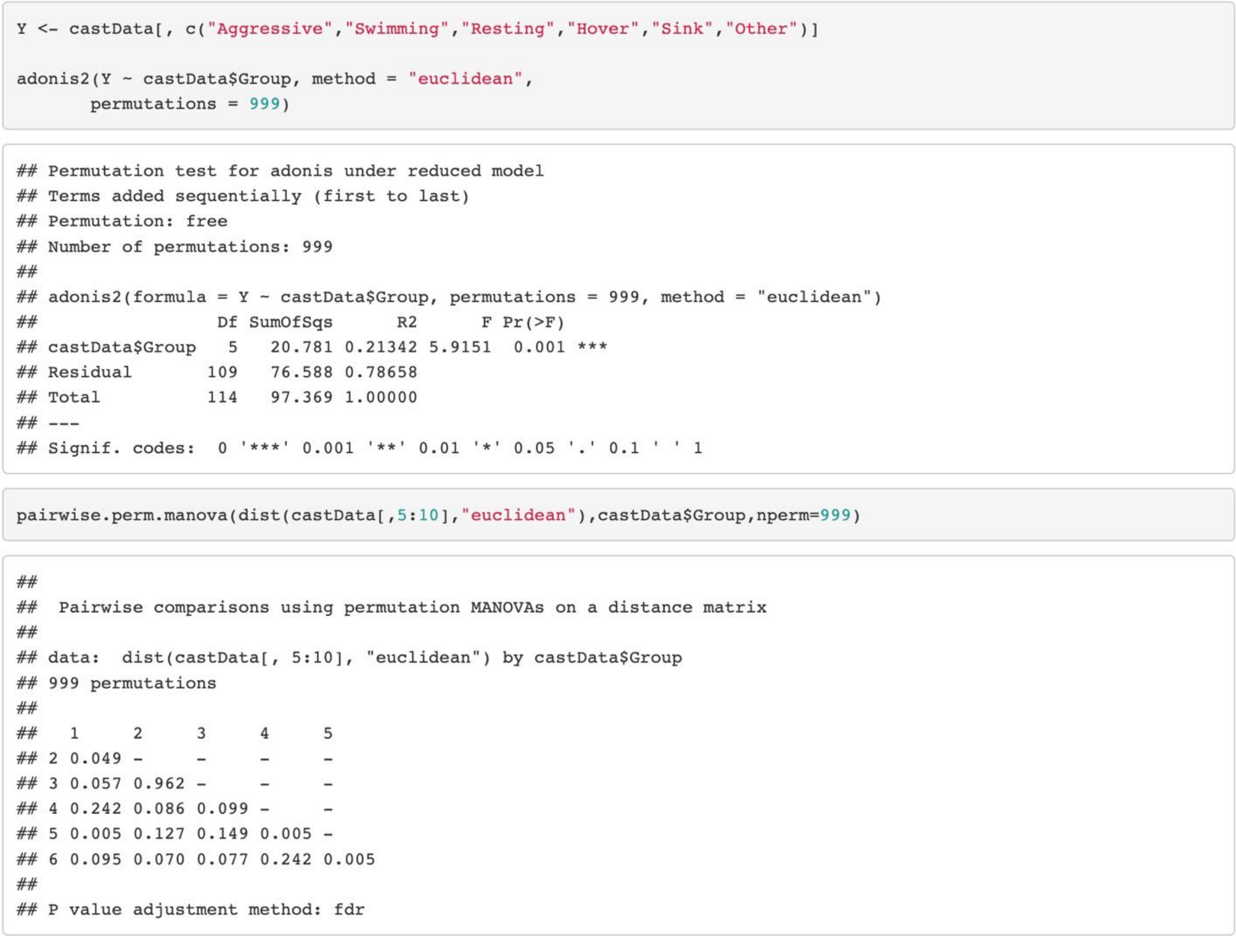
```
pairwise.perm.manova(dist(castData[,5:10],"euclidean"),castData$Hour,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(castData[, 5:10], "euclidean") by castData$Hour
## 999 permutations
##
##    09   11   13   15
## 11 0.88 -    -    -
## 13 0.75 0.88 -    -
## 15 0.97 0.88 0.75 -
## 17 0.97 0.88 0.75 0.97
##
## P value adjustment method: fdr
```

Experimental treatment effects among groups

The nonparametric MANOVA comparing all six groups with experimental treatments pooled indicated there were significant differences among the groups of fish used in the trials (Table 6). Specifically, aggressive behavior was higher in groups 4, 5, and 6.

Table 6. Results from One-Way MANOVA testing for a difference in behaviors with group.

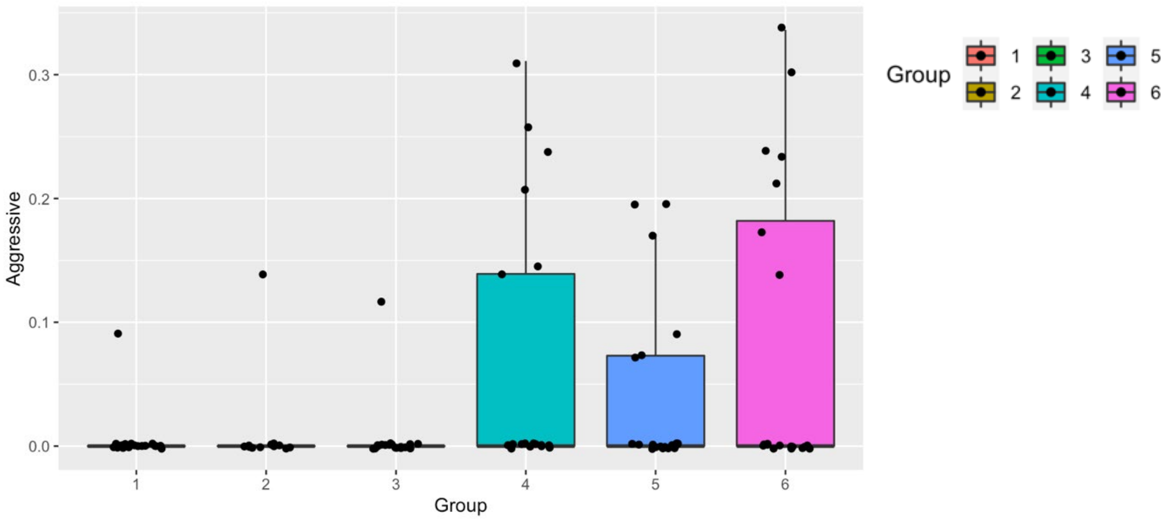


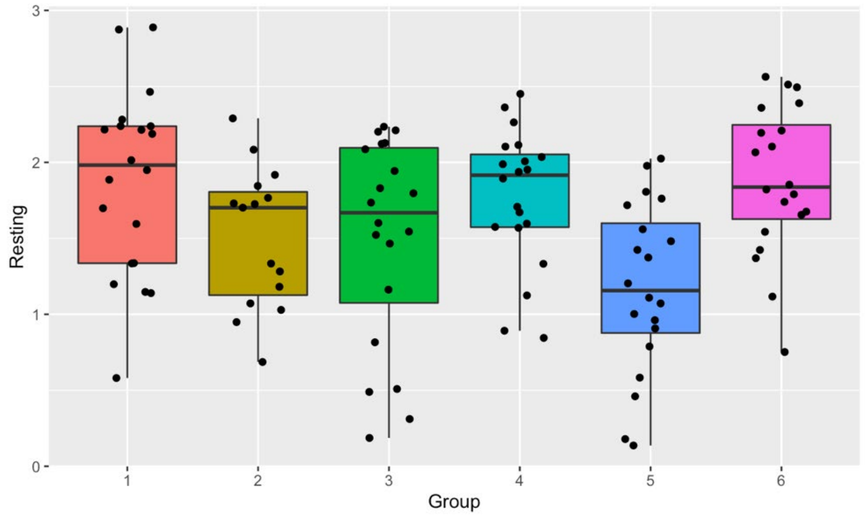
```
Y <- castData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ castData$Group, method = "euclidean",
       permutations = 999)
```

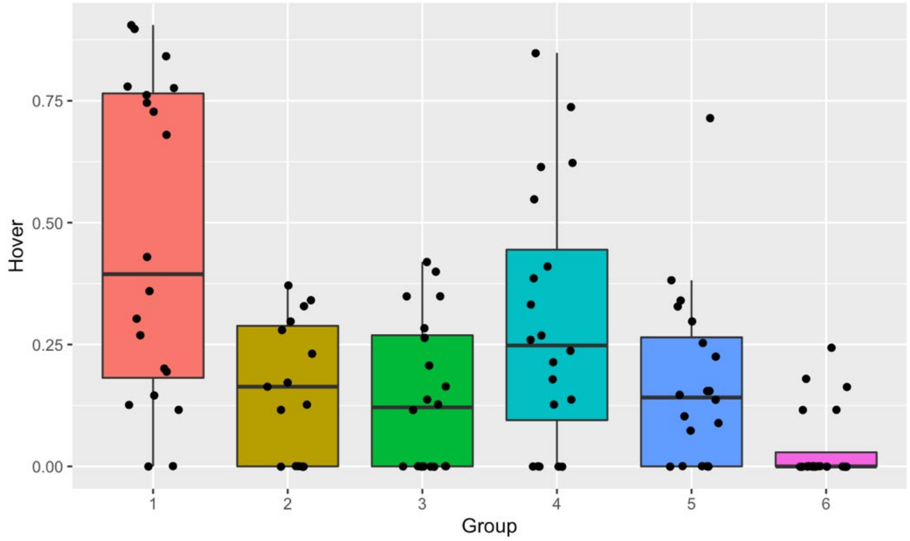
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ castData$Group, permutations = 999, method = "euclidean")
##                  Df SumOfSqs      R2      F Pr(>F)
## castData$Group   5   20.781 0.21342 5.9151  0.001 ***
## Residual       109   76.588 0.78658
## Total          114   97.369 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.perm.manova(dist(castData[,5:10],"euclidean"),castData$Group,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(castData[, 5:10], "euclidean") by castData$Group
## 999 permutations
##
##   1     2     3     4     5
## 2 0.049 -     -     -     -
## 3 0.057 0.962 -     -     -
## 4 0.242 0.086 0.099 -     -
## 5 0.005 0.127 0.149 0.005 -
## 6 0.095 0.070 0.077 0.242 0.005
##
## P value adjustment method: fdr
```


Aggressive
0.0
0.1
0.2
0.3
Group
1
2
3
4
5
6
Group
1
2
3
4
5
6


Resting
0
1
2
3
Group
1
2
3
4
5
6


Hover
0.00
0.25
0.50
0.75
Group
1
2
3
4
5
6

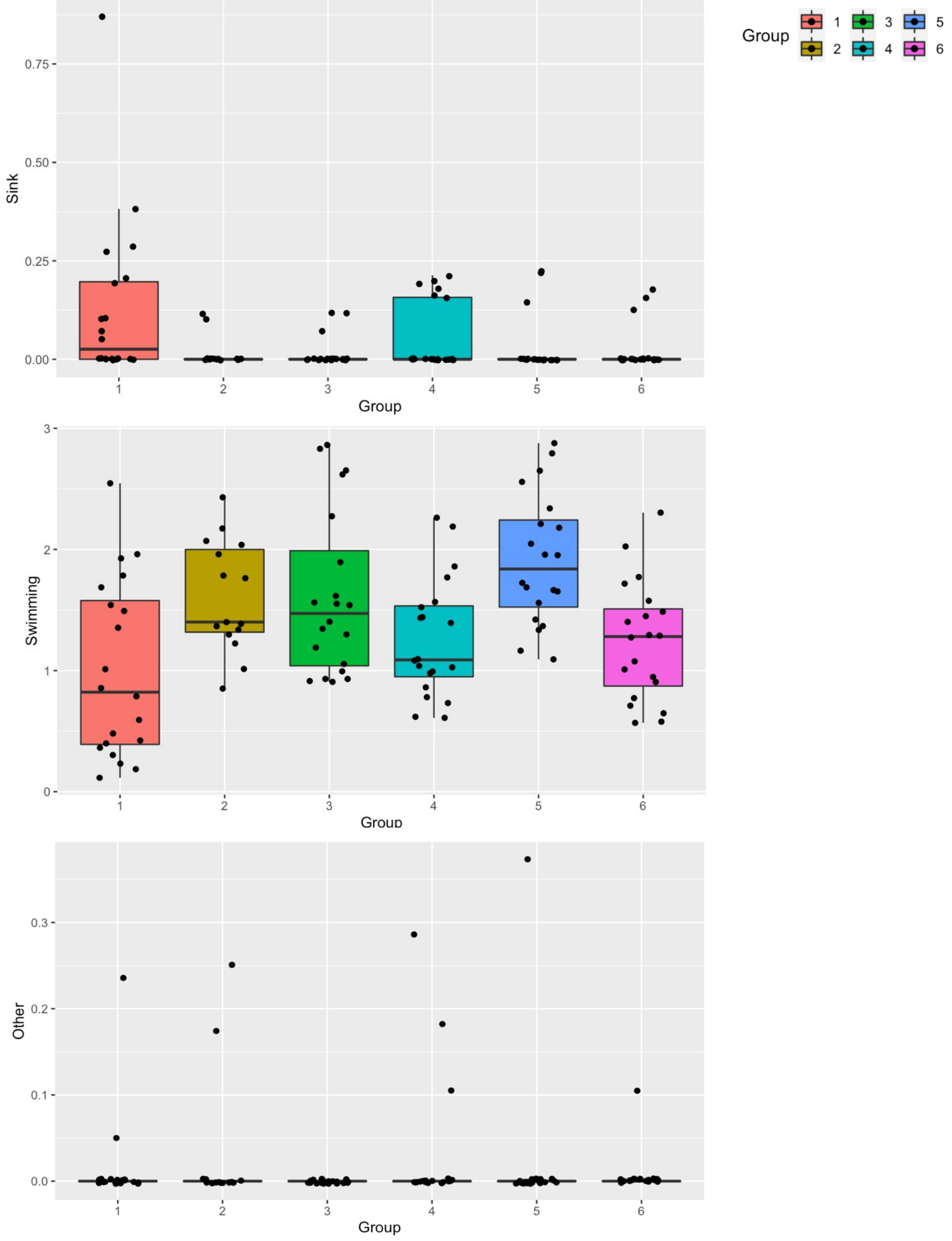


Figure 19. Boxplots showing proportion of time spent in each behavior by each group of fish tested (1 – 6) with all experimental treatment pooled.

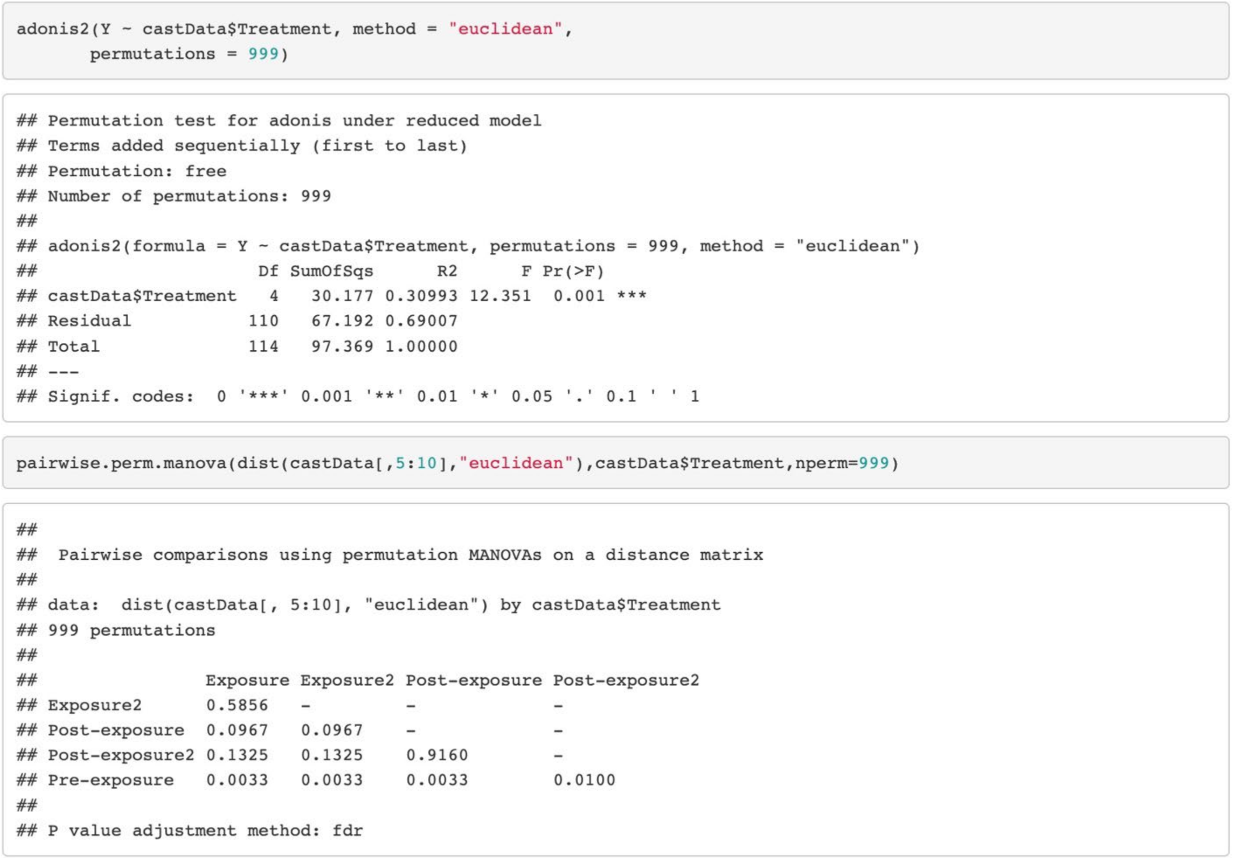
Experimental treatment effects among groups

Results with Groups pooled

There were also highly significant differences among experimental treatment days within the trials when pooling groups (Table 7).

Note. Care must be taken when considering group pooled results as there was differences in behavior detected among groups. However, this was largely the ‘Aggressive’ behavior which increased in groups 4, 5 and 6.

Table 7. Results from One-Way MANOVA testing for a difference in treatments with groups pooled.



```r
adonis2(Y ~ castData$Treatment, method = "euclidean",
       permutations = 999)
```

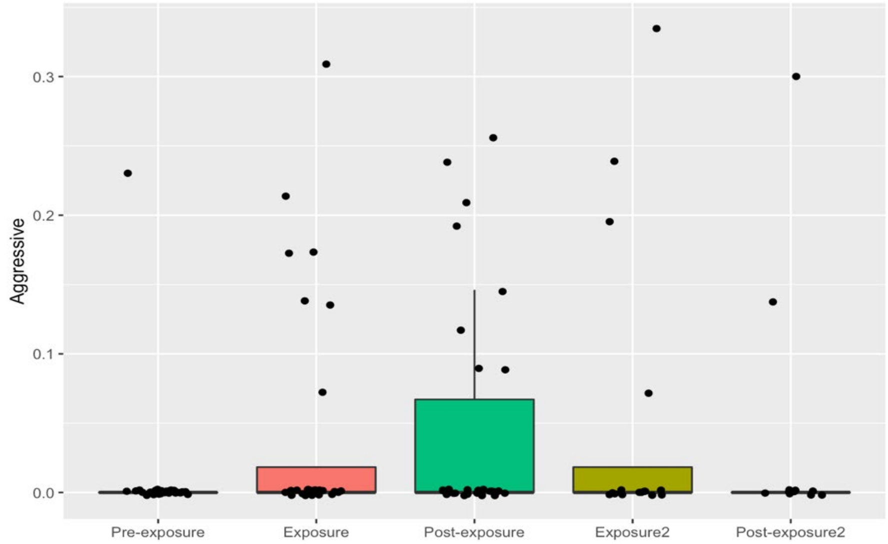
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ castData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2      F Pr(>F)
## castData$Treatment   4   30.177 0.30993 12.351  0.001 ***
## Residual           110   67.192 0.69007
## Total              114   97.369 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

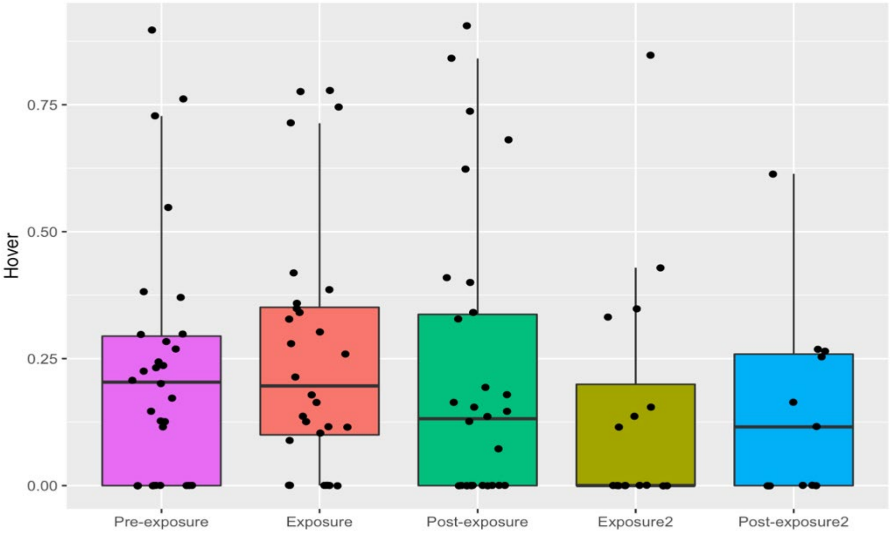
```r
pairwise.perm.manova(dist(castData[,5:10],"euclidean"),castData$Treatment,nperm=999)
```

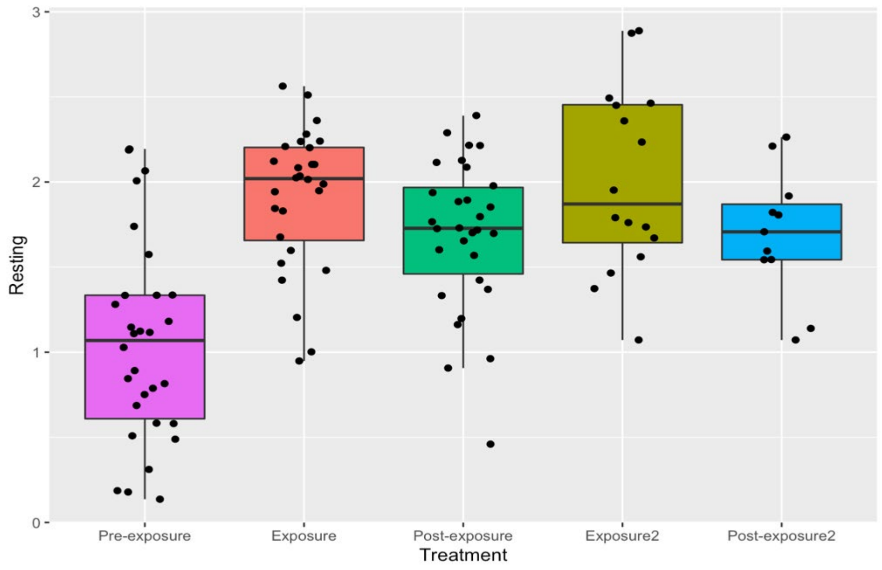
```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(castData[, 5:10], "euclidean") by castData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      0.5856   -         -             -
## Post-exposure  0.0967   0.0967    -             -
## Post-exposure2 0.1325   0.1325    0.9160        -
## Pre-exposure   0.0033   0.0033    0.0033        0.0100
##
## P value adjustment method: fdr
```

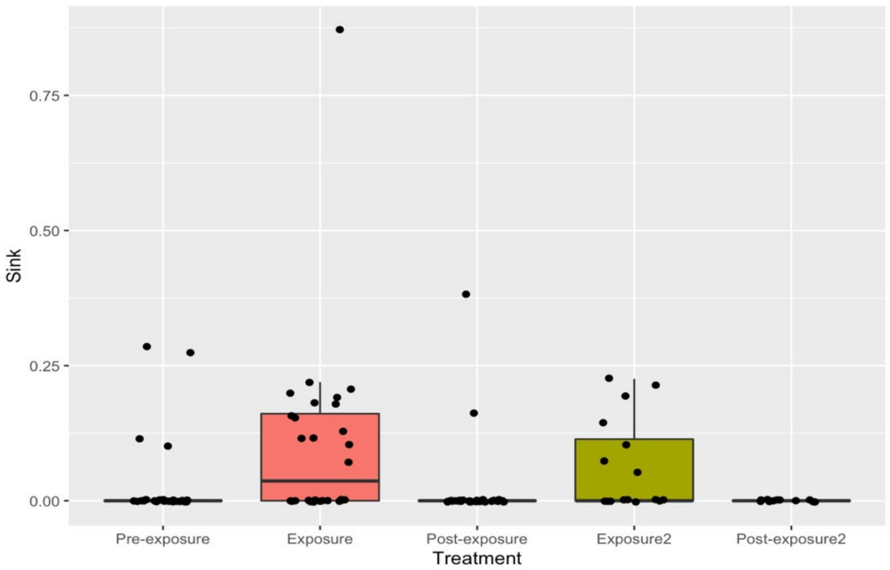
Pairwise comparisons among the experimental treatment days showed that Pre-exposure behavior (before exposure to pile driving sound) was significantly different (all $P \leq 0.01$) from the behavior observed during all other experimental treatments (Exposure, Exposure 2, Post-exposure, and Post-exposure 2) (Table 7). The least significant was the difference between Pre-exposure and Post-exposure 2, potentially indicating a habituation effect of the exposure to pile driving on the fish’s behavior.

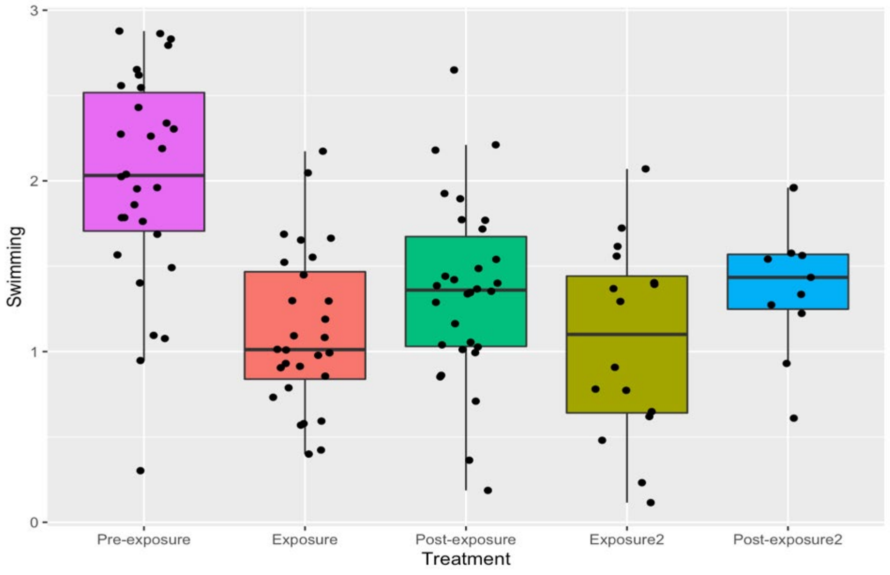
Swimming and resting were the most common behaviors observed by the black sea bass regardless of exposure conditions. Swimming was significantly higher during Pre-exposure than any other treatment. With exposure to pile driving, Swimming behavior decreased and Sinking and Resting behaviors increased. Aggression behavior increased during Post-exposure 1. Hovering did not appear to change due to exposure to sound.






Resting
0
1
2
3
Pre-exposure
Exposure
Post-exposure
Exposure2
Post-exposure2
Treatment


Sink
0.00
0.25
0.50
0.75
Pre-exposure
Exposure
Post-exposure
Exposure2
Post-exposure2
Treatment


Swimming
0
1
2
3
Pre-exposure
Exposure
Post-exposure
Exposure2
Post-exposure2
Treatment

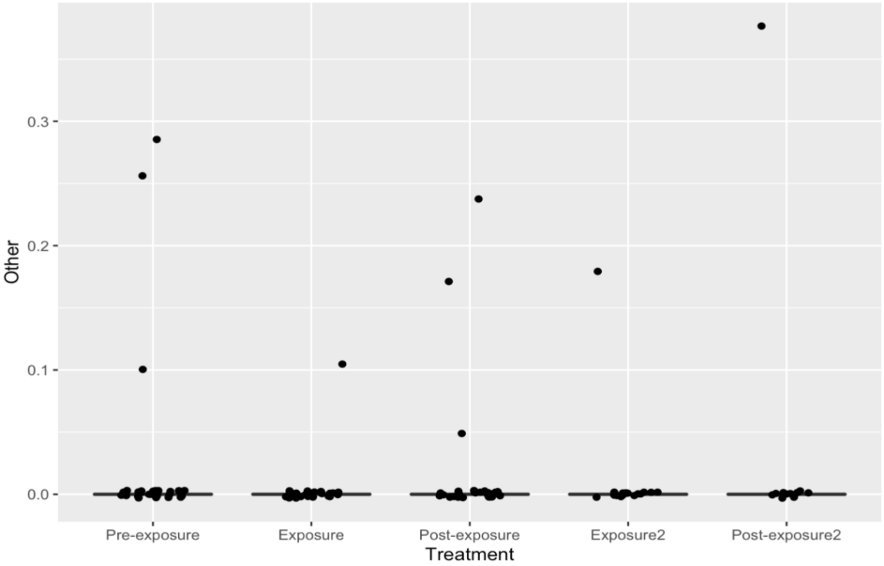


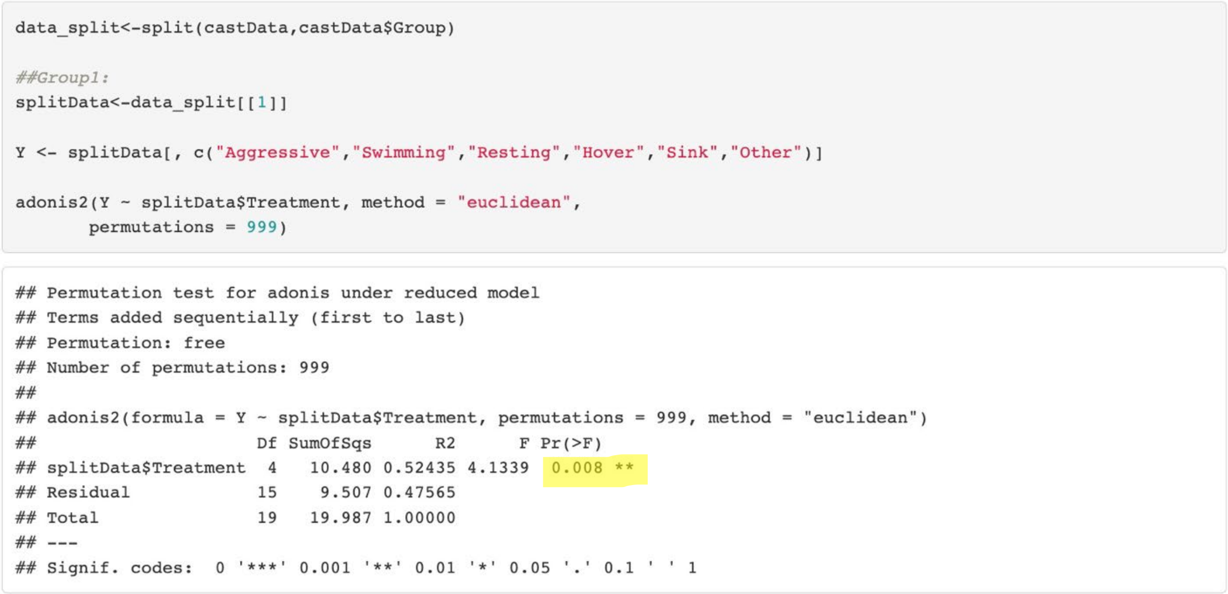
Figure 20. Boxplots showing proportion of time spent in each behavior during each experimental treatment with all fish groups pooled.

Results with Groups independently tested

The nonparametric MANOVA comparing behavior among treatments within individual groups indicated there were some groups that showed highly significant differences among experimental treatment days (Treatments) within the trials; Group 1 ($P = 0.008$) and Group 3 ($P = 0.008$) (Tables 8 & 10). Groups 2, 4 and 5 showed significant differences among experimental treatment days ($P = 0.037$, $P = 0.048$ and $P = 0.03$ respectively) (Tables 9, 11 & 12). Group 6 showed no statically significant difference in behavior among treatment groups ($P = 0.144$) (Table 13), however

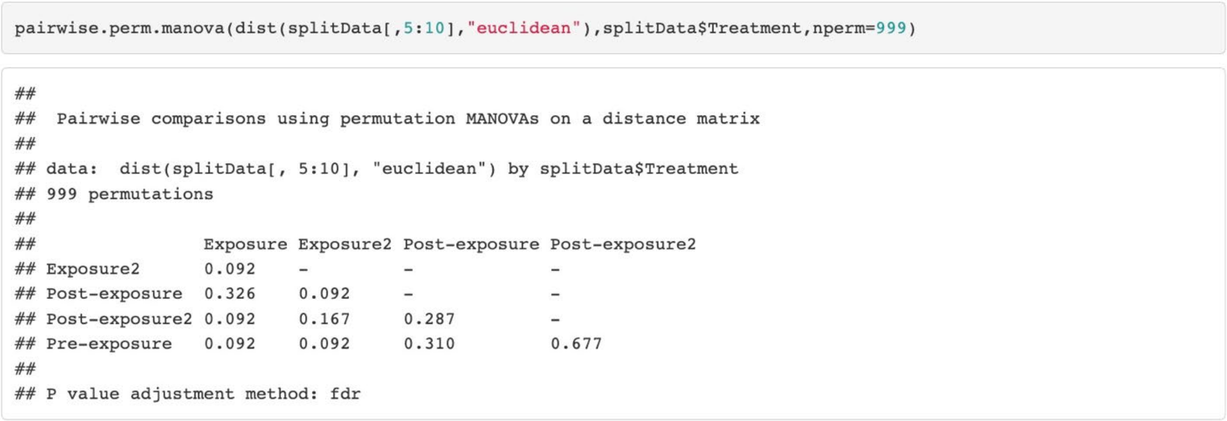
Furthermore, the post-hoc pairwise analysis did not in every instance detect significant differences among the treatments (Table 8 – 13). However, this result is likely due to a lack of statistical power as the amount of time spent in each behavior during each treatment was fairly low. The largest differences occurred between Pre-exposure and both Exposure and Exposure 2, and sometimes Post-exposure ($P = 0.019 – 0.09$).

Table 8. Results from One-Way MANOVA testing for a difference in treatment effect in Group 1.



```
data_split<-split(castData,castData$Group)

##Group1:
splitData<-data_split[[1]]

Y <- splitData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ splitData$Treatment, method = "euclidean",
       permutations = 999)
```

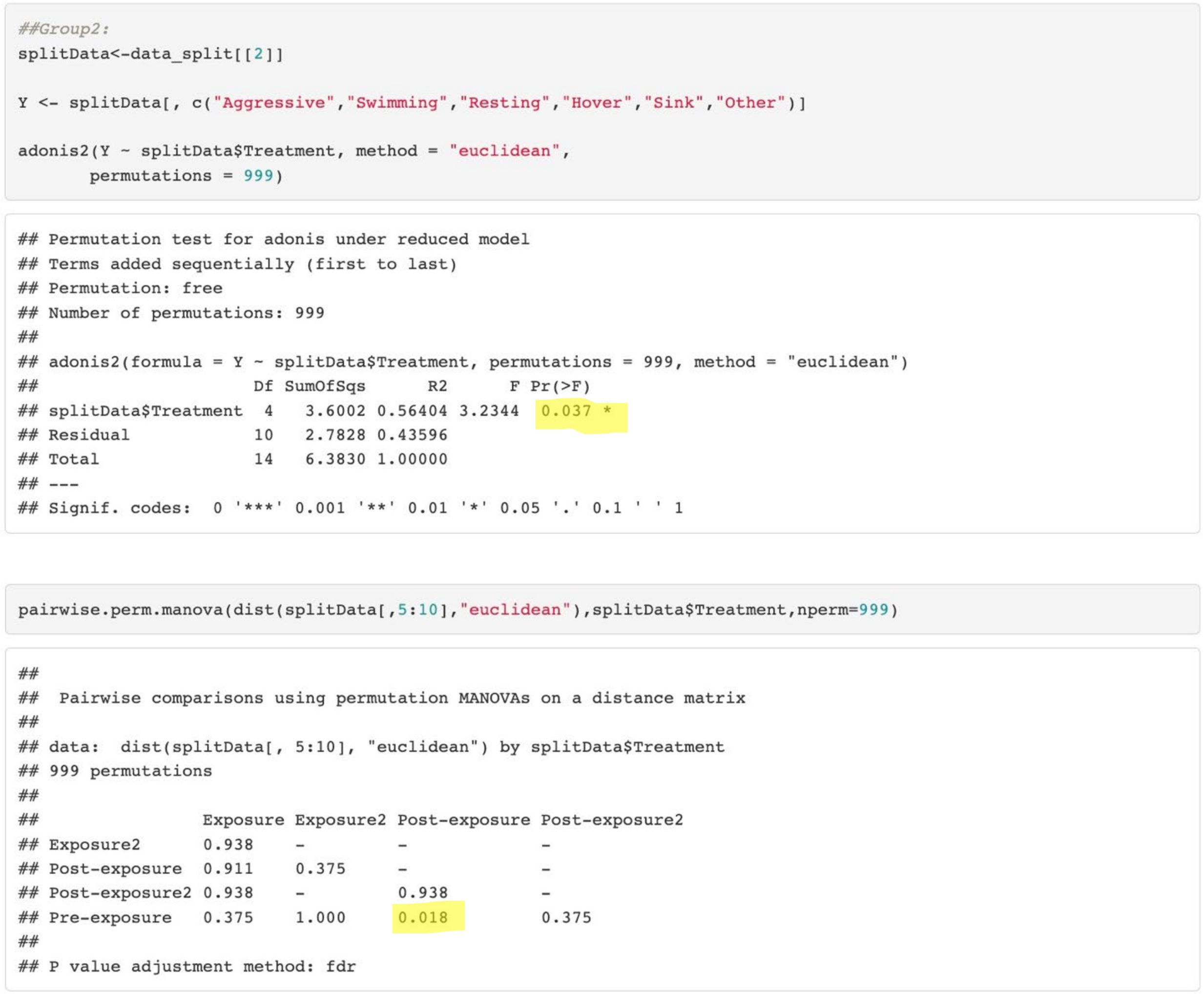
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ splitData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2      F Pr(>F)
## splitData$Treatment  4   10.480 0.52435 4.1339  0.008 **
## Residual            15    9.507 0.47565
## Total               19   19.987 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



```
pairwise.perm.manova(dist(splitData[,5:10],"euclidean"),splitData$Treatment,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(splitData[, 5:10], "euclidean") by splitData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      0.092    -         -             -
## Post-exposure  0.326    0.092     -             -
## Post-exposure2 0.092    0.167     0.287         -
## Pre-exposure   0.092    0.092     0.310         0.677
##
## P value adjustment method: fdr
```

Table 9. Results from One-Way MANOVA testing for a difference in treatment effect in Group 2.



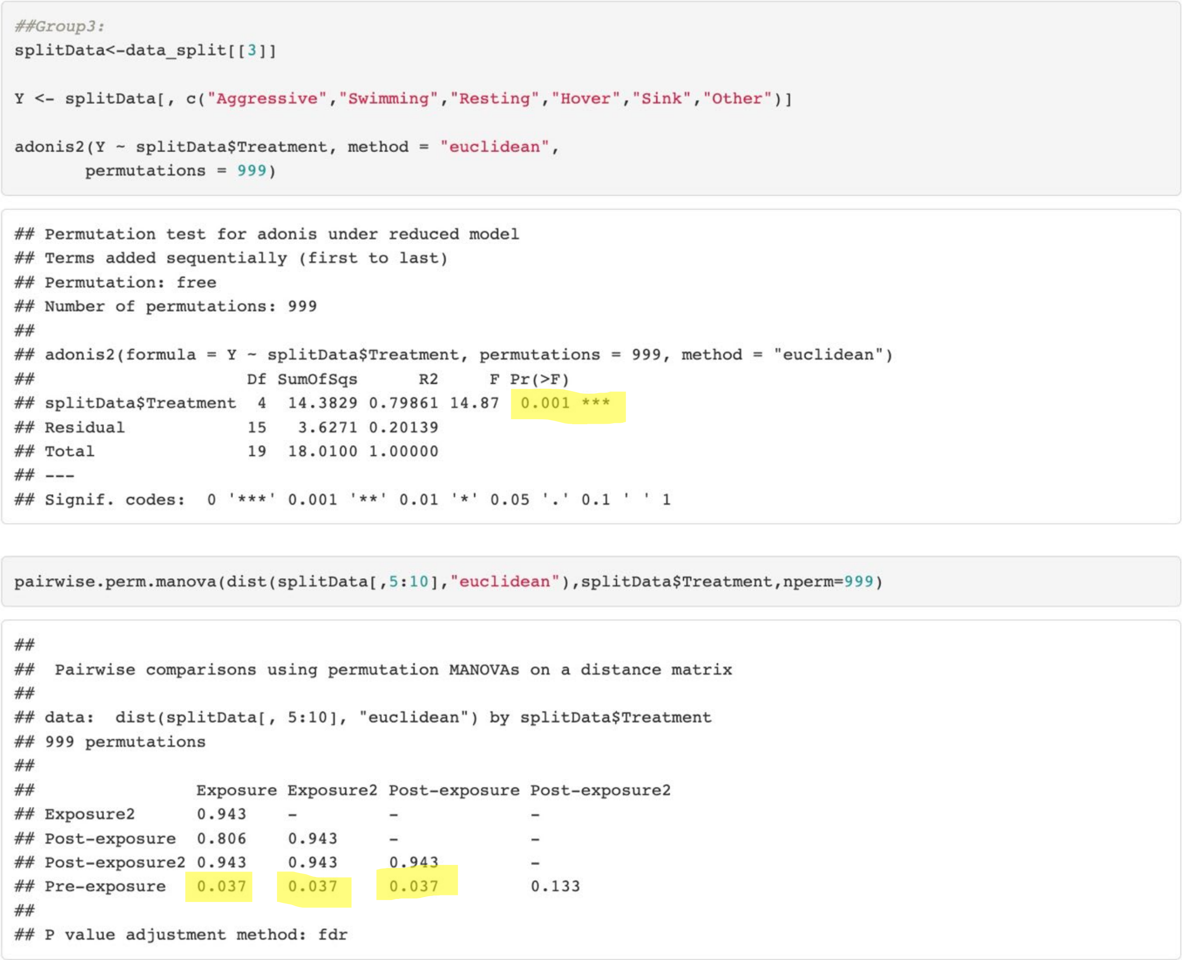
```
##Group2:
splitData<-data_split[[2]]

Y <- splitData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ splitData$Treatment, method = "euclidean",
       permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ splitData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2      F Pr(>F)
## splitData$Treatment  4   3.6002 0.56404 3.2344  0.037 *
## Residual            10   2.7828 0.43596
## Total               14   6.3830 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.perm.manova(dist(splitData[,5:10],"euclidean"),splitData$Treatment,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(splitData[, 5:10], "euclidean") by splitData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      0.938    -         -             -
## Post-exposure  0.911    0.375     -             -
## Post-exposure2 0.938    -         0.938         -
## Pre-exposure   0.375    1.000     0.018         0.375
##
## P value adjustment method: fdr
```

Table 10. Results from One-Way MANOVA testing for a difference in treatment effect in Group 3.



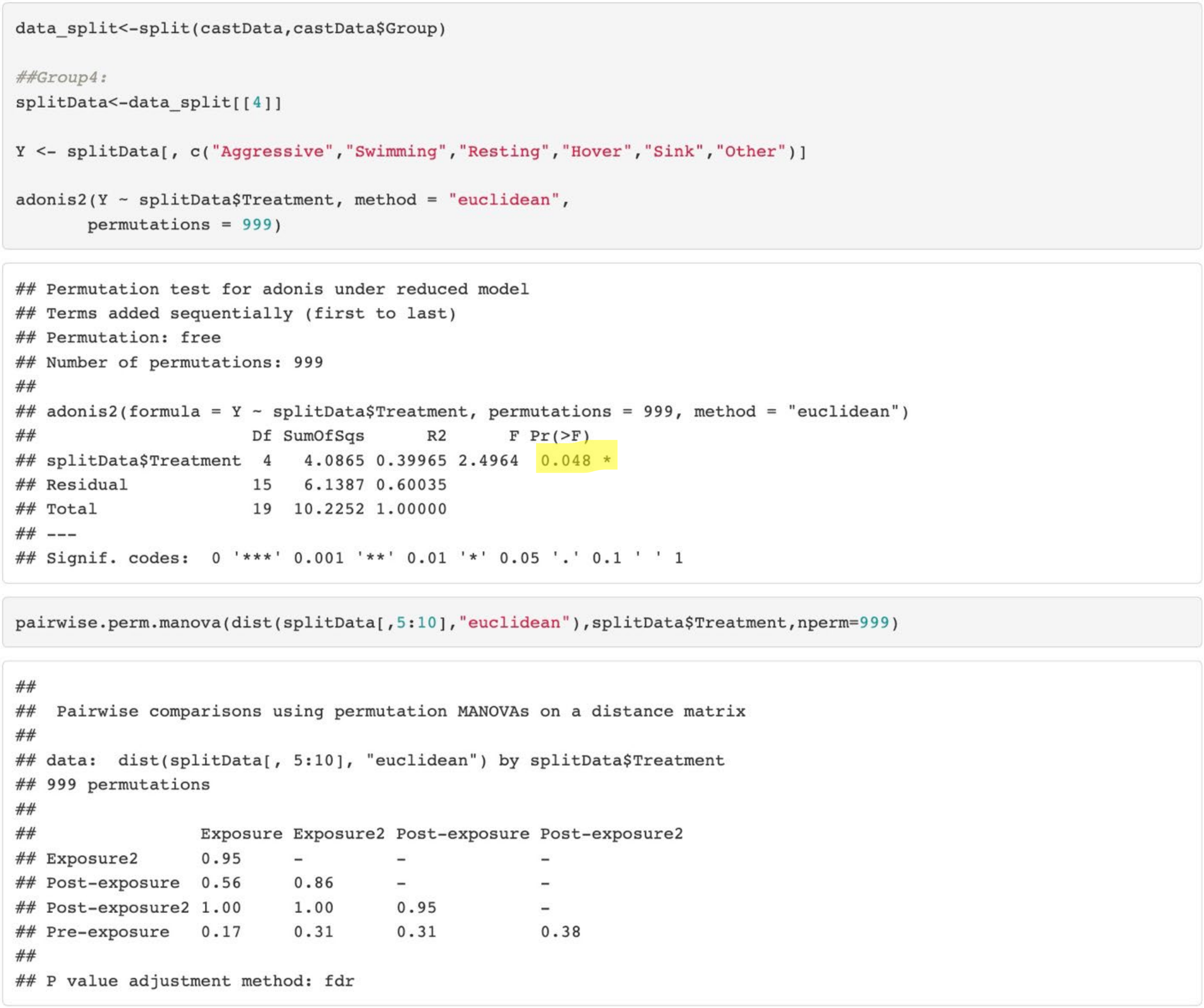
```
##Group3:
splitData<-data_split[[3]]

Y <- splitData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ splitData$Treatment, method = "euclidean",
       permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ splitData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2     F Pr(>F)
## splitData$Treatment  4  14.3829 0.79861 14.87  0.001 ***
## Residual            15   3.6271 0.20139
## Total               19  18.0100 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.perm.manova(dist(splitData[,5:10],"euclidean"),splitData$Treatment,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(splitData[, 5:10], "euclidean") by splitData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      0.943    -         -             -
## Post-exposure  0.806    0.943     -             -
## Post-exposure2 0.943    0.943     0.943         -
## Pre-exposure   0.037    0.037     0.037         0.133
##
## P value adjustment method: fdr
```

Table 11. Results from One-Way MANOVA testing for a difference in treatment effect in Group 4.



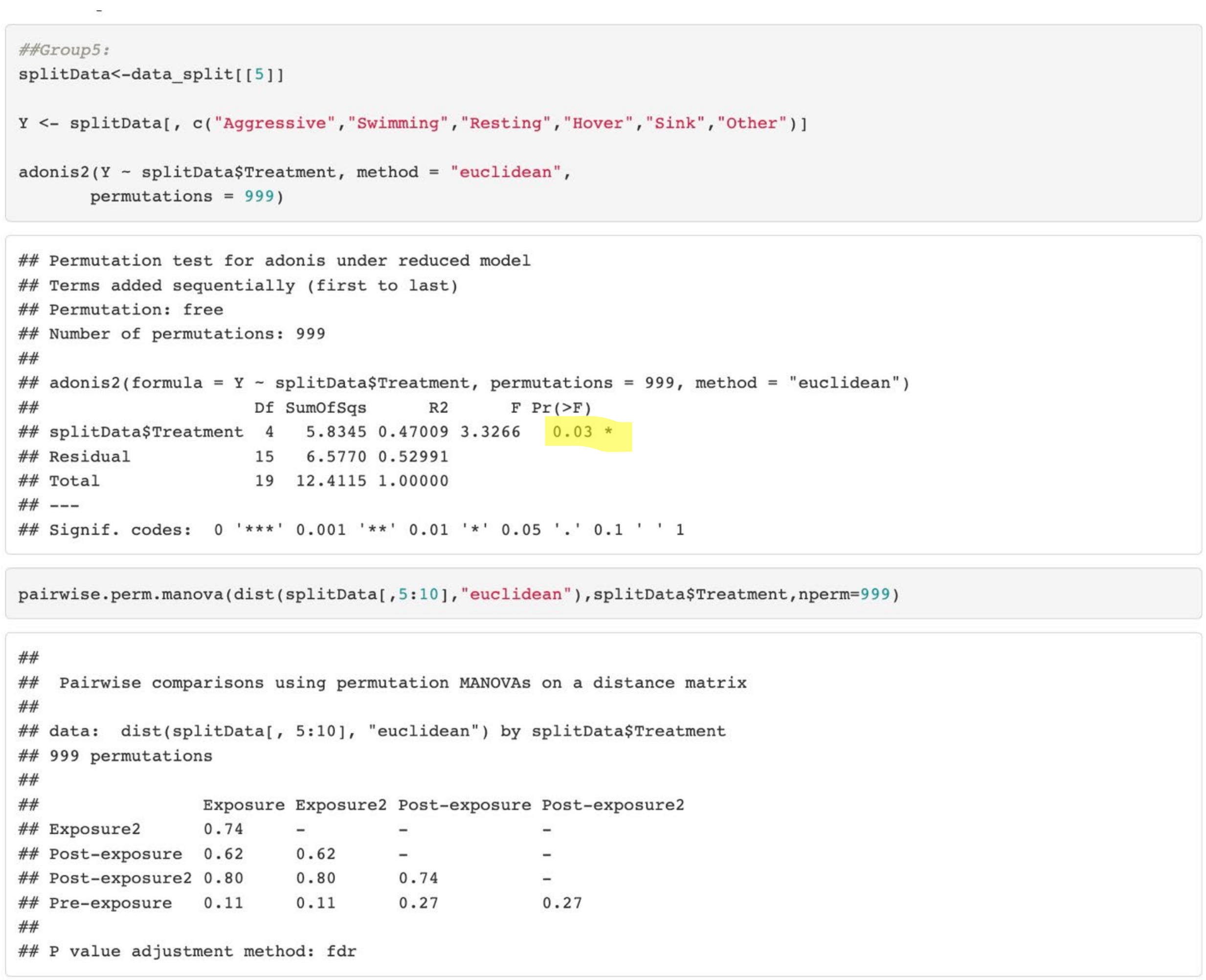
```
data_split<-split(castData,castData$Group)

##Group4:
splitData<-data_split[[4]]

Y <- splitData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ splitData$Treatment, method = "euclidean",
       permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ splitData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2      F Pr(>F)
## splitData$Treatment  4   4.0865 0.39965 2.4964  0.048 *
## Residual            15   6.1387 0.60035
## Total               19  10.2252 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.perm.manova(dist(splitData[,5:10],"euclidean"),splitData$Treatment,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(splitData[, 5:10], "euclidean") by splitData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      0.95     -         -             -
## Post-exposure  0.56     0.86      -             -
## Post-exposure2 1.00     1.00      0.95          -
## Pre-exposure   0.17     0.31      0.31          0.38
##
## P value adjustment method: fdr
```

Table 12. Results from One-Way MANOVA testing for a difference in treatment effect in Group 5.



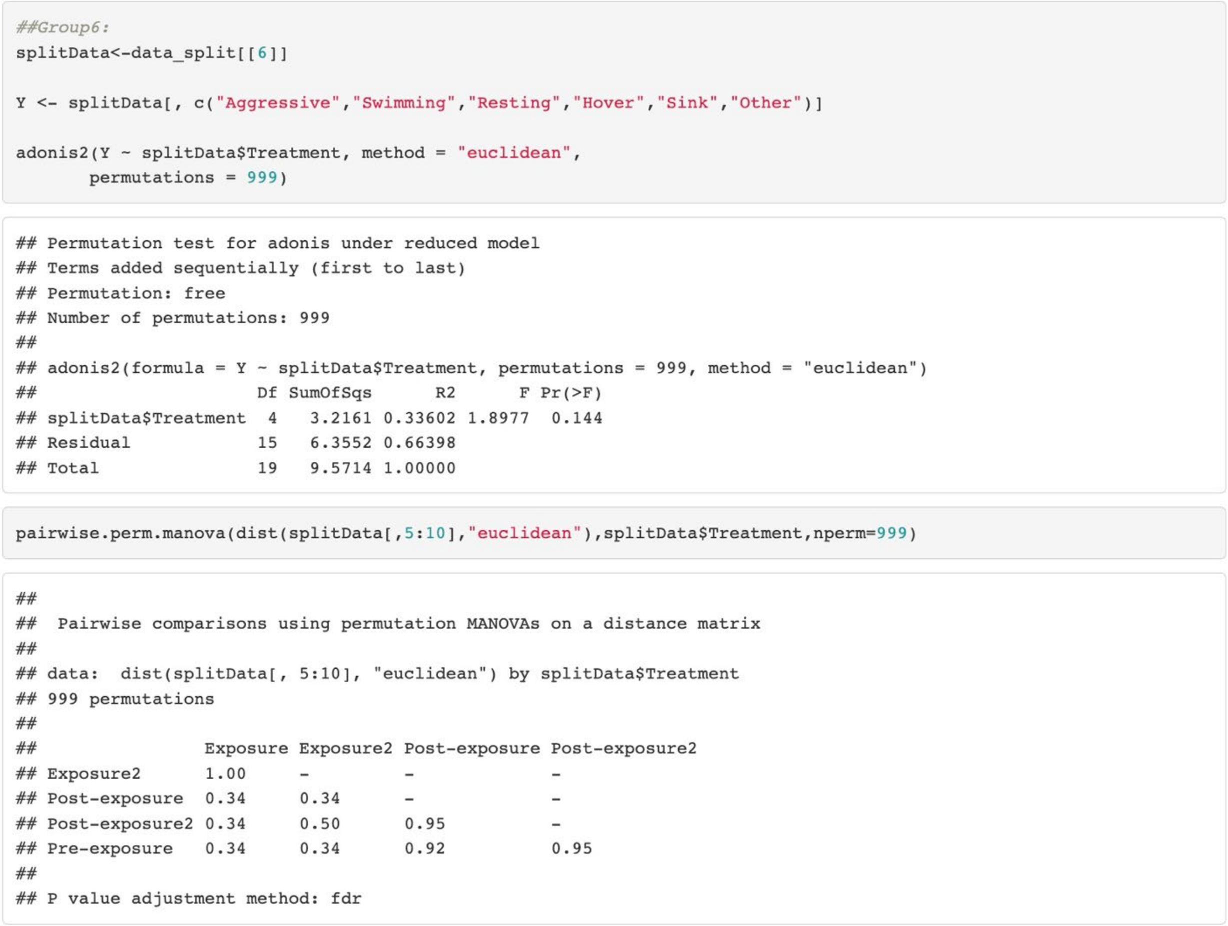
```
##Group5:
splitData<-data_split[[5]]

Y <- splitData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ splitData$Treatment, method = "euclidean",
       permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ splitData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2      F Pr(>F)
## splitData$Treatment  4   5.8345 0.47009 3.3266   0.03 *
## Residual            15   6.5770 0.52991
## Total               19  12.4115 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
pairwise.perm.manova(dist(splitData[,5:10],"euclidean"),splitData$Treatment,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(splitData[, 5:10], "euclidean") by splitData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      0.74     -         -             -
## Post-exposure  0.62     0.62      -             -
## Post-exposure2 0.80     0.80      0.74          -
## Pre-exposure   0.11     0.11      0.27          0.27
##
## P value adjustment method: fdr
```

Table 13. Results from One-Way MANOVA testing for a difference in treatment effect in Group 6.



```
##Group6:
splitData<-data_split[[6]]

Y <- splitData[, c("Aggressive","Swimming","Resting","Hover","Sink","Other")]

adonis2(Y ~ splitData$Treatment, method = "euclidean",
       permutations = 999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = Y ~ splitData$Treatment, permutations = 999, method = "euclidean")
##                     Df SumOfSqs      R2      F Pr(>F)
## splitData$Treatment  4   3.2161 0.33602 1.8977  0.144
## Residual            15   6.3552 0.66398
## Total               19   9.5714 1.00000
```

```
pairwise.perm.manova(dist(splitData[,5:10],"euclidean"),splitData$Treatment,nperm=999)
```

```
##
##  Pairwise comparisons using permutation MANOVAs on a distance matrix
##
## data:  dist(splitData[, 5:10], "euclidean") by splitData$Treatment
## 999 permutations
##
##                Exposure Exposure2 Post-exposure Post-exposure2
## Exposure2      1.00     -         -             -
## Post-exposure  0.34     0.34      -             -
## Post-exposure2 0.34     0.50      0.95          -
## Pre-exposure   0.34     0.34      0.92          0.95
##
## P value adjustment method: fdr
```

Experimental treatment effects among individuals

A principal component analysis was used to determine the behavioral changes in response to treatment to sound exposure over the days of treatments. The first two PC axes (two showing the most variance) explained 54.2 % of the variation in the behavior (Dim1: 34.9 % and Dim2: 19.3 %) (Figure 21, 22 & 23).

Swimming was correlated to PC2, while Resting and Aggressive, was positively correlated with PC2 and Sink and Hover were negatively correlated with PC2, but not to the same extent as Resting or Swimming (see color contribution scale). Behaviors Swimming and Resting were negatively correlated to each other, while Sinking and Hovering were correlated to each other. (Figure 22).

Differences between the five treatments were visualized on the PC axes (Figure 23). The ordination of the observations in the Pre-exposure treatment group was consistent with the pair-wise perMANOVA test and displayed marginally different patterns in behavior that the other five treatment groups based on visual inspection. The Pre-exposure treatment was strongly associated with swimming and not associated with Resting or Aggressive behavior (Figure 23). The other four treatments were more strongly associated with resting and sinking behavior.

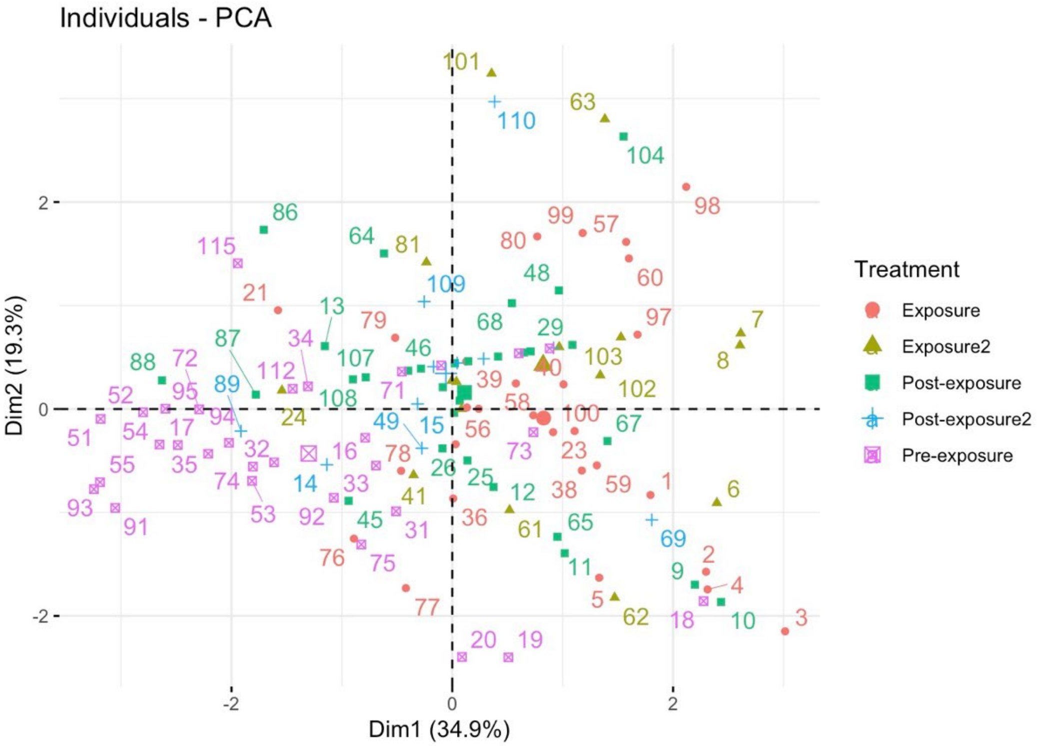


Figure 21. Multivariate ordination of black sea bass behavior during experimental treatments.

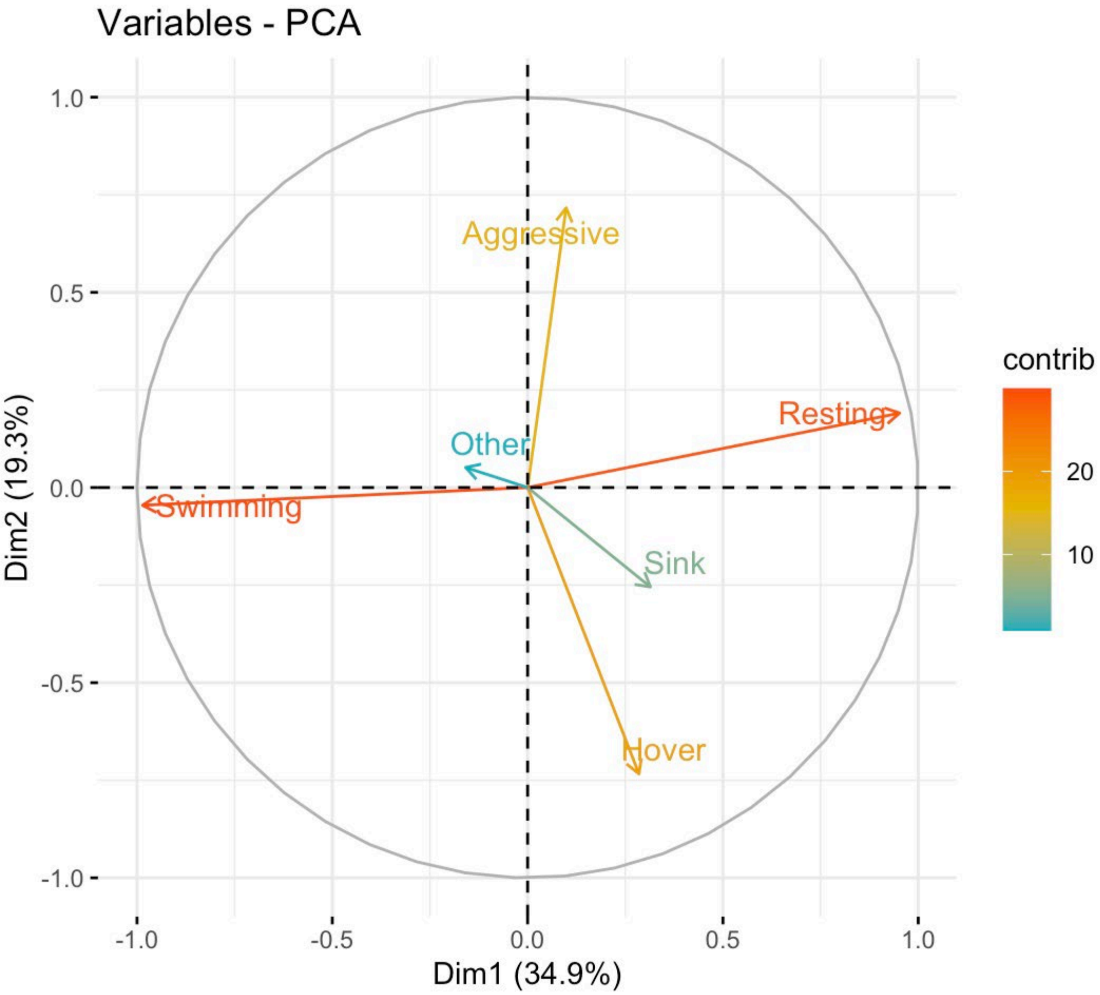


Figure 22. Multivariate ordination of individual black sea bass behavior during experimental treatments. Behavioral variable loadings on PC axes 1 and 2. The length of the vectors indicates the strength of the associated variable for describing the principle components. The direction of the vectors indicates the direction of the associated variable gradients in ordination space.

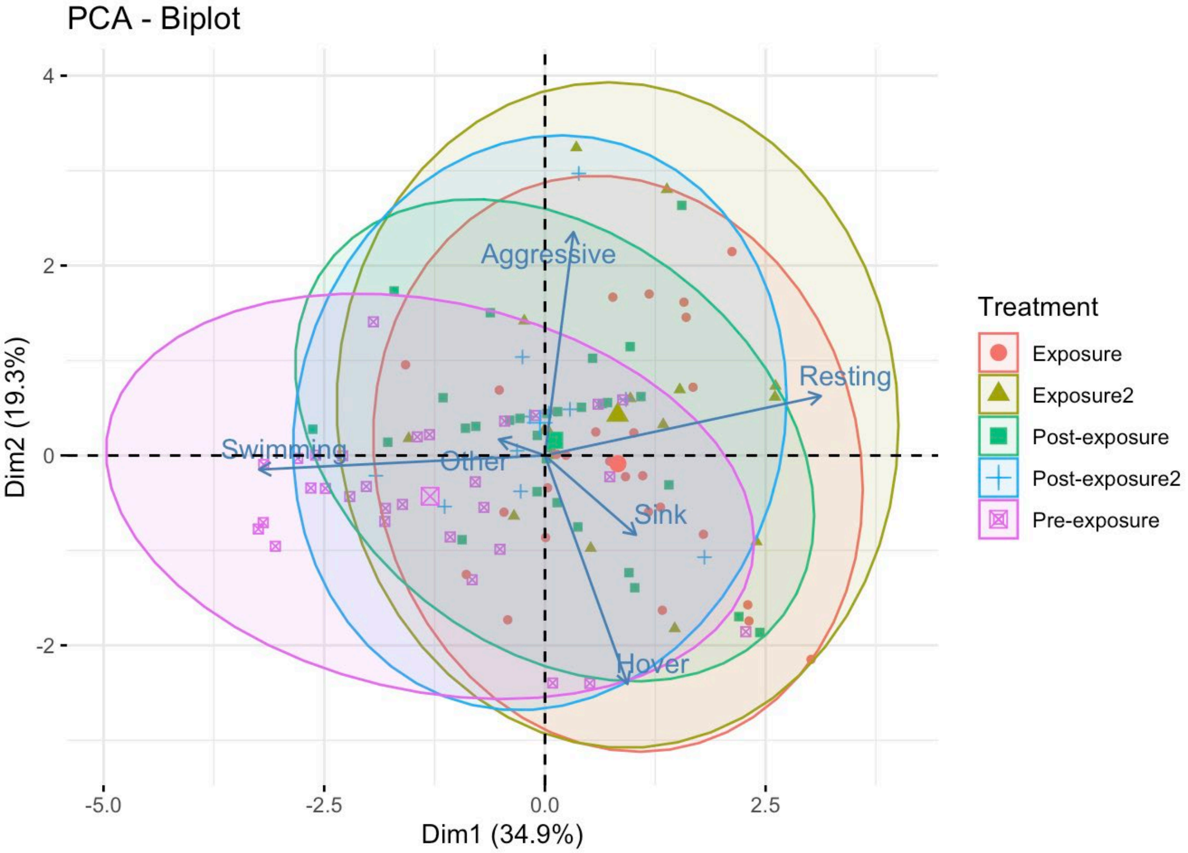


Figure 23. Principle Component Analysis biplot of behaviors and variables (treatments). Ordination of the behavior in multivariate space. The observations are color-coded by treatment, and 95 % confidence ellipses are included.

3.4.4. Discussion

This study used a behavioral response experimental design to examine the effect of replayed pile driving audio signal on black sea bass behavior in a 121,133 L controlled experimental tank environment. The large research tank has been successfully used to observe and record large commercial fish behavior in other studies (Stoner et al. 1999, Fabrizio et al. 2005, Stehlik 2009). Time spent exhibiting five recognized behaviors was recorded before and after exposure to pile driving sounds recorded at the Block Island Wind Farm construction site and adjusted for playback in the tank.

The non-parametric multivariate analysis used examine differences in behavioral patterns for the six behavioral trials with the non-spawning black sea bass indicated significant differences among the experimental treatments and six groups of fish (aggressive behavior). Of the five behaviors observed and measured, swimming and resting were the most common behaviors observed during the study. However, all six groups consistently showed decreased swimming behavior and increased resting behavior (swimming down to the substrate and resting in one position) when exposed to the replayed pile driving Signal. Shelledy *et al.,* (In Prep) showed similar reduction in active behaviors like swimming in their experiments with black sea bass in smaller tanks. Secor *et al*., 2021 saw bottom seeking behavior associated with vessel noise. These behaviors could indicate avoidance and/or “sheltering” behaviors, but certainly highlight a divergence from normal behavior due to the onset of the sound. In the wild, this could result in reduced foraging and intraspecific in interactions. Aquatic noise, depending largely on the source, has the potential to disturb species ability to interact with conspecifics and forage efficiently (Cox et al. 2018). Pelagic schooling fish like European seabass swim faster, deeper and away from pile-driving playback (Neo et al. 2016). Reduced activity could have evolutionary and ecological implications for black sea bass including altered predator avoidance behavior and feeding behavior (Mercer 1989). Additionally, altered behavior could impact black sea bass fisheries directly by causing changes in distribution of the black sea bass in the water column where they are commonly targeted.

Interestingly, aggressive behavior was higher in the last three groups used in the study. Aggression is an indication of spawning territoriality which was documented as a male black sea bass behavior during spawning in captivity (Fabrizio et al. 2014). We did hold temperature and light levels constant during the months of experimentation in an effort to reduce any/all reproductive behaviors. However, our last trial was later in the calendar year and closer to the spawning period for black sea bass in nature. Interestingly, 29 days after ending the trials, the last group of black sea bass used in the study started to spawn despite the prior pile driving sound exposure the month previously and people entering the tank room on a regular basis. Which indicates there may be no long-lasting effects of the changes in behavior due to exposure. Hovering, sinking and swimming behaviors were lower in Group 1 which was observed earliest in the calendar year possibly reflecting post wintertime period when water temperatures in nature are lowest and activity is consequently lower.

Time of day was not a significant factor in the multivariate analysis either during a no pile driving day or during a pile driving day. This indicates the effect of pile driving is established during the first day’s exposure, and subsequent exposures during the same day continued to elicit the same responses. Furthermore, differences in behavior were found among the first exposure (known as Exposure 1) and the day after the first exposure (known as Post-exposure), and after the second exposure to pile driving (known as Exposure 2). However, Exposure 1 had a greater effect on behavior than Exposure 2, indicating the black sea bass may show acclimation to repeated environmental noise like pile driving.

Due to the nature of experiments in a controlled environment fish in these experiments did not have the ability to significantly move away from the pile driving noise, as there were only relatively small changes in intensity in the replayed sound field within the tank. Fish showed a very even distribution within the tank for the duration of the experiments. There was no indication that there were “preferred” locations or areas that were used as “acoustic relief”. In the field, black sea bass have the option of leaving the area of disturbance as shown in acoustic tagging studies to storm disturbance (Wiernicki et al. 2020, Secor et al. 2021). Further studies in the spatiotemporal scale of displacement are needed to determine how far fish displacement takes place from the source and if fish return to areas from which they are displaced (Popper 2021). Unsurprisingly, swimming and resting had opposite placement on the first principal component. Sinking and hovering were related and were correlated and in the wild may not be easily distinguishable.

Opportunistically in 2021, 10 black sea bass were observed to spawn in the large experimental tank. Pile driving sound was broadcast the subsequent two days and the group continued to spawn. The sample size was too small to conduct any analysis and there were no longer any remaining fish to conduct further experiments, however, these initial observations indicate that spawning would take place during replayed pile driving exposure in at least this group of fish. During previous years, in NOAA’s Sandy Hook Laboratories, black sea bass have spawned in the large experimental tank on multiple occasions, and it always involved the male and female fish swimming rapidly up to the surface before releasing milt and eggs (Phelan *et al.,* unpublished). A similar behavior was observed in winter flounder during spawning which assisted in the analysis of acoustic tag data collected in the wild (Grothues et al. 2008) and was important in a subsequent tagging effort that used acoustic tags with pressure sensors to identify spawning locations of winter flounder (Grothues and Bochenek 2010). Secor et al (2021) presented evidence of persistent diurnal vertical behaviors from June to September in acoustically tagged black sea bass consistent with courtship and spawning behaviors of other Serrandidae. He believed this was strongly indicative of pelagic spawning. Our laboratory observations and video recordings corroborate field evidence of pelagic spawning.

Black sea bass transit through offshore areas during seasonal migrations and likely will move through the new wind energy development areas so it is important to determine if construction noise such as pile driving and if the wind energy areas themselves may affect this migration (Fabrizio et al. 2005). The method that most likely led to the greatest understanding of this would be the use of acoustic telemetry. Tagging a large number of individuals and using an fixed acoustic array, and/or a remotely operated vehicle with incorporated telemetry receiver, would likely determine if black sea bass would use other routes or migrate away from construction areas (Popper 2021). Fabrizio et al (2005) used an acoustic grid array with 72 receivers to passively record transmission from 129 acoustically tagged black sea bass off the New Jersey coast for 1 year to determine habitat use and residence time. They determined black sea bass were patchily distributed at the site and that some moved through and out of the site during early summer months and late fall. Male black sea bass had dispersal likelihoods that were significantly different from non-males. Laboratory observations of black sea bass as part of that study showed that fish resting, and swimming were common behaviors. Also, that males were observed displaying to other males and maintaining territories often with aggression during the spawning season. However, Fabrizio et al. (2005) suffered a loss of 13 receivers due to interactions with commercial and recreational vessels illustrating that while valuable data collection devices acoustic telemetry in complex ecological sites can increases the logistics and technological limitations. Further, variation in space-use among individuals or of particular individuals over time can be greater than the variability explained by changing environmental conditions (Biesinger et al. 2013a, Biesinger et al. 2013b).

4 Study Species 2: Longfin Squid (*Doryteuthis pealeii*)

4.1. Study 5: Resting and individual squid: Impulsive pile driving noise elicits alarm responses in squid (*Doryteuthis pealeii*)

Published as: Jones, I. T., Stanley, J. A., & Mooney, T. A. (2020). Impulsive pile driving noise elicits alarm responses in squid (*Doryteuthis pealeii*). *Marine Pollution Bulletin, 150,* 110792. https://doi.org/10.1016/j.marpolbul.2019.110792.

4.1.1. Background

Regardless of the natural function of their sound-sensitivity, squid and other cephalopods may be vulnerable to adverse behavioral and physiological effects of anthropogenic noise. Laboratory studies utilizing ferry noise elicited apparent behavioral stress responses in cuttlefish (*Sepia officinalis*) including increases in frequency of visual displays and time spent swimming (Kunc et al. 2014). In situ exposure of caged squid (*Sepioteuthis australis*) to impulsive noise from air guns induced behavioral alarm responses such as jetting (Fewtrell and McCauley, 2012). Though results from this small handful of studies suggest adverse effects, noise sources and cephalopod species are diverse, and little is known regarding how *D. pealeii* or other cephalopod species may be behaviorally responding to anthropogenic noise.

This first study sought to determine how *D. pealeii* individuals behaviorally respond to water-borne pile driving noise. To limit extraneous stimuli, experiments were conducted in a relatively quiet, controlled laboratory environment. The playback stimuli were pile driving impulse sounds recorded during the construction of the Block Island Wind Farm (BIWF) in Rhode Island. Our goal was to categorize the types of responses demonstrated and their occurrence rate with respect to noise duration and received sound level in a controlled, consistent, and well-calibrated environment. Response rates were first quantified during initial (15 min) noise exposures to pile driving noise. Exposures were repeated after a 24-h rest period to evaluate potential long-term habituation. Both particle motion and sound pressure were quantified in order to describe received levels of the sound component relevant to squid (particle motion) and to support the use of these data for potential management needs.

4.1.2. Methods

4.1.2.1. Experimental tank and playback setup

Experiments took place in 2017 from September 2nd to October 28th (n = 23 trials), and in 2018 from June 15th to June 19th (n = 11 trials). All experiments were conducted in a 110 cm diameter circular tank filled to a 50 cm depth with ambient flowing seawater (Figure 17). The tank was isolated from nearby vibrations with cinderblocks, plywood, and two layers of neoprene between the tank and the concrete floor of the lab. An UW-30 underwater speaker (Electro-Voice, Fairport, NY) was suspended, facing horizontally, 23 cm from the surface and 15 cm from the closest tank wall. To ensure squid did not swim into or behind the speaker, a plastic mesh barrier attached to a PVC frame was placed 15 cm in front of the speaker. To monitor ambient tank sound and noise playbacks during experiments a hydrophone (High-Tech Inc., Long Beach, MS) was placed just behind the mesh barrier 1 cm from the tank wall, 44 cm from the speaker, and 35 cm deep. The hydrophone was attached to a Song Meter SM2 (Wildlife Acoustics, Maynard, MA) data acquisition device. Two cameras were used to record squid behavioral responses to pile diving stimuli. An overhead camera (HDRCX440 Handycam, Sony) was used for all quantitative video analyses and a GoPro mounted above and to the side of the tank allowed a closer view of subtle behaviors post-hoc. Two 15 cm long rulers were placed on the top and bottom of the tank to determine depth of squid in video analyses. A red LED light was clamped to the side of the tank in view of the camera but not visible to the squid, to indicate in subsequent video analyses when pile noise or control playbacks were on.

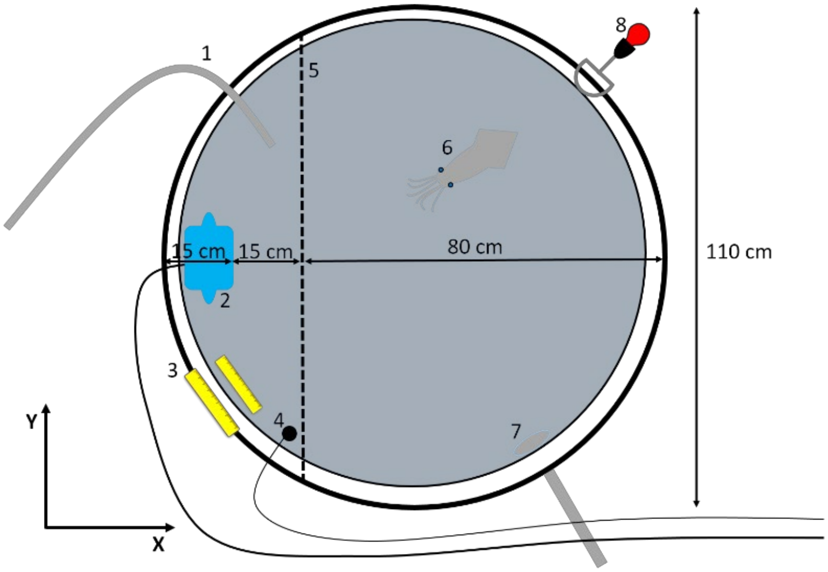


Figure 24. Top-down view of the experimental tank setup
1) Water inflow hose, 2) underwater speaker, 3) calibration rulers (one on top edge of tank, one on bottom of tank), 4) hydrophone, 5) mesh barrier, 6) squid, 7) water outflow opening and pipe (in grey), 8) LED light clamped to the top edge of the tank. Measurements, but not all objects shown, are to scale.

Audio files of pile driving noise used for experiments were recorded during construction of BIWF on October 25th, 2015 (between 16:00 and 20:00 UTC), from a hydrophone (High Tech Inc., model: HTI-94-SSQ, sensitivity: -203.8 dB re 1 V/μPa, gain: 6 dB, flat frequency response from 2 Hz to 30 kHz) on a benthic sled located 26 m deep and 0.5 km away from a pile driving site (Amaral et al., 2018). The hydrophone was about 1 m above the seabed, and part of a tetrahedral array of hydrophones used to calculate particle acceleration via pressure differentials between hydrophones along three orthogonal axes. The hydrophones were spaced 0.5 m apart and were the same model and had the same sensitivity and frequency response noted above. These acceleration data, in m/s^2, were provided to the authors (see acknowledgements). These files were recorded at a 9766 Hz sample rate. The steel, hollow pile had a diameter of 127.0 cm, wall thickness of 3.8 cm, a rake of 13.27° with respect to vertical, and was driven up to 76.2 m deep into the seabed.

To prevent pseudoreplication of playback stimuli, three 15- min long recordings, hereafter referred to as Pile1, Pile2, and Pile3, were extracted from two different pile driving bouts and edited in Adobe Audition (Adobe Systems, San Jose, CA) prior to playback. A 3-s fade-in of the sound file (before the pile driving sound was emitted) was applied to each recording to prevent artifacts that could result from the playback beginning at a higher amplitude. Each recording was amplified by a custom magnitude to obtain the highest playback sound levels possible without clipping, with the goal to match received zero-to-peak sound pressure levels in the tank with those present 0.5 km from the BIWF pile driving site, i.e*.*, 190–194 dB re 1µPa. For Pile1, Pile2, and Pile3 respectively, median inter-pulse intervals were 1.53 s (IQR: 1.52–1.55 s), 1.81 s (IQR: 1.80–1.82 s) and 2.35 s (IQR: 2.32–2.42 s), median zero-to-peak pulse amplitudes were 190.6 dB (IQR: 190.0–191.2 dB), 193.8 dB (IQR: 193.6–194.0 dB), and 194.2 dB (IQR: 193.6–194.5 dB) re 1 µPa, and median root-mean-square inter-pulse amplitudes were 140.7 dB (IQR: 140.5–141.0 dB), 138.4 dB (IQR: 138.2–138.7 dB), and 137.8 dB (IQR: 137.6–138.0 dB) re 1 µPa. Notably, pulse amplitude, inter-pulse time interval, and inter-pulse interval amplitude within a pile driving event will vary slightly from impact to impact and as the pile is driven into the sediment. Here, playback amplitudes and inter-pulse interval were not manipulated to be identical within or across the three pile driving files because we were interested in studying behavioral impacts of pile driving noise at a simulated distance from the pile, rather than studying responses dependent on these specific metrics. The variability in these metrics across impulses reflects that which a wild squid may experience near an offshore pile driving site. For use in control trials, a 15- min long silent file was played.

4.1.2.2. Experimental procedures

All trials were conducted during the daytime, and squid were tested individually for each trial (no pairs or groups of squid were tested in a given trial). Trial order for experimentally naïve squid was randomized between controls and the three pile driving noise files. Noise files for retests were also randomly selected regardless of the file used for a squid's first noise exposure. Individual squid (mean mantle length ± SD: 16.5 ± 4.1 cm**,** n=23) were transferred by net into a container with a volume of at least 19 L, for transport to the experimental tank. Only squid that displayed normal behaviors (i.e*.*, normal body patterning, swimming normally in the water column) and were without major skin damage (Hanlon and Messenger 2018) were used for experiments. Squid were then hand-transferred into the experimental tank, and hydrophone and video recordings began immediately thereafter. To allow squid to acclimate to the new tank and be able to compare their behaviors before and during noise, squid were allowed 5 min to acclimate in the tank, followed by an additional 15 min (termed the "pre-exposure" period) before control or pile driving files were played. Playback of pile driving or silent control files ran for 15 min (the “exposure” period). Trials for which pile driving was played to experimentally naïve squid are hereafter referred to as “Day 1” trials. To examine the effects of repeated exposures, a subsample of squid was retested 24 h after the first exposure (“Day 2” trials). To maintain these animals in between trials, they were housed in a separate building in separate, 1.2 m diameter tanks with opaque covers, but otherwise with the same conditions as pre-experiment holding tanks. The experimental procedure on Day 2 trials was identical to that for Day 1 trials.

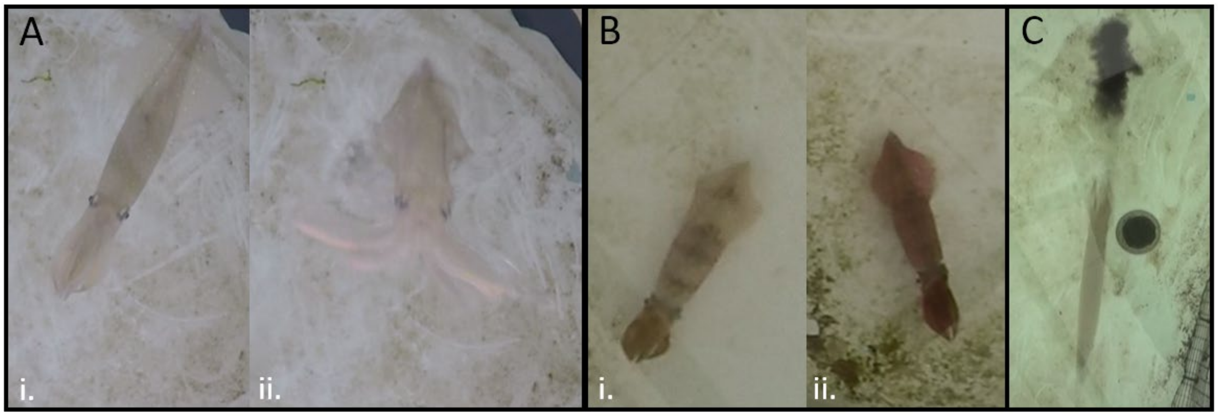


Figure 25. Examples of alarm responses observed during pile driving noise exposure
A) Calm, swimming squid with a lightly-shaded (termed *Clear*) body pattern before noise onset (i) that subsequently exhibited a startle response, in this case a forward lunge, during the first noise impulse (ii). B) Example of a body pattern change in another squid, in which the squid changed from a *Bands* (i) between noise impulses to an *All dark* body pattern (ii) during the next impulse, indicating a change from a cryptic to an alarmed state. C) Example of simultaneous inking and jetting. See Hanlon et al. (1999) for a detailed ethogram of this species .

4.1.2.3. Acoustic calibration of the experimental tank

The experimental tank was calibrated in 10 cm increments in all 3 dimensions (224 positions total) without animals present, creating a 3D array of received sound levels. At each position, the first minute of each of the three pile driving noise files was played and recorded at a 48 kHz sampling rate by a triaxial ICP accelerometer (Model W356B11, PCB Piezotronics) and Reson TC4013 hydrophone (Teledyne Marine) spaced 10 cm apart. The accelerometer was wired through a signal conditioner (Model 480B21, Piezotronics). The accelerometer signal and hydrophone were input to two analog filters (Model 3382, Krohn-Hite Corporation), which each applied an anti-aliasing low-pass filter at 24 kHz and a 20 dB gain. See (Jones et al. 2020) for further details on data analyses. Example images of analyzed behaviors are in Figure 19.

4.1.3. Results

4.1.3.1. Tanks acoustics and sound exposures

Background sound levels of the tank were low, starting at a PSD of ca. 80 dB re 1 uPa^2/Hz at 20–100 Hz and decreasing in a logarithmic fashion down to ca. 50 dB re 1 uPa^2/Hz at 600–1000 Hz (Figure 20A). The acceleration levels of ambient sound in the tank and silent playback were below the self-noise floor of the accelerometer, evidenced by flat power spectra of these recordings at -70 dB re 1 (m s^{-2})^2/Hz (not shown). This relatively quiet tank environment enabled us to isolate and assess potential effects of water-borne pile driving noise on squid while minimizing extraneous stimuli that are found in natural field environments. The PSD curves of pile driving pulses in the tank indicated that noise playback created a substantially elevated sound field (by up to 50 dB) and this noise was generally similar among the three playback files, with some differences in spectral shape between pressure and acceleration metrics (Figure 20) The highest received pile driving pulse energy was between 100–300 Hz, a range within which *D. pealeii* has relatively high sound sensitivity (Mooney et al., 2016, 2010). Across the frequency range of 20–1000 Hz, acceleration PSD of pile driving pulses was 20–30 dB higher than that of inter-pulse intervals. Spectral curves of sound pressure of pile driving pulses received in the tank had roughly similar shapes but lower amplitudes (by 20–40 dB) compared to those received in the field 0.5 km from the pile installation. These sound pressure levels in the tank were in the range of levels typically present in the water column 2–4 km away from the Block Island Wind Farm piles (Amaral et al., 2018). Acceleration PSD of pile driving pulses in the tank was about 20 dB higher than acceleration in the field at most frequencies from 20-1000 Hz, and about 40 dB higher near 300 Hz. Median peak particle velocity (Euclidian norm) across all tank recording positions and files was -40 (IQR: -43 to -37) dB re 1 m s^{-1}, compared with measured peak particle velocity of -70 dB re 1 m s^{-1} at about 1 m above the seabed and -60 dB re 1 m s^{-1} at the seabed, 0.5 km from the pile (Amaral et al., 2018). A prior study that modeled peak particle velocity propagation from simulated pile driving sound predicted -40 dB re 1 m s^{-1} at the seabed, 150 m away from the pile (Miller et al., 2016).

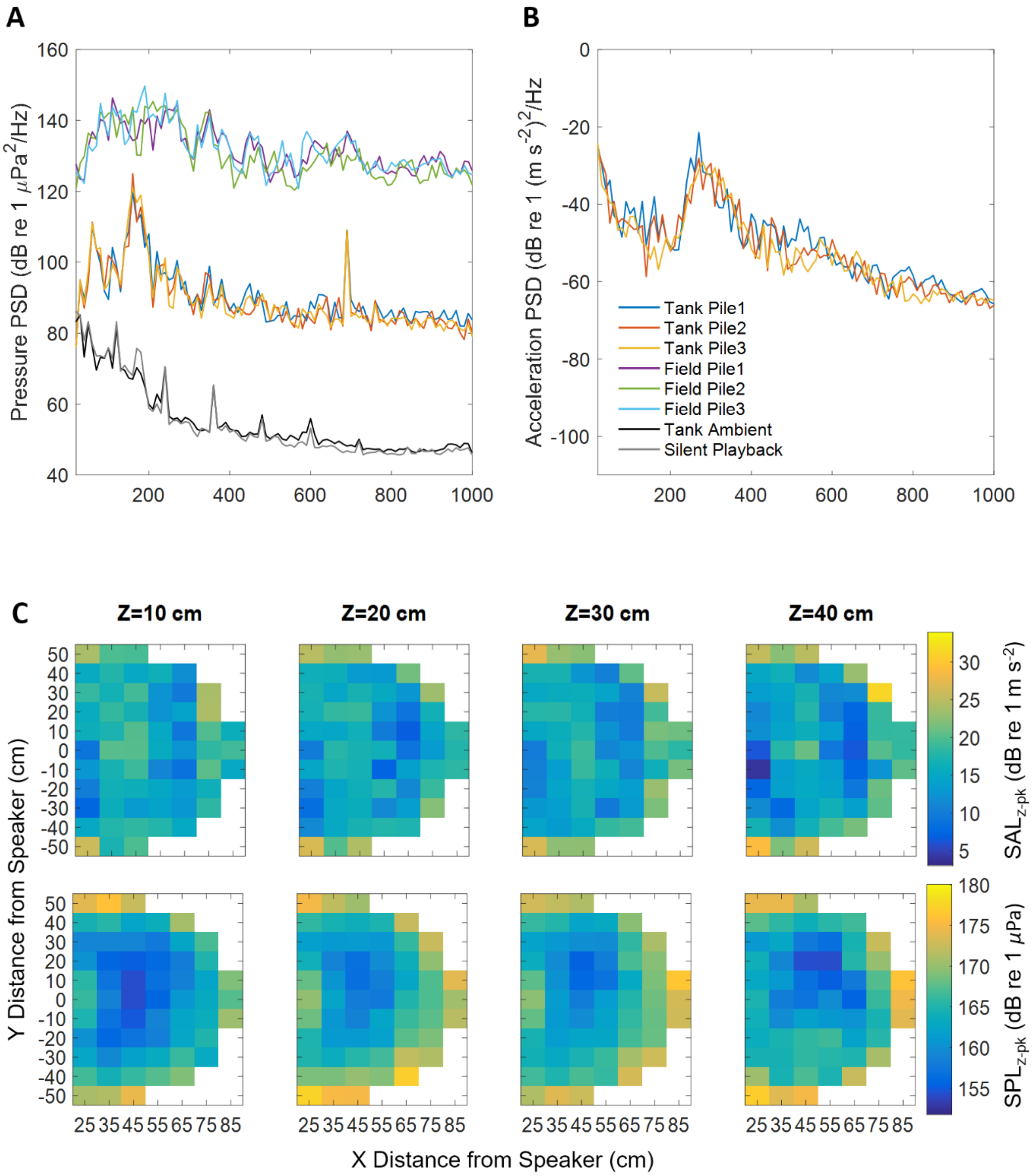


Figure 26. Spatial maps of pile driving playback recorded in the experiment tank
Power spectral density (PSD) curves, in 10 Hz bins, for sound pressure (A) and acceleration data (B) of each pile driving file as received in the experiment tank ("Tank Pile"). Also shown are spectra for the original files used for playbacks, as received in the field 0.5 km away from a pile installation ("Field Pile"), and mean ambient noise in the experiment tank during the pre-exposure period ("Tank Ambient") and during the 'exposure' period of control trials ("Silent Playback"). The PSD curves of pile driving noise in the experiment tank are from the calibration position squid were most frequently closest to (X=45, Y=20, Z=40; as shown in C), and are

integrated over the pulse length (‘Pulse’) as defined in the methods, or inter-pulse intervals (at times outside the pulse length). The PSD curves for the field data were also calculated over the first minute of each file. PSD levels for “Tank Ambient” and “Silent Playback” curves were calculated over the 15 min pre-exposure and 15 min control exposure periods, respectively. C) Maps of mean received zero-to-peak (z-pk) acceleration (top row) and z-pk sound pressure (bottom row) in the tank from the acoustic calibration, at each water depth (columns). The median z-pk level across pile impulses for the first minute of each of the three pile files was found, then for each recording position the mean value across files was calculated, shown here. The origin for the XY plane is the speaker.

4.1.3.2. Alarm responses and repeated exposures

Nearly all squid (15 out of 16 individuals) exhibited at least one of the recorded alarm behaviors (inking, jetting, startle, body pattern change) during the first 30 pile driving noise impulses (Figure 21A). These 30 impulses amounted to 45 to 69 s of playback, depending on inter-pulse interval. In contrast, only one control squid exhibited any of these behaviors during the first minute of silent playback. This control squid displayed one body pattern change, a brief flash of Bands, 7 s into silent playback. Proportions of squid exhibiting each of the four alarm behavior types during the first 30 impulses did not differ significantly between Day 1 and Day 2 trials, reflecting similar responses despite a ca. 24-hr no-playback period (inking: $P = 0.66$; jetting: $P = 1.00$; startle: $P = 1.00$; body pattern change: $P = 0.45$; Fisher’s exact test). Over the first minute of playback, a significantly greater proportion of control squid had ‘No response’ compared to Day 1 squid ($P < 0.001$; Fisher’s exact test) and compared to Day 2 squid ($P < 0.01$). Notably, inking only occurred during experimental trials. Relatively few alarm behaviors occurred at times outside the first 30 pile impulses.

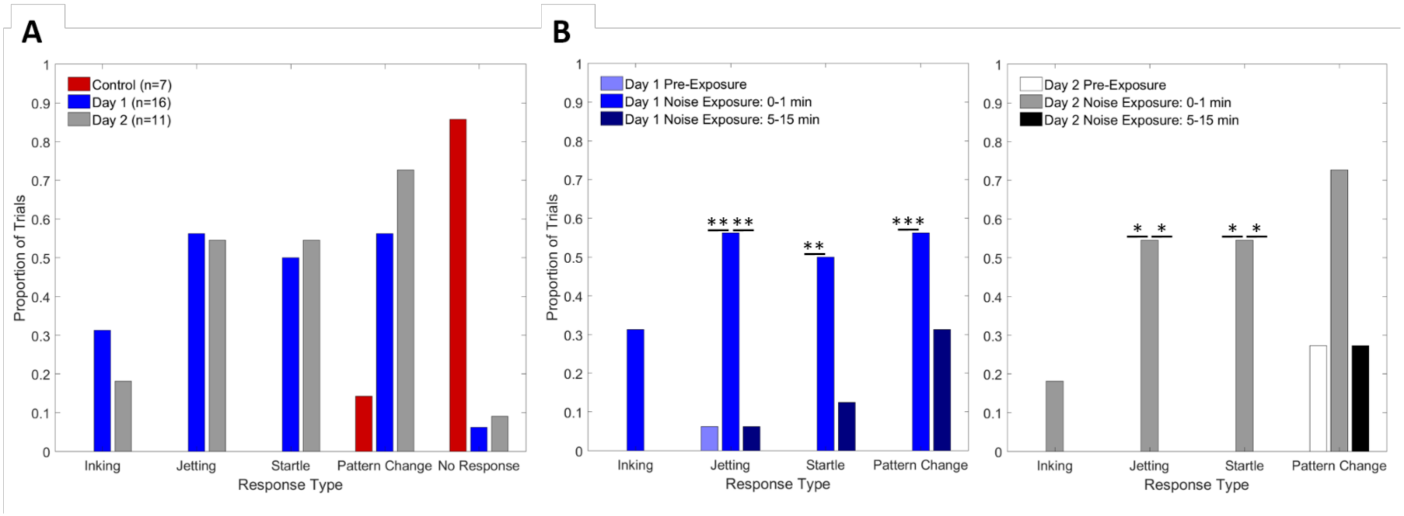


Figure 27. Histograms showing behavioral responses
A) Proportions of trials in which squid exhibited alarm behaviors, or no alarm behaviors (“No Response”) at least once during the first 30 pile driving noise impulses (for Day 1 and Day 2 noise exposure trials: n=16 and n=11, respectively) or during the first minute of silent file

playback (for control trials: n=7). There were no significant differences between Day 1 and Day 2 noise exposure trials for each response type, including “no response”. Over the first minute of playback, a significantly greater proportion of control squid had ‘No Response’ compared to Day 1 squid ($P < 0.001$; Fisher’s exact test) and compared to Day 2 squid ($P < 0.01$). B) Proportions of trials in which squid exhibited each behavior at least once during three different periods, for Day 1 (left) and Day 2 (right) trials. “Pre-exposure” refers to the last minute of the pre-exposure period, just prior to the start of noise playback. “Noise Exposure: 5-15 min” refers to three, pooled periods of 30 pile impulses encompassing the 5th, 10th, and 15th minute of noise. Asterisks denote significant differences between the first 30 impulses of noise exposure and other periods: * $P < 0.017$, ** $P < 0.01$, *** $P < 0.001$.

During the first minute of playback, inking and jetting behaviors were confined entirely to the first 18 impulses, i.e., up to about 40 s of playback, and the majority of recorded behaviors occurred during the first eight impulses (Figure 22). The greatest proportion of each alarm response type occurred at the first impulse and responses quickly diminished with successive impulses. There were no significant differences in proportions of any alarm behavior between Day 1 and Day 2 trials in any of the first 30 impulse time bins ($P > 0.05$; Fisher’s exact test). Pile driving impulse number was a significant predictor of the number of each alarm behavior over the first 30 impulses for Day 1 (inking: $P < 0.05$; jetting: $P < 0.001$; startle: $P < 0.001$; body pattern change: $P < 0.01$; Poisson GLMs) and for Day 2 (inking: $P < 0.05$; jetting: $P < 0.001$; startle: $P < 0.01$; body pattern change: $P < 0.001$; Poisson GLMs).

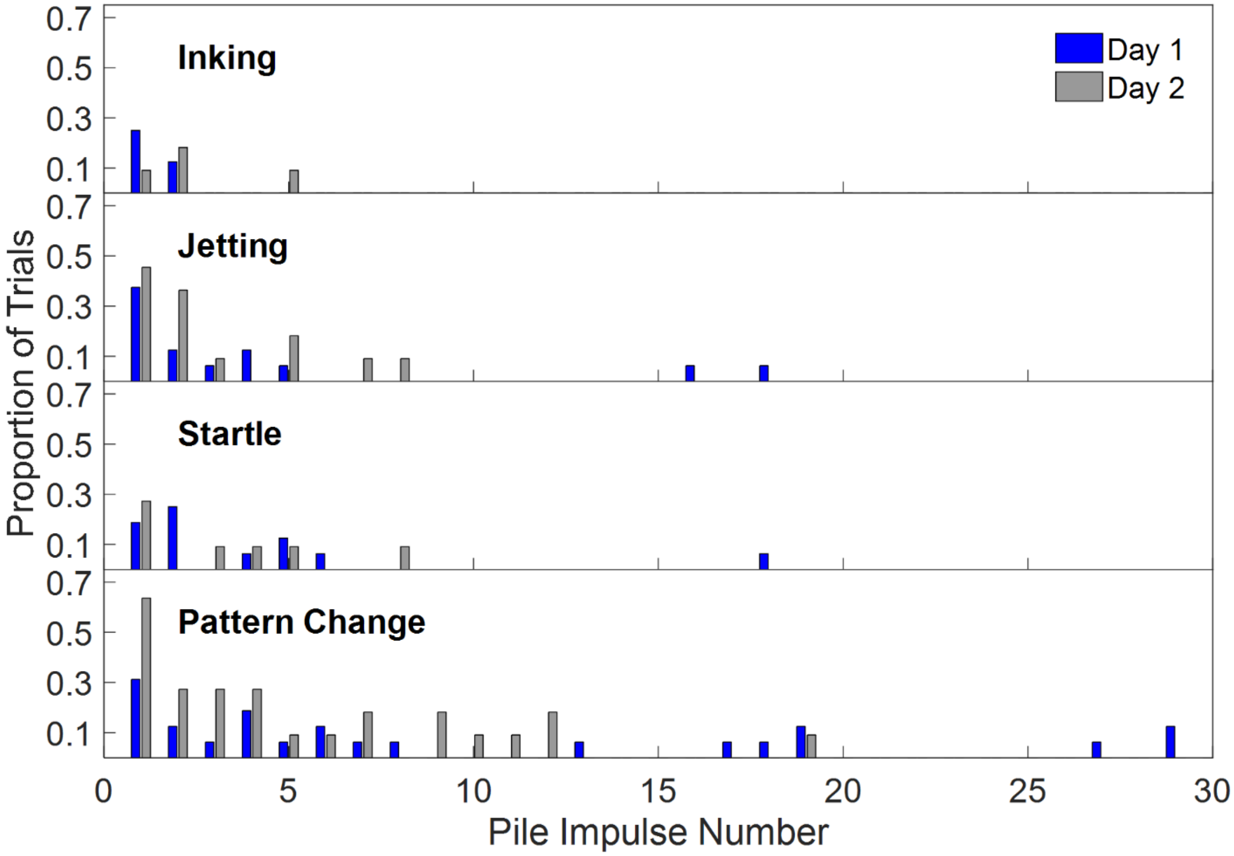


Figure 28. Behavioral responses observed per impulse number
Proportions of trials with squid that responded with inking, jetting, startle, and body pattern change behaviors in given pile impulse time bins over the first 30 impulses, for Day 1 and Day 2 trials.

4.1.3.3. Alarm responses verses acceleration levels

Analyzing over the first minute of pile driving noise playback, for Day 1 and Day 2 trials, median received zero-to-peak particle acceleration level ($SAL_{z\text{-pk}}$) at which squid inking occurred was greater than for any other alarm response behavior (Fig. 23A). Distributions of $SAL_{z\text{-pk}}$ for jetting and startle responses, followed by body pattern changes, spread to lower values than those for inking. For Day 2 trials, median $SAL_{z\text{-pk}}$ was lowest for impulses at which "No response" occurred. A Kruskal-Wallis test indicated significant differences in $SAL_{z\text{-pk}}$ among these five response categories for Day 2 ($P < 0.05$) but not for Day 1 ($P > 0.05$). Pairwise tests indicated no significant differences in received $SAL_{z\text{-pk}}$ among the four alarm response types and 'No response' for Day 1 or Day 2 trials (Wilcoxon rank sum tests, $P > 0.005$). Regression analyses of these same data were performed after ranking the ordinal alarm response categories with "Inking" being the 'strongest' response, followed by "Jetting", "Startle", "Body pattern change", and "No response" being the 'weakest' response (Fig. 21B). This revealed a significant, monotonic relationship between received acceleration level and behavioral response for Day 2, with the received amplitude decreasing as the 'strength' of the response decreased

(Spearman’s Rho: $r = -0.16$, $p < 0.01$). For Day 1, this regression analysis resulted in a similar trend with weaker correlation and a non-significant result (Spearman’s Rho: $r = -0.03$, $p > 0.05$).

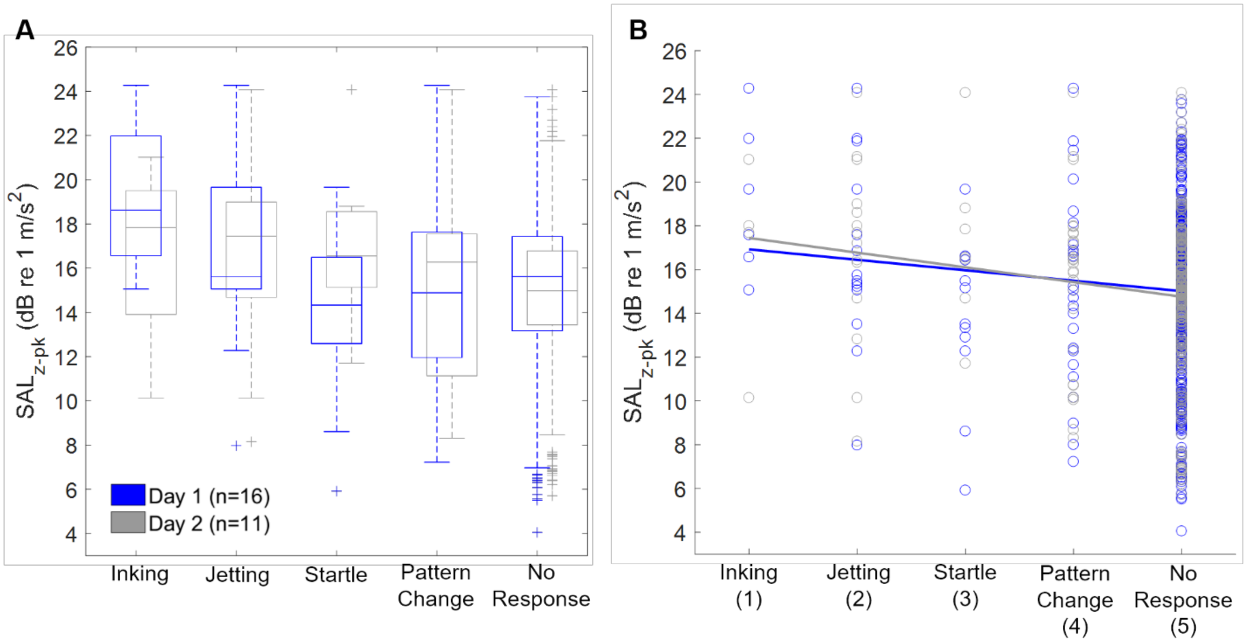


Figure 29. Received zero-to-peak sound acceleration levels (not normalized) at which squid exhibited each alarm response type or no response.

Data are shown for Day 1 trials (blue symbols) and Day 2 trials (gray symbols) for the first minute of pile driving impulses (30 impulses for Pile1 and Pile2, or 26 impulses for Pile3). All behaviors are shown here, with more than one alarm response sometimes occurring on a given impulse during a given trial. A) Horizontal lines in the middle of the boxes indicate median values, bottom and top of the boxes indicate 25th and 75th percentiles, whiskers extend to ranges 1.5 times the 25th and 75th percentiles, and crosses indicate outliers (outliers are defined for data that is greater than $q_3 + 1.5 \times (q_3 - q_1)$ or less than $q_1 - 1.5 \times (q_3 - q_1)$, where q_1 and q_3 are the 25th and 75th percentiles of the sample data, respectively). B) Points indicate received acceleration levels at which each response type occurred, and linear, least-squares lines of best fit are shown for Day 1 (blue) and Day 2 (gray) trials.

4.1.4. Discussion

All squid exposed to pile driving noise responded with alarm behaviors, indicating squid detected and were dramatically influenced by this noise. The responses (inking, jetting and pattern change) were clearly identifiable. Such behaviors are used for escaping predators and communication with conspecifics, including in reproductive contexts (Hanlon et al., 1999; Shashar and Hanlon, 2013). This study sought to focus on individual squid to assess the overall likelihood of responses without the complexities of ecological interactions (yet). Hence we did not test for disruption to inter- or intraspecific communication in the present study, yet we found noise affected fundamental behaviors (e.g., body pattern changes, evasion) typically enacted by squid as part of communication and ecological interactions. Therefore, noise exposure could potentially influence these fundamental communication pathways. Given the ecological and

commercial importance of squid, the potential of such an impact should be of concern to fishers, and those who seek to manage squid stocks and the ecosystems with which they interact.
The files of pile driving used for playback in the present study were from hydrophones about 1 m from the bottom at 500 m away from pile driving sites. Particle acceleration values in our experiment tank reflected those squid may experience at and near the seabed within 500 m away from offshore pile driving sites, based on the fact that particle acceleration values in our experiment tank exceeded those measured in the field both at and just above the benthos 500 m away. Notably, given the inherent complexity of acoustic propagation in small tanks (Rogers et al., 2016), it was not possible to recreate the exact pile driving noise field present in the squids' natural environment. The noise field in our tank was our best approximation of water-borne noise from pile driving, given available resources. Future tests should examine squid behavior in the field with signals from real pile installations.

Due to the high spatial variation of sound acceleration in the tank, the squid probably could not seek refuge in locations of the tank subject to lower particle acceleration levels. The highly variable trends in normalized $SAL_{z\text{-}pk}$ received by the squid (estimated based on squids' location at the onset of each noise pulse) suggest squid did not, or could not, remain in locations of the tank with lower particle acceleration magnitude.
The alarm and escape behaviors observed during noise playback are characteristic squid responses to perceived predatory threats (Hanlon and Messenger, 2018; Staudinger et al., 2011). Squid rely on these 'secondary defenses' to survive predatory attacks when primary defenses, e.g., camouflage, fail to prevent detection by the predator (Staudinger et al., 2011). Based on the low observed number of inking behaviors per squid in this study, and field observations of squid inking repeatedly (for up to an hour, (R. Hanlon, personal communication), squid could not have depleted their ink stores in this study".

Squid exhibited similar proportions of alarm responses between Day 1 and Day 2 trials over the first 30 impulses, and in both groups alarm responses diminished quickly over time, with GLMs indicating all recorded behavior types but body pattern changes were extinguished by the 30th impulse. Regression analysis predicted near (but not total) extinction of alarm-type body pattern changes by the 30th impulse, but this model did not consider body pattern changes occurring at later subsamples of the noise exposure period. This suggests that responses, although rare, are likely to still occur across the entire pile driving exposure, making squid show these alarm responses, and their visual communication systems potentially disrupted, for much of the pile driving event. Further, there may be disruption to their predator-avoidance system, even if escape responses are not clearly exhibited.

The reduction in alarm responses over several noise impulses indicates increased tolerance over time to the noise source and suggests these squid may have behaviorally habituated.

In addition to its dependence on the number of noise impulses over time, there was a dependence of alarm response occurrence on received acceleration levels, with squid exhibiting "No response” having received significantly lower $SAL_{z\text{-}pk}$ during analyzed impulses than on impulses they exhibited an alarm response. Lower received $SAL_{z\text{-}pk}$ correlated with ranked alarm responses of lower ‘strength’ (i.e., minor alarm responses such as startle and body pattern changes or no response, compared to ‘stronger’ inking and jetting responses). This suggests that higher-amplitude (or potentially closer) pile driving noise is more likely to elicit locomotor escape responses including inking and jetting, which are naturally employed to avoid capture by predators, versus lower-amplitude impulses that are more likely to elicit body pattern changes or no response.

4.1.4.1. Conclusions

Our study is the first to demonstrate behavioral effects of pile driving noise on any cephalopod species and focused on a commercially important squid species whose range overlaps with areas where marine construction projects involving pile driving will occur in the near future. We observed a variety of alarm responses associated with anti-predator reactions at the onset of pile driving noise. Squid had rapidly diminished alarm responses within the first minute of noise exposure in all trials and had re-sensitized to the noise after a 24-h rest period. Diminished predator-defense and escape behaviors may alter squid susceptibility to predation, depending on potential factors such as squids’ habituation specificity and their predators’ responses to noise. Although caution must be taken when extrapolating lab-based results to free-swimming wild animals that may potentially escape a noise source, controlled lab-based studies are useful for analyzing such interactions without the influence of confounding environmental variables. That said, to conclusively test ecological implications of noise exposure, field studies are also needed, in which acoustic conditions are more realistic and squid are less confined, and thus allowed to behave more naturally. The present findings of the influence of pile driving noise on squid alarm responses will leverage future studies on behavioral and physiological effects of anthropogenic noise on squid and other cephalopods, as well as impacts on these animals’ ecology.

4.2. Study 6: Feeding Squid: Changes in feeding behavior of longfin squid (*Doryteuthis pealeii*) during laboratory exposure to pile driving noise.

Published as: Jones, I. T., Peyla, J. F., Clark, H., Song, Z., Stanley, J. A., & Mooney, T. A. (2021). Changes in feeding behavior of longfin squid (Doryteuthis pealeii) during laboratory exposure to pile driving noise. *Marine Environmental Research, 165*, 105250. doi.org/10.1016/j.marenvres.2020.105250.

4.2.1. Background

Though studies investigating effects of anthropogenic noise on invertebrates' feeding behavior are limited, several studies have indicated adverse effects of anthropogenic noise on other behaviors, and physiology of cephalopods and other invertebrate taxa. Hair cells in hearing structures of the cuttlefish *Sepia officinalis*, and squids *Loligo vulgaris* and *Illex coindetii* suffered damage after 2 h continuous exposures of noise (Solé et al., 2017, 2018, 2019). Southern reef squid, *Sepioteuthis australis*, exhibited alarm responses, i.e., inking and jetting, during impulsive air gun noise (Fewtrell & McCauley, 2012). Pile driving noise in a prior laboratory study elicited alarm responses in longfin squid, *Doryteuthis pealeii*, including inking, jetting, and body pattern changes (Jones et al., 2020). Rock lobsters, *Jasus edwardsii*, exposed to air gun noise had an impaired behavioral righting reflex (employed to escape predation), and had damage to statocyst structures important in controlling this righting response (Day et al., 2019). Further, bay scallops, *Pecten fumatus*, exposed to air gun noise had higher mortality rates, higher rates of recessing behavior, and changes in haemolymph biochemistry suggestive of reduced capacity for homeostasis (Day et al., 2017). These studies indicate diverse potential noise impacts on cephalopods and other invertebrates, though the extent of impacts on these taxa are only just beginning to be understood.

The current study focused on the longfin squid, which inhabits continental shelf waters in the Western Atlantic Ocean, ranging from Venezuela to Newfoundland. The species is most abundant in the Northeast U.S., between Cape Hatteras, NC, and Georges Bank (Hanlon et al., 2013). In that region, offshore wind farms are planned for construction in the 2020s and 2030s within multiple established lease areas (Musial et al., 2019). Longfin squid are commercially important, with average annual landings of about 11,000 mt and values of $30 million since 2010 (NMFS, 2020). They are opportunistic predators that feed on a wide variety of fish and invertebrate species throughout their lifetime (Hunsicker & Essington, 2006; Vovk, 1985). Small, young juveniles feed primarily on copepods, and they consume increasingly larger fish prey as they grow into adults. Squid rely heavily on visual cues for communication and finding prey, and are more likely to pursue mobile prey than stationary prey (Hanlon & Messenger, 2018). Longfin squid are known to feed during the daytime and at night (Macy, 1982; Vovk, 1985). They have fast metabolisms, rapid digestion rates, and limited energy stores; thus it is suspected they need to frequently consume prey to survive in the wild (Hanlon et al., 2013; Hatfield et al., 2001).

In the present study, we examined how playbacks of sounds from impact pile driving influenced predation by the ecologically key squid *Doryteuthis pealeii*. In both daytime and nighttime trials, live killifish, *Fundulus heteroclitus*, were added to the experimental tank to quantify squids' prey capture rates, failed predation attempts, and latencies to predation behaviors. We also quantified the mobility level of the killifish as a potential covariate in squid feeding behaviors, and we measured the hearing range of *F. heteroclitus* using neurophysiological auditory evoked potential (AEP) methods to assess their ability to detect the sounds. This study intends to elucidate how pile driving noise may alter feeding behaviors critical for individual squid's survival.

4.2.2. Methods

Experiments during the daytime ("Day" trials) were conducted between June 23 and July 27, 2018 (n = 54 trials). Experiments during the nighttime ("Night" trials) were conducted between September 4 and October 21, 2018 (n = 32 trials). Day trials took place during daylight (between 09:00 and 18:45 local time), and Night trials took place after astronomical twilight (between 20:00 and 02:45 local time). Experiments took place in a 1.1 m diameter cylindrical tank, filled to 0.5 m depth with ambient flowing seawater (Figure 24). An Aqua-30 speaker (Theunissen Technical Trading, Malden, The Netherlands; frequency response: 100 Hz–10 kHz) was suspended, facing horizontally, at 25 cm depth and 15 cm forward of the closest tank wall, projecting the pile driving sound. Audio files of pile driving sounds used for the experiments were recorded during construction of the Block Island Wind Farm. To avoid pseudoreplication of playback stimuli, three 10-min long recordings were generated from one pile driving bout and edited in Adobe Audition prior to playback.

There were three playback treatment types, designated "Onset", "5min", and "Control". Treatment was randomly selected for each trial. In Onset trials, the experimenter raised the PVC cap to reveal the killifish prey after the acclimation period, and waited for the squid to start pursuing it, at which time the pile driving noise was immediately started. The noise exposure lasted for 10 min or until the squid captured and began consuming the fish. In 5min trials, the pile driving playback was started after the acclimation period but five minutes before the fish was revealed. After revealing the fish, the playback continued for five more minutes or until the squid captured and began consuming the fish. Control trials had the same protocol as Onset trials, except that a 10-min long silent file was played instead of the pile driving noise file. For all trials, if the squid did not pursue the fish within 10 min after the fish was revealed, the trial was ended. The noise exposure duration was chosen based on observations of squid in preliminary noise trials that consumed prey less than 10 min, often within 1 min, after its reveal. Though durations of individual pile driving periods are variable in the field, this experimental duration was within the range of those observed for BIWF construction (Amaral et al., 2018). The experimental tank was calibrated in 10 cm increments in all three dimensions (280 positions total) without animals present, creating a 3D array of received sound levels. See Jones et al. (2021) for more methodological details, including data analysis methods.

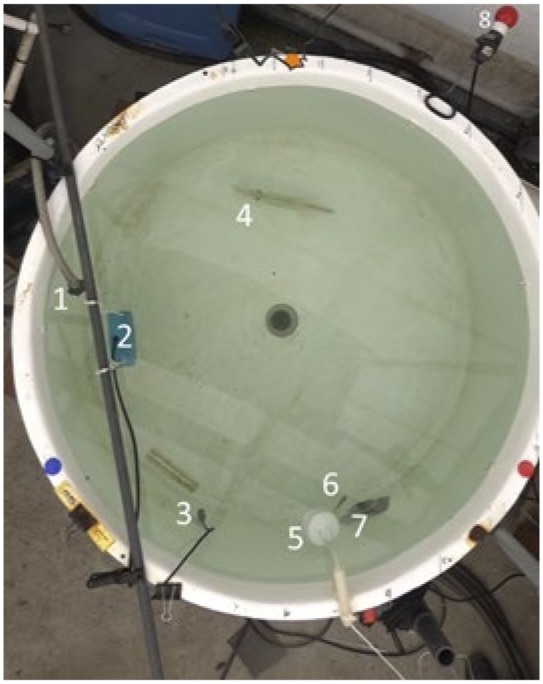


Figure 30. View of experiment tank from top-down video camera
1) Inflow hose, 2) underwater speaker (Aqua-30), 3) hydrophone to monitor ambient sound and playbacks during experiments, 4) squid (*Doryteuthis pealeii*) 5) PVC container lifted to reveal fish, 6) fish prey (*Fundulus heteroclitus*), 7) flow outlet, covered with mesh, 8) LED to indicate start of playbacks (used only in Day trials). The dark circle in the center of the tank is a plugged outflow pipe.

4.2.3. Results

4.2.3.1. Experimental acoustic field

The confines of the tank provided a quiet and isolated background environment for the study. Pressure spectra of ambient sound in the tank were at least 30 dB lower than those of the pile driving noise, and spectral levels of the silent Control playbacks were similar to these ambient levels. Accelerometer recordings of ambient sound and silent playbacks resulted in flat spectra at the self-noise floor of the accelerometer, i.e., 55 dB re 1 µm s-2 (not shown), thus these conditions were likely at lower acceleration levels.

$SAL_{z\text{-}pk}$ and $SPL_{z\text{-}pk}$ of the pile driving playbacks were highly variable throughout the tank, ranging from about 130 to 150 dB re 1 µm s-2 and 160–180 dB re 1 µPa, respectively (Figure 25A). For both metrics, amplitudes were more variable in the horizontal plane than across depths, though generally, higher amplitudes were recorded at 20 and 30 cm depths than at 10 and 40 cm depths. Particle acceleration followed a complex, non-monotonic pattern with distance from the speaker. Sound pressure was higher closer to the speaker and dropped off with distance from the speaker along the X and Y axes, increasing again near the tank boundaries.

Acceleration PSD in the tank exceeded that recorded in the field, by up to 40 dB at frequencies below 400 Hz (Figure 25B). Pressure spectra of noise pulses in the tank were generally lower than, and within 30 dB of PSD of the field recordings (Figure 25C). Below 400 Hz, the pressure spectra of pulses in the tank were closer to those in the field, generally within 20 dB.

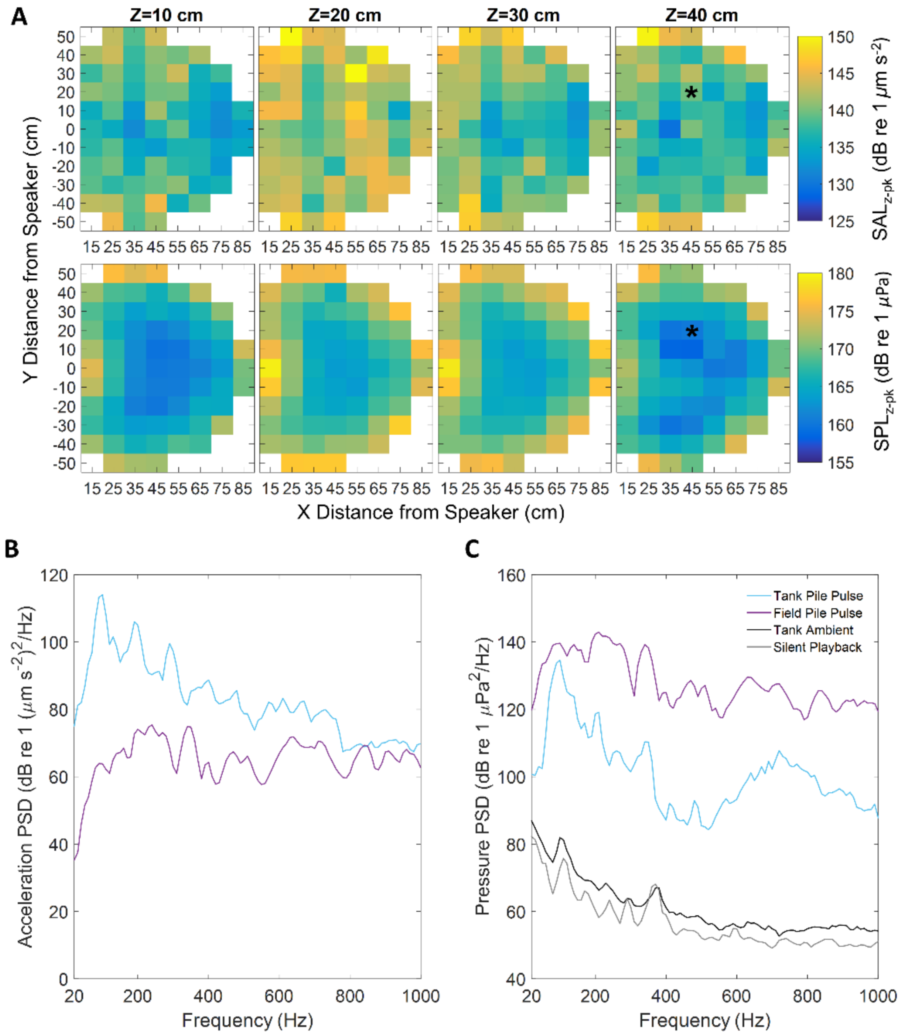


Figure 31. Tank calibrations

A) Spatial maps of zero-to-peak acceleration (top) and pressure (bottom) for four water depths (columns), shown from top-down in the horizontal plane, with the front-center of the speaker set as the origin. Data were band-pass filtered to 20-1000 Hz and median zero-to-peak values of pile pulses (across 30 pulses, i.e. 1 min) are shown for each recording location. The asterisks indicate the recording location at which spectra are shown in B and C. Power spectral densities are shown for particle acceleration (B) and sound pressure (C) in time windows covering pile driving pulses of in-tank playbacks and original field recordings. The median spectra of 30 pulses are shown. Spectra of ambient tank sounds (no playback) and the silent playback file are also shown in pressure, but not in acceleration since these conditions were below the noise floor of the accelerometer.

4.2.3.2. Prey capture rates: ‘day trials’

The proportion of squid that pursued or attacked fish without capture (‘failed attempt’) was greater in the Onset treatment, but not significantly so (Figure 26). Between Onset and Control treatments, there were no significant differences in rates of ‘failed attempt’ (odds ratio (OR) = 0.25, 95% confidence interval (CI): 0.04–1.58, p = 0.198) or ‘capture’ (OR = 4.06, 95% CI: 0.63–26.1, p = 0.198; Fisher’s Exact tests). A lower proportion of squid in the 5min treatment captured the prey than in Control and Onset. In 5min trials, some squid made no attempt to pursue or attack the fish (‘no attempt’). We sought to compare the proportion of squid that made no predation attempts in the 5min treatment, with that of the other treatments. In this analysis, the ‘no attempt’ category in the Control and Onset treatments defines squid that were likely not motivated to feed in the experiment tank, as these squid did not pursue the fish and were not exposed to any audio playback. Thus, a similar ‘no attempt’ proportion in 5min treatments compared to the Control and Onset treatments suggests squid with ‘no attempt’ in the 5min treatment were not motivated to feed prior to playback. Conversely, a higher ‘no attempt’ proportion in the 5min treatments suggests reduction in feeding behavior caused by noise playback. In Day trials, there was no significant difference in the proportion of squid that made ‘no attempt’ in the 5min treatment, compared to either Control (OR = 0.16, 95% CI: 0.02–1.46, p = 0.104) or Onset (OR = 0.90, 95% CI: 0.21–3.66, p = 1.000; Fisher’s Exact tests).

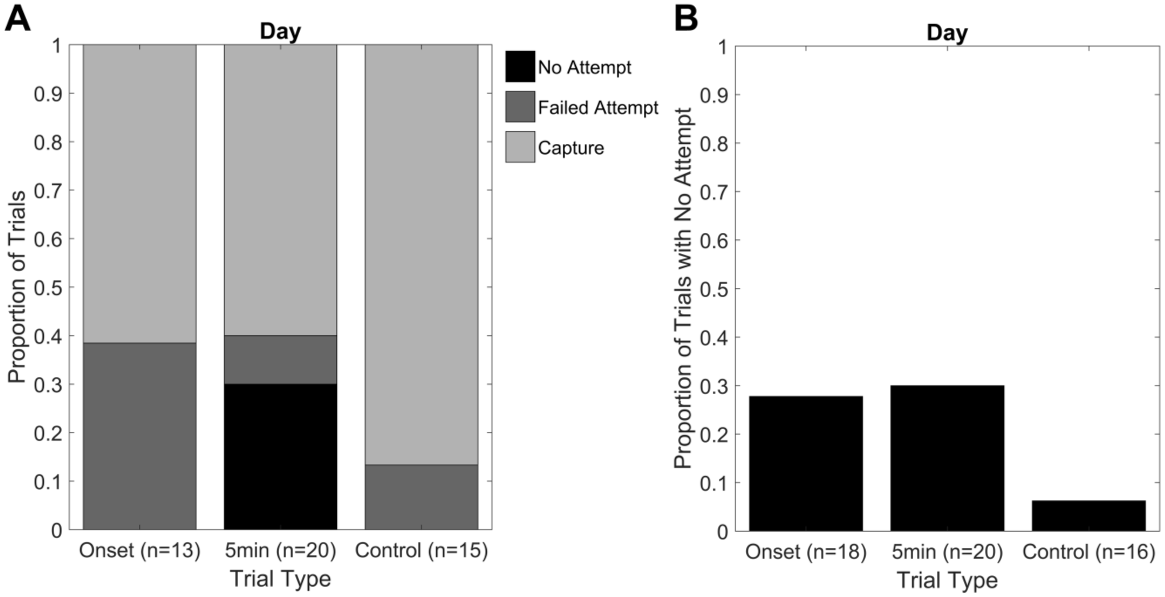


Figure 32. Stacked histogram showing capture rates
A) Proportions of trials in the ‘Day’ dataset, in which squid successfully captured and consumed the fish (‘Capture’), attempted to capture (with pursuit and/or attack) but did not successfully capture (‘Failed Attempt’), or made no attempt to pursue or capture the fish (‘No Attempt’) during playback, for each playback treatment. Only trials in which a silent or pile driving playback was started are included here. B) Proportions of squid that made no attempt to feed in

the ‘Day’ dataset**.** The proportions of ‘No Attempt’ in Onset and Control treatments represent squid that received no noise exposure or control playback, respectively, as they did not pursue prey during the trial. Sample sizes for Onset and Control treatments are greater than in 25A because trials in which no playback was started are included here. All squid in 5min trials received noise exposure, starting 5 min before the prey was revealed.

4.2.3.3. Prey capture rates: ‘night trials’

In the ‘Night’ dataset, there were similar rates of ‘failed attempt’ or ‘capture’ between Onset and Control treatments, though low sample sizes in these treatments, limited to the number of squid that pursued the fish, precluded our ability to perform statistical comparisons. Analyzing all Night trials performed, the ‘no attempt’ proportion was similar (40–50%) in Night Control and Night Onset treatments and was higher (83%) in the Night 5min treatment than the control (though not significantly so: p = 0.074, Fisher’s Exact tests).

4.2.3.4. Failed predation attempts

The median number of failed attempts was highest for squid in Onset trials (Figure 4). There were significant differences among the three treatments ($\chi^2 = 8.08$, df = 2, n = 42, p = 0.018; Kruskal-Wallis; Figure 27). Pairwise tests revealed a significant difference in the median number of failed attempts between Onset and 5min treatments (z = 2.43, U = 138; p = 0.015; MWU with Holm’s procedure; lowest p value of the three comparisons), and between Onset and Control treatments (z = -2.36, U = 50, p = 0.018; second lowest p value). The failed attempt rate was statistically similar between squid in Control and 5min treatments (z = 0.39, U = 113, p = 0.697). In Day Onset trials, 53% of failed attempts stopped at the pursuit stage, and the other 47% of failed attempts were missed attacks (15 total failed attempts). About 83% of failed attempts ended at the pursuit stage and 17% ended at the attack stage in Day 5min trials (6 total failed attempts), and 43% and 43% in Control trials, respectively (7 total failed attempts). The remaining 14% in the Control trials represents one squid that captured then immediately released the fish.

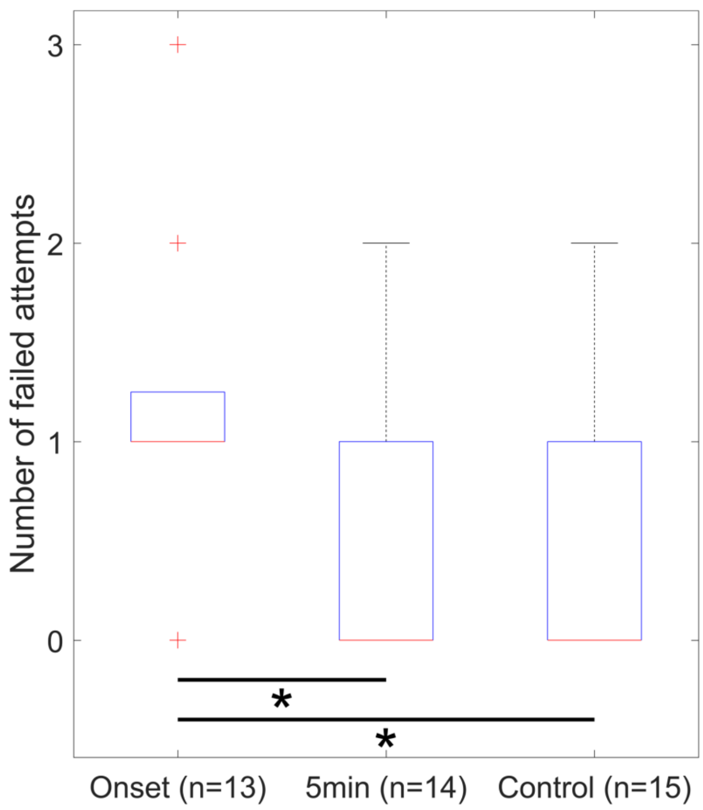


Figure 33. Failed predation attempts
Number of failed predation attempts (defined as a pursuit and/or attack without capture during a predation sequence) for Day trials. Only trials in which a silent or pile playback was started are shown. Sample sizes are reduced here for the 5min treatment compared to data presented in Fig. 3, because only trials in which squid made at least one predation attempt were included. Squid that captured the fish on their first predation sequence were assigned a failed attempt count of zero. Outliers (crosses) are defined outside the range $q_3 + 1.5 \times (q_3 - q_1)$ and $q_1 - 1.5 \times (q_3 - q_1)$, where q_1 and q_3 are 25th and 75th percentiles, respectively. Lines in the middle of boxes indicate medians, and whiskers extend to integer data points furthest from the median that are not outliers. *$p<0.017$ for Onset vs. 5min, or $p<0.025$ for Onset vs. Control (Mann-Whitney U tests with Holm's procedure).

4.2.3.5. Predation latency

Comparing the three playback trial types in Day trials, there were no significant differences in the time elapsed from when the fish was revealed to the squids' _first display of each predation sequence behavior (orient, pursuit, attack, capture; $p > 0.05$, Kruskal-Wallis tests). Assessing the three playback treatments together, median latencies for orient, pursuit, attack, capture were 13, 15, 23, and 23 s respectively, with interquartile ranges of 3–37, 4–41, 9–80, and 11–100 s, respectively.

4.2.3.6. Squid alarm response

We looked for potential effects of squids' engagement in hunting on their alarm responses to pile driving noise playbacks by comparing alarm response rates when the killifish was revealed, and squid were pursuing it (Onset) to when the killifish was hidden (5min) at the beginning of playback.

In Day Onset and Day 5min treatments, there were larger proportions of each of the four alarm response types during the first minute of the playback period compared to the pre-playback period (Figure 26). Proportions of 'no response' were higher in the pre-playback period compared to the first minute of playback. In the Day 5min treatment, proportions of inking, jetting, startle, and body pattern change were significantly higher in the playback period ($p < 0.001$, $p < 0.001$, $p = 0.007$, and $p = 0.010$, respectively, Fisher's Exact tests). In Day Onset, only the proportion of jetting was significantly higher in the playback period ($p = 0.002$). During the first minute of playback, a higher proportion of squid in Onset and 5min treatments showed alarm responses compared to Controls, and a higher proportion of squid had no response in the Controls. In Day trials, there was a significantly lower proportion of squid with 'no response' in the 5min treatment compared to the Control treatment (OR = 0.05, 95% CI: 0.00–0.58, $p = 0.015$, Fisher's Exact test), and there were no significant differences in any response type between Onset and 5min treatments. Pooling 5min and Onset treatments and comparing them with Controls, there were significant differences in the proportions of inking (OR = 9.60, 95% CI: 1.00–92.0, $p = 0.039$), jetting (OR = 10.50, 95% CI: 1.56–70.76, $p = 0.016$), and no response (OR = 0.10, 95% CI: 0.01–0.67, $p = 0.023$).

Alarm responses per pile driving impulse were analyzed for the first minute (30 impulses) of pile driving noise playback. The same patterns in alarm responses over time were observed in Day Onset trials, during which the squid was hunting at the start of playback, and Day 5min trials, during which the fish had not yet been revealed. Squid displayed alarm responses at the highest rates within the first 5 pile driving impulses. Inking behaviors were extinguished first, followed by jetting and startle behaviors, with body pattern changes persisting the longest. Quasipoisson GLMs indicated that pile impulse number was a significant predictor of each of the four alarm response types ($p < 0.001$), and that noise treatment was a significant predictor of inking behaviors ($p < 0.001$; higher rate for 5min) jetting behaviors ($p < 0.05$; higher rate for Onset), and startle behaviors ($p < 0.001$) and not body pattern change behaviors ($p > 0.05$). However, the low number of counts of inking behaviors and strong overlap in 95% confidence regions for GLMs of each alarm response type suggest similar initial response rates on the first impulse and similar rates of decreased response over time between 5min and Onset trials.

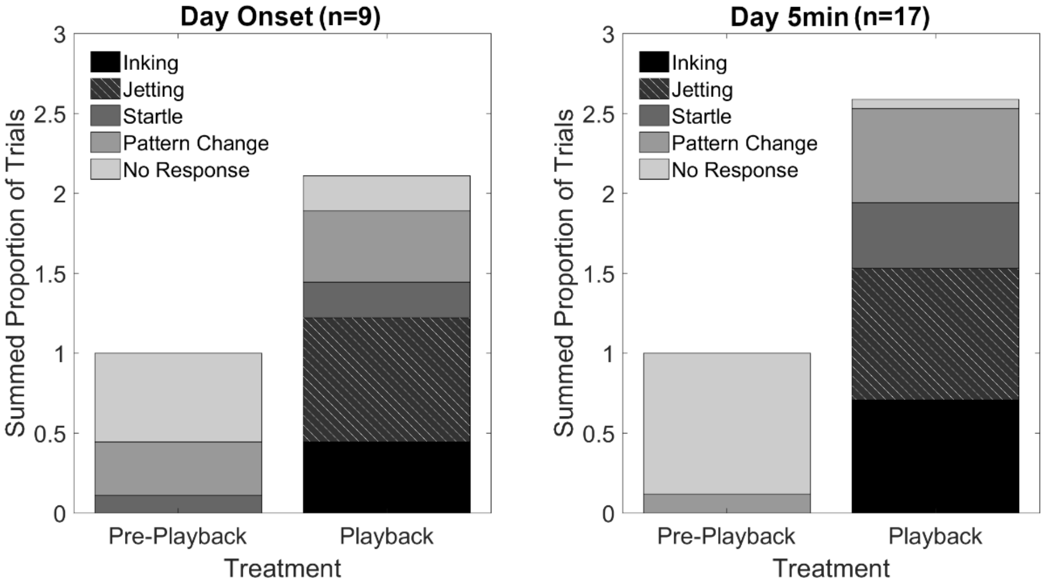


Figure 34. Alarm responses during feeding trials
Summed proportion of trials in which squid exhibited each alarm response type, or no response, during the last minute of the pre-playback period ("Pre-Playback") and during the first minute (first 30 pulses) of pile driving playback ("Playback"), for the Day Onset (left plot) and Day 5min (right plot) treatments. Only trials for which data were available for 30 pulses are shown here (playback for several Onset and Control trials was stopped before 30 pulses elapsed because the squid had captured the fish). Proportions were found individually for each behavior category, which, aside from 'No Response', are not mutually exclusive. Therefore, summed proportions may exceed 1.

4.2.4. Discussion

Squid exposed to pile driving noise playbacks generally had lower prey capture rates, and squid were more likely to abandon pursuit of prey if noise started during their pursuit. Prey mobility significantly negatively correlated with squids' predation latency, whereas noise did not have a significant effect on predation latency. Squid exhibited similar alarm response rates during noise whether or not they were hunting at the start of noise. Together, these results suggest that pile driving noise seems to alter the feeding activity of squid and reduce squids' capacity to hunt. The extent, or duration of this has yet to be addressed. Similar to the distracted prey hypothesis, noise may shift squid predators' attention away from feeding tasks and toward the noise, which, given the observed alarm responses, appears to be perceived as a threat.

Though in-tank particle acceleration levels of the pile driving noise playback were spatially variable, they remained near or above physiological hearing thresholds for longfin squid. There were overall trends of lower proportions of squid capturing prey in noise treatments compared to controls. Importantly however, lack of statistical significance in differences of these proportions prevents conclusive interpretations.

The present study uniquely demonstrates how pile driving noise can alter the feeding behavior of squid. To the authors' knowledge, this study is the first to demonstrate changes in feeding behaviors of cephalopods during anthropogenic noise. These data underscore the importance of accounting for noise-induced behavioral changes of both predator and prey species, and for ecosystem dynamics, when assessing noise effects. Squid were significantly more likely to abandon pursuit of prey and have failed capture attempts when noise playback started during squids' predation sequences. In addition, a lower proportion of squid captured live killifish prey in noise exposure trials compared to silent Control trials, though these differences were not statistically significant. Missed opportunities for prey capture and lower feeding rates during anthropogenic noise could lead to reductions in growth or survival of individuals, particularly for longfin squid, with their high-metabolic rates that require frequent feeding; this could be especially damaging to squid survival when prey resources are limited. Future work should address the potential longer term metabolic consequences of noise exposure. Squids' latency to capture prey was significantly negatively correlated with fish locomotion, emphasizing the importance of considering natural covariates at play when investigating effects of anthropogenic stressors on predator-prey relationships. Further, at the onset of noise exposure, when squid were engaged in hunting they had similar alarm response rates compared to when they were not hunting; this indicated that both in feeding and non-feeding contexts, individual squid were similarly alert to threat stimuli. Changes in feeding behaviors reported here have potential implications for reduced feeding activity of squid exposed to construction noise of marine pile driving operations. However, behaviors and acoustics observed in the laboratory may differ from those *in situ*. Thus, future comparative field studies are needed to further investigate influences of pile driving noise on foraging behaviors of squid. Further, the present results raise questions regarding how other key longfin squid behaviors such as breeding, shoaling, predator avoidance, and habitat selection may be impacted by noise.

4.3. Study 7: Reproductive and group dynamics: Context is key – squid reproductive behaviors and spawning withstand replayed wind farm pile driving noise

4.3.1. Background

Humans are increasingly utilizing and developing coastal environments, with many activities having the potential to impact marine taxa. The offshore wind energy industry is expanding globally, inducing concern over how anthropogenic noise pollution associated with construction, operation, and maintenance of wind farms will adversely impact aquatic wildlife (Mooney et al. 2020b). Support vessels, sonars used for seismic surveys, construction equipment, and operational turbines emit anthropogenic noise during offshore wind farm development (Mooney et al. 2020b). Impact pile driving during construction is considered the most intense and pervasive (Amaral et al. 2020). Pile driving involves repeated hammering of cylindrical piles into the seabed to support foundations for wind turbines, generally for those in water shallower than 60 m (Musial et al. 2018). It is a widespread noise source as pile driving is used in not just in the installation of windfarm turbine pilings, but also within harbors and coastal areas for a myriad of construction activities, in sum making it a sound of primary concern. To minimize negative impacts to marine life, studies investigating effects of pile driving noise on animal behaviors are crucial, especially behaviors that have direct implications for survival and reproduction.

Many studies have demonstrated effects of pile driving and other noise sources on the behavior of marine mammals and fish (Bruintjes et al. 2016a, Nedelec et al. 2017, Graham et al. 2019), but far fewer have studied effects on marine invertebrates (Morley et al. 2014). Among fish, impulsive noise alters group cohesion and swimming dynamics of European seabass shoals (*Dicentrarchus labrax*); (Neo et al. 2016, Herbert-Read et al. 2017). Fish exposed to pile driving noise also suffer temporary injuries to hearing structures and organs in close proximity to swim bladders (Casper et al. 2013a, Casper et al. 2013b). For invertebrates (a diverse group of taxa), substrate vibrations from simulated pile driving cause filter-feeding mussels (*Mytilus edulis*) to reduce valve gape (Roberts et al. 2015), elicit startle behaviors in hermit crabs (*Pagurus bernhardus*) (Roberts et al. 2016a) and impair hermit crabs' (*Pagurus acadianus*) abilities to find key resources, i.e. empty shells (Roberts and Laidre 2019).

There are no comparable data regarding impacts of far-reaching water-borne pile driving sound on invertebrates. The limited knowledge regarding impacts on numerous ecologically vital invertebrate taxa is both a concern and a risk as offshore windfarm construction progresses (Gedamke et al. 2016, Mooney et al. 2020b). Furthermore, while ecological and behavioral contexts have been argued as critical to the management of anthropogenic noise pollution faced by marine mammals (Ellison et al. 2012, Harris et al. 2018), such context-based approaches have not been addressed for most other marine taxa, including invertebrates.

Given this paucity of data, cephalopods (including squid, cuttlefish, and octopuses) are particularly important to examine anthropogenic noise impacts on, in part because of their high ecological and commercial relevance. These squid detect and behaviorally respond to sounds at frequencies below 1000 Hz (Mooney et al. 2010, Mooney et al. 2016), suggesting sound-sensitivity overlapping with the typical peak frequencies of pile driving noise. Ecological functions of cephalopods' sound detection are unknown; however, they are thought to utilize sound and vibration for predator avoidance and possibly for developing an "auditory scene" to orient to and navigate in their environment, as has been discussed for fishes (Fay 2009, York and Bartol 2014).

Moreover, key studies of their behavior, reproduction and sound-sensitivity provide a vital foundation enabling this taxon to act as a model to address invertebrate noise impacts. Several studies have reported disruptive behavioral and physical effects of anthropogenic noise on cephalopods. Squid display alarm responses normally utilized for predator evasion, such as inking and jetting, during air gun (*Sepioteuthis australis*) and pile driving noise (*D. pealeii*) (Fewtrell and McCauley 2012, Jones et al. 2020). Pile driving noise also causes D. pealeii to cease their pursuit of prey (Jones et al. 2021). Additionally, noise may cause physical damage to cephalopods' sound detection structures (statocysts) (Solé et al. 2013, Solé et al. 2017). To the authors' knowledge, to date no studies have addressed impacts of anthropogenic noise on reproductive behaviors of cephalopods, nor any marine invertebrate.

Like many cephalopods, *D. pealeii* is short-lived, and has an average lifespan of less than a year (Brodziak and Macy 1996, Macy and Brodziak 2001), mating for a short time period from May to October. Although they can mate and lay eggs multiple times over several weeks (Maxwell et al. 1998, Hanlon et al. 2013a) they are considered semelparous because they only breed for one season then senesce, making mating events highly important to individual fitness (Shashar and Hanlon 2013). Complex behavioral dynamics are associated with inshore mating and spawning of *D. pealeii* around communal egg beds and spawning dynamics are well-described (Shashar and Hanlon 2013). Briefly, females lay gelatinous capsules containing eggs on the substrate, which form large bundles called "egg mops". Males are visually attracted to and approach egg mops. The capsules contain a contact pheromone that elicits heightened aggression in males, leading to subsequent agonistic (fighting) behaviors (Buresch et al. 2003, Buresch et al. 2004, Cummins et al. 2011). Males take on flexible mating roles according to their size relative to nearby males, and their success or failure in agonistic bouts. "Consort males" (usually larger males) will pair with and swim parallel to a female, and "guard" her from other males. Consort males perform the majority of successful mating. "Sneaker males" are usually smaller and keep their distance from larger males, but may quickly jet toward a female, attempting to mate with her.

The present study investigated anthropogenic noise impacts on reproductive behaviors of small mixed-sex groups of longfin squid *Doryteuthis pealeii*, via controlled laboratory experiments using underwater playbacks of pile driving sound recorded during wind farm construction. To determine if this noise impacted reproduction, we measured a suite of reproductive behaviors including: mate guarding (when consort males actively position themselves between a female and other males and defend that female resource), as well as male-male agonistic (fighting) behaviors, mating, and egg laying. To evaluate context-dependent effects, we compared the response rates during reproductive activities to those of parallel studies with similar methods addressing noise-induced disturbances during feeding and resting.

4.3.2. Methods

4.3.2.1. Experimental setup

Experiments occurred in a cylindrical, 1.8-m-diameter tank in the ESL during daylight hours. Water was maintained at a depth of 0.82 m and temperature of 18.0 + 1.2 °C (mean + SD). A slow water inflow-maintained tank temperature and high dissolved oxygen levels. The bottom of the tank had a 3 cm layer of sand. An Aqua-30 speaker (DNH, Netherlands; frequency response: 0.08–20 kHz) and hydrophone (High Tech Inc., USA; sensitivity: -165 dB re 1 V/μPa; frequency response: 0.002–30 kHz) were inserted into the tank to project and monitor sound respectively during experiments (Figure 11). The hydrophone was connected to a SoundTrap ST4300 acoustic recorder (Ocean Instruments, NZ; 4 dB gain) located outside the tank. Trials were recorded with an overhead camera (Sony Handycam HDR-XR550), and two underwater cameras (GoPro Hero 4) facing each other from opposing sides of the tank at mid-depth, so that video of the entire inside of the tank was captured.

4.3.2.2. Mate guarding experimental design

During experiments, squid were exposed to either 5-min long pile driving playbacks or 5-min long silent control playbacks (hereafter referred to as “pile” and “control” trials, respectively). Originally, pile driving files had an inter-pulse interval of 1.8 s, and were recorded from a hydrophone array 500 m away from a pile driven for the Block Island Wind Farm, 1 m above the seabed, in water 26 m deep (Amaral et al. 2018, Amaral et al. 2020). These field data, along with particle acceleration calculated from hydrophones in the field, were provided to the authors (see Acknowledgements). Three distinct pile driving playback files were prepared using Adobe Audition (version 3.0), with pulses of equal amplitude arranged in randomized order for a 1 min loop, which was looped five times for a total 5 min duration. All files were edited to have a consistent inter-pulse interval of 2 s.

Squid were tested in groups of three, consisting of a large male, a small male, and a female, with respective mean + SD mantle lengths of 22.5 + 3.3 cm, 15.6 + 2.2 cm, and 14.3 + 1.5 cm. Mantle lengths of each large male and small male pair differed by at least 3.5 cm (mean + SD: 6.9 + 2.1). The large males were expected to act as consort males, and small males were expected to act as sneakers (Shashar and Hanlon 2013). All cameras were in place and recording before squid were added to the experiment tank. Squid were added sequentially to the tank at intervals of two minutes in a consistent order of large male, small male, then female. Immediately following the addition of the female to the tank, the SoundTrap was turned on to start monitoring sound levels in the tank. Two minutes after the female was added, an egg mop was added to the center of the tank, and a second egg mop was added near the tank wall (approximately a quarter of the tank's circumference away from the speaker). The addition of the egg mops marked the start time of each trial. Egg mops induced male aggression and mate guarding (Buresch et al. 2003, Cummins et al. 2011).

For all trials, squid were given at least ten minutes to habituate to the experiment tank before starting playback. An experimenter was stationed out of view of the squid and watched the trial on a screen to monitor the occurrence of mate guarding. Criteria for mate guarding were that the male remained within two body lengths of the female and actively positioned itself between the female and other male (Shashar and Hanlon 2013). Playback was started when mate guarding was observed occurring for 15 s continuously (after the 10 min habituation period had passed). Although this meant the duration between the start of the trial and start of the first playback varied (median [IQR]: 14.3 [11.1–21.3] min), this playback start criterion was chosen because the experimental design was primarily focused on testing whether noise disrupted mate guarding. Playbacks were repeated up to three times if squid resumed mate guarding after the first and second exposure, with a minimum of 10 min of quiet (i.e., no playback) in between. Agonistic behaviors, including the number of chases (forward acceleration in pursuit of another squid) and lunges (forward acceleration followed by attempt to grab another squid) by males were also quantified, as defined previously (Cummins et al. 2011).

Fifty-five trials were conducted. Of these, 30 trials had males that mate guarded (15 pile trials, 15 control trials) and were used for analyses. One control trial and one pile trial had a single playback period; the remaining 28 trials had three playback periods. Two trained observers (50% overlap of analyzed trials) watched videos and recorded time spent mate guarding, number of chases, and number of lunges during 5-min-long "playback" periods, and 5-min-long periods just preceding the second and third playback periods ("quiet1" and "quiet2", respectively). Inter-observer reliability was high for both mate guarding and agonistic behaviors (r > 0.96, Pearson correlation). Mating and egg-laying events were also scored by an observer who watched the entire (1–2 h) duration of each trial.

4.3.2.3. Acoustic calibration of the experimental tank

Mapping of the sound field in the experimental tank involved similar methods and instrumentation used in prior studies (Jones et al. 2020, Jones et al. 2021) and was the same tank and set up as Study 3 in this report. Briefly, 1 min of a pile driving file (same as used in experiments) was played through the speaker and recorded in 20 cm increments in all three dimensions (240 recording positions total). Cephalopods detect acoustic particle acceleration rather than pressure, therefore, particle acceleration was recorded, as well as sound pressure for comparison with other studies. Recording instruments were affixed at the end of a PVC probe in the following configuration: a PCB triaxial accelerometer (model W356B11, frequency response: 0.5 Hz–5 kHz, sensitivity of each axis: 1.04 mV / m s-2) was centered at the recording position, and a Reson hydrophone (model TC4013, frequency response: 1 Hz–170 kHz, sensitivity: -211 dB re 1 V/µPa) was spaced 1.5 cm to the left of the accelerometer (facing the speaker).

Acoustic data analyses were performed following previous methods (Jones et al. 2020), and are briefly summarized here. Zero-to-peak levels of individual pile pulses, in decibels (dB), were calculated for particle acceleration ($SAL_{z\text{-}pk}$) and sound pressure ($SPL_{z\text{-}pk}$) as follows:

$$SAL_{z-pk} \text{ or } SPL_{z-pk} = 20 * \mathrm{Log}_{10}(X_{pk})$$

where X_{pk} is the ratio of the maximum absolute acceleration (µm s-2) or pressure (µPa) to the reference level, 1 µm s^{-2} or 1 µPa respectively. For simplicity, the 3D vector (Euclidean) norm of particle acceleration was calculated, and its magnitude is reported. Power spectral density (PSD) was calculated to visualize these magnitudes across frequencies. All acoustic metrics were limited to 20–1000 Hz, encompassing the hearing range of *D. pealeii*.

4.3.2.4. Statistical methods

Statistics were performed in R version 4.0.4 using the lme4 (Bates et al. 2015), car (Fox and Weisberg 2019), and glmmTMB (Brooks et al. 2017) packages. The significance threshold for all tests was $\alpha = 0.05$. Generalized linear mixed effects models (GLMMs) were used to fit mate guarding and agonistic data with repeated measures. Proportion of time spent mate guarding was fit with a beta distribution and counts of chases and lunges were fit with a negative binomial distribution. Treatment (pile vs. control) and period ("playback" periods 1, 2 and 3, and "quiet" periods 1 and 2) were included as main effects and squid individual was a random effect. Interactions between treatment and period were kept in final models if they were significant. Model best fit was selected according to Akaike information criteria (AIC). Post-hoc Wald type II χ^2 tests were used to test significance of fixed factors.

4.3.3. Results

4.3.3.1. Mate guarding

Large males mate guarded the female squid in 25 of the 30 trials (12 control, 13 pile), and the small male mate guarded in eight trials (5 control, 3 pile). In three trials (2 control, 1 pile), the small male mate guarded first, and the large male later took over as consort and mate guarded for the rest of the trial. These consort role shifts took place earlier in the trials, either before playback1, during quiet1, or during quiet2 periods. The number of mate-guarding small males was too low to perform GLMMs including all period and treatment groups, thus statistics were limited to large males.

There was no significant effect of the pile driving sound on time spent mate guarding by large males. Inter-quartile ranges of time spent mate guarding in pile and control treatments largely overlapped, whether comparing these treatments within playback periods, within quiet periods, or comparing playback and quiet periods within a given sound treatment (Figure 29a). This reflects the similarity of behaviors in noise vs. quiet conditions, despite the high amplitude pile driving sound. Analyzing all large males (Figure 29a), period was a significant factor (χ^2 = 10.01, df = 4, p = 0.04), treatment was not significant (χ^2 = 0.88, df = 1, p = 0.35), and the interaction between period and treatment was significant (χ^2 = 14.0, df = 4, p < 0.01). For small males, mate guarding between pile and control treatments was more variable (Figure 29b); differences between pile and control proportions (within a period) were likely due to individual effects of different small males rather than noise effects, since proportions within either sound treatment were similar between playback and quiet periods. We analyzed large males in trials where small males did not mate guard to determine whether the significant period effect may have been due to consort role shifts from the small male to large male. In this subset of trials (n=10 control, n=12 pile) period was not a significant factor (χ^2 = 2.69, df = 4, p = 0.61), nor was treatment (χ^2 = 0.01, df = 1, p = 0.93). This suggests that the delay of several large males (n=3) in taking on a consort role led to the significant time period factor for data in Figure 29a.

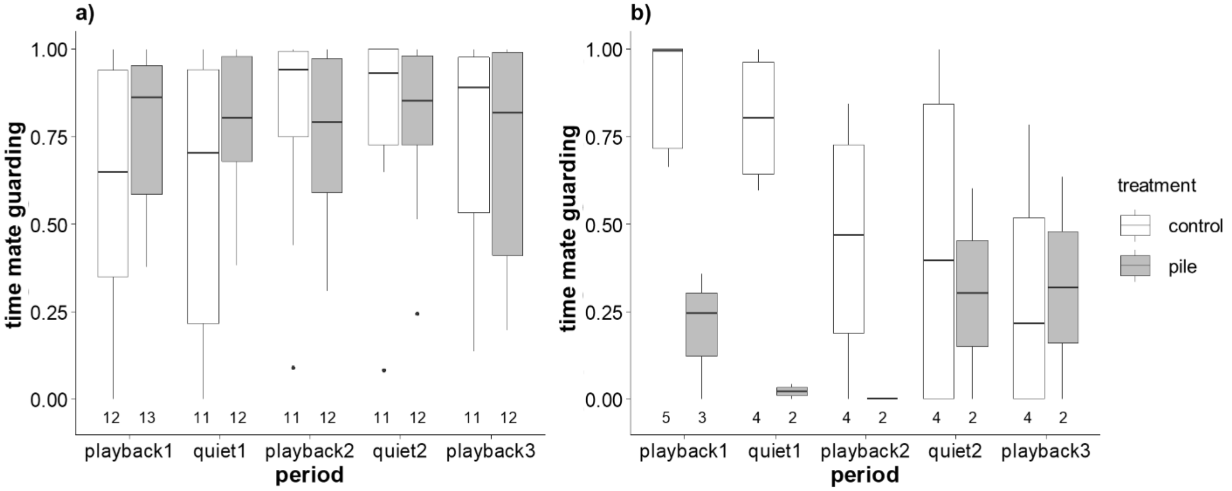


Figure 35. Mate guarding
Proportion of time spent mate guarding in playback and between-playback (quiet) time periods for a) large males, and b) small males. Periods each had 5 min duration and are listed in the sequence they were presented to the squid. Numbers under each box are sample sizes. Horizontal lines represent medians, boxes extend from the 25th to 75th percentile, and dots represent outliers, defined as values smaller than 1.5 times the inter-quartile range.

4.3.3.2. Agonistic behaviors

Pile driving noise did not have a significant effect on the agonistic behaviors of large males, in terms of number of chases and number of lunges toward competing males (Figure 30). For number of chases, neither period (χ^2 = 1.43, df = 4, p = 0.84) nor treatment (χ^2 = 1.88, df = 1, p = 0.17) were significant factors. Similarly, for number of lunges, period (χ^2 = 1.05, df = 4, p = 0.90) and treatment (χ^2 = 0.19, df = 1, p = 0.67) were not significant. There were no significant interactions between periods and treatment for chase or lunge behaviors. Small males did not chase or lunge at large males. Overall, these results indicated that aggressive behaviors of male squid were unaffected by the noise treatment.

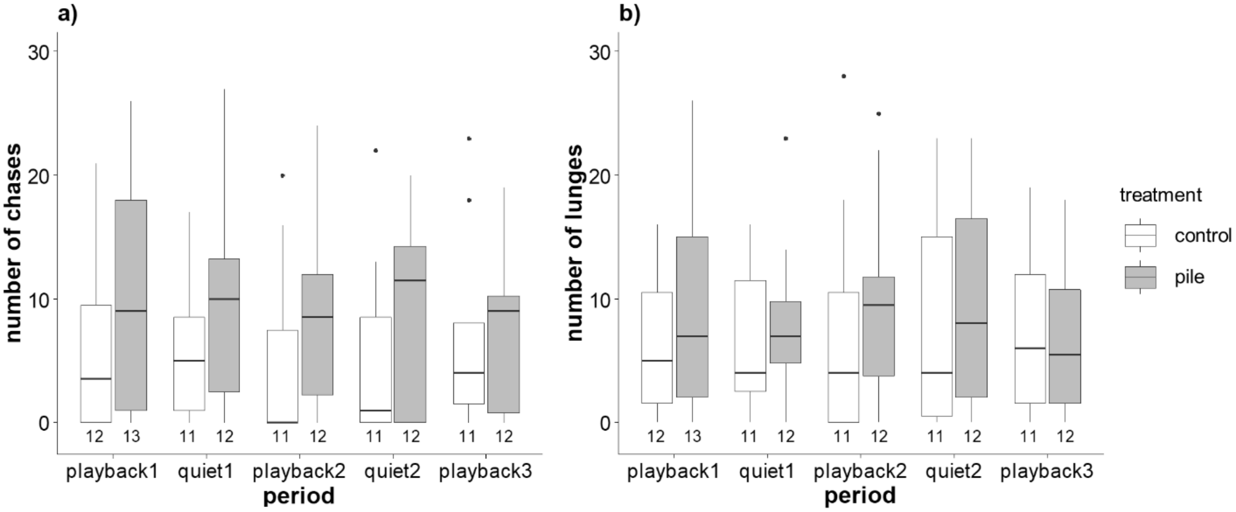


Figure 36. Agonistic behaviors

a) Number of chases, and b) number of lunges toward small males by the same large males shown in Figure 29a, in playback and between-playback (quiet) time periods. Periods each had 5 min duration and are listed in the sequence they were presented to the squid. Numbers under each box are sample sizes. Horizontal lines represent medians, boxes extend from the 25th to 75th percentile, and dots represent outliers, defined as values greater than 1.5 times the inter-quartile range.

4.3.3.3. Mating and egg-laying

Mating and egg-laying continued to occur during and after noise exposure. Mating occurred in 30% of trials (5 control, 4 pile), and was always between the large male and female. Mating between a given consort pair occurred once in six trials, twice in two trials, and thrice in one trial. There was no significant difference in duration of mating events between pile and control trials (U = 18, p = 0.10, Mann-Whitney U test). Overall, the median duration of the typical male parallel mating position was 17 s (IQR: 15–18), and durations ranged from 9–33 s, within those observed in wild squid (Shashar and Hanlon 2013). In two pile trials, noise playback (playback1 or playback3) started during mating and mating continued. The durations of these two mating events were 18 and 28 s, within the duration range of mating events that occurred in "quiet" periods and control playbacks. Egg laying (by females) also occurred after mating at statistically similar rates in pile and control trials (mean ± SD: 0.90 ± 0.30, and 0.74 ± 0.14 eggs capsules min^{-1}, respectively; U= 21, p = 0.41, Mann-Whitney U test).

4.3.4. Discussion

In the present study, trios of squid exposed to high intensity noise did not demonstrate any significant changes in reproductive behaviors. Collectively, these studies emphasize the importance of behavioral context when predicting anthropogenic noise effects on marine taxa.

This theme has been demonstrated across vertebrate and invertebrate taxa (Parvulescu 1964, Ellison et al. 2012, Lin et al. 2019). For example, boat noise caused cichlids (*Neolamprologus pulcher*) to change rates of digging and attack behaviors when eggs were not present, but there were no significant changes to these behaviors when eggs were present (Lin et al. 2019). In the case of longfin squid, evidence from laboratory experiments indicate noise exposure is potentially more disruptive to anti-predator responses and feeding behaviors than to reproductive activities (Figure 31). Notably, possible noise effects on feeding behavior may indirectly influence reproductive behaviors in ways not yet assessed; for instance, a reduced caloric intake could lead to reduced energy put towards reproduction.

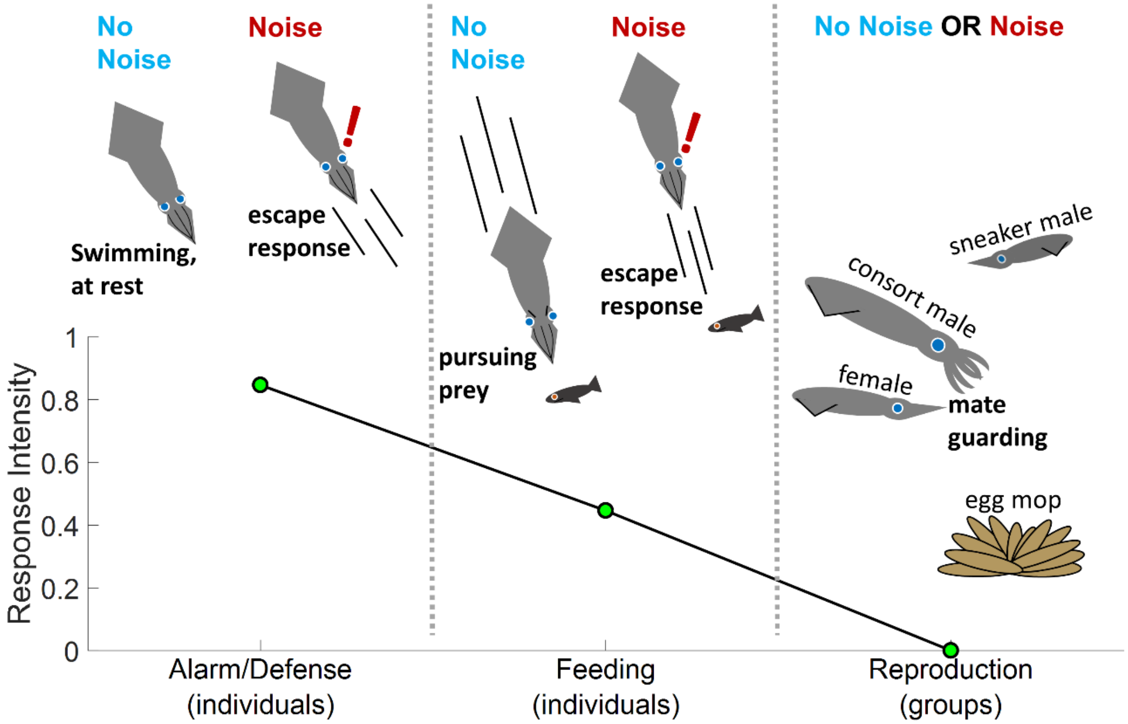


Figure 37. Conceptual comparison of behavioral responses across studies
Comparison of relative response rates of D. pealeii to pile driving noise across studies measuring alarm/defense behaviors *(*Jones et al. 2020*)*, feeding behaviors *(*Jones et al. 2021*)*, and reproductive behaviors (present study). Response intensity is the quantified proportion of noise exposure trials with a response subtracted by the proportion of control trials with a response. For the alarm/defense study, a response was defined as at least one occurrence of inking, jetting, startle, or body pattern change. For the feeding study, a response was defined as at least one missed predation attempt. Since there were no significant noise effects on any reproductive behaviors, response intensity for the present study is set at zero. Example videos showing these behaviors during noise are in the electronic supplementary material.

4.3.4.1. Conclusions and future directions

The present study indicates that reproductive behaviors of longfin squid may be less at risk of being disrupted by noise relative to anti-predator defense and feeding behaviors. Together with previous studies on *D. pealeii*, these results reinforce the importance of considering multiple ecological contexts in which animals' responses to anthropogenic noise may differ. To address noise effects in more ecologically and acoustically relevant scenarios, complementary field studies on squid behavioral responses to noise are needed. Laboratory studies have provided valuable information on the contexts in which squid behavior may be adversely affected by anthropogenic noise and can inform research foci for field studies addressing in-situ and population-level impacts. These research efforts and the results of the present study are of central importance to the fishing industry, regulators, and energy industry seeking to assess and address risks that offshore energy expansion poses to ecologically and commercially key marine fauna.

5 General Conclusions and Future Directions

5.1. Overview of results

5.1.1. Black Sea Bass (*Centropristis striata*)

5.1.1.1. Ecological implications: What the overlap between sound detection bandwidth and pile driving signal parameters means for *C. striata*

The northeast coast of the United States is the first region (in the U.S.) to begin extensive offshore wind energy development, covering an area spanning from the ocean south of Cape Cod, Massachusetts to Virginia. This was followed by the lease of the Massachusetts wind energy area beginning approximately 12 nm south of Martha's Vineyard and 13 nm southwest of Nantucket. It covers an area of approximately 300,672 hectares (https://catalog.data.gov/dataset/boem-wind-planning-areas-June2019). With the first approved construction and operations plan belonging to Vineyard Wind for the construction of an 800-megawatt (MW) wind farm with 80 to 100 wind turbines is set to start in the near future (https://www.boem.gov/renewable-energy/state-activities/consultation-documents-associated-vineyard-wind-construction-and - Dec 2019).

Construction noise, both water-borne and substrate borne or "ground-roll", especially that produced through pile driving of the foundations and support structure of wind turbines, could be significant when the scope of the east coast development is considered. Peak underwater sound levels (both particle motion and pressure) vary substantially, dependent on many factors of the construction, including pile diameter, size of impact hammer, substrate etc. However, those measured from field examples are in the order of 220 dB re 1 µPa at a range of ~10 m, 200 dB re 1 µPa at a range of 300 m from 0.75 m and 5 m diameter piles respectively (Reinhall and Dahl 2011a, Lippert and von Estorff 2014). The predominant energy is below 500 Hz, with some energy extending past 1 kHz, and with sharp rise times to maximum energy (Popper and Hawkins 2019). The particle motion component and substrate transmission has been far less reported than sound pressure, however, it is likely far more relevant to many fish species than the pressure component. Using a four-hydrophone tetrahedral array and a three-axis geophone, Miller et al. (2018) measured and estimated the particle velocity and sound pressure levels from the pile driving activity installing the foundations of wind turbine in the Block Island Wind Farm, measured 1 m above the seabed in 26 m of water. The authors reported zero-to-peak total sound velocity levels of ~110 dB re 1 nm/s (vector sum) (tetrahedral hydrophone array), ~124 dB re 1 nm/s (geophone) and peak-to-peak received sound pressure levels of ~185 dB re 1 µPa (tetrahedral hydrophone array) from one hammer strike, 500 m from the activity (Miller et al. 2018).

With multiple construction efforts occurring along the northeast coast (U.S.), potentially concurrent, the sound not just from the nearest development regions, but those at a distance, may affect the fishes inhabiting these regions. In many respects, fishes of the region are likely naïve to such noise. There have been very few experimental examples of loud, impulsive underwater sounds causing death or mortal injury to fishes. Nonetheless, anthropogenic sound at levels far lower than those causing death can have substantial effects on fish physiology, behavior (e.g., changes in migration routes, feeding or breeding grounds), cause physical injury, and/or acoustic masking (see Introduction). As *C. striata* make annual offshore migrations across the continental shelf and southward, facilitating both overwinter survival and connectivity between juvenile and adult populations, these potential adverse effects are relevant to *C. striata* populations in the northeast (Massachusetts to New York). The migration will likely have the species crossing wind energy development areas and subsequently being exposed to the sounds associated with construction at these sites (Drohan et al. 2007, Miller et al. 2016). The current research (see Study 1; Stanley *et al.* 2020) suggests that the most sensitive range of this species' sound detection capabilities directly overlaps with the highest sound energy created from pile driving activity. This suggests *C. striata* will be able to hear the sounds made during the construction phase in many circumstances which could potentially lead to short- or long-term changes in behavior around the sites.

There is also anecdotal evidence from fishermen that (Perso. Comm. 2019) black sea bass are soniferous (acoustically active). Conversely, to date there is no published data describing their vocalizations and on closer inspection of a range of size classes there of both sexes, they do not appear to have developed sonic muscles associated with their swim bladder (Stanley, unpublished data). During the duration of this project, holding tanks were monitored for vocalizations and to date none were detected. However, during a recent group spawning experiment where a group of sexually mature individuals were observed over a four-week period, video and audio were recorded on underwater cameras and cabled hydrophones and acoustic signals were detected at the moment of gamete release. These are yet to be formally analyzed but on initial inspection, the signals are of low frequency (30 – 300 Hz), low amplitude and of very short durations (< 2 seconds) (Stanley *et al.*, unpublished data in prep). These signals may be critically important in the timing and synchronization of spawning in this species in habitats where other cues such as vision and olfaction is limited, and may be masked or misinterpreted in the presence of loud anthropogenic noise, especially multiple sources (Stanley et al. 2017).

However, extensive behavioral and physiology *in situ* research is needed to best identify how this species will be most vulnerable to potential effects. For example, changes in migration, feeding and breeding grounds, interruption of critical activities, or stress-induced reduction in growth and/or reproductive output seen in response to short-term acute, long-term chronic exposures, or multiple sources. These results together could have significant impact for individuals and populations, and industries relying on this species.

5.1.1.2. Comparison with related noise studies

To our knowledge, this project represents the first work to test effects of aspects of pile driving signal on the behavior and physiology of *C. striata*. The only previous study to date which included any kind of acoustic aspect investigated the potential for acoustic ranching (Lindell et al. 2012). Here fish were trained enclosed in a sea cage by feeding with a sound cue (280 Hz pure tone). Once training was complete, fish could exit the sea cage at will, with the sound cue being used for feeding times when the fish can re-enter the cage to feed. The study indicated that black sea bass could detect the tone and were readily adaptable to acoustic training.

The current studies showed significant changes in the behavioral patterns displayed by black sea bass in Control vs Treatment (exposure to pile driving signal) groups, with the most common observation of decreased activity and increased association with the substrate or any structure in the tank. This observation of decreased activity is consistent with responses reported in other studies on fish and anthropogenic sounds. Knudsen *et al.* 1992 described a similar swim down response in Atlantic salmon to 10 Hz tones, but with no response at 150 Hz. Similarly, Neo *et al.,* 2015 tested four pulse repetition intervals (PRI) of filtered brown noise (200 – 1000 Hz; matching hearing range of European seabass) on European seabass swimming patters in an outdoor basin. They found that at sound onset fish initial increased swimming speed to dive deeper and in tighter shoals, and that PRI effected the immediate and delayed behavioral changes but not recovery time in these species (Neo et al. 2015b). In the same species, Neo *et al.* 2019 also observed that groups of fish increased their swimming speed, depth, and cohesion in greater effect during the night vs during the day in response to impulsive sound exposure. These findings suggest that the impact of impulsive anthropogenic noise may be stronger at night than during the day for some fish species (Neo et al. 2018). This study also observed a suggested inter-trial habituation with respect to swimming depth.

There are few studies reporting empirical data on the potential effects of anthropogenic noise on foraging and feeding behavior in fishes, especially in a controlled environment. Magnhagen *et al.* 2017 reported Eurasian perch *Perca fluviatilis* made fewer feeding attempts at pieces of saithe when exposed to sounds from an outboard motor, similar to the results of the current study of black sea bass. However, as the experiment progressed, they reported increased feeding both with and without noise, which is in contrast to the current study with black sea bass. In a laboratory study Voellmy *et al.* 2014 investigated how exposure to playback of noise originally recorded from ships affects the feeding behaviour of the three-spined stickleback, *Gasterosteus aculeatus*, and the European minnow, *Phoxinus phoxinus*. Both species consumed significantly fewer live Daphnia and showed startle responses significantly more often during playback of additional noise than during control conditions. However, whereas minnows showed a qualitative shift in activity away from foraging behavior (greater inactivity, more social behaviour) under increased noise conditions, consistent with a classic stress- or fear-related defense cascade, sticklebacks maintained foraging effort but made more mistakes, which may result from an impact of noise on cognition. These findings indicate that additional noise in the environment can lead to reduced food consumption, but that the effects of elevated noise are species specific (Magnhagen et al. 2017).

The U.S. National Marine Fisheries Service (NMFS) currently uses 150 dB re 1 μPa (rms) as the sound pressure level that may result in onset of behavioral effects (Caltrans 2015). This is based on a recent NMFS guidance document (www.go.umd. edu/Ucs) that suggest sound pressures above this 150 dBrms level are expected to cause temporary changes in behavior, such as eliciting startle responses, feeding disruption, area avoidance, etc.

Though the present study only considered water-borne particle motion, pile driving also produces large substrate-borne vibration (Roberts and Elliott 2017). *Centropristis straiata* naturally rests on the substrate and would also potentially sense this "ground-roll". Accordingly, future studies on this species should examine the influence of substrate-borne vibration.

5.1.2. Longfin Squid (*Doryteuthis pealeii*)

5.1.2.1. Comparison with related noise studies

To our knowledge, this is the first study to test effects of aspects of pile driving signal on any cephalopod, and follows a comparative study that examined impacts of a separate type of anthropogenic noise, air gun pulses, on squid (Fewtrell and McCauley 2012). Their work found that seismic air gun arrays, which passed over caged groups of *Sepioteuthis australis*, elicited alarm responses similar to those in the present study, including inking, jetting, and body pattern changes. In a study with cuttlefish (*Sepia officinalis*), in-lab playbacks of boat noise elicited body pattern changes, specifically increased frequency of the *Raised arms* posture and changes in chromatic components, as well as time spent swimming (Kunc et al. 2014). These changes suggested an increased state of alarm and stress during noise playback. Startle responses to substrate-borne vibration have also been observed in the mussel *Mytilus edulis* and hermit crab *Pagurus bernhardus* (Roberts et al. 2015, Roberts et al. 2016b). Though the present study only considered water-borne particle motion, pile driving also produces large substrate-borne vibration (Roberts and Elliott 2017). *Doryteuthis pealeii* naturally rests on the substrate and would potentially sense this "ground-roll" as well; accordingly, future studies on this species should examine the influence of substrate-borne vibration.

Squid schooling and shoaling behaviors could be disrupted during impulsive noise, as has been demonstrated in fish. For example, schools of European sea bass (*Dicentrarchus labrax*) responded to anthropogenic noise impulses by increasing swimming depth and school cohesion, both considered primary stress responses (Neo et al. 2014, Neo et al. 2015b). Decreased school cohesion has also been observed in *D. labrax* during pile driving noise, which might increase predation risk (Herbert-Read et al., 2017). Future studies with groups of squid should monitor changes in schooling behaviors, in addition to fighting, visual displays, and other behaviors naturally occurring in agonistic and reproductive scenarios. Since pile driving activity could occur during the day or night and wild *D. pealeii* occupy different parts of the water column between day and night (more benthic and schooling during the day, more pelagic and dispersed at night), differences in behavior during these periods should also be considered. Diel differences in responses to simulated noise impulses have been found in fish that naturally exhibit diel differences in schooling behavior (Hawkins et al., 2014).

5.1.2.2. Ecological implications

Short-term effects such as startle responses and subsequent increased tolerance or habituation are often dismissed as ecologically irrelevant but there is an increasing awareness of the effects such responses may incur (Bejder et al. 2009). We suggest several hypotheses for ecological impacts that can be drawn based on the present study and previous work, which are worth investigating in future studies. First, as squid become more tolerant of a noise stimulus they might fail to exhibit alarm and escape responses in order to evade predators, possibly due to: 1) learned unresponsiveness to threat stimuli, 2) distraction by the noise source (the "distracted prey" hypothesis, see Chan et al., 2010), or 3) failure to detect particle motion associated with important signals (e.g.*,* predator sounds) in the presence of interfering noise, a phenomenon termed "masking". Squids' initial natural responses to a predator are often crypsis or schooling; short-term startle responses have the potential to disrupt schooling or cause animals to reveal themselves to predators. In both cases they are more vulnerable to predation. The aforementioned habituation study on the squid *L. brevis* found habituation specificity of this species to visual predator models, meaning that after habituating to one model, the squid showed elevated jetting and body pattern change responses when presented with a new, different model (Long et al. 1989). Squid that have diminished responses to pile driving noise may restore behaviors in response to new sound or visual stimuli, such as those of predators, however this mechanism should be empirically evaluated. Additionally, exposure to noxious stimuli may increase squids' alertness to predators. After physical injury, *D. pealeii* exhibited increased responsiveness to black sea bass (*Centropristis striata*) attacks, initiating alert and flight reactions sooner and at longer distances from the predator (Crook et al. 2014). It is yet unclear whether squid would similarly be sensitized (or desensitized) to predatory threats during or after noise exposure.

Although it is not known how squid utilize natural sound cues, squid possess sets of hair cells along the external surface of their skin which detect nearby water movements and play an important role in successful predator evasion (York and Bartol, 2014). Squid also possess internal statocyst structures known to detect particle motion, specifically acceleration (Budelmann 1992, Mooney et al. 2010). Exposure to pile driving noise could potentially interfere with the detection of particle motion cues from nearby, swimming predators via masking through both these sensory systems, though masking effects have not yet been investigated in any cephalopod.

Although the present studies did not test for noise-amplitude dependent alarm responses, inking and jetting are expected to occur more frequently in squid as the amplitude of low-frequency noise increases (Mooney et al., 2016). In future studies, when significant impacts of pile driving noise on ecologically relevant behavioral and physiological variables are found, attempts should be made to establish amplitude and duration (for a given inter-pulse-interval) thresholds for adverse effects to likely occur. Such thresholds would better inform regulations for acceptable limits on pile driving activity in areas utilized by ecologically and economically important species such as *D. pealeii*.

Our studies are the first to demonstrate behavioral effects of pile driving noise on any cephalopod species and focused on a commercially important squid species whose range overlaps with areas where marine construction projects involving pile driving may occur in the near future. We observed a variety of alarm responses associated with anti-predator reactions at the onset of pile driving noise. Squid had rapidly diminished alarm responses within the first minute of noise exposure in all trials and had re-sensitized to the noise after a 24-h rest period. Diminished predator-defense and escape behaviors may alter squid susceptibility to predation, depending on potential factors such as squids' habituation specificity and their predators' responses to noise. Although caution must be taken when extrapolating lab-based results to free-swimming wild animals that may potentially escape a noise source, controlled lab-based studies are useful for analyzing such interactions without the influence of confounding environmental variables. That said, to conclusively test ecological implications of noise exposure, field studies are also needed, in which acoustic conditions are more realistic and squid are less confined, and thus allowed to behave more naturally. Potential physiological changes, e.g., in respiration rate and energy expenditure, remain to be investigated. The present findings of the influence of pile driving noise on squid alarm responses will leverage future studies on behavioral and physiological effects of anthropogenic noise on squid and other cephalopods, as well as impacts on these animals' ecology.

5.1.2.3. Potential resilience of reproductive behaviors during noise

Here we present the first empirical dataset addressing the potential effects of anthropogenic noise on reproductive behaviors of a marine invertebrate. Overall, there was no indication that the high-intensity, repeated pile driving noise impacted any of the suite of reproductive behaviors measured including agonistic, mate guarding, mating, or egg-laying behaviors. Typical behavioral dynamics (Shashar and Hanlon 2013) of sexually active squid continued to occur despite the repeated, high-intensity, impulsive noise treatment. These results are perhaps surprising given the array of impacts seen in other behaviors of cephalopods and in other taxa (Mooney et al. 2020a); however, they underscore the exceptionally strong motivation of these squid to reproduce. Squid engaging in these behaviors are nearing the end of their lifespan; females may continue to mate with multiple males and spawn over a few weeks, but both sexes will soon senesce (Maxwell and Hanlon 2000, Hanlon et al. 2013a). From an evolutionary standpoint, persistence of reproductive behaviors during environmental stressors is advantageous for species with limited opportunity to reproduce in their lifetime. The present results are consistent with theory that reproductive behaviors of semelparous species should be relatively uninfluenced from potentially inhibitory effects of stress (Wingfield and Sapolsky 2003, de Jong et al. 2020). Based on these data, one might conclude that mating behaviors of semelparous species are at a lower risk of adverse effects from noise exposure.

Of course, it is not possible to generalize these trends to all semelparous species, all cephalopods, or all noise types, since responses may be specific to species and noise characteristics. For instance, females of one semelparous goby species (*Pomatoschistus microps*) significantly delayed their inspection of nests, delayed spawning, and laid fewer eggs during noise from airstones (Blom et al. 2019a). These differences only occurred when gobies were presented with continuous, rather than impulsive, noise. It is possible that squid behaviors in the present study may have differed if presented a different noise type (e.g., boat noise). Continuous noise with irregular amplitude and frequency characteristics is thought to be more likely to cause stress compared to impulsive sounds that have more consistent amplitude and frequency spectra (de Jong et al. 2020). Impulses played in the present study were similar to each other in amplitude and spectra and may be considered "regular," although amplitudes received by squid varied spatially in the tank. Effects of continuous or irregular noise on squid behavior remain to be tested. Considering species with currently published particle motion audiograms, cephalopods tend to have lower particle acceleration sensitivities (higher thresholds) than many fish species (Wysocki et al. 2009, Mooney et al. 2010, Wright et al. 2011, Samson et al. 2014, Mooney et al. 2016, Blom et al. 2019b). Such differences in hearing sensitivity could also contribute to different responses to noise across taxa, though this should not be assumed *a priori* (Rogers et al. 2016b).

Laboratory-based studies allow detailed observation of behavior in a well-controlled environment without confounding influence of extraneous noise sources and other environmental factors. Peak particle acceleration levels in the experiment tank exceeded those measured at 500 m from Block Island Wind Farm piles (1 m above seabed, 26 m depth) thus representing acceleration levels predicted within a 500 m radius (Amaral et al. 2020). Importantly, sound propagation from piles depends on multiple engineering and environmental factors, including pile dimensions, angle with respect to the seabed, hammer strike energy, bathymetry, sediment properties, and seasonally-dependent sound speed profiles; however, propagation of particle acceleration from piles is poorly understood (Lippert and von Estorff 2014, Tsouvalas and Metrikine 2016, Lin et al. 2019). In-tank underwater acoustics cannot exactly replicate *in-situ* acoustic propagation (Rogers et al. 2016b, Jézéquel et al. 2019, Jones et al. 2019). Yet the experimental acoustic field can be measured precisely in high spatial resolution (perhaps more so than in the field), and careful effort was made to represent similar amplitudes and frequency spectra that squid may experience from *in-situ* pile driving.

5.1.2.4. Behavioral context-dependent noise impacts on squid: cross-study comparisons

Comparisons to response rates of squid in other behavioral conditions (but similar methodological setups) underscores that behavioral context of the noise exposure greatly affects responses exhibited. Previous studies of individual squid either simply swimming, resting or otherwise not engaged in specific tasks (Jones et al. 2020), or during feeding events, showed substantially higher rates of alarm and flight responses (Jones et al. 2021) (see above for summaries). Alarm responses included inking, jetting, other locomotor startle behaviors, and body pattern changes, which are all employed by squid as anti-predator defenses. Comparatively, noise effects on feeding behaviors were more nuanced. There were no statistically significant differences between noise and control treatments in the proportion of squid that ultimately captured prey during the trial. Yet noise played during squids' pursuit of prey led to a significant increase in missed or abandoned prey capture attempts.

5.2. General conclusions

Together, the results of these studies suggest that general, antipredator and feeding behaviors may be altered during exposure to noise; conversely, squid engaged in reproductive behaviors are highly motivated to continue these behaviors, even during this noise stressor.
Ecological and population-level consequences of anthropogenic noise are inherently difficult to measure. Our laboratory-based experiments on black sea bass and squid have allowed identification of several important processes regarding their responses to noise. These include: response and habituation rates, identification of natural behaviors susceptible to change due to noise stressors, and identification of particle motion levels at which they may be expected to occur. These are key initial steps to discerning potential noise impacts. From these findings, one can make educated hypotheses on how, when, and where wild animals may react to pile driving. We hypothesized that after habituating to noise, squid might similarly become desensitized to predator threats; yet we also point out that research on another squid species revealed habituation specificity to stimuli, i.e., squid that became sensitized to one visual predator model would still respond to a new, different visual predator model (Long et al. 1989). Squid habituation to visual threat cues may or may not carry over to acoustic threat cues. Future experiments modeled after Long et al., (1989) could be conducted to test this.

Further, we suggested that that missed opportunities for prey capture and lower feeding rates could lead to reduced growth and survival. Considering the metabolic requirements of both species, especially in *D. pealeii* to feed often, there exists the potential for population level reductions in abundance if wild animals similarly are disrupted from feeding due to a sudden onset of anthropogenic noise. Yet in a reproductive context, *D. pealeii* retain appropriate reproductive behaviors during noise. Therefore, pile driving noise is not expected to reduce the reproductive output of wild populations as far as behaviors up through egg laying are concerned. Potential noise effects on early development have not yet been investigated (see next section). Overall, our results indicate that for *C. Striata* and *D. pealeii*, responses to sound are most likely to occur at the onset of noise, rapid habituation is expected, with some re-sensitization, and reproductive behaviors may be relatively resilient to noise stressors for semelparous species that have limited opportunity to reproduce.

5.3. Future directions

The present studies indicate that reproductive behaviors of longfin squid may be less at risk of being disrupted by noise relative to anti-predator defense and feeding behaviors. Together with previous studies on *D. pealeii*, these results reinforce the importance of considering multiple behavioral contexts in which animals' responses to anthropogenic noise may differ. To address noise effects in more ecologically and acoustically relevant scenarios, complementary field studies on squid and black sea bass behavioral responses to noise, with longer noise exposures, are needed. This should include some studies utilizing controlled, but *in situ* (actual) pile driving, preferably prior installation of at least some U.S. windfarms that will be developed in squid and black sea bass habitats. This goal would be to provide key data regarding how windfarm pile driving may affect these squid and black sea bass species, as well as potential mitigation measures such as ramp-ups. Additional work would ideally include evaluating impacts from windfarm construction activities. In both study frameworks, this would examine key ecological relationships such as: movement and displacement from a breeding or feeding aggregation area; schooling, communication and breeding activities; hearing, hearing loss and auditory physiology; and overall ecosystem-related influence (on the study species, their prey and related taxa). These methods would allow new questions to be addressed, such as whether squid will actively avoid and swim away from noise sources; this question is highly relevant to the fishing industry's concerns about possible reduced catch near wind farms. Ideally, these studies will utilize before-during-and-after gradient (BAG) design (Methratta 2020), where potential significant changes from baseline in the variables of interest are assessed using statistical methods that allow for the exploration of changes in spatial relationships over time (Brandt et al. 2011). This BAG method reduces some challenges that may occur with finding unequal and unsuitable control sites, and spillover effects (due to impacts). Rather, BAG leverages the notion that distance from source and propagation are important factors, and concurrently addresses the potential scale of effects at increasing distance from the construction site.

Finally, laboratory studies should not be ignored. They have provided valuable information on the behavioral contexts in which squid and black sea bass may be adversely affected by anthropogenic noise and can inform research foci for field studies addressing *in-situ* and population-level impacts. There are a number of additional experiments on squid and fish behavior that would fill some key remaining knowledge gaps, which could occur in controlled tanks or in outdoor enclosures. They can provide key data which are otherwise not feasible to address in the field, such as many studies on impacts to early life. However, pre-juvenile stages of many invertebrates, including cephalopods, have variable sensitivities to environmental stressors that may differ from those of later stages (Zakroff & Mooney, 2020). Noise has been found to increase mortality, delay development, and cause malformations of sea hare embryos and scallop larvae (de Soto et al., 2013; Nedelec et al., 2014). Regular and random noise exposure experiments were also used to on larval Atlantic cod and behavior, growth and development was monitored. Over the short-term, noise caused startle responses in newly hatched fish, irrespective of rearing noise. Two days of exposure to both regular and random noise reduced growth, and regular noise also caused faster yolk sac utilization. After 16- days growth converged with control groups, however noise groups had lower body width-length ratios, which in predator avoidance experiments led to increased capture rates (Nedelec et al. 2015).

Although egg laying rates of *D. pealeii* appear unaffected by noise, potential influences of noise exposure on egg hatching and behavior and development of paralarvae (hatched pre-juveniles) have not been investigated. Thus, future work should also include laboratory studies on developing or young animals. Experiments could be conducted to test whether fish predators' feeding behavior on live squid and squids' ability to escape predation are altered during noise. These experiments would help address whether squid alarm responses and subsequent habituation to noise would affect their vigilance and responsiveness to actual predator threats. Such studies could be conducted with natural predators of *D. pealeii* including flounder, black sea bass, or bluefish, and be modelled after previous experiments detailing predator-prey interactions among these species (Crook et al., 2014; Staudinger et al., 2011).

Most squid and back sea bass experiments utilized the same pile driving noise playbacks, with playback files being relatively consistent in acoustic properties such as the length of noise impulses and inter-pulse-interval. Yet, several studies on fishes have revealed varied behavioral responses to noise depending on inter-pulse interval, continuous (e.g., white noise or boat noise) versus impulsive (e.g. pile driving, air gun) sounds, and whether sounds have regular or irregular amplitudes and frequencies across time (Blom et al., 2019; Neo et al., 2014, 2015). Based on prior pile driving work and observing, we would generally expect wind farm installation pile driving impulses to have a fairly regular inter-pulse-interval. Still, similar studies on cephalopods could help predict their responses to a wider variety of anthropogenic noises, including those associated with offshore wind farm development stages other than construction (Mooney, Andersson, et al., 2020). These research efforts and the results of the present study are of central importance to the fishing industry, regulators, and energy industry seeking to assess and address risks that offshore energy expansion poses to ecologically and commercially key marine fauna.

6 References

Abbott, R., J. Reyff, and G. Marty. 2005. Final Report: Monitoring the Effects of Conventional Pile Driving on Three Species of Fish. Manson Construction Company, Richmond, CA.

Akamatsu, T., T. Okumura, N. Novarini, and H. Y. Yan. 2002. Empirical refinements applicable to the recording of fish sounds in small tanks. The Journal of the Acoustical Society of America **112**:3073 - 3082.

Allison, T. D., J. E. Diffendorfer, E. F. Baerwald, J. A. Beston, D. Drake, A. M. Hale, C. D. Hein, M. M. Huso, S. R. Loss, J. E. Lovich, D. Strickland, K. A. Williams, and V. L. Winder. 2019. Impacts to Wildlife of Wind Energy Siting and Operation in the United States. Issues in Ecology **21**:23.

Amaral, J. L., R. Beard, R. J. Barham, A. G. Collett, J. Elliot, A. S. Frankel, D. Gallien, C. Hager, A. A. Khan, Y.-T. Lin, T. Mason, J. H. Miller, A. E. Newhall, G. R. Potty, K. Smith, and K. Vigness-Raposa. 2018. Field Observations During Wind Turbine Foundation Installation at the Block Island Wind Farm, Rhode Island. Appendix D: Underwater Sound Monitoring Reports.

Amaral, J. L., J. H. Miller, G. R. Potty, K. J. Vigness-Raposa, A. S. Frankel, Y.-T. Lin, A. E. Newhall, D. R. Wilkes, and A. N. Gavrilov. 2020. Characterization of impact pile driving signals during installation of offshore wind turbine foundations. The Journal of the Acoustical Society of America **147**:2323-2333.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**:32-46.

Andersson, M. H., S. Andersson, J. Ahlsén, B. L. Andersson, J. Hammar, L. K. G. Persson, J. Pihl, P. Sigray, and A. Wikström. 2016. A framework for regulating underwater noise during pile driving. A technical Vindval report, ISBN 978-91-620-6775-5. Swedish Environmental Protection Agency, Stockholm, Sweden.

Bailey, H., K. L. Brookes, and P. M. Thompson. 2014. Assessing environmental impacts of offshore wind farms: lessons learned and recommendations for the future. Aquatic Biosystems **10**:8.

Bailey, H., B. Senior, D. Simmons, J. Rusin, G. Picken, and P. M. Thompson. 2010. Assessing underwater noise levels during pile-driving at an offshore windfarm and its potential effects on marine mammals. Marine Pollution Bulletin **60**:888-897.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software **67**:1 - 48.

Bejder, L., A. Samuels, H. Whitehead, H. Finn, and S. Allen. 2009. Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Marine Ecology Progress Series **395**:177-185.

Biesinger, Z., B. M. Bolker, D. Marcinek, T. M. Grothues, J. A. Dobarro, and W. J. Lindberg. 2013a. Testing an autonomous acoustic telemetry positioning system for fine-scale space use in marine animals. Journal of Experimental Marine Biology and Ecology **448**:46-56.

Biesinger, Z., B. M. Bolker, D. Marcinek, and W. J. Lindberg. 2013b. Gag (*Mycteroperca microlepis*) space-use correlations with landscape structure and environmental conditions. Journal of Experimental Marine Biology and Ecology **443**:1-11.

Blom, E.-L., C. Kvarnemo, I. Dekhla, S. Schöld, M. H. Andersson, O. Svensson, and M. C. P. Amorim. 2019a. Continuous but not intermittent noise has a negative impact on mating success in a marine fish with paternal care. Scientific Reports **9**:1-9.

Blom, E.-L., C. Kvarnemo, I. Dekhla, S. Schöld, M. H. Andersson, O. Svensson, and M. C. P. Amorim. 2019b. Continuous but not intermittent noise has a negative impact on mating success in a marine fish with paternal care. Scientific Reports **9**:5494.

Bolle, L. J., C. A. F. de Jong, S. M. Bierman, P. J. G. van Beek, O. A. van Keeken, P. W. Wessels, C. J. G. van Damme, H. V. Winter, D. de Haan, and R. P. A. Dekeling. 2012. Common Sole Larvae Survive High Levels of Pile-Driving Sound in Controlled Exposure Experiments. PLoS ONE **7**.

Bowen, B. W., and J. C. Avise. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon - influence of zoogeographic factors and life-history patterns. Marine Biology **107**:371-381.

Boyle, P., and P. Rodhouse. 2005. Cephalopods: Ecology and fisheries. Blackwell Science, Oxford, UK.

Brandt, M. J., A. Diederichs, K. Betke, and G. Nehls. 2011. Responses of harbour porpoises to pile driving at the Horns Rev II offshore wind farm in the Danish North Sea. Marine Ecology Progress Series **421**:205-216.

Braun, C. B., and T. Grande. 2008. Evolution of peripheral mechanisms for the enhancement of sound reception. Pages 99-144 *in* J. F. Webb, R. R. Fay, and A. N. Popper, editors. Fish Bioacoustics. Springer, New York.

Brodziak, J., and W. K. Macy. 1996. Growth of long-finned squid, Loligo pealei, in the northwest Atlantic. . Fishery Bulletin **94**:212–236.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal **9**:378–400.

Bruintjes, R., J. Purser, K. A. Everley, S. Mangan, S. D. Simpson, and A. N. Radford. 2016a. Rapid recovery following short-term acoustic disturbance in two fish species. Royal Society Open Science **3**.

Bruintjes, R., and A. N. Radford. 2013. Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. Animal Behaviour **85**:1343-1349.

Bruintjes, R., S. Simpson, H. Harding, T. Bunce, T. Benson, S. Rossington, and D. Jones. 2016b. The impact of experimental impact pile driving on oxygen uptake in black seabream and plaice. Proceedings of Meetings on Acoustics **27**:010042.

Budelmann, B. U. 1992. Hearing in Crustacea. Pages 131 - 139 *in* D. B. Webster, R. R. Fay, and A. N. Popper, editors. Evolutionary Biology of Hearing. Springer, Berlin Heidelberg New York.

Bullock, T. H., and J. T. Corwin. 1979. Acoustic evoked activity in the brain in sharks. Journal of Comparative Physiology **129**:223-234.

Buresch, K. C., J. G. Boal, J. Knowles, J. Debose, A. Nichols, A. Erwin, S. D. Painter, G. T. Nagle, and R. T. Hanlon. 2003. Contact Chemosensory Cues in Egg Bundles Elicit Male–Male Agonistic Conflicts in the Squid Loligo pealeii. Journal of Chemical Ecology **29**:547-560.

Buresch, K. C., J. G. Boal, G. T. Nagle, J. Knowles, R. Nobuhara, K. Sweeney, and R. T. Hanlon. 2004. Experimental evidence that ovary and oviducal gland extracts influence male agonistic behavior in squids. Biol Bull **206**:1-3.

CA Dept of Fish & Wildlife. 2016. 2016 California Legislative Fisheries Forum: Annual Marine Fisheries Report. Dept of Fish and Wildlife **54**:35.

Caiger, P. E., J. C. Montgomery, M. Bruce, J. Lu, and C. A. Radford. 2013. A proposed mechanism for the observed ontogenetic improvement in the hearing ability of hapuka (*Polyprion oxygeneios*). Journal of Comparative Physiology A Neuroethology, Sensory, Neural, and Behavioral Physiology **199**:653-661.

Caltrans. 2015. Technical guidance for assessment and mitigation of the hydroacoustics effects of pile driving on fish. Sacramento, CA.

Carroll, A. G., R. Przeslawski, A. Duncan, M. Gunning, and B. Bruce. 2017. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Marine Pollution Bulletin **114**:9-24.

Casper, B. M., M. B. Halvorsen, T. J. Carlson, and A. N. Popper. 2017. Onset of barotrauma injuries related to number of pile driving strike exposures in hybrid striped bass. The Journal of the Acoustical Society of America **141**:4380-4387.

Casper, B. M., M. B. Halvorsen, F. Matthews, T. J. Carlson, and A. N. Popper. 2013a. Recovery of Barotrauma Injuries Resulting from Exposure to Pile Driving Sound in Two Sizes of Hybrid Striped Bass. PLoS ONE **8**:e73844.

Casper, B. M., M. E. Smith, M. B. Halvorsen, H. Sun, T. J. Carlson, and A. N. Popper. 2013b. Effects of exposure to pile driving sounds on fish inner ear tissues. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology **166**:352-360.

Chan, A., P. Giraldo-Perez, S. Smith, and D. T. Blumstein. 2010. Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biology Letters **6**:458-461.

Chapman, C. J., and A. D. Hawkins. 1973. Field study of hearing in the Cod, *Gadus morhua* L. Journal of Comparative Physiology **85**:147-167.

Clarke, M. R. 1977. Beaks, nets and numbers. Symposia of the Zoological Society of London **38**.

Clarke, M. R. 1996. Cephalopods as Prey. III. Cetaceans. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **351**:1053-1065.

Corwin, J. T., T. H. Bullock, and J. Schweitzer. 1982. The auditory brain-stem response in 5 vertebrate classes. Electroencephalography and Clinical Neurophysiology **54**:629-641.

Cox, K., L. P. Brennan, T. G. Gerwing, S. E. Dudas, and F. Juanes. 2018. Sound the alarm: A meta-analysis on the effect of aquatic noise on fish behavior and physiology. Global Change Biology **24**:3105-3116.

Crook, R. J., K. Dickson, R. T. Hanlon, and E. T. Walters. 2014. Nociceptive sensitization reduces predation risk. Current Biology **24**:1121-1125.

Cummins, S. F., J. G. Boal, K. C. Buresch, C. Kuanpradit, P. Sobhon, J. B. Holm, B. M. Degnan, G. T. Nagle, and R. T. Hanlon. 2011. Extreme aggression in male squid induced by a β-MSP-like pheromone. Curr Biol **21**:322-327.

de Jong, K., M. C. P. Amorim, P. J. Fonseca, C. J. Fox, and K. U. Heubel. 2018a. Noise can affect acoustic communication and subsequent spawning success in fish. Environmental Pollution **237**:814-823.

de Jong, K., M. C. P. Amorim, P. J. Fonseca, and K. U. Heubel. 2018b. Noise Affects Multimodal Communication During Courtship in a Marine Fish. Frontiers in Ecology and Evolution **6**.

de Jong, K., T. N. Forland, M. C. P. Amorim, G. Rieucau, H. Slabbekoorn, and L. D. Sivle. 2020. Predicting the effects of anthropogenic noise on fish reproduction. Reviews in Fish Biology and Fisheries **30**:245-268.

Debusschere, E., K. Hostens, D. Adriaens, B. Ampe, D. Botteldooren, G. De Boeck, A. De Muynck, A. K. Sinha, S. Vandendriessche, L. Van Hoorebeke, M. Vincx, and S. Degraer. 2016. Acoustic stress responses in juvenile sea bass Dicentrarchus labrax induced by offshore pile driving. Environmental Pollution **208**:747-757.

Domjan, M. 2015. The Principles of Learning and Behaviour 7th edition. Cengage Learning, USA.

Dooling, R. J., and S. H. Blumenrath. 2016. Masking Experiments in Humans and Birds Using Anthropogenic Noises. Advances in Experimental Medicine and Biology **875**:239-243.

Drohan, A. F., J. P. Manderson, and D. B. Packer. 2007. Essential Fish Habitat Source Document: Black Sea Bass, *Centropristis striata*, Life History and Habitat Characteristics. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, Massachusetts.

Dvorak, M. J., B. A. Corcoran, J. E. Ten Hoeve, N. G. McIntyre, and M. Z. Jacobson. 2013. US East Coast offshore wind energy resources and their relationship to peak-time electricity demand. Wind Energy **16**:977-997.

Egner, S. A., and D. A. Mann. 2005. Auditory sensitivity of sergeant major damselfish Abudefduf saxatilis from post-settlement juvenile to adult. Marine Ecology Progress Series **285**:213-222.

Ellison, W. T., B. L. Southall, C. W. Clark, and A. S. Frankel. 2012. A new context-based approach to assess marine mammal behavioral responses to anthropogenic sounds. Conserv Biol **26**:21-28.

Fabrizio, M. C., J. P. Manderson, and J. P. Pessutti. 2014. Home Range And Seasonal Movements Of Black Sea Bass (*Centropristis Striata*) During Their Inshore Residency At A Reef In The Mid-Atlantic Bight. Fishery Bulletin **112**:82 - 97.

Fabrizio, M. C., J. P. Pessutti, J. P. Manderson, A. F. Drohan, and B. A. Phelan. 2005. Use of the Historic Area Remediation Site by black sea bass and summer flounder. Northeast Fisheries Science Center, U.S. Department of Commerce, Woods Hole, MA.

Fay, R. 2009. Soundscapes and the sense of hearing of fishes. Integrative Zoology **4**:26-32.

Fay, R. R., and A. N. Popper. 1974. Acoustic stimulation of the ear of the goldfish (*Carassius auratus*). J. Exp. Biol. **61**:243-260.

Fewtrell, J., and R. D. McCauley. 2012. Impact of air gun noise on the behaviour of marine fish and squid. Marine pollution bulletin **64 5**:984-993.

Fish, M. P., and W. H. Mowbray. 1970. Sounds of West North Atlantic Fishes. The John Hopkins Press, Baltimore and London.

Fox, J., and S. Weisberg. 2019. An {R} companion to applied regression. . Sage, Thousand Oaks, CA.

Garabon, J. R., and D. M. Higgs. 2017. The effects of stimulus parameters on auditory evoked potentials of *Carassius auratus*. Journal of Comparative Physiology A Neuroethology, Sensory, Neural, and Behavioral Physiology **203**:945-951.

Gedamke, J., J. Harrison, L. Hatch, R. Angliss, J. Barlow, C. Berchok, C. Caldow, M. Castellote, D. Cholewiak, M. L. DeAngelis, R. Dziak, E. Garland, S. Guan, S. Hastings, M. Holt, B. Laws, D. Mellinger, S. Moore, T. J. Moore, E. Oleson, J. Jearson-Meyer, W. Piniak, J. Redfern, T. Rowles, A. Scholik-Schlomer, A. Smith, M. Soldevilla, J. Stadler, S. Van Parijs, and C. M. Wahle. 2016. Ocean Noise Strategy Noise Roadmap. National Oceanic and Atmospheric Administration NOAA.

Graham, I. M., N. D. Merchant, A. Farcas, T. R. Barton, B. Cheney, S. Bono, and P. M. Thompson. 2019. Harbour porpoise responses to pile-driving diminish over time. Royal Society Open Science **6**:190335.

Grothues, T. M., and E. A. Bochenek. 2010. Winter flounder telemetry in the Navesink River Estuary, New Jersey, during the 2009 spawning season. Final Report to the New Jersey Grant Program of the New Jersey Department of Transportation Office of Maritime Resources.

Grothues, T. M., B. A. Phelan, and E. A. Bochenek. 2008. Telemetry of spawning winter flounder, Pseudopleuronectes americanus, in a New Jersey estuary. Final Report to the New Jersey Grant Program of the New Jersey Department of Transportation Office of Maritime Resources.

Guida, V., A. Drohan, H. Welch, J. McHenry, D. Johnson, V. Kentner, J. Brink, D. Timmons, and E. Estela-Gomez. 2017. Habitat Mapping and Assessment of Northeast Wind Energy Areas. US Department of the Interior, Bureau of Ocean Energy Management, Sterling, VA.

Halvorsen, M. B., B. M. Casper, F. Matthews, T. J. Carlson, and A. N. Popper. 2012. Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker. Proceedings of the Royal Society B: Biological Sciences **279**:4705-4714.

Halvorsen, M. B., L. E. Wysocki, and A. N. Popper. 2006. Effects of high-intensity sonar on fish. The Journal of the Acoustical Society of America **119**:3283-3283.

Hanlon, R., K. Buresch, H. Moustahfid, and M. Staudinger. 2013a. Doryteuthis pealeii, Longfin Inshore Squid. Pages 205-240 *in* R. Rosa, R. O'Dor, and P. GJ, editors. Advances in Squid Biology, Ecology and Fisheries. Nova Science Publishers, Inc., Hauppauge, New York.

Hanlon, R. T., K. Buresch, H. Moustahfid, and M. D. Staudinger. 2013b. Doryteuthis pealeii, Longfin inshore squid. Pages 205 - 240 *in* R. Rosa, G. Pierce, and R. O'Dor, editors. Advances in Squid Biology, Ecology, and Fisheries. Nova Science Publishers Inc., Hauppauge, NY.

Hanlon, R. T., M. R. Maxwell, N. Shashar, E. R. Loew, and K. L. Boyle. 1999. An Ethogram of Body Patterning Behavior in the Biomedically and Commercially Valuable Squid Loligo pealei off Cape Cod, Massachusetts. The Biological Bulletin **197**:49-62.

Hanlon, R. T., and J. B. Messenger. 2018. Cephalopod Behaviour. 2nd edition. Cambridge University Press, New York.

Harris, C. M., L. Thomas, E. A. Falcone, J. Hildebrand, D. Houser, P. H. Kvadsheim, F.-P. A. Lam, P. J. O. Miller, D. J. Moretti, A. J. Read, H. Slabbekoorn, B. L. Southall, P. L. Tyack, D. Wartzok, and

V. M. Janik. 2018. Marine mammals and sonar: Dose-response studies, the risk-disturbance hypothesis and the role of exposure context. Journal of Applied Ecology **55**:396-404.

Hawkins, A. D., and C. J. Chapman. 1975. Masked auditory thresholds in the cod, Gadus morhua L. Journal of Comparative Psychology **103**:209-226.

Hawkins, A. D., C. Johnson, and A. N. Popper. 2020. How to set sound exposure criteria for fishes. The Journal of the Acoustical Society of America **147**:1762-1777.

Hawkins, A. D., A. E. Pembroke, and A. N. Popper. 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Reviews in Fish Biology and Fisheries **25**:39-64.

Hawkins, A. D., and A. N. Popper. 2016. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES Journal of Marine Science **73**:635 - 651.

Hawkins, A. D., L. Roberts, and S. Cheesman. 2014. Responses of free-living coastal pelagic fish to impulsive sounds. The Journal of the Acoustical Society of America **135**:3101-3116.

Herbert-Read, J. E., L. Kremer, R. Bruintjes, A. N. Radford, and C. C. Ioannou. 2017. Anthropogenic noise pollution from pile-driving disrupts the structure and dynamics of fish shoals. Proceedings of the Royal Society B: Biological Sciences **284**.

Higgs, D., and C. A. Radford. 2013. The contribution of the lateral line to "hearing" in fish. Journal of Experimental Biology **216**:1484-1490.

Higgs, D. M., and C. A. Radford. 2016. The potential overlapping roles of the ear and lateral line in driving "acoustic" responses. Advances in Experimental Medicine and Biology **877**:255-270.

Higgs, D. M., M. J. Souza, H. R. Wilkins, J. C. Presson, and A. N. Popper. 2002. Age- and size-related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*). Jaro **3**:174-184.

Hildebrand, J. A. 2009. Anthropogenic and natural sources of ambient noise in the ocean. Marine Ecology Progress Series **395**:5-20.

Holles, S., S. D. Simpson, A. N. Radford, L. Berten, and D. Lecchini. 2013. Boat noise disrupts orientation behaviour in a coral reef fish. Marine Ecology Progress Series **485**:295-300.

Hunsicker, M. E., T. E. Essington, R. Watson, and U. R. Sumaila. 2010. The contribution of cephalopods to global marine fisheries: can we have our squid and eat them too? Fish and Fisheries **11**.

Iafrate, J. D., S. L. Watwood, E. A. Reyier, D. M. Scheidt, G. A. Dossot, and S. E. Crocker. 2016. Effects of Pile Driving on the Residency and Movement of Tagged Reef Fish. PLoS ONE **11**:e0163638.

International Organization for Standardization. 2017. Underwater acoustics — Measurement of radiated underwater sound from percussive pile driving 18406:2017(en). Retrieved from: https://www.iso.org/obp/ui/#iso:std:iso:18406:ed-1:v1:en.

Jézéquel, Y., J. Bonnel, J. Coston-Guarini, and L. Chauvaud. 2019. Revisiting the bioacoustics of European spiny lobsters Palinurus elephas: comparison of antennal rasps in tanks and in situ. Marine Ecology Progress Series **615**:143-157.

Jones, I. T., J. F. Peyla, H. Clark, Z. Song, J. A. Stanley, and T. A. Mooney. 2021. Changes in feeding behavior of longfin squid (Doryteuthis pealeii) during laboratory exposure to pile driving noise. Marine Environmental Research **165**:105250.

Jones, I. T., J. A. Stanley, J. Bonnel, and T. A. Mooney. 2019. Complexities of tank acoustics warrant direct, careful measurement of particle motion and pressure for bioacoustic studies. Page 010005 *in* Proceedings of Meetings on Acoustics 5ENAL. Acoustical Society of America.

Jones, I. T., J. A. Stanley, and T. A. Mooney. 2020. Impulsive pile driving noise elicits alarm responses in squid (*Doryteuthis pealeii)*. Marine Pollution Bulletin **150**:110792.

Kastelein, R. A., S. van der Heul, J. van der Veen, W. C. Verboom, N. Jennings, D. de Haan, and P. J. H. Reijnders. 2007. Effects of acoustic alarms, designed to reduce small cetacean bycatch in gillnet fisheries, on the behaviour of North Sea fish species in a large tank. Marine Environmental Research **64**:160-180.

Kastelein, R. A., S. van der Heul, W. C. Verboom, N. Jennings, J. van der Veen, and D. de Haan. 2008. Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Marine Environmental Research **65**:369-377.

Kenyon, T. N. 1996. Ontogenetic changes in the auditory sensitivity of damselfishes (Pomacentridae). Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology **179**:553-561.

Kenyon, T. N., F. Ladich, and H. Y. Yan. 1998. A comparative study of hearing ability in fishes: the auditory brainstem response approach. Journal of Comparative Physiology A Sensory Neural and Behavioral Physiology **182**:307-318.

Kight, C. R., and J. P. Swaddle. 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecology Letters **14**:1052-1061.

Knudsen, F. R., P. S. Enger, and O. Sand. 1992. Awareness reactions and avoidance responses to sound in juvenile Atlantic salmon, *Salmo sarar*. Journal of Fish Biology **40**:523-534.

Knudsen, F. R., P. S. Enger, and O. Sand. 1994. Avoidance responses to low frequency sound in downstream migrating Atlantic salmon smolt, *Salmo salar*. Journal of Fish Biology **45**:227-233.

Kunc, H. P., G. N. Lyons, J. D. Sigwart, K. E. McLaughlin, and J. D. R. Houghton. 2014. Anthropogenic Noise Affects Behavior across Sensory Modalities. The American Naturalist **184**:E93-E100.

Ladich, F., and R. R. Fay. 2013. Auditory evoked potential audiometry in fish. Reviews in Fish Biology and Fisheries **23**:317-364.

Langhamer, O. 2012. Artificial reef effect in relation to offshore renewable energy conversion: state of the art. TheScientificWorldJOURNAL **2012**:386713-386713.

Lin, Y.-T., A. E. Newhall, J. H. Miller, G. R. Potty, and K. J. Vigness-Raposa. 2019. A three-dimensional underwater sound propagation model for offshore wind farm noise prediction. The Journal of the Acoustical Society of America **145**:EL335-EL340.

Lindell, S., S. Miner, C. A. Goudey, H. Kite-Powell, and S. Page. 2012. Acoustic conditioning and ranching of Black Sea Bass *Centropristis striata* in Massachusetts USA. Bulletin of Fisheries Research Agency **35**:103-111.

Lippert, T., and O. von Estorff. 2014. The significance of parameter uncertainties for the prediction of offshore pile driving noise. The Journal of the Acoustical Society of America **136**:2463-2471.

Long, T. M., R. T. Hanlon, A. T. Maat, and H. M. Pinsker. 1989. Non-associative learning in the squid lolliguncula brevis (Mollusca, Cephalopoda). Marine Behaviour and Physiology **16**:1-9.

Macy, W. K., III, and J. K. T. Brodziak. 2001. Seasonal maturity and size at age of Loligo pealeii in waters of southern New England. ICES Journal of Marine Science **58**:852-864.

Magnhagen, C., K. Johansson, and P. Sigray. 2017. Effects of motorboat noise on foraging behaviour in Eurasian perch and roach: a field experiment. Marine Ecology Progress Series **564**:115-125.

Mann, D., D. Higgs, W. Tavolga, M. Souza, and P. AN. 2001. Ultrasound detection by clupeiform fishes. Journal of the Acoustical Society of America **109**:3048-3054.

Markus, T., and P. P. S. Sánchez. 2018. Managing and Regulating Underwater Noise Pollution. Pages 971-995 *in* M. Salomon and T. Markus, editors. Handbook on Marine Environment Protection: Science, Impacts and Sustainable Management. Springer International Publishing, Cham.

Maxwell, M. R., and R. T. Hanlon. 2000. Female reproductive output in the squid Loligo pealeii: multiple egg clutches and implications for a spawning strategy. Marine Ecology Progress Series **199**:159-170.

Maxwell, M. R., W. K. Macy, S. Odate, and R. T. Hanlon. 1998. Evidence for Multiple Spawning by Squids (*Loligo pealei*) in Captivity. Biol Bull **195**:225-226.

McCauley, R., J. Fewtrell, and A. N. Popper. 2003. High intensity anthropogenic sound damages fish ears. J. Acoust. Soc. Am. **113**:638-642.

McGarigal, K. R. p. h. w. u. e. l. t. e. l. b. p. 2016. Biostats. R Package.

Mercer, L. P. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic): black sea bass. Coastal Ecology Group, U.S. Army Corps of Engineers, Waterways Experiment Station.

Methratta, E. T. 2020. Monitoring fisheries resources at offshore wind farms: BACI vs. BAG designs. ICES Journal of Marine Science **77**:890-900.

Miller, A. S., G. R. Shepherd, and P. S. Fratantoni. 2016. Offshore Habitat Preference of Overwintering Juvenile and Adult Black Sea Bass, *Centropristis striata*, and the Relationship to Year-Class Success. PLoS ONE **11**:e0147627.

Miller, J. H., G. R. Potty, Y.-T. Lin, A. E. Newhall, K. J. Vigness-Raposa, J. L. Amaral, and A. S. Frankel. 2018. Underwater Acoustic Measurements of the Construction of the Block Island Wind Farm. U.S. Department of the Interior Bureau of Ocean Energy Management Office of Renewable Energy Programs.

Montgomery, J. C., N. Tolimieri, and O. S. Haine. 2001. Active habitat selection by pre-settlement reef fishes. Fish and Fisheries **2**:261 - 277.

Mooney, T. A., M. H. Andersson, and J. Stanley. 2020a. Acoustic impacts of offshore wind energy on fishery resources. Oceanography **33**:82-95.

Mooney, T. A., M. H. Andersson, and J. A. Stanley. 2020b. Acoustic impacts of offshore wind energy on fishery resources: An evolving source and varied effects across a wind farm's lifetime. Oceanography **4**:82-85.

Mooney, T. A., R. T. Hanlon, J. Christensen-Dalsgaard, P. T. Madsen, D. R. Ketten, and P. E. Nachtigall. 2010. Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. The Journal of Experimental Biology **213**:3748-3759.

Mooney, T. A., J. E. Samson, A. D. Schlunk, and S. Zacarias. 2016. Loudness-dependent behavioral responses and habituation to sound by the longfin squid (Doryteuthis pealeii). Journal of Comparative Physiology A **202**:489-501.

Morley, E. L., G. Jones, and A. N. Radford. 2014. The importance of invertebrates when considering the impacts of anthropogenic noise. Proceedings of the Royal Society B: Biological Sciences **281**:20132683.

Moser, J., and G. R. Shepherd. 2009. Seasonal Distribution and Movement of Black Sea Bass (*Centropristis striata*) in the Northwest Atlantic as Determined from a Mark-Recapture Experiment. Journal of Northwest Atlantic Fishery Science **40**:17-28.

Musial, W., and S. Butterfield. 2004. Future for Offshore Wind Energy in the United States. EnergyOcean, Palm Beach, Florida.

Musial, W. D., P. C. Beiter, P. Spitsen, J. Nunemaker, and V. Gevorgian. 2018. Offshore Wind Technologies Market Report.

Musick, J. A., and L. P. Mercer. 1977. Seasonal distrubution of black sea bass, *Centropristis striata,* in mid-Atlantic Bight with comments on ecology and fisheries of species. Transactions of the American Fisheries Society **106**:12-25.

National Research Council. 2003. Ocean Noise and Marine Mammals. National Academic Press, Washington, DC.

Nedelec, S. L., S. C. Mills, D. Lecchini, B. Nedelec, S. D. Simpson, and A. N. Radford. 2016. Repeated exposure to noise increases tolerance in a coral reef fish. Environmental Pollution.

Nedelec, S. L., A. N. Radford, L. Pearl, B. Nedelec, M. I. McCormick, M. G. Meekan, and S. D. Simpson. 2017. Motorboat noise impacts parental behaviour and offspring survival in a reef fish. Proceedings of the Royal Society B: Biological Sciences **284**.

Nedelec, S. L., S. D. Simpson, E. L. Morley, B. Nedelec, and A. N. Radford. 2015. Impacts of regular and random noise on the behaviour, growth and development of larval Atlantic cod (Gadus morhua). Proceedings of the Royal Society B-Biological Sciences **282**.

Neo, Y. Y., J. Hubert, L. Bolle, H. V. Winter, C. Ten Cate, and H. Slabbekoorn. 2016. Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure. Environmental Pollution **214**:26-34.

Neo, Y. Y., J. Hubert, L. J. Bolle, H. V. Winter, and H. Slabbekoorn. 2018. European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environmental Pollution **239**:367-374.

Neo, Y. Y., L. Parie, F. Bakker, P. Snelderwaard, C. Tudorache, M. Schaaf, and H. Slabbekoorn. 2015a. Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. Frontiers in Behavioral Neuroscience **9**:28.

Neo, Y. Y., J. Seitz, R. A. Kastelein, H. V. Winter, C. ten Cate, and H. Slabbekoorn. 2014. Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biological Conservation **178**:65-73.

Neo, Y. Y., E. Ufkes, R. A. Kastelein, H. V. Winter, C. ten Cate, and H. Slabbekoorn. 2015b. Impulsive sounds change European seabass swimming patterns: Influence of pulse repetition interval. Marine Pollution Bulletin **97**:111-117.

NOAA. 2010. 51st Northeast Regional Stock Assessment Review. Stock Assessment Review Committee **54**.

NOAA. 2011. http://www.nero.noaa.gov/ro/fso/reports/reports_frame.htm.

Oksanen, J. 2018. Vegan: Ecological Diversity. R Package Version 2.5-2.

Overholtz, W., J. S. Link, and L. E. Suslowicz. 2000. The impact and implications of fish predation on pelagic fish and squid on the eastern USA shelf. ICES Journal of Marine Science **57**.

Parvulescu, A. 1964. Problems of propagation and processing. Pages 87–100 Marine bio-acoustics. Bimini, Baham.

Paxton, A. B., J. C. Taylor, D. P. Nowacek, J. Dale, E. Cole, C. M. Voss, and C. H. Peterson. 2017. Seismic survey noise disrupted fish use of a temperate reef. Marine Policy **78**:68-73.

Pearson, W. H., J. R. Skalski, and C. I. Malme. 1992. Effects of sounds from a geophysical survey device on behavior of captive rockfish (Sebastes Spp) Canadian Journal of Fisheries and Aquatic Sciences **49**:1343-1356.

Pine, M. K., K. Nikolich, B. J. Martin, C. Morris, and F. Juanes. 2020. Assessing auditory masking for management of underwater anthropogenic noise. The Journal of the Acoustical Society of America **147**:3408-3417.

Popper, A., Hawkins, A.D. (eds). 2012. The Effects of Noise on Aquatic Life. Springer, New York.

Popper, A., N,, A. D. Hawkins, O. Sand, and J. A. Sisneros. 2019. Examining the hearing abilities of fishes. The Journal of the Acoustical Society of America **146**:948-955.

Popper, A. N., and R. R. Fay. 1973. Sound detection and processing by teleost fishes: a critical review. J Acoust Soc Am **53**:1515-1529.

Popper, A. N., and R. R. Fay. 1999. The auditory periphery in fishes. Pages 43 - 100 *in* R. R. Fay and A. N. Popper, editors. Comparative Hearing: Fish and Amphibians. Springer, Berlin Heidelberg New York.

Popper, A. N., and R. R. Fay. 2011. Rethinking sound detection by fishes. Hearing Research **273**:25-36.

Popper, A. N., R. R. Fay, C. Platt, and O. Sand. 2003a. Sound Detection Mechanisms and Capabilities of Teleost Fishes. Pages 3-38 Sensory Processing in Aquatic Environments.

Popper, A. N., J. Fewtrell, M. E. Smith, and R. D. McCauley. 2003b. Anthropogenic sound: Effects on the behavior and physiology of fishes. Marine Technology Society Journal **37**:35-40.

Popper, A. N., and M. C. Hastings. 2009a. The effects of anthropogenic sources of sound on fishes. Journal of Fish Biology **75**:455 - 489.

Popper, A. N., and M. C. Hastings. 2009b. The effects of anthropogenic sources of sound on fishes. Journal of Fish Biology **75**:455-489.

Popper, A. N., and A. D. Hawkins. 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. Journal of Fish Biology.

Popper, A. N., and A. D. e. Hawkins. 2016. The effects of noise on aquatic life II. Springer Science+Business Media, New York.

Popper, A. N., L. Hice-Dunton, K.A. Williams, and E. Jenkins. . 2021. Workgroup Report on Sound and Vibration Effects on Fishes and Aquatic Invertebrates for the State of the Science Workshop on Wildlife and Offshore Wind Energy 2020: Cumulative Impacts. Report to the New York State Energy Research and Development Authority (NYSERDA). Albany, NY.

Poulton, D. A., C. S. Porteus, and S. D. Simpson. 2016. Combined impacts of elevated CO2 and anthropogenic noise on European sea bass (*Dicentrarchus labrax*). ICES Journal of Marine Science: Journal du Conseil.

Purser, J., R. Bruintjes, S. D. Simpson, and A. N. Radford. 2016. Condition-dependent physiological and behavioural responses to anthropogenic noise. Physiology & Behavior **155**:157-161.

Purser, J., and A. N. Radford. 2011. Acoustic Noise Induces Attention Shifts and Reduces Foraging Performance in Three-Spined Sticklebacks (<italic>Gasterosteus aculeatus</italic>). PLoS ONE **6**:e17478.

Radford, A. N., L. Lebre, G. Lecaillon, S. L. Nedelec, and S. D. Simpson. 2016. Repeated exposure reduces the response to impulsive noise in European seabass. Glob Chang Biol **22**:3349-3360.

Radford, C. A., J. C. Montgomery, P. Caiger, P. Johnston, J. Lu, and D. M. Higgs. 2013. A novel hearing specialization in the New Zealand bigeye, *Pempheris adspersa*. Biology Letters **9**.

Rankin, C. H., T. Abrams, R. J. Barry, S. Bhatnagar, D. F. Clayton, J. Colombo, G. Coppola, M. A. Geyer, D. L. Glanzman, S. Marsland, F. K. McSweeney, D. A. Wilson, C.-F. Wu, and R. F. Thompson. 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiology of learning and memory **92**:135-138.

Reinhall, P. G., and P. H. Dahl. 2011a. Underwater Mach wave radiation from impact pile driving: theory and observation. Journal of the Acoustical Society of America **130**:1209-1216.

Reinhall, P. G., and P. H. Dahl. 2011b. Underwater Mach wave radiation from impact pile driving: Theory and observation. The Journal of the Acoustical Society of America **130**:1209-1216.

Roberts, L., S. Cheesman, T. Breithaupt, and M. Elliott. 2015. Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically generated noise. Marine Ecology Progress Series **538**:185-195.

Roberts, L., S. Cheesman, M. Elliott, and T. Breithaupt. 2016a. Sensitivity of *Pagurus bernhardus* (L.) to substrate-borne vibration and anthropogenic noise. Journal of Experimental Marine Biology and Ecology **474**:185-194.

Roberts, L., and M. Elliott. 2017. Good or bad vibrations? Impacts of anthropogenic vibration on the marine epibenthos. Science of The Total Environment **595**:255-268.

Roberts, L., H. R. Harding, I. Voellmy, R. Bruintjes, S. D. Simpson, A. N. Radford, T. Breithaupt, and M. Elliott. 2016b. Exposure of benthic invertebrates to sediment vibration: from laboratory experiments to outdoor simulated pile-driving. Proceedings of Meetings on Acoustics **27**.

Roberts, L., and M. E. Laidre. 2019. Finding a home in the noise: cross-modal impact of anthropogenic vibration on animal search behaviour. Biology Open **8**:bio041988.

Rogers, P. H., A. D. Hawkins, A. N. Popper, R. R. Fay, and M. D. Gray. 2016a. Parvulescu Revisited: Small Tank Acoustics for Bioacousticians. Advances in Experimental Medicine and Biology **875**:933-941.

Rogers, P. H., A. D. Hawkins, A. N. Popper, R. R. Fay, and M. D. Gray. 2016b. Parvulescu revisited: small tank acoustics for bioacousticians. Pages 933-941 The Effects of Noise on Aquatic Life II. Springer.

Salas, A. K., P. S. Wilson, and L. A. Fuiman. 2019. Ontogenetic change in predicted acoustic pressure sensitivity in larval red drum (*Sciaenops ocellatus*). J Exp Biol **222**.

Samson, J. E., T. A. Mooney, S. W. S. Gussekloo, and R. T. Hanlon. 2014. Graded behavioral responses and habituation to sound in the common cuttlefish Sepia officinalis. Journal of Experimental Biology **217**:4347-4355.

Sand, O., P. S. Enger, H. Karlsen, and F. Knudsen. 2001. Detection of infrasound in fish and behavioral responses to intense infrasound in Juvenile Salmonids and European Silver Eels: a minireview. American Fisheries Society Symposium **26**:183-193.

Sara, G., J. M. Dean, D. D'Amato, G. Buscaino, A. Oliveri, S. Genovese, S. Ferro, G. Buffa, M. Lo Martire, and S. Mazzola. 2007. Effect of boat noise on the behaviour of bluefin tuna *Thunnus thynnus* in the Mediterranean Sea. Marine Ecology Progress Series **331**:243-253.

Scholik, A. R., and H. Y. Yan. 2001. Effects of underwater noise on auditory sensitivity of a cyprinid fish. Hearing Research **152**:17-24.

Schulz-Mirbach, T., B. Metscher, and F. Ladich. 2012. Relationship between Swim Bladder Morphology and Hearing Abilities–A Case Study on Asian and African Cichlids. PLoS ONE **7**:e42292.

Secor, D. H., H. Bailey, A. Carroll, V. Lyubchich, M. H. P. O'Brien, and C. J. Wiernicki. 2021. Diurnal vertical movements in black sea bass (Centropristis striata): Endogenous, facultative, or something else? Ecosphere **12**:e03616.

Shashar, N., and R. T. Hanlon. 2013. Spawning behavior dynamics at communal egg beds in the squid *Doryteuthis (Loligo) pealeii*. Journal of Experimental Marine Biology and Ecology **447**:65-74.

Shelledy, K., A. G. Keller, B. Phelan, J. Rosendale, and J. A. Stanley. In Prep. Effects of replayed pile driving on the general behaviour of black sea bass (Centropristus striata) in a controlled environment. Marine Pollution Bulletin.

Shepherd, G. R., and M. Terceiro. 1994. The summer flounder, scup and black sea bass fishery of the Middle Atlantic Bight and Southern New England waters. National Marine Fisheries Service.

Simpson, S. D., A. N. Radford, S. Holles, M. C. O. Ferrari, D. P. Chivers, M. I. McCormick, and M. G. Meekan. 2016a. Small boat noise impacts natural settlement behaviour of coral reef fish larvae. Springer, New York.

Simpson, S. D., A. N. Radford, S. L. Nedelec, M. C. O. Ferrari, D. P. Chivers, M. I. McCormick, and M. G. Meekan. 2016b. Anthropogenic noise increases fish mortality by predation. Nat Commun **7**.

Skalski, J. R., W. H. Pearson, and C. I. Malme. 1992. Effects of Sounds from a Geophysical Survey Device on Catch-per-Unit-Effort in a Hook-and-Line Fishery for Rockfish (Sebastes spp.). Canadian Journal of Fisheries and Aquatic Sciences **49**:1357-1365.

Smith, M. E., A. S. Kane, and A. N. Popper. 2004a. Acoustical stress and hearing sensitivity in fishes: does the linear threshold shift hypothesis hold water? Journal of Experimental Biology **207**:3591-3602.

Smith, M. E., A. S. Kane, and A. N. Popper. 2004b. Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). Journal of Experimental Biology **207**:427-435.

Snyder, B., and M. Kaiser. 2009. Ecological and Economic Cost-Benefit Analysis of Offshore Wind Energy. Renewable Energy **34**:1567 - 1578.

Solé, M., M. Lenoir, M. Durfort, M. López-Bejar, A. Lombarte, and M. André. 2013. Ultrastructural Damage of Loligo vulgaris and Illex coindetii statocysts after Low Frequency Sound Exposure. PLOS ONE **8**:e78825.

Solé, M., P. Sigray, M. Lenoir, M. van der Schaar, E. Lalander, and M. André. 2017. Offshore exposure experiments on cuttlefish indicate received sound pressure and particle motion levels associated with acoustic trauma. Scientific Reports **7**:45899.

South East Data, A. a. R. 2018. South Atlantic Black Seabass Assessment Report. SEDAR, North Charleston, SC.

Southall, B. L., A. E. Bowles, W. T. Ellison, J. J. Finneran, R. L. Gentry, C. R. G. Jr., D. Kastak, D. R. Ketten, J. H. Miller, P. E. Nachtigall, W. J. Richardson, J. A. Thomas, and P. L. Tyack. 2009. Marine mammal noise exposure criteria: Initial scientific recommendations. The Journal of the Acoustical Society of America **125**:2517-2517.

Spiga, I., N. Aldred, and G. S. Caldwell. 2017. Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.). Marine Pollution Bulletin **122**:297-305.

Stanley, J. A., P. E. Caiger, B. Phelan, K. Shelledy, T. A. Mooney, and S. M. Van Parijs. 2020. Ontogenetic variation in the auditory sensitivity of black sea bass (*Centropristis striata*) and the implications of anthropogenic sound on behavior and communication. The Journal of Experimental Biology **223**:jeb.219683.

Stanley, J. A., S. M. Van Parijs, and L. T. Hatch. 2017. Underwater sound from vessel traffic reduces the effective communication range in Atlantic cod and haddock. Scientific Reports **7**:14633.

Stehlik, L. L. 2009. Effects of seasonal change on activity rhythms and swimming behavior of age-0 bluefish (*Pomatomus saltatrix*) and a description of gliding behavior.

Steimle, F. W., C. A. Zetlin, P. L. Berrien, and S. Chang. 1999. Essential Fish Habitat Source Document: Black Sea Bass, *Centropristis striata*, Life History and Habitat Characteristics. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, Massachusetts.

Stoner, A. W., A. J. Bejda, J. P. Manderson, J. P. Jeffrey P. Pessutti, B. A. Phelan, and L. S. Stehlik. 1999. Behavior of winter flounder, Pseudopleuronectes americanus, during the reproductive season: laboratory and field observations on spawning, feeding, and locomotion. Fisheries Bulletin **97**:999 - 1016.

Strobel, S. M., and T. A. Mooney. 2012. Detection of low-frequency tones and whale predator sounds by the American sand lance *Ammodytes americanus*. Journal of Fish Biology **81**:1646-1664.

Thomsen, F., K. Ludemann, R. Kafemann, and W. Piper. 2006. Effects of offshore wind farm noise on marine mammals and fish, biola, Hamberg, Germany on behalf of COWRIE Ltd.

Tsouvalas, A., and A. V. Metrikine. 2016. Structure-borne wave radiation by impact and vibratory piling in offshore installations: From sound prediction to auditory damage. Journal of Marine Science and Engineering **4**:44.

Voellmy, I. K., J. Purser, D. Flynn, P. Kennedy, S. D. Simpson, and A. N. Radford. 2014a. Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Animal Behaviour **89**:191-198.

Voellmy, I. K., J. Purser, S. D. Simpson, and A. N. Radford. 2014b. Increased Noise Levels Have Different Impacts on the Anti-Predator Behaviour of Two Sympatric Fish Species. PLoS ONE **9**:e102946.

Webb, J. F., and W. L. Smith. 2000. The laterophysic connection in chaetodontid butterflyfish: morphological variation and speculations on sensory function. Philosophical Transactions of the Royal Society of London B **355**:1125-1129.

Wiernicki, C. J., M. H. P. O'Brien, F. Zhang, V. Lyubchich, M. Li, and D. H. Secor. 2020. The recurring impact of storm disturbance on black sea bass (Centropristis striata) movement behaviors in the Mid-Atlantic Bight. PLoS ONE **15**:e0239919.

Williams, R., A. J. Wright, E. Ashe, L. K. Blight, R. Bruintjes, R. Canessa, C. W. Clark, S. Cullis-Suzuki, D. T. Dakin, C. Erbe, P. S. Hammond, N. D. Merchant, P. D. O'Hara, J. Purser, A. N. Radford, S. D. Simpson, L. Thomas, and M. A. Wale. 2015. Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean & Coastal Management **115**:17-24.

Wingfield, J., and R. Sapolsky. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology **15**:711-724.

Wright, K. J., D. M. Higgs, A. J. Belanger, and J. M. Leis. 2005. Auditory and olfactory abilities of pre-settlement larvae and post-settlement juveniles of a coral reef damselfish (Pisces: Pomacentridae). Marine Biology **147**:1425-1434.

Wright, K. J., D. M. Higgs, A. J. Belanger, and J. M. Leis. 2008. Auditory and olfactory abilities of larvae of the Indo-Pacific coral trout Plectropomus leopardus (Lacepède) at settlement. Journal of Fish Biology **72**:2543-2556.

Wright, K. J., D. M. Higgs, D. H. Cato, and J. M. Leis. 2010. Auditory sensitivity in settlement-stage larvae of coral reef fishes. Coral Reefs **29**:235-243.

Wright, K. J., D. M. Higgs, and J. M. Leis. 2011. Ontogenetic and interspecific variation in hearing ability in marine fish larvae. Marine Ecology Progress Series **424**:1-13.

Wysocki, L. E., A. Codarin, F. Ladich, and M. Picciulin. 2009. Sound pressure and particle acceleration audiograms in three marine fish species from the Adriatic Sea. Journal of the Acoustical Society of America **126**:2100-2107.

York, C. A., and I. K. Bartol. 2014. Lateral line analogue aids vision in successful predator evasion for the brief squid, Lolliguncula brevis. J Exp Biol **217**:2437-2439.

Zar, J. H. 1999. Biostatisical analysis. 3 edition. Prentice Hall, New Jersey.

Zeddies, D. G., R. R. Fay, M. D. Gray, P. W. Alderks, A. Acob, and J. A. Sisneros. 2012. Local acoustic particle motion guides sound-source localization behavior in the plainfin midshipman fish, *Porichthys notatus*. The Journal of Experimental Biology **215**:152-160.

A.1 Study Products List

Publications:

Jones, IT, Schumm, M, Stanley, JS, Hanlon, RT, **Mooney, TA**. Context is key: squid reproductive behaviours and spawning withstand wind farm pile driving noise. Submitted.

Jones, I. T., Peyla, J. F., Clark, H., Song, Z., Stanley, J. A., Mooney, T. A. (2021). Changes in feeding behavior of longfin squid (*Doryteuthis pealeii*) during laboratory exposure to pile driving noise. *Marine Environmental Research*. *165*. https://doi.org/10.1016/j.marenvres.2020.105250.

Jones, I. T., Stanley, J. A., Mooney, T. A. (2020). Impulsive pile driving noise elicits alarm responses in squid (*Doryteuthis pealeii*). *Marine Pollution Bulletin*, *150*. 110792. https://doi.org/10.1016/j.marpolbul.2019.110792

Mooney, TA, Andersson, MH, and Stanley, J. 2020. Acoustic impacts of offshore wind energy on fishery resources. Oceanography. Special issue on understanding the effects of offshore wind energy development on fisheries. 33(4): 82-95. doi: www.jstor.org/stable/26965752.

Stanley, J, Caiger, PE, Mooney, TA, Phelan, B, Shelledy, K, and Van Parijs, SM. 2020. Ontogenetic variation in the hearing sensitivity of black sea bass (*Centropristis striata*) and the implications of anthropogenic sound on behavior and communication. *Journal of Experimental Biology*. 223: jeb219683 doi: 10.1242/jeb.219683

Jones, I. T., Stanley, J. A., Bonnel, J., Mooney, T. A. (2019). Complexities of tank acoustics warrant direct, careful measurement of particle motion and pressure for bioacoustic studies. *Proceedings of Meetings on Acoustics*, *37*, 010005. https://doi.org/10.1121/2.0001073

Presentations:

Stanley, J, Caiger, P, Shelledy, K, Phelan, B., van Parijs, S., Mooney, TA Hearing in black sea bass (*Centropristis striata*) and the effects of pile driving signals on their behaviour in controlled environments. New Zealand Marine Sciences Society Annual Conference. Tauranga, New Zealand. July, 2021.

Schumm M, Jones I, Mooney, TA. The effect of pile driving noise on mating behavior in squid (*Doryteuthis pealeii*). Ocean Sciences Meeting, San Diego, Ca, USA, February 16-21, 2020.

Jones, I. T. Impacts of underwater construction noise on defense, feeding, and reproductive behaviors of longfin squid. Biology Department Seminar, Woods Hole, MA, USA. February 6, 2020.

Jones, I. T., Stanley, J. A., Mooney, T.A. Influences of pile driving noise exposure on feeding and reproductive behaviors of longfin inshore squid (*Doryteuthis pealeii*). 5th International Conference on the Effects of Noise on Aquatic Life, Dan Haag, The Netherlands. July 7-2, 2019.

Jones, I. T., Stanley, J. A., Mooney, T.A. Assessing impacts of offshore pile driving noise on the antipredator defense and shoaling behaviors of squid (*Doryteuthis pealeii*). 176th Meeting of the Acoustical Society of America, Victoria, Canada. November 5-9, 2018.

Jones, I. T., Stanley, J. A., Mooney, T.A. Offshore pile driving noise elicits alarm responses in longfin squid (*Doryteuthis pealeii*). 148th Meeting of the American Fisheries Society, Atlantic City, NJ, USA. August 19-23, 2018.

Shelledy, K., Phelan, B., Stanley, J. A., Black Sea Bass (*Centropristis striata*) behavioral response to pile-driving in small tank environment. 148th Meeting of the American Fisheries Society, Atlantic City, NJ, USA. August 19-23, 2018.

Mooney, TA. Loud and clear: Cephalopod behavioral responses, predator evasion and habituation to sounds. WHOI Acoustics Seminar Series. June 14, 2018.

Schumm, M, Jones, I, Mooney, TA. The effect of pile driving noise on mating behavior in squid. WHOI Biology Department Summer Student Research Forum. Woods Hole, MA. August 8, 2019. (speed talk and poster)

Stanley, J, Caiger, P, Shelledy, K, Mooney, TA, Phelan, B, and van Parijs, S. Hearing in black sea bass (*Centropristis striata*) and the effects of pile driving signals on their behaviour in controlled environments. 5th Conference on the Effects of Noise on Aquatic Life. Den Haag, Netherlands. 7-12 July, 2019.

Reese, DN, Caruso, F, Jones, I, Perelman, J, and Mooney, TA. Analysis of *Doryteuthis pealeii* Squid Locomotion Using a Novel Bio-logging Tag: Effects of Attachment, Water Flow, and Noise. Marine Biological Laboratory Research Experience for Undergraduates, Woods Hole, Ma, USA. August 17 2018. (talk and poster)

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).