

# Ontogenetic variation in the hearing sensitivity of black sea bass (*Centropristis striata*) and the implications of anthropogenic sound on behavior and communication

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## Summary statement

This research provides the first published information on the hearing abilities of the black sea bass, *Centropristis striata*, an ecologically, recreationally and commercially important fish species.

## Abstract

Black sea bass (*Centropristis striata*) is an important fish species in both commercial and recreational fisheries of southern New England and the mid-Atlantic Bight. Due to the intense urbanization of these waters, this species is subject to a wide range of anthropogenic noise pollution. Concerns that *C. striata* are negatively affected by pile driving and construction noise predominate in areas earmarked for energy development. However, as yet, the hearing range of *C. striata* is unknown, making it hard to evaluate potential risks. This study is a first step in understanding the effects of anthropogenic noise on *C. striata* by determining the auditory bandwidth and thresholds of this species using auditory evoked potentials (AEPs), creating pressure and acceleration audiograms. These physiological tests were conducted on wild-caught *C. striata* in three size/age categories. Results showed that juvenile *C. striata* significantly had the lowest thresholds, with hearing sensitivity decreasing in the larger size classes. Furthermore, *Centropristis striata* has fairly sensitive hearing relative to other related species. Preliminary investigations into the mechanisms of their hearing ability were undertaken with gross dissections and an opportunistic micro computed tomography image to address the auditory structures including otoliths and swimbladder morphology. Crucially, the hearing range of *C. striata*, and their most sensitive frequencies, directly overlap with high-amplitude anthropogenic noise pollution such as shipping and underwater construction.

## Introduction

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 (Kight and Swaddle, 2011; National Research Council, 2003; Popper and Hastings, 2009). 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., 2004), 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., 2014), predator avoidance (Simpson et al., 2016b), group cohesion (Bruitjes and Radford, 2013; Sara et al., 2007), 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 (Dvorak et al., 2013; Musial and Butterfield, 2004; Snyder and Kaiser, 2009) 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 (Bruitjes et al., 2016), 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).

A principal target species in the western North Atlantic is black sea bass (*Centropristis striata* Linnaeus 1758). 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 (SEDAR, 2018). There is some circumstantial evidence that *C. striata* communicate acoustically (Fish and Mowbray, 1970), and potentially during spawning events. 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, 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 measure neural responses in a subject to a given sound stimulus, it permits rapid evaluation of hearing 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 hearing 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' hearing 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., 2016), 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 hearing 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 hearing ability through ontogeny (e.g., Caiger et al., 2013; Higgs et al., 2002).

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 (Radford et al., 2013; Webb and Smith, 2000). 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 hearing range 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 bandwidth or thresholds vary with size or age were assessed by testing hearing across three size groups, from juvenile to adult. Additionally, the mechanism responsible for the hearing ability in this species was investigated via gross dissections and micro CT imaging of the internal morphology. The potential implications for the assessed hearing range were discussed in relation to the pervasive anthropogenic noises that share acoustic space with this species.

## Methods

### 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 ( $14^{\circ}\text{C} \pm 2^{\circ}$ ) to eliminate any potential temperature effects on hearing 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.

### Auditory evoked potentials

Hearing 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 hearing 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 (Caiger et al., 2013; Higgs et al., 2002; Strobel and Mooney, 2012; Wright et al., 2005).

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 hearing 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.

Auditory stimuli were digitally generated using custom Labview software (National Instruments; [www.ni.com](http://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](http://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  $\mu$ Pa 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 Fig. 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).

### **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 1V/ $\mu$ Pa) (Teledyne Marine), an HTI-96 Min Series Hydrophone (High Tech Inc.) (sensitivity -165 dB re 1V/ $\mu$ Pa) 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.

### **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).

### **Micro Computer 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  $\mu$ m in reflection mode and 2  $\mu$ m 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  $\mu$ A 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. Extremely care was taken to observe any connective tissues or musculature leading to the inner ear region.

### **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 hearing 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.

SPL<sub>z-pk</sub> and zero-to-peak sound acceleration levels (SAL<sub>z-pk</sub>) 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. SPL<sub>z-pk</sub> and SAL<sub>z-pk</sub> were calculated as:

$$\text{SPL}_{z-pk} \text{ or } \text{SAL}_{z-pk} = 20 * \log_{10} \left( \frac{X_{peak}}{X_0} \right)$$

with  $X_0 = 1 \text{ uPa}$  or  $1 \text{ m s}^{-2}$  respectively

where  $X_{peak}$  was the maximum absolute  $\mu\text{Pa}$  or  $\text{m s}^{-2}$  over a given measurement period, for pressure and acceleration respectively, with units of dB re 1  $\mu\text{Pa}$  and dB re 1  $\text{m s}^{-2}$  for SPL<sub>z-pk</sub> and SAL<sub>z-pk</sub>, respectively. To quantify sound energy distribution over frequencies from 20-20000 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.

## Results

### Hearing 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, 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 1  $\mu\text{Pa}$  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 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, which ranged from 75–116 dB re 1 $\mu$ Pa. 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 (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 1 $\mu$ Pa, 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. Fish in the Large class were found to be the least sensitive, ranging from 90 – 108 dB re 1 $\mu$ Pa 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 in all size/age classes, 150, 200 and 100 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 from 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* (Fig. 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).

### **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 1 $\mu$ Pa) with an inter-pulse interval of 1.46 s  $\pm$  0.092 SE (over 30 minutes of activity). This was within the range which *C. striata* had the greatest sensitivity to sound pressure (Fig. 4). The recording had a median  $X_{z-pk}$  of 187.8 dB re 1 $\mu$ Pa and 14.1 dB re 1 m s<sup>-2</sup>.

## **Discussion**

### **Hearing in *Centropristis striata***

This study represents the first published record of the hearing ability of *C. striata*. The audiogram of *C. striata* ranged from 80 to 1000 Hz, with the most sensitive hearing 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, hearing sensitivity 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., 2003; 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 hearing sensitivity, relative to other fishes without specializations *C. striata* appears to have good hearing. At the most sensitive frequency (150 Hz) the mean threshold was ~75-90 dB, 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 hearing 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  $\mu$ Pa. 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  $\mu$ Pa, which is dependent not only on species, but also on age and the design of AEP setup (Ladich and Fay, 2013; Popper and Fay, 2011). 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 hearing is predominantly particle motion derived at the lower frequencies, which is well documented (Popper and Hawkins, 2019). However, the only way to completely remove 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 acousticolateralis 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 hearing thresholds were 50 dB less sensitive in the 150 – 300 Hz range, the acoustic signal from pile driving activity would still be detectible.

### ***Ontogenetic variation in hearing ability***

Hearing sensitivity decreased with increasing size in *C. striata*. The negative correlation with *C. striata* size class and hearing 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 (Caiger et al., 2013; Kenyon, 1996; Schulz-Mirbach et al., 2012), 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 showed no defined projections but with evidence of projections beginning to form, 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 also 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).

### **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 hearing 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 hearing 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 (Garabon and Higgs, 2017; Higgs and Radford, 2013). 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 hearing ability in a species. Further work to better understand the species true sensitivities and/or their behavioral thresholds could include treatments that separate pressure from particle motion (i.e. using shaker tables), behavioral conditioning using very large tanks, or preferably, *in situ* fish cages (Popper et al., 2019).

### **Ecological implications: What the overlap between hearing range and pile driving means for *Centropristis 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. The construction of a single wind farm off Block Island, Rhode Island in 2016 marked the first commercial

offshore wind farm in the US (<http://dwwind.com/project/block-island-wind-farm/>). 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 lease block sold to Vineyard Wind 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). Further lease areas in Massachusetts waters, as well as off New York, New Jersey, Maryland, Delaware and Virginia are under review and are at various stages along the process towards offshore wind energy development.

Construction noise, especially that produced through pile driving of the foundations and support structure of wind turbines, and the disruption of the bottom sediment layers could be significant when the scope of the east coast development is considered. Impact pile driving is a loud, high-energy, impulsive sound and is widely used for marine construction, including wind energy development, bridges, marinas, harbors and docks, and other offshore structures (Dahl et al., 2015; Popper and Hawkins, 2019). The striking of the hammer to the pile results in vibration of the pile in the water and vibration in the substrate. 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  $\mu$ Pa at a range of ~10 m, 200 dB re 1  $\mu$ Pa at a range of 300 m from 0.75 m and 5 m diameter piles respectively (Lippert and Estorff, 2014; Reinhall and Dahl, 2011). 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 1m 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  $\mu$ Pa (tetrahedral hydrophone array) from one hammer strike, 500 m from the activity (Miller et al., 2018). Much less is known about the possible effects of the continuous sound produced by the operating wind farms (Cheesman, 2016). The reported sound levels of operation are generally below 700 Hz and centered around 180 Hz, however, like pile driving, sound levels vary substantially dependent on a number of factors including power rating, wind speed and substrate type etc. (Pangerc et al., 2016; Sigray and Andersson, 2011). All operational sounds reported are relatively low in sound level compared to that of pile driving, although within the frequency range and intensity thresholds seen currently of *C. striata* hearing.

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 (e.g., increased stress response consequences), behavior (e.g., changes in migration routes, feeding or breeding grounds), cause physical injury (e.g., temporary or permanent hearing impairment, barotrauma), and/or acoustic masking (e.g., intraspecific communication, cues for orientation) (see detailed review by Popper and Hawkins, 2019). 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 suggests that the most sensitive range of this species' hearing directly overlaps with the highest sound energy created from pile driving activity. This suggests *C. striata* will be able to hear this noise in many circumstances. However, extensive behavioral and physiology

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.

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## **Competing interests**

No competing interest declared.

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## **Data availability**

All data is available upon request.

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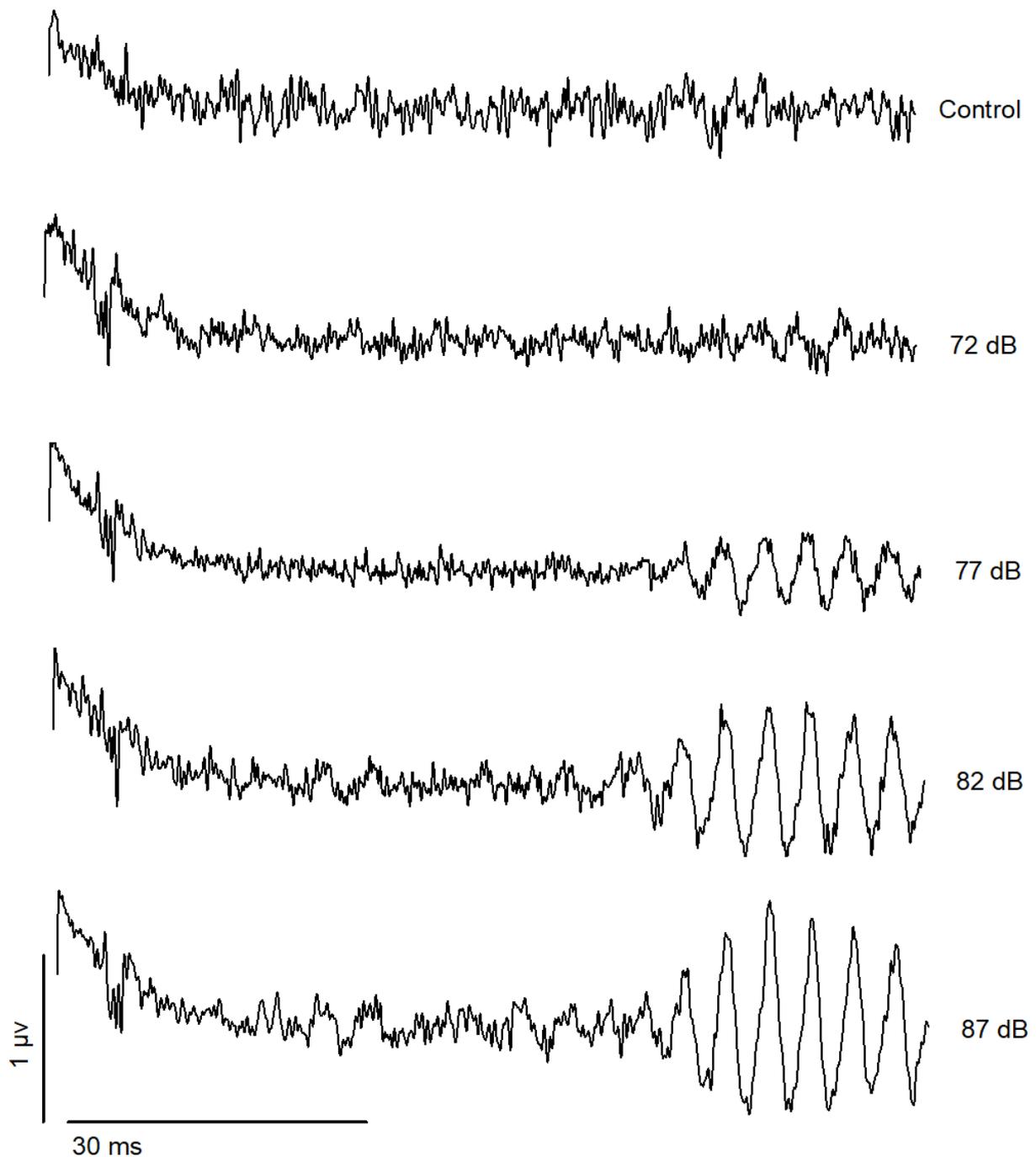
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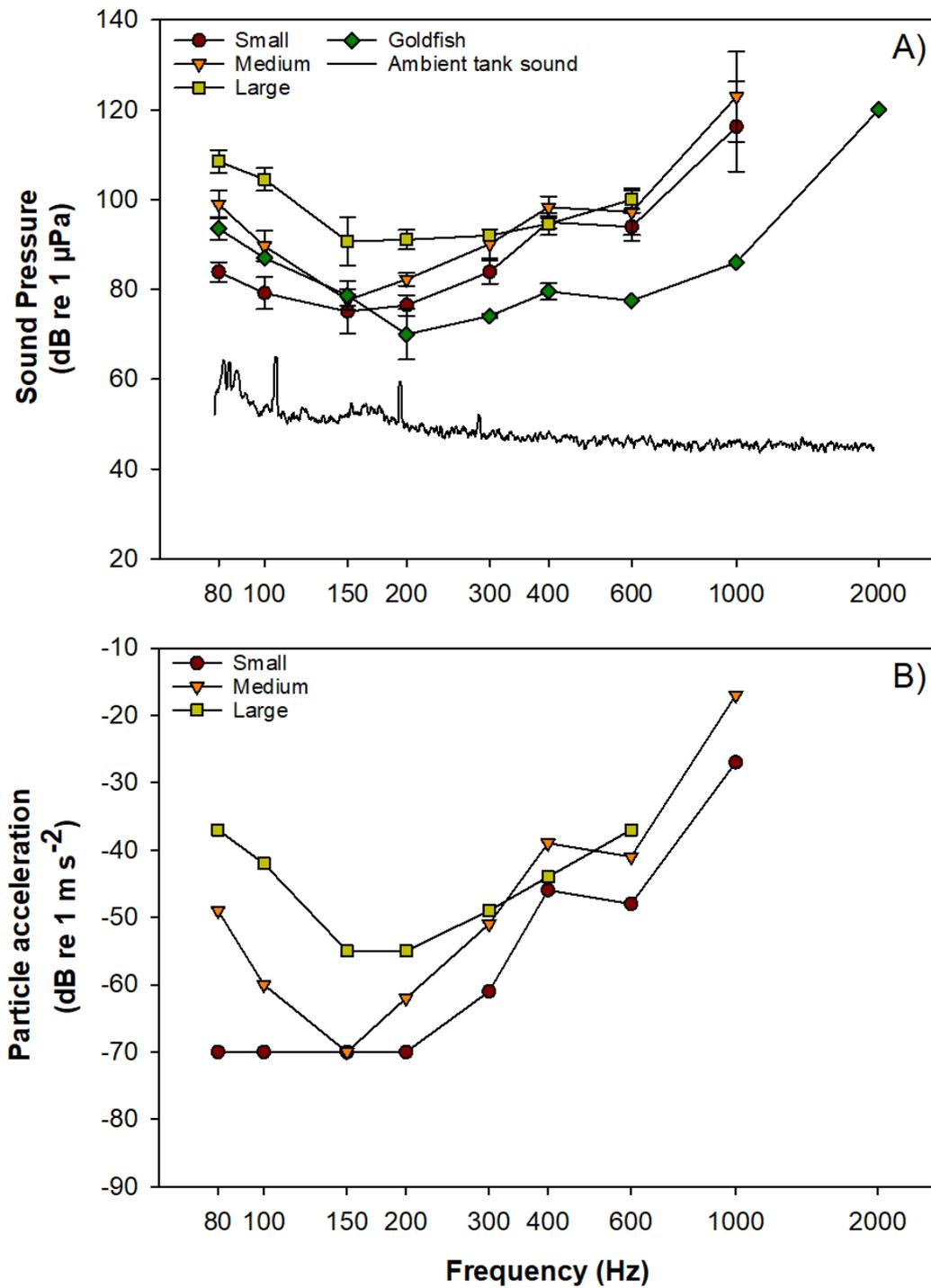
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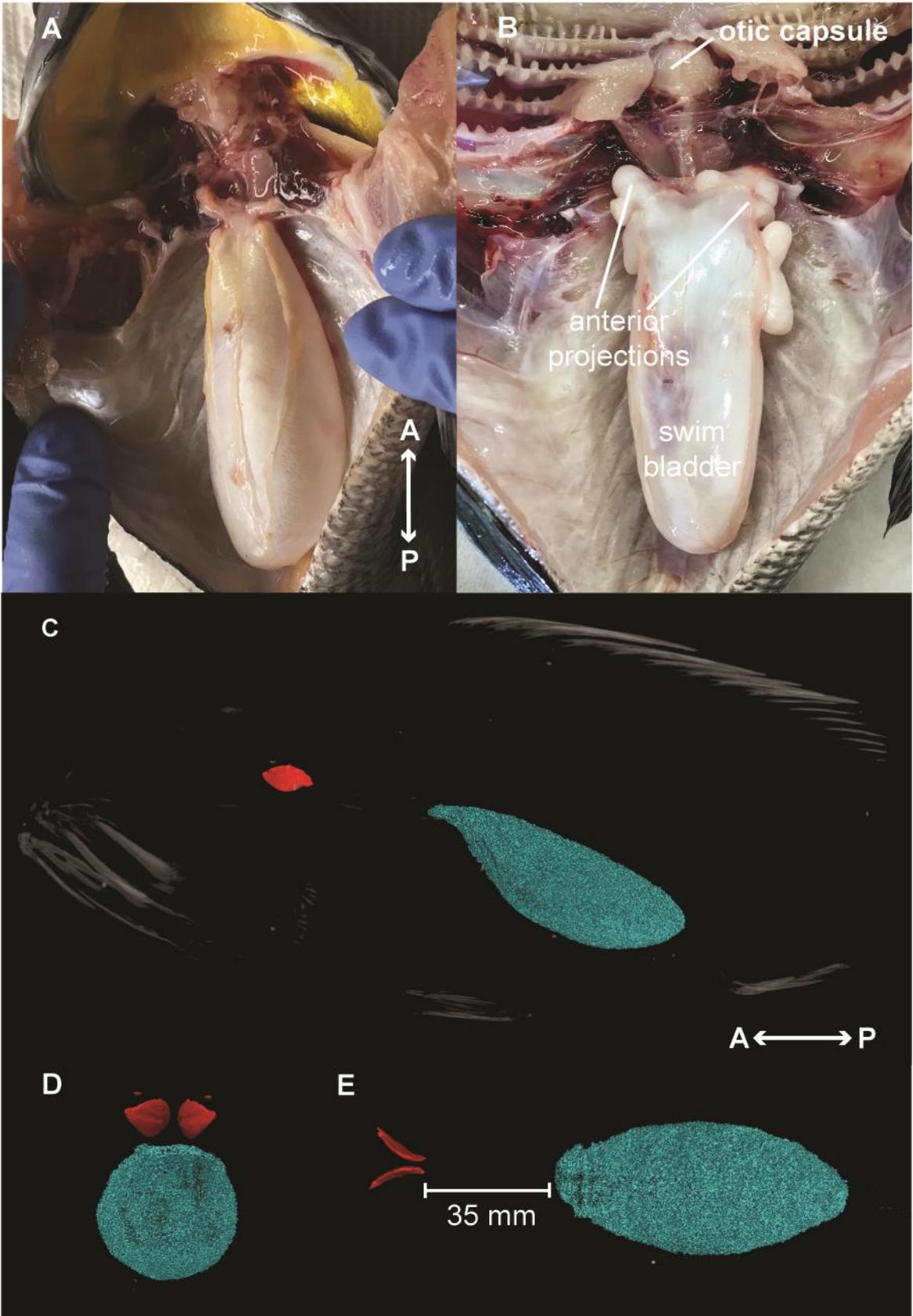
## Figures



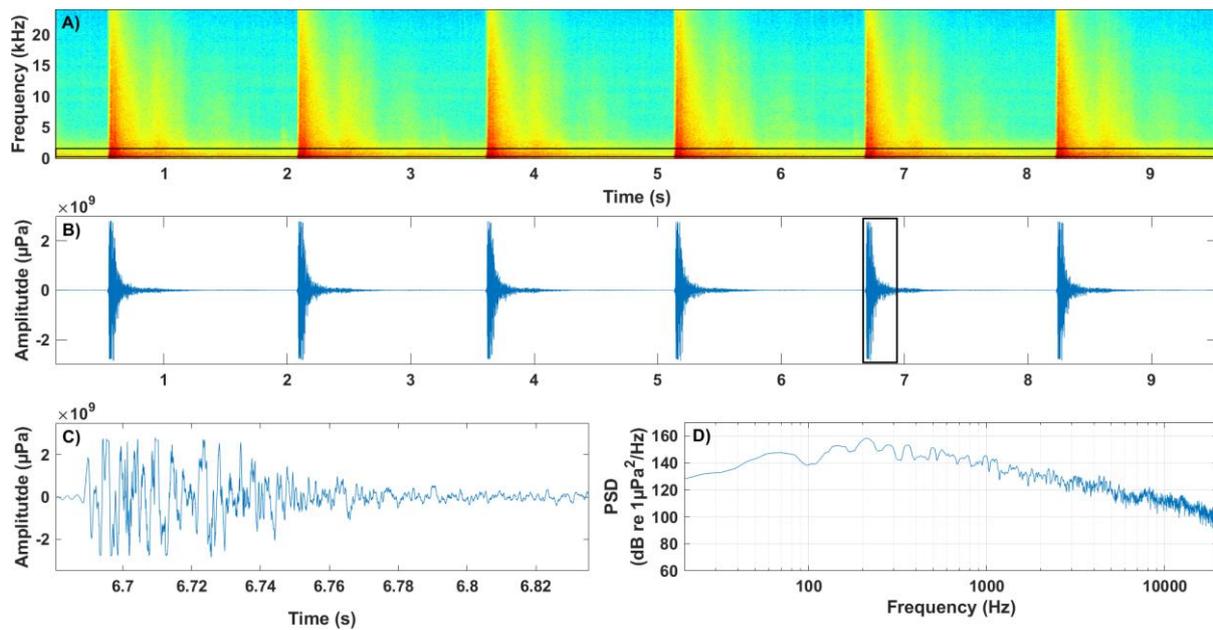
**Figure 1. Example of auditory evoked potential waveforms from an individual *Centropristis striata* (93 mm TL) from the Small size class, in response to sound stimulus of 100 Hz pure tone bursts.** The lowest sound pressure level to show a definitive response occurred at 77 dB in this example. Stimulus 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.



**Figure 2. Sound pressure levels and measured particle acceleration at auditory thresholds of *Centropomus striata* (n=20) and *Carassius auratus* (n=4). A)** Mean ( $\pm$ 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.



**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 hearing in *Centropristus striata*, **B)** wave form of events, **C)** increased time resolution of waveform to illustrate one pulse, **D)** power spectral density (PSD) of impact driving event, red box indicates most sensitive hearing range. 48 kHz sampling rate, spectrogram computed using a 1024-point fast Fourier transform (FFT), Hann-window, 80% overlap.