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Effects of exposure to pile driving sounds on fish inner ear tissues

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ABSTRACT

Impulsive pile driving sound can cause injury to fishes, but no studies to date have examined whether such injuries include damage to sensory hair cells in the ear. Possible effects on hair cells were tested using a specially designed wave tube to expose two species, hybrid striped bass (white bass *Morone chrysops* × striped bass *Morone saxatilis*) and Mozambique tilapia (*Oreochromis mossambicus*), to pile driving sounds. Fish were exposed to 960 pile driving strikes at one of three treatment levels: 216, 213, or 210 dB re 1 $\mu Pa^2 \cdot s$ cumulative Sound Exposure Level. Both hybrid striped bass and tilapia exhibited barotraumas such as swim bladder ruptures, herniations, and hematomas to several organs. Hybrid striped bass exposed to the highest sound level had significant numbers of damaged hair cells, while no damage was found when fish were exposed at lower sound levels. Considerable hair cell damage was found in only one out of 11 tilapia specimens exposed at the highest sound level. Results suggest that impulsive sounds such as from pile driving may have a more significant effect on the swim bladders and surrounding organs than on the inner ears of fishes, at least at the sound exposure levels used in this study.

1. Introduction

Sound provides fishes, as other animals, with a substantial amount of information about their environment (Fay and Popper, 2000; Fay, 2009; Slabbekoorn et al., 2010; Ladich and Fay, 2013). This “acoustic scene” is an acoustic “view” of their environment that often extends for distances well beyond the range of other senses. Indeed, it has been suggested that hearing evolved in fishes (and thus in vertebrates) to extend the animals' spatial range of sensory input and increase chances for detecting predators and prey, and for increasing awareness of general acoustic cues in the environment (Popper and Fay, 2011). Thus, anything that interferes with the ability of an animal to detect and use its acoustic scene is likely to decrease fitness and the chance of survival.

While exposure to lower sound levels may interfere with detection of the acoustic scene by masking (Slabbekoorn et al., 2010), exposure to high intensity sound has the potential to damage sensory hair cells of the inner ears of vertebrates, thereby temporarily or permanently impairing detection of all sounds (Hu, 2012). This damage has been widely documented in birds and mammals (Rubel et al., 2013), but damage also occurs in some fish species (Enger, 1981; Hastings et al., 1996; McCauley et al., 2003; Smith et al., 2004a,b; Smith, 2012). However, unlike in mammals, fishes and birds are able to repair and/or replace damaged hair cells (Lombarte et al., 1993; Lombarte and Popper, 1994; Smith et al., 2004a,b, 2006; Schuck and Smith, 2009; Smith et al., 2011; Smith, 2012; Rubel et al., 2013). Fishes exhibit a constant addition of hair cells over their lives (Corwin, 1981; Popper and Hoxter, 1984; Lombarte and Popper, 1994, 2004). It is currently unclear how the process of regeneration following damage differs from the process of normal hair cell production during growth in fishes (Lanford et al., 1996; Presson et al., 1996). Although fishes can regenerate lost hair cells following trauma to their inner ears, the process of hair cell and functional hearing recovery takes at least seven days following the insult (Smith et al., 2004a, 2006; Schuck and Smith, 2009). How such hearing loss might affect the survival and fitness of fishes during this recovery time has not been directly tested.

There is evidence that exposure to high intensity sound sources, such as low and mid-frequency sonars and seismic air guns, does not necessarily result in damage to sensory hair cells in fishes, at least at the sound levels used in previous studies (Popper et al., 2007; Song et al., 2008; Kane et al., 2010). Still, generalizations about the potential impact of high intensity sounds on fish ears are impossible to make since there are so few relevant data and no clear indication as to the characteristics of sound that might result in inner ear damage. Moreover, based on the inter-specific variation seen in temporary hearing threshold shifts (TTS) resulting from loud sounds, it is possible that

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hair cell damage resulting from exposure to loud sounds will also be species-specific (Popper et al., 2005, 2007).

The biggest concern for potential harm to the tissues of the fish ear comes from exposure to seismic air guns used in off-shore oil and gas exploration and impact pile driving used in the construction of off-shore wind farms and oil and gas platforms. Both seismic air guns and impact pile driving are capable of producing sound levels that far exceed 200 dB re 1 μPa RMS close to the source, and both exhibit very sharp rise times (onset times) (Normandeau Associates, Inc., 2012a,b).

A number of recent studies have documented that exposure to multiple pile driving strikes can result in substantial damage to internal organs of fishes including the swim bladder, liver, kidney, and gonads (Halvorsen et al., 2011; Casper et al., 2012; Halvorsen et al., 2012a,b; Casper et al., in press). To date, however, no studies examined the effects of exposure to pile driving on tissues of the inner ear. Thus, the purpose of this study was to examine the effects of pile driving sounds on the inner ear tissues in two species, the hybrid striped bass (white bass *Morone chrysops* × striped bass *Morone saxatilis*, Moronidae) and the Mozambique tilapia (*Oreochromis mossambicus*, Cichlidae). Previous studies have shown tissue damage as a result of exposure to intense pile driving sounds in hybrid striped bass and in Nile tilapia (*O. niloticus*, Cichlidae), a member of the same genus as the Mozambique tilapia (Halvorsen et al., 2012b; Casper et al., in press).

Both species were exposed to high intensity sounds in a specially designed lab-based device, the HICI-FT (High Intensity Controlled Fluid-filled wave Tube), that allows presentation of free field pile driving sounds that are equivalent to those that a fish might experience at 10 m from an actual pile driving operation. The fishes were examined for evidence of barotrauma damage to external and internal tissues as well as for any potential effects on the sensory cells of the inner ear.

2. Materials and methods

2.1. Fish

Hybrid striped bass (white bass *Morone chrysops* × striped bass *Morone saxatilis*, Moronidae; 80.6 ± 6.5 mm SL and 9.8 ± 2.4 g) were obtained in June–November, 2011 from Keo Fish Farms, Inc. (Keo, AR, USA). The Mozambique tilapia (*Oreochromis mossambicus*, Cichlidae; 72.9 ± 7.6 mm SL and 11.9 ± 3.2 g) were obtained in January–February, 2012 from a breeding colony in the laboratory of Dr. Thomas Kocher of the Department of Biology at University of Maryland. Fishes were acclimated for a minimum of two weeks following arrival in the lab and before being used in experiments. Fishes were maintained on a 14:10 light/dark cycle in 890-L round tanks. The hybrid striped bass were maintained at 18 °C and the Mozambique tilapia at 28 °C. Fishes scheduled for experiments were not fed prior to a treatment so that their digestive systems would be void of food during sound exposure. Experiments were conducted under supervision and approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland (protocol #R-09-23).

2.2. Pile driving exposure equipment and signal presentation

Exposure to pile driving sounds was done using the HICI-FT. The HICI-FT, which is described in detail by Halvorsen et al. (2011, 2012a), is a 45 cm long, 25 cm internal diameter water-filled cylindrical holding chamber with 3.81 cm-thick stainless steel walls. Large shakers on either end of the chamber create sounds that accurately reproduce the acoustic characteristics and sound levels of pile driving under far-field plane wave acoustic conditions.

Signal generation and data acquisition for the HICI-FT are also described in detail in Halvorsen et al. (2011, 2012a). The pile driving sounds used in this study were derived from field recordings taken at a range of 10 m from a 76.2 cm steel shell pile (outer diameter) driven using a diesel hammer at the Eagle Harbor Maintenance Facility (MacGillivray and Racca, 2005). Eight different recordings of pile driving strikes were normalized to the same sound exposure level (SEL) with twelve repetitions of each of the 8 strikes used to generate a file of 96 strikes that were randomized each day using MATLAB (The MathWorks, Inc., Natick, MA, USA). That file was then repeated 10 times for a 960-strike presentation and used for all exposures.

2.3. Fish exposure

Four fish for each exposure or control treatment were allowed to acclimate in an acrylic chamber mounted around the opening of the HICI-FT exposure chamber for 20 min. Following acclimation, the fish were gently corralled into the exposure chamber which was then sealed, the acrylic chamber was drained, and the HICI-FT was rotated into the horizontal position for each exposure or control treatment.

Following the completion of each treatment fish were removed from the HICI-FT and either immediately necropsied for physiological effects to non-auditory tissues (barotrauma assessment) or returned to their tanks for a recovery period of 2 days before necropsy. The 2 days post-exposure was selected on the basis of a pilot study designed to determine the extent of hair cell damage at 0, 2, 5, and 10 days post-exposure (recovery periods based on previous pile driving, barotrauma injury recovery studies) (Casper et al., 2012, in press). Results showed unequivocally that at day 0, hair cell damage had not yet appeared, while fish necropsied on days 5 and 10 already showed evidence of hair cell recovery, thereby making it difficult to assess the extent of inner ear damage. Therefore day 2 post-exposure, which yielded clear evidence of hair cell injury, was designated as the recovery time period for these experiments and will be the time period analyzed in the results and discussion.

During this recovery time, fish were fed on their normal schedules (three days per week). Buoyancy was documented in all fishes as done in previous studies (Halvorsen et al., 2011; Casper et al., 2012; Halvorsen et al., 2012a,b; Casper et al., in press) and both species always displayed neutral buoyancy, indicating that the swim bladder was filled during sound exposure. Throughout the study there was no evidence of a lack of feeding or abnormal swimming behavior in any of the exposed or control fish during the recovery periods.

In total, 164 hybrid striped bass (132 exposed, 32 control) and 28 Mozambique tilapia (14 exposed, 14 control) were used. Control fish were subject to the identical process as exposed fish but without the pile driving sound. The highest exposure sound levels (treatment 1) for each species began with a cumulative sound exposure level (SEL_{cum}) over the course of 960 pile strikes of 216 dB re 1 $\mu Pa^2 \cdot s$, with a single strike sound exposure level (SEL_{ss}) of 186 dB re 1 $\mu Pa^2 \cdot s$. The SEL_{cum} and SEL_{ss} were decreased in 3 dB steps for each subsequent treatment of 960 pile strikes as summarized in Table 1. From here forward, the

Table 1
Experimental design showing sample sizes and cumulative and single strike sound exposure level (SEL_{cum} and SEL_{ss}) for each of the three treatments.

Treatment name	SEL_{cum}	SEL_{ss}	pile strikes	Hybrid striped bass n	Mozambique tilapia n
Treatment 1	216 dB re 1 $\mu Pa^2 \cdot s$	186 dB re 1 $\mu Pa^2 \cdot s$	960	16 exp/ 6 con	11 exp/ 14 con
Treatment 2	213 dB re 1 $\mu Pa^2 \cdot s$	183 dB re 1 $\mu Pa^2 \cdot s$	960	102 exp/ 20 con	n/a
Treatment 3	210 dB re 1 $\mu Pa^2 \cdot s$	180 dB re 1 $\mu Pa^2 \cdot s$	960	16 exp/ 6 con	n/a

exp = exposed; con = control.