THE EFFECTS OF UNDERWATER NOISE ON AUDITORY SENSITIVITY OF FISH

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1. ABSTRACT

The underwater acoustic environment is inherently loud as a result of ambient sounds and an increasing amount of noise from anthropogenic sources. The report summarizes two sets of experiments which examined how white noise and boat engine noise impact auditory physiology of the fathead minnow (Pimephales promelas), a freshwater fish that is widely distributed in the North America. The results show that threshold elevation and recovery after noise exposure are frequency and exposure duration dependent.

2. INTRODUCTION

The auditory system is one of the most important sensory systems for an aquatic animal because it provides a multitude of information about the environment (e.g. prey items, competitors, predators, and potential mates) [1]. In addition to these sources, it has been hypothesized that fish may be listening to ambient sounds, created from sound scattering objects, to interpret changes in their acoustic environment. These subtle changes in ambient noise levels may be as important to a fish as the sounds they use for communication [2].

The underwater acoustic environment is inherently loud and has the potential to interupt important acoustic signals for an organism or degrade its ability to receive these signals. Many origins of sound result from ambient sources, but the primary concern is due to the increasing amount of noise generated from anthropogenic sources [3]. In addition, most human activities associated with the underwater acoustic environment produce noise with low frequency components less than 1.0 kHz [3]. These low frequency sounds are not only in the hearing range of most fish, but also in their most sensitive hearing frequency range [2, 4-6]. Thus, it is imperative to better understand how underwater noise affects auditory sensitivity of fish, since their ability to accurately interpret the acoustic environment is essential for survival.

A major source of underwater noise for fish comes from commercial vessels and recreational boats, which is due to their expansive distribution and increasing numbers [7]. The National Marine Manufactures Association [8] reported, in 1999, that there were over 12.7 million recreational boats registered in the United States alone, and Greene and Moore [7] report that even small boats with large outboard motors can produce sound pressure levels in excess of 175 dB (re: 1μPa). In addition, commercial shipping, in the Northern Hemisphere, has been implicated in increasing oceanic noise levels by 10-100 fold [9].

The primary source of noise associated with boats and vessels largely comes from high-speed engines and propellers. Sources of noise generated strictly from propeller rotation are relatively low in frequency and depend on several factors including number of blades and rotation speed [10]. In addition to these low frequency sounds, cavitation, from bubbles being produced from the movement of the propeller in the water, contributes greatly to the boat's noise production. Noise created by cavitation usually results in higher frequencies [10].

There is very little data on how these sounds and others sources (e.g. geophysical surveys, sonar, underwater explosions, dredging, construction, hydroelectric dams, and power plants) affect the inner ear and auditory sensitivity of aquatic animals, especially fish. Most of the studies have focused, specifically, on the anatomical effects of noise exposure on the fish inner ear [11-12}. However, the direct relationship between anatomical damage and auditory sensitivity is still unclear. To this date, no studies, in fish, have correlated shifts in auditory threshold with damage to the inner ear.

Until recently, only one study examined effects of noise on auditory threshold in fish. Popper and Clarke [13] used the goldfish (*Carassius auratus*) as a model species to measure, behaviorally, changes in auditory threshold after exposure to pure tone sounds. This study provided information about the effects of 4 hours of noise exposure (149 dB re: 1μPa), but only examined two auditory frequencies (0.5 and 0.8 kHz). Temporary threshold shifts were observed immediately after exposure, but were found to have returned to pre-exposed threshold levels within 24 hours.

From the aforementioned studies, it is obvious that our understanding of how noise affects fish hearing and the inner ear is rather limited. Issues like the characteristics and sources of noise, the effects of exposure duration, and recovery time after noise exposure are just a few issues that are crucial to the understanding of noise-induced hearing loss in fish. Our studies [14-15] try to address these issues by using the auditory brainstem response (ABR) technique and the fathead minnow (*Pimephales promelas*) as a model species.

The purpose of these experiments were threefold in design: 1) to examine immediate effects of white noise exposure on auditory sensitivity with varying exposure times (1-24 hrs), 2) to see if recovery was possible after exposure to this white noise (1-14 days), and 3) to look at a source of noise, i.e., boat engine noise**,** found in the fathead minnow's natural environment and its effects on hearing thresholds.

3. MATERIALS AND METHODS

Two different types of noise were used for playback: artificial, computer generated white noise and noise from a 55 horsepower outboard boat engine recorded in the field (Bullock Pen Lake in Northern Kentucky). White noise is defined as a broadband noise in which all frequencies in the noise spectrum are of the same sound pressure level [16]. Both, white noise and boat engine noise, were played to the fish at 142 dB (re: 1μPa) so the results could be compared. The white noise had sound pressure levels that were equal at all frequencies (0.3-4.0 kHz), while the boat engine noise had varying pressure levels at different frequencies (peak at 1.3 kHz).

3.1 The Fathead Minnow as a Model Species

The fathead minnow is an ideal fish model species for studying the effects of noise exposure on hearing thresholds for several reasons. First, the fathead minnow is a cosmopolitan species found in a variety of habitats with its natural range extending from southern Canada to southern United States [17]. As a result of the fathead minnow's wide distribution, it has the potential to be exposed to a variety of different acoustic environments. Secondly, the fathead minnow is considered a hearing specialists, i.e. it has enhanced auditory sensitivity (wide frequency range and low hearing threshold) due to the presence of accessory structures, the Weberian ossicles coupling the inner ear and gasbladder [18]. Therefore, it has the ability to hear across a wide auditory range and may be more sensitive to intense noise exposure than fish without this enhanced hearing capability.

3.2 ABR Technique

Baseline auditory thresholds and threshold changes after white and boat engine noise exposure, were measured using the auditory brainstem response (ABR) technique [14-15,19-23]. The ABR technique is an electrophysiological far-field recording of synchronous neural activity in the eighth cranial nerve and brainstem auditory nuclei in response to an acoustic stimulus [24]. Details of this technique were originally reported in Kenyon et al. [19], and therefore, for this study, only a brief summary of the technique is given.

The sound stimuli presented and the ABR waveforms recorded, for these experiments, used a Tucker-Davis Technologies (TDT) modular rack system. This modular rack system was controlled by an optically-linked Pentium III, 350 MHz desktop computer consisting of a TDT board and ran TDT BioSig™ software. Sound stimuli, used to determine auditory thresholds, were presented for 20 ms as a tone bursts of specified frequency (0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, and 4.0 kHz, each for 2000 sweeps per test) and pressure levels using TDT BioSig^{TM} software. For frequencies under 3.0 kHz, a 30-cm diameter speaker (Pioneer) was used, and a 12-cm midrange speaker (Pyle MR 516) was used to present acoustic stimuli above 3.0 kHz. Both the speakers used were mounted 1 m above the fish. The highest pressure level was presented first and then attenuated in 5 dB steps for frequencies between 0.3-2.0 kHz and 3 dB steps for 2.5 and 4.0 kHz until a repeatable ABR waveform was no longer visible. For these studies, auditory threshold was defined as the lowest sound level where a repeatable ABR trace can be obtained. This was based on visual inspection of the waveform and cross-correlation coefficient examination [14-15, 19-23].

3.3 Test Conditions

To examine the effect of noise exposure and to identify frequencies that exhibited noise effects, audiograms (i.e., all frequencies in hearing range of fish examined, 0.3-4.0 kHz) were compared between fish exposed to noise for 24 hours (n=6) and baseline fish (not exposed, n=5). Each frequency was compared using an unpaired t-test (one-tailed) (SigmaStat). Critical alpha values were adjusted, to account for multiple comparisons, using the sequential Bonferroni technique [25].

To examine the effect of exposure duration on auditory sensitivity, fish were exposed to white noise for different durations (1, 2, 4, and 8 hours each with an n=6), and thresholds were measured immediately thereafter. The effect of exposure duration was then compared at noise-sensitive frequencies (See results). To examine variations in recovery of auditory sensitivity, the hearing thresholds of fish were measured at 1, 2, 4, 6, and 14 days following 24 hours of exposure to white noise. Noise-sensitive frequencies were compared between baseline fish and fish exposed to noise. Separate one-way ANOVAs were used to compare exposure duration and recovery effects for each frequency (SigmaStat). Auditory thresholds were then compared against baseline thresholds using Dunnett tests (Bonferroni adjusted).

To examine the relationship between exposure duration and recovery, frequencies that did not recover after 14 days (24 hours of exposure) were examined when exposure duration was reduced to 2 hours. This comparison was made at day 6 and 14 only. Separate one-way ANOVAs, with multiple comparisons, were used to compare recovery times for each frequency.

Finally, to examine the effect of boat engine noise exposure and to identify frequencies that exhibited noise effects, audiograms were compared between fish exposed to noise for 2 hours of boat engine noise and baseline fish (not exposed to boat engine noise, n=5) from our previous study [14]. Each frequency was compared using an unpaired t-test (one-tailed) (SigmaStat). Critical alpha values were adjusted, to account for multiple comparisons, using the sequential Bonferroni technique [25].

4. RESULTS

Exposure to an intense white noise for 24 hours significantly elevated the fathead minnow's auditory threshold at five of the eight frequencies tested when compared to the audiogram of the baseline group, which received no noise exposure (Fig. 1). Four (0.8,1.0, 1.5, and 2.0 kHz) of these five frequencies were chosen to be used to further assess the effects of varying exposure duration on hearing thresholds. These four frequencies were chosen because they are in the fish's best hearing range and demonstrated the most significant elevation in threshold, and thus are considered noise-sensitive frequencies.

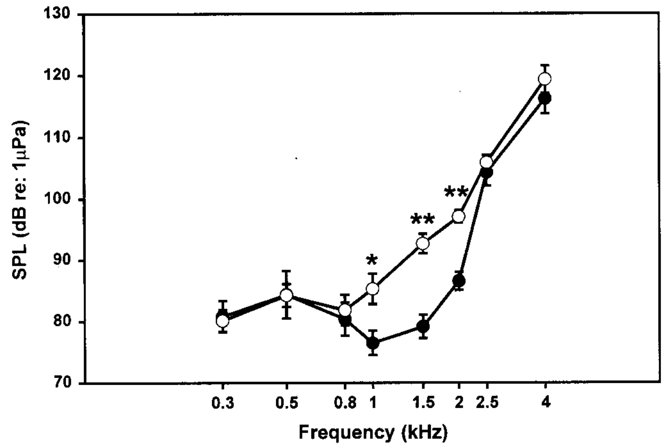

Figure 5. Audiogram of fathead minnow showing threshold shifts due to boat noise expsoure. Solid circles: baseline data of control group of fish. Open circles: experimental group of fish exposed to 2 hrs of boat noise. * indicates a $P < 0.05$ level and ** indicates a $P < 0.001$level.

It was found that the auditory effect of noise exposure on fish was dependent on duration of exposure to white noise. One hour of noise exposure significantly elevated threshold in 3 out of the 4 noise-sensitive frequencies examined (1.0, 1.5, 2.0 kHz). Additionally, two hours of exposure lead to a significant threshold shift for all 4 noise-sensitive frequencies examined, with this shift being comparable to a fish exposed to noise for 4, 8, and even 24 hours (Figure 2). This is quite a dramatic effect showing that just two hours of noise exposure can elevate threshold as much as 24 hours or 12 times the exposure duration.

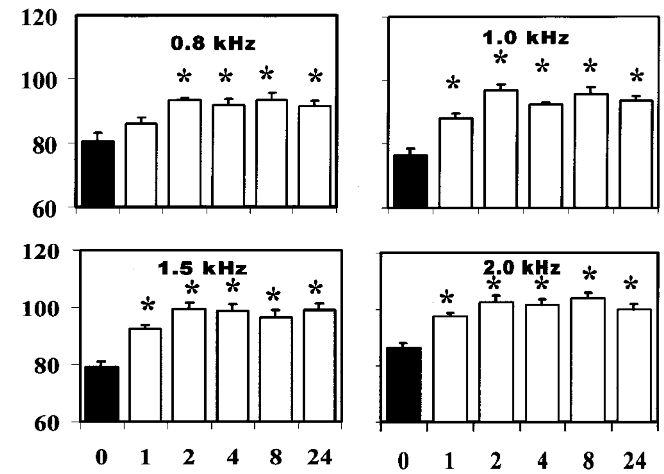

Figure 2. Immediate effects of noise exposure duration on shifts in auditory threshold with SPL on Y axis (dB re: 1µPa) and Duration of Exposure on X axis (hours). Shown are threshold values immediately after noise exposure at four noise-sensitive frequencies. Asterisks (*) indicate a significant difference to baseline thresholds (P<0.001).

The second goal was to examine how long this temporary shift in threshold lasted. Recovery was defined as the auditory threshold level, after noise exposure, which was no longer significantly different from the baseline threshold. It was found that there were frequency-specific effects associated with recovery. For example, at 0.8 and 1.0 kHz recovery was observed one day following exposure to 24 hours of noise, but 1.5 and 2.0 kHz saw no recovery even 14 after exposure (Figure 3). Whether some sort of damage had occurred to the inner ear in relation to this effect, remains to be determined. Nevertheless, it shows that fish may be encoding higher frequencies (1.5 and 2.0 kHz) differently from the lower ones (0.8 and 1.0 kHz).

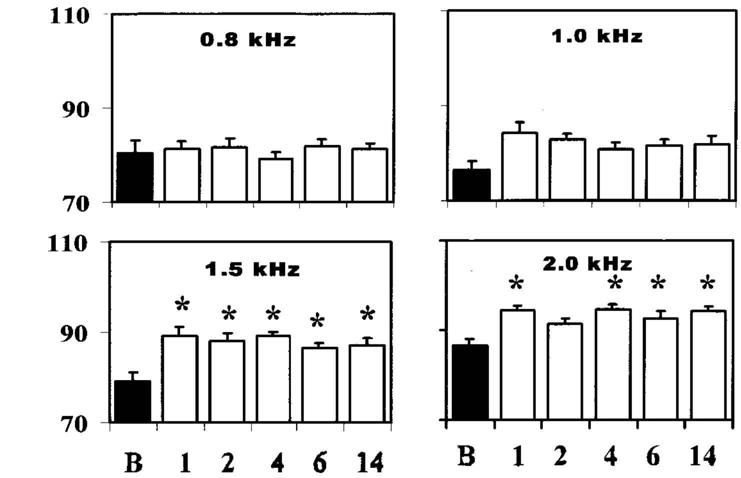

Figure 3. Effects of 24 hours of noise exposure on recovery of hearing threshold with with SPL on Y axis (dB re: 1μPa) and Recovery Time (days) on X axis (hours). Asterisks (*) indicate a significant difference to baseline thresholds (P<0.001).

To further examine how recovery is related to exposure duration, recovery was examined at 1.5 and 2.0 kHz after only 2 hours of exposure instead of 24 hours of exposure. Despite the fact that the immediate effects of 2 hours of exposure were comparable to 24 hours of exposure, recovery time was different. For these two frequencies, recovery was seen within 6 days after exposure to 2 hours of noise, compared to no recovery even after 14 days after exposure to 24 hours of noise. This shows that recovery is not only frequency specific but also it depends on duration of exposure.

The final goal of these studies was to compare the effects of noise on auditory threshold using a source of noise that a fish would experience in its natural acoustic environment. Boat engine noise was recorded in the field from a 55 horsepower outboard engine. The noise from the motor covered a range of frequencies from 0.3 to 10.0 kHz with a peak frequency of 1.3 kHz (Figure 4). One notices that the peak frequency of the boat engine noise is in the fish's most sensitive auditory range (0.8-2.0 kHz).

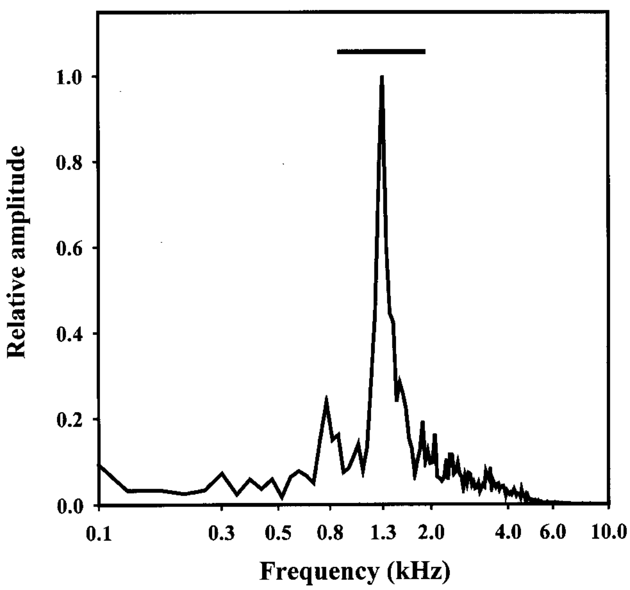

Figure 4. Power spectrum of noise produced by a 55 horsepower outboard motor idling at neutral position. The peak frequency is 1.3 kHz. The horizontal solid line indicates the fathead minnow's most sensitive hearing frequency range.

This sound was played back to a group of fish for two hours and the sound pressure level was adjusted so that the peak frequency would match the sound pressure level of the white noise (142 dB re: 1μPa) presented in earlier experiments. The two hour duration of boat engine noise exposure was chosen because, during white noise exposure, 2 and 24 hours of exposure had the same immediate effects on threshold elevation.

Of the eight frequencies tested, after

exposure to boat engine noise, 3 showed a significant elevation in threshold when compared to the audiogram of the baseline fish (no boat noise exposure) (Fig. 5). The three frequencies (1.0, 1.5, and 2.0 kHz) were in the fish's most sensitive hearing range and in the range of the peak frequency of the boat noise. Thus this shows that as little as 2 hours of boat noise exposure has the potential to elevate auditory thresholds in the fathead minnow's most sensitive hearing range.

Thus far, the two studies [14-15] mentioned here have provided a link between noise exposure and elevated auditory thresholds in the fathead minnow. In addition, hearing threshold elevations and recovery, after noise exposure, have been demonstrated to be specifically related to duration of exposure and frequency. Also, noise produced by a boat engine, which is found in the fish's natural acoustic environment, has been shown to adversely effect hearing.

5. DISCUSSION

Along with the pioneering work of Popper and Clarke [13], our studies have provided evidence that either white noise or boat engine noise exposure has the ability to significantly elevate the auditory threshold of fish. In addition, we have demonstrated that noises present in the fathead minnow's acoustic environment has the ability to adversely impact hearing thresholds. Even though we tested just one species of fish, there is also a concern associated with the potential damage these types of noises may have on not only the fathead minnow but other fish with similar auditory capabilities (i.e. other hearing specialists). Since the fathead minnow audiogram is very similar, in terms of auditory thresholds and frequency range, as the goldfish, which is another cyprinid fish, it can be hypothesized that boat engine noise could have similar effects on auditory sensitivity of most cyprinid fish [14, 19, 22].

The studies discussed, thus far, only examined the effect of noise on auditory thresholds. What effects this has on fish behavior or overall physiology requires further investigation. This is an issue of high concern because of the increasing use of sound as acoustic barriers or in applications to modify fish behavior [26]. Acoustics barriers are often preferred over physical barriers to reduce physical stress to the fish. In addition, Nestler et al. [27] advocate the possible use of sound over other methods to modify fish behavior citing several advantages, including the fact that most fish are easily startled by sound, short-range propagation is minimally affected by turbidity, and sounds can be used during both day and night.

Knudsen et al. [28] examined responses of salmon smolt (*Salmo salar*) to low-frequency pure tone noise, but most work in this area has dealt with clupeids and the use of ultrasonic deterrents as repellents [27, 29-31]. Nevertheless, more studies need to be done focusing on not only behavioral effects of this noise exposure, but also hearing threshold effects since this and other studies have demonstrated intense noise can result in hearing loss in fish [13-14]. In addition, studies by Enger [11] and Hastings et al. [12] have found anatomical damage of the inner ear associated with exposure to intense noise.

Even though physical stress is reduced using acoustic barriers, one needs to consider auditory stresses induced by intense noise exposure [32-33]. Cudahy et al. [34] ask the pertinent question: "is chronic exposure to anthropogenic sound from any source, or combination of sources, causing psychological or physiological stress that is reducing the average longevity or average number of offspring produced by individual animals and thus causing a decrease in the productivity (biological fitness) or size of the affected stocks (e.g. by suppressing the immune systems of individual animals, making them more vulnerable to disease)?"

In addition to noise effects associated with elevated auditory threshold or mechanical damage to the inner ear, there is also a multitude of behavioral responses associated with underwater noise. Most research in the area of underwater noise has focused on cetaceans, which have many reactions to noise including such responses as avoidance, alterations in feeding and resting, swimming patterns, vocalizations and breathing rate [3, 16, 35-37]. Richardson and Würsig [3] described behavioral reactions of cetaceans to noise as ranging from attraction and short-term changes in behavior to long-term displacement. They hypothesize that anthropogenic noise could have numerous deleterious effects such as disturbance reactions, masking of calls from conspecifics, temporary or permanent hearing impairment, and noise-induced physiological stress. In addition, Bowles [38] list several areas of concern in regards to the effects of noise on wildlife, including stress and effects on activity and energy consumption. Noise could also effect habitat use, courtship and mating, social communication, and predation and predator avoidance behaviors [38]. These deleterious effects associated with noise exposure should also be considerations when examining behavioral effects of noise on fish because fish are potentially being exposed to the same sources of noise and are perhaps of greater concern because of the amount of boat traffic associated with freshwater environment. As emphasized by Erbe and Farmer [39], long-term behavioral effects of noise exposure on aquatic organisms still remain unknown and require future studies.

Many studies examining fish behavioral responses to noise that have focused on the potential loud sound has to induce startle and alarm responses. Schwartz and Greer [40] examined Pacific Herring (*Harengus pallasi*) and their reactions to noise from various sources including a number of vessel types and found that abrupt changes in temporal characteristics of the sound, associated with sudden changes in vessel speed, were more effective at eliciting an alarm response. Boussard [41] found that sound from a 260 horsepower high-speed boat produced sound that elicited a flight response in two cyprinid species (roach, *Rutilus rutilus*, and rudd, *Scardinius erythrophthalamus*) with noise levels as low as 120-125 dB (re: 1 Pa). Additional studies [42-44] have examined this phenomenon with herring on a behavioral level only, disregarding the potential deleterious effects on hearing thresholds.

The intricate combination of noise's effect on auditory sensitivity, behavior, and physiology make it increasingly laborious to institute safe exposure levels of aquatic and marine organisms. Nevertheless, this is an area of research that begs for further studies, especially with the recent dramatic increases in noise levels due to anthropogenic sources. This and future studies, resulting from this work, will ultimately lead to a more complete understanding of how the underwater acoustic environment shapes the lives of its inhabitants in terms of hearing thresholds, physiology, and behavior.

In summary, our results [13-14] indicate that the fathead minnow's auditory system may be processing acoustic signals in a more complicated manner than previously realized. From these initial studies on the effects of noise exposure on auditory sensitivity of the fathead minnow, several important conclusions can be made. Most important is that intense white noise exposure has the ability to elevate auditory thresholds in the fathead minnow's most sensitive hearing range (0.8-2.0 kHz). In addition, recovery after this intense noise exposure is frequency and duration specific and threshold shifts can be long-term (>14 days). Finally, noise generated from a 55 horsepower outboard motor is not only in the hearing range of the fathead minnow but has the potential to significantly elevate auditory threshold.

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