Effects of underwater noise on auditory sensitivity of a cyprinid fish

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Abstract

The ability of a fish to interpret acoustic information in its environment is crucial for its survival. Thus, it is important to understand how underwater noise affects fish hearing. In this study, the fathead minnow (Pimephales promelas) was used to examine: (1) the immediate effects of white noise exposure (0.3–4.0 kHz, 142 dB re: 1 μPa) on auditory thresholds and (2) recovery after exposure. Audiograms were measured using the auditory brainstem response protocol and compared to baseline audiograms of fathead minnows not exposed to noise. Immediately after exposure to 24 h of white noise, five out of the eight frequencies tested showed a significantly higher threshold compared to the baseline fish. Recovery was found to depend on both duration of noise exposure and auditory frequency. These results support the hypothesis that the auditory threshold of the fathead minnow can be altered by white noise, especially in its most sensitive hearing range (0.8–2.0 kHz), and provide evidence that these effects can be long term (>14 days). © 2001 Elsevier Science B.V. All rights reserved.

Key words: Auditory brainstem response; Noise induced hearing loss; Temporary threshold shift; Fathead minnow

1. Introduction

The auditory system is one of the most important sensory systems for an aquatic animal because it provides information about food, competitors, predators, and potential mates through the perception of intended and/or unintended acoustic signals in the environment (Myrberg, 1978). It has been hypothesized that fish may be listening to ambient sounds, from sound scattering objects, to interpret changes in their acoustic environment, and that these ambient noises may be as important to a fish as sounds used for communication (Popper and Fay, 1993). The underwater acoustic environment is inherently loud as a result of ambient sounds and an increasing amount of noise from anthropogenic sources (Richardson and Würsig, 1997). Thus, it is important to understand how noise affects fish auditory sensitivity, since their ability to accurately interpret the underwater acoustic environment is essential for survival.

There have been few experiments examining noise and its relation to fish audition. Most of the studies have focused, specifically, on the anatomical effects of noise exposure on the fish inner ear (e.g. Enger, 1981; Hastings et al., 1996). However, the direct relationship between anatomical damage and auditory sensitivity is still unclear. For example, is it possible that auditory thresholds will be elevated, after noise exposure, but show no dramatic signs of inner ear anatomical damage?

Popper and Clarke (1976) have provided the only examination of hearing threshold effects after noise exposure in fish (goldfish, Carassius auratus). However, auditory thresholds for only two frequencies (0.5 and 0.8 kHz) were tested after exposure to pure tones. In addition, only one exposure duration (4 h) was examined. In their study, temporary threshold shifts were observed immediately after exposure, but were found to have returned to pre-exposed threshold levels within 24 h.

Long-term effects and recovery, after noise exposure, are other issues that have not been thoroughly investigated in regards to fish audition. Hastings et al. (1996) observed limited sensory hair cell loss after exposure to intense noise, and attributed this effect to the unknown timetable of inner ear damage. They suggested that hair
cell damage, caused by acoustic trauma, might take a certain amount of time to fully manifest and that 4 days post-treatment, used in their experiment, may not have been long enough to wait before observing complete damage. From the aforementioned studies, it is obvious that our understanding of how noise affects fish hearing is rather limited. Issues like the characteristics of noise, duration of exposure, and recovery time after noise exposure are crucial to the understanding of noise-induced hearing loss. Our study tries to address these issues using the fathead minnow as a model species.

The fathead minnow, *Pimephales promelas* (Cyprinidae), is a cosmopolitan species found in a variety of habitats including creeks, headwaters, small rivers, shallow ponds, and lakes ranging from southern Canada to southern USA (Trautman, 1981). As a consequence, of its diverse habitat range, *Pimephales* has the potential to be exposed to a variety of different acoustic environments. Fathead minnows are also hearing specialists, i.e., they have enhanced auditory sensitivity (wide frequency range and low hearing threshold) due to the presence of accessory structures, the Weberian ossicles (von Frisch, 1938). Thus, they are an ideal fish model species for studying the effects of noise on hearing thresholds across a wide auditory range.

The two main objectives of this study were to (1) examine the immediate effects of white noise using a variety of exposure durations (1–24 h) and to (2) assess if auditory threshold shifts were permanent or temporary, and if temporary, to determine recovery time after exposure. This study uses white noise as an acoustic stimulus to examine effects over a wide frequency range rather than discrete frequencies using pure tone stimulation because ambient noises are not likely to be of pure tone origin. The merits of this study are that it examines not only the role of exposure duration on auditory thresholds, but also recovery and the long-term effects of noise exposure over the entire hearing range of the fathead minnow.

2. Materials and methods

2.1. Subjects

Fathead minnows (*P. promelas*) used for this study (43.2–80.8 mm total length (TL); 0.6–5.2 g body weight) were obtained from the Frankfort State Hatchery (Frankfort, KY, USA). These fish were spawned naturally and grown in hatchery ponds, which are considered ‘quiet’ environments (75–80 dB re: 1 µPa; Yan, unpublished data). In the laboratory, the fish were fed commercially prepared food (Tetra Standardmix®) and kept in filtered aquaria at 25 ± 1°C. The animal-use protocol for this study was approved by the University of Kentucky Institutional Animal Care Committee (98008L).

2.2. Auditory brainstem response (ABR) technique

For this study, auditory thresholds were measured using the ABR technique. This approach is preferred to behavioral means of obtaining auditory thresholds because it eliminates extraneous factors associated with behavioral methods and allows one to focus specifically on what the fish is hearing (Kenyon et al., 1998; Ladich and Yan, 1998; Yan, 1998; Yan et al., 2000; Yan and Curtsinger, 2000). In addition, auditory thresholds can be obtained immediately after noise exposure, which is a limitation of behavioral studies, as used in Popper and Clarke (1976).

The ABR technique used on fish hearing has been well documented (Kenyon et al., 1998; Ladich and Yan, 1998; Yan, 1998; Yan et al., 2000; Yan and Curtsinger, 2000), therefore, only a brief summary of the technique will be given. During testing, the fish was mildly sedated with Flaxedil, (gallamine triethiodide, Sigma Chemical Co.), a neuromuscular junction blocker, to reduce myogenic noise and restrained in a mesh sling. The dosages of Flaxedil, 15–25 µl (0.07 mg/ml), were given by intramuscular injections and adjusted so that the fish was still capable of producing slight opercular movement on its own. The fish, wrapped in a mesh sling, was then held in a metal clamp attached to a glass rod that was fixed in a micromanipulator (M330, World Precision Instruments, Sarasota, FL, USA). During testing, the fish was placed in a plastic tub (38 × 24.5 × 14.5 cm) filled with water. The fish was positioned so that the nape of the head was approximately 1 mm above the water surface and a respiration pipette was inserted in the fish’s mouth. Tissue paper was placed on the portion of the head above the water surface to keep the surface from drying out. On top of the tissue paper, a recording electrode and reference electrode was placed firmly against the skin. The recording electrode was placed in the approximate area of the brainstem, specifically the region of the medulla (along midline of skull), with the reference electrode placed 5 mm anteriorly. The recording and reference electrode were made of Teflon-insulated silver wire (0.25 mm in diameter) with 1 mm of exposed tip and adjusted using micromanipulators. A hydrophone (Celsesco LC-10) was used to monitor sound pressure levels of the stimulus and was placed near the head of the subject. This entire apparatus was on a vibration-free air table (Kinetic Systems, model 1201) and placed inside a walk-in soundproof chamber (2 × 3 × 2 m, Industrial Acoustics Company, Inc., NY, USA).

Sound stimuli were presented and ABR waveforms were recorded using a Tucker-Davis Technologies
running TDT BioSig

1 MHz desktop computer consisting of a TDT board and was controlled by an optically linked Pentium III, 350

MHz computer board (Pyle MR 12 cm midrange speaker (Pioneer, frequency 19 Hz–5 kHz) for testing. For frequencies under 2.5 kHz a 30 cm 1 m above the subject was used to produce the sounds for testing. For frequencies under 2.5 kHz a 30 cm diameter speaker (Pioneer, frequency response 19–15

kHz) suspended 1 m above the plastic tub. This was the same speaker used for the ABR technique.

Up to eight fathead minnows were exposed at a time and different fish were used for each experimental treatment, such that the same fish was never used twice. Sound exposure consisted of placing the fathead minnows in a plastic tub (same tub used for ABR) with 5.5 cm depth of water. The fish were free to swim about the tub during exposure, and a fine mesh screen was placed over the tub to keep the fish from jumping out of the tub during the duration of exposure. The plastic tub sat upon the same vibration-free air table in the sound proof chamber used for ABR.

After noise exposure, fish were kept in aquaria in an isolated area of the laboratory where auditory disturbances were kept minimal (87 dB re: 1 μPa, Scholik, unpublished data) until auditory testing could be completed, and aquaria filters were shut off to eliminate excess noise. Since testing for the immediate effects of noise exposure on fish was complete within 12 h of exposure, this did not compromise the fish’s health. When assessing recovery after exposure, aquaria filters were operational briefly for 30 min per day (99 dB re: 1 μPa, Scholik unpublished data) during the duration of the recovery period (1–14 days) to reduce extraneous noise, but not compromise the overall water quality of the tank.

2.4. Experiment 1: effect of noise exposure

To assess the immediate effects of noise exposure on fish hearing, a group of fathead minnows (n = 6) were exposed to 24 h of noise. Immediately following exposure, thresholds were measured using the ABR technique. To examine the effect of noise exposure and to identify frequencies that exhibited noise effects, complete audiograms (i.e., all frequencies in the hearing range of fish examined) were compared between fish exposed to noise for 24 h and baseline fish (not exposed, n = 5). Threshold for each frequency was compared using an unpaired t-test (one-tailed) (SigmaStat). Critical α values were adjusted, to account for multiple comparisons, using the sequential Bonferroni technique (Rice, 1989).

2.5. Experiment 2: effect of exposure duration

To examine the effect of exposure duration on auditory sensitivity, fish were exposed to white noise for different durations (1, 2, 4, and 8 h each with an n = 6), and thresholds were measured immediately thereafter. The effect of exposure duration was then compared at noise-sensitive frequencies (see Section 3.1). Separate one-way ANOVAs were used to compare exposure duration effects for each frequency (SigmaStat).
Auditory thresholds were then compared against baseline thresholds using Dunnett tests (Bonferroni adjusted).

2.6. Experiment 3: recovery and exposure duration

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 h of exposure to white noise. Noise-sensitive frequencies (see Section 3.1) were compared between baseline fish and fish exposed to noise. Separate one-way ANOVAs were used to compare recovery time 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 h of exposure) were examined when exposure duration was reduced to 2 h. 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.

3. Results

3.1. Experiment 1: effect of noise exposure

To assess the immediate effects of noise exposure on auditory thresholds, audiograms were measured for a group of fathead minnows exposed to noise for 24 h and compared to baseline thresholds. Fig. 1 shows that five out of eight frequencies tested (0.3, 0.8, 1.0, 1.5, and 2.0 kHz) yielded a significant increase in auditory threshold after noise exposure when compared to baseline. This noise-induced hearing loss effect can also be seen by comparing acoustically evoked brainwaves (tested at 1.0 kHz at 105 dB) of a baseline and noise-exposed fish (Fig. 2). The nonsignificant correction ($r^2 = 0.1839, P > 0.05$) between the waveforms of baseline and noise-exposed fish indicates that noise-exposed fish showed significant loss of hearing ability when compared to baseline fish. For the remainder of the experiment and data analysis, it was decided to focus on four of these frequencies, specifically 0.8, 1.0, 1.5, and 2.0 kHz, instead of all eight frequencies tested. These four particular frequencies were chosen because they all had a $P$ value of 0.01 or less when compared to baseline thresholds. In addition, the audiogram showed that these frequencies were considered in the fathead minnows most sensitive hearing range. Thus, these four frequencies were considered to be the most ‘noise-sensitive’ frequencies and thus, most important to understand.

3.2. Experiment 2: effect of exposure duration

To further examine the immediate effects of exposure duration, various durations of noise exposure, ranging from 1 to 8 h, were examined with noise-sensitive thresholds (0.8, 1.0, 1.5, 2.0 kHz) being measured immediately after exposure. After as short as 1 h of ex-
posure (Table 1) the hearing threshold was elevated significantly above that of baseline fish in three out of the four frequencies examined (1.0, 1.5, and 2.0 kHz). Two hour of exposure to noise yielded a significant elevation in threshold for all four frequencies measured. Not only was there a significant elevation after 2 h of exposure, but the elevation in threshold was comparable to that after 4, 8, and even 24 h of noise exposure.

3.3. Experiment 3: recovery and exposure duration

To examine if recovery of threshold occurred after exposure to intense noise, fish auditory thresholds were measured at various intervals after exposure. Audiograms for fish exposed to noise were obtained from 1 to 14 days after exposure to 24 h of noise. For this experiment, recovery was defined as the point where the threshold, after noise exposure, was no longer significantly different from the baseline threshold at a particular frequency.

Table 2 shows recovery for the noise-sensitive frequencies (0.8, 1.0, 1.5, and 2.0 kHz). The data in Table 2 reveal that recovery seems to vary with frequency, i.e., frequency dependent. For 0.8 and 1.0 kHz, recovery was seen as early as 1 day after exposure. Conversely, for thresholds at 1.5 kHz and 2.0 kHz, the timetable for recovery seemed to be very different. Measuring threshold 2 weeks (day 14) post-noise exposure showed that the threshold was still elevated significantly above the threshold of the baseline fish. The only exception was for 2 days after noise exposure for 2.0 kHz, which may be due to individual variability associated with the fish.

To further examine the interaction between recovery time and exposure duration, a comparison was made between thresholds after 2 h of exposure versus 24 h of exposure, specifically at 1.5 kHz and 2.0 kHz. Despite that the immediate threshold elevation after just 2 h of exposure was comparable to 24 h of exposure, recovery for these two exposure durations was significantly different. Recovery, after 2 h of exposure, was seen by day 6 for both of these frequencies (Table 2). After 24 h of exposure, recovery was never observed even 14 days after exposure. This indicates that elevation of threshold after noise exposure is not only frequency dependent but also dependent on initial exposure duration.

4. Discussion

4.1. The fathead minnow as a model species

The fathead minnow as a cosmopolitan species with enhanced hearing capabilities, is an ideal model animal to use for better understanding how noise affects the auditory system of fish. In addition, this study provides the first baseline audiogram for this species. The fathead minnow’s audiogram is very similar, in terms of auditory thresholds and frequency range, as the goldfish, which is another cyprinid fish (Kenyon et al., 1998;
Yan et al., 2000). As a result of having similar auditory capabilities and hearing enhancement structures (Weberian ossicles), it can be hypothesized that noise could have similar effects on auditory sensitivity of most cyprinid fish.

4.2. Immediate effects of noise exposure

Exposure to an intense white noise for 24 h significantly elevated the 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. The frequencies most affected by noise exposure were those in the minnow’s most sensitive auditory range (0.8–2.0 kHz), despite the fact that white noise was played at equal SPL at all frequencies (0.3–4.0 kHz). This can be seen in Fig. 1 where white noise is more than 60 dB above threshold at 1.0 kHz but less than 20 dB above threshold at 4.0 kHz. This phenomenon is of biological concern to the fish because frequencies showing the lowest thresholds are often associated with the dominant frequencies of the sounds produced by fish for acoustic communication (Ladich and Yan, 1998). We do not know yet if fathead minnows make sounds. Nevertheless, these frequencies may be of great importance to this species in assessing changes in their acoustic environment (Popper and Fay, 1993). Since the frequencies in the minnow’s most sensitive hearing range (0.8–2.0 kHz) demonstrated the most dramatic immediate threshold shift, they were specifically examined in relation to recovery and the effects of exposure duration on auditory thresholds.

It was found that the auditory effects of noise exposure on fish were dependent on duration of exposure to white noise. On average threshold shifts were consistently lower for 0.8 kHz (10.8 dB) compared to the other three frequencies measured (1.0 kHz = 16.8 dB; 1.5 kHz = 18.1 dB; 2.0 kHz = 14.6 dB). One hour of noise exposure significantly elevated threshold in three out of the four frequencies examined, while 2 h of noise exposure elevated all four frequencies. The elevation in threshold after 2 h of exposure was also comparable to a fish exposed to noise for 4, 8, and 24 h. This is known as the asymptotic threshold shift (ATS) or upper limit of the threshold shift (Yost, 1994). This is quite a dramatic effect indicating that with as little as 2 h of noise exposure, one can see elevations in threshold that are equivalent to that of fish exposed 12 times as long.

These results show that white noise, of various exposure durations, significantly elevated the auditory threshold in the minnow’s most sensitive hearing range (0.8–2.0 kHz) and that the ATS is reached rather quickly. This could have dramatic consequences on fish, which potentially could be exposed to noise in their natural environment from anthropogenic sources. Most human activities associated with the underwater acoustic environment produce noise with low frequencies components less than 1.0 kHz (Richardson and Wursig, 1997). Thus, there is a real potential of auditory threshold elevation in fish with exposure to noise in their environment, and this is an area where further investigations are needed.

4.3. Recovery after noise exposure, frequency effects

The second goal of this study was to determine the duration of noise exposure on the threshold shift. Frequency specific effects were found associated with recovery. For example, at 0.8 and 1.0 kHz recovery was seen one day following exposure to noise for 24 h, but 1.5 and 2.0 kHz saw no recovery even 14 days after exposure (Table 2). This suggests that inner ear damage may be associated with these frequencies resulting in a permanent threshold shift. Further research is required to examine if there is a correlation between inner ear damage and threshold shifts. Nevertheless, this shows that there are frequency-specific effects associated with recovery time, which might mean that the fish are encoding these higher frequencies (1.5 and 2.0 kHz) differently than the lower frequencies (0.8 and 1.0 kHz).

Though we do not know the exact mechanism responsible for these threshold shift results, some speculations can be made since hair cells function is comparable in all vertebrates (Popper and Fay, 1999). Thus, studies examining noise effects on hair cells in other vertebrates could be applicable also to fish. However, these suggested mechanisms are areas that remain to be further investigated with the fish auditory system.

A review on threshold shifts and acoustic injury by Saunders et al. (1991), suggests that damage to hair cell bundles, tip links, and intracellular organelles affect hair cell channel transduction. In addition, Saunders et al. (1991) propose that different types of noise exposure, in terms of frequency and duration, leave distinct ‘footprints’ in terms of injury to the hair cell.

Zhao et al. (1996) specifically examined the time course for tip link (protein structures connecting sensory hair cells) regeneration in chickens after treatment with a calcium chelator. Their results suggest that tip link damage and the their rapid recovery (within several hours, with almost complete recovery by 24 h) may offer an explanation for temporary threshold shifts. Husbands et al. (1999) found that tip link recovery in chicks, after noise exposure, occurred within 24 h. The same mechanism perhaps could also account for the relatively rapid recovery rate seen in the fathead minnows at their lower hearing frequency range (0.8 and 1.0 kHz). Canlon (1988) found that permanent shifts in auditory thresholds were correlated with swelling of the
afferent dendrite below the hair cell, which may be occurring with the higher frequencies in the fathead minnow (1.5 and 2.0 kHz) where recovery is not seen.

Popper and Clarke (1976) suggest that the teleost ear may have a mechanism, though not as specific as that found in the mammalian ear with its tonotopic cochlea, to discriminate frequencies differentially. They found that threshold shifts, due to pure tone stimulation, were not equal for all frequencies tested and that the amount threshold shifts, after stimulation with these tones, were grouped (0.3 and 0.5 kHz; 0.8 and 1.0 kHz) which indicates some degrees of frequency differentiation mechanism. Our results corroborate their finding.

Popper and Clarke (1976) offer some suggestions to explain the phenomena of frequency differential effects of noise exposure. They suggest that it could result from specific neurons in the inner ear, which respond to only particular frequencies and which may fatigue differentially. In addition, it could be due to a difference in hair cell morphology, which is a likely possibility since hair cell stereocilia are known to be especially prone to damage as a result of overstimulation (Canlon, 1988).

It is known that in the fish ear, the hair cell ciliary bundles have varying lengths along the epithelium from the rostral to caudal end. Platt and Popper (1981) have also described at least seven distinct hair cell bundle types based on kinocilia and stereocilia length and size. The significance of the different hair cell lengths has not been determined in fish (Popper and Fay, 1999), but Sugihara and Furukawa (1989) found that the shorter hair cell bundles responded higher frequencies while longer bundles respond optimally to lower frequencies of sound.

In the guinea pigs, it was seen that differential susceptibility of hair cell bundles to acoustic stimulation might be due to differences in stereocilia height with short hair cells being more vulnerable to overstimulation by noise than longer hair cells (Canlon, 1988; Chan et al., 1998). This might be because taller hair cell bundles need less angular displacement to reach threshold than shorter bundles. This might also explain why we saw no recovery with the higher frequencies examined (1.5 and 2.0 kHz). However, this hypothesis remains to be tested.

Recently it has been discovered that there is heterogeneity within the ears of fish by having type I-like (longer) and type II (shorter) vestibular sensory hair cells like other amniote vertebrates (Saidel et al., 1995; Chang et al., 1992). These two distinct hair cell types are found in different regions of the endorgans and respond differently to ototoxic drugs (Lombarte et al., 1993; Yan et al., 1991). Whether these two types of sensory hair cells respond differentially to white noise remains to be examined.

4.4. Recovery after noise exposure, exposure duration effects

In addition, we examined how recovery varies with exposure duration by looking specifically at thresholds at 1.5 and 2.0 kHz after 2 h of exposure instead of 24 h of exposure. The role exposure duration plays on threshold shifts is not a new idea. Johnson et al. (1975), reported that in humans, 24 and 48 h of noise exposure had similar magnitudes of threshold shifts, but they observed subjects exposed to 48 h having a significantly longer recovery time than those only exposed to 24 h of noise. Their study concluded that recovery was dependent on duration and intensity of noise exposure. We observed a similar result in fish recovery. Despite the fact that the immediate effects of 2 h of exposure on auditory thresholds are comparable to 24 h of exposure, recovery time was also different. For these two frequencies, recovery was seen within 6 days after 2 h of noise exposure, compared to no recovery even 14 days after exposure to 24 h of noise (Table 2). Our study shows that, in fish, recovery is not only frequency specific but also is exposure duration dependent.

The two main conclusions of this study are that: (1) white noise has the potential to elevate auditory thresholds to the point where they are significantly different from baseline fish, and (2) that recovery, after noise exposure, is not only frequency specific but also exposure duration specific. In summary, our results indicate that fish auditory system may be processing acoustic signals in a more complicated manner than previously realized. In addition, noise has the potential to elevate thresholds, in the fish’s most sensitive hearing range, after a mere 1 h of exposure and depending on exposure duration, threshold shifts could be long-term (> 14 days).

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