Start/end page
–
COLOUR Page nos.
1st Proof:
Press:
Issue
MS order
Page total
Reprints



The Journal of Experimental Biology 207, 000-000
Published by The Company of Biologists 2004
doi:10.1242/jeb.00755

# Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*)

Michael E. Smith[1,*], Andrew S. Kane[2] and Arthur N. Popper[1,3]

[1]*Department of Biology and Center for Comparative and Evolutionary Biology of Hearing, University of Maryland, College Park, MD 20742, USA,* [2]*Aquatic Pathobiology Program, Department of Veterinary Medicine, University of Maryland, College Park, MD 20742, USA* and [3]*Neuroscience and Cognitive Science Program, University of Maryland, College Park, MD 20742, USA*

*Author for correspondence (e-mail: mesmith@umd.edu)

*Accepted 20 October 2003*

## Summary

**Fishes are often exposed to environmental sounds such as those associated with shipping, seismic experiments, sonars and/or aquaculture pump systems. While efforts have been made to document the effects of such anthropogenic (human-generated) sounds on marine mammals, the effects of excess noise on fishes is poorly understood. We examined the short- and long-term effects of increased ambient sound on the stress and hearing of goldfish (*Carassius auratus*; a hearing specialist). We reared fish under either quiet (110 dB re 1 μPa) or noisy (white noise, 170 dB re 1 μPa) conditions and examined animals after specific durations of noise exposure. We assessed noise-induced alterations in physiological stress by measuring plasma cortisol and glucose levels and in hearing capabilities by using auditory brainstem responses. Noise exposure did not produce long-term physiological stress responses in goldfish, but a transient spike in plasma cortisol did occur within 10 min of the noise onset. Goldfish had significant threshold shifts in hearing after only 10 min of noise exposure and these shifts increased linearly up to approximately 28 dB after 24 h of noise exposure. Further noise exposure did not increase threshold shifts, suggesting an asymptote of maximal hearing loss within 24 h. After 21 days of noise exposure, it took goldfish 14 days to fully recover to control hearing levels. This study shows that hearing-specialist fishes may be susceptible to noise-induced stress and hearing loss.**

Key words: threshold shift, hearing, noise, cortisol, glucose, ABR, recovery, fish, *Carassius auratus*.

## Introduction

Sound is an important means of communication in aquatic environments because it can be propagated rapidly (five times faster than in air) over great distances and it is not attenuated as quickly as other signals such as light or chemicals (Hawkins and Myrberg, 1983). Thus, it is not surprising that fishes and marine mammals make considerable use of sound for communication, for detection of predators and prey and for learning about their environment (Au and Nachtigall, 1997; Edds-Walton, 1997; Zelick et al., 1999; Fay and Popper, 2000).

Within the past decade, there has developed an increased awareness that underwater anthropogenic (human-generated) sounds may be detrimental to marine organisms by masking the detection of biologically relevant signal and/or even damaging the exposed animals (NRC, 2000, 2003). These sounds may be associated with shipping, dredging, drilling, seismic surveys, sonar, recreational boating and many other human-made sources. As a result of the human-generated sounds, ambient noise levels in the ocean are thought to be growing (NRC, 2003). Indeed, early estimates by Ross (1993) suggest a 10 dB increase from 1950 to 1975 alone, or more than a doubling in noise level. This is likely to have risen further with increases in shipping and uses of other acoustic sources in parts of the oceans (NRC, 2003). Indeed, recent forecasts by the National Oceanographic and Atmospheric Administration (NOAA)'s Marine Transportation System indicate that foreign ocean-borne trade is expected to double by the year 2020 (US Department of Transportation, 1999), and this could result in even greater ocean noise levels in shipping lanes unless there are dramatic changes in ship acoustics.

Substantial exposure of fish to acoustical stress is also found in many aquaculture facilities (Bart et al., 2001) that are important sources of food, ornamental species and stock enhancement of wild populations. While considerable effort has been made to optimize growth of aquaculture species by manipulating many environmental parameters such as temperature, food quality, photoperiod, water chemistry and stock density, little or no concern has been directed to determining the appropriate acoustic environment for optimal growth and development. Rearing conditions in aquaculture tanks can produce sound levels that are 20–50 dB higher within the frequency range of fish hearing than in natural habitats

(Bart et al., 2001). The few studies that have examined the effects of sound levels on aquacultured species show that high levels of ambient sound can potentially be detrimental and result in reduced egg survival and reduced reproductive and growth rates (Banner and Hyatt, 1973; Lagardère, 1982). Clearly, these studies need to be replicated and extended to additional species and include analysis of additional parameters that could be indicative of the effects of noise on developing fish.

While most research efforts to date, and public interest, have focused on how underwater noise affects the behavior of marine mammals, the effects of this noise pollution on fishes has rarely been examined (Myrberg, 1990; NRC, 2000, 2003). It is known that intense sounds can cause temporary hearing threshold shifts (Popper and Clark, 1976; Scholik and Yan, 2001) and damage to the sensory cells of the ears of the few fish species that have been studied (Enger, 1981; Hastings et al., 1996; McCauley et al., 2003). Besides damage to the inner ear, high levels of background noise may also create physiological and behavioral stress responses in fishes similar to those found in mammals (Welch and Welch, 1970).

In the present study, we investigated the effect of high levels of continuous white noise exposure on the physiological stress levels (measured by plasma cortisol and glucose concentrations) and hearing loss (utilizing the auditory brainstem response technique) of goldfish (*Carassius auratus*). Our goal was to examine the effects of noise duration on the physiological stress responses and hearing shifts in order to elucidate a potential relationship between hearing loss and noise-induced physiological stress. We also examined the time course of hearing recovery.

In order to examine a broad range of noise exposure durations, we exposed goldfish to noise in two separate sets of experiments – a short-term experiment in which exposure durations ranged from 10 min to 24 h and a long-term experiment that ranged from 1 day to 21 days. We found that intense noise can produce initial physiological stress responses as well as short- and long-term hearing loss in goldfish.

We chose goldfish as a model hearing specialist because of their known hearing sensitivity and the available literature database about hearing in this species (Fay and Popper, 1974; Fay, 1988). Goldfish are otophysan fishes, which possess Weberian ossicles (modified cervical vertebrae that abut the ear; von Frisch, 1938). These bones acoustically couple movement of the swim bladder imposed by impinging sound pressure waves to the inner ear, leading to enhanced hearing sensitivity that includes a broadened frequency range of hearing and lower auditory thresholds when compared with fishes without such specializations.

## Materials and methods

### *Experimental animals and design*

Goldfish (*Carassius auratus* L.?) were obtained from a local hatchery and maintained at the Aquatic Pathobiology Center at the University of Maryland. Standard length and wet mass means (± S.E.M.) for goldfish were 10.5±0.1 cm and 34.8±0.8 g, respectively. For the long-term noise exposure experiment, 42 goldfish were maintained in each of two 600-liter all-glass aquaria with corner filters and 65% water changes thrice weekly. These two aquaria were kept in separate rooms. One aquarium was for control animals and the other for noise-exposed animals. The effects of long-term noise exposure was examined using groups of six fish that were noise-exposed for either 1, 3, 7, 14 or 21 days.

Two sets of experiments were performed to assess the effects of short-term noise exposure. One experiment examined the time course of physiological stress responses and the other characterized the effect of exposure duration on temporary hearing threshold shifts. In the stress experiment, six fish were noise-exposed in each of three 76-liter glass aquaria that were visually isolated from one another. Each tank was randomly assigned an exposure duration time (0 min, 10 min or 60 min). In the short-term hearing study, groups of six fish were noise-exposed for each of four exposure durations (0 min, 10 min, 1 h or 24 h) in a 19-liter bucket with an underwater speaker resting on the bottom. All work was done under the supervision of the Institutional Animal Care and Use Committee of the University of Maryland.

### *White noise exposure*

All experiments were done using a white noise with a bandwidth ranging from 0.1 kHz to 10 kHz at 160–170 dB re 1 μPa total sound pressure level (SPL). The sound was presented *via* a Sony MiniDisc player through an amplifier (5.2 A monoblock; AudioSource, Town, State, Country) to an underwater speaker (UW-30; Underwater Sound Inc., Oklahoma City, OK, USA) placed centrally on the bottom of the aquarium. The white noise, which is defined as having a flat power spectrum across the entire bandwidth (i.e. all frequencies are presented at the same SPL), was computer-generated using Igor Pro software (Supplier, Town, State, Country). Characteristics of the noise exposure (bandwidth and SPL) were similar in both long- and short-term noise exposure experiments, with transduction in the tanks having little effect on the digitally generated flat, white noise spectra (Fig. 1). For the short-term experiments, the SPL of the noise exposure varied within the bucket from 170 dB re 1 μPa 1 cm directly above the speaker to 166–169 dB re 1 μPa at 8–14 cm above the speaker. For the long-term experiments, the SPL of the noise exposure varied slightly within an aquarium, with a maximum (170 dB re 1 μPa) in the center right above the underwater speaker and a minimum (161–168 dB re 1 μPa) near the sides farthest from the speaker. The SPL of the control aquaria was in the range of 110–125 dB re 1 μPa. These SPLs are equivalent to power spectral densities ranging from approximately 80 db re 1 $\mu Pa^2$/Hz (for controls) to 122 db re 1 $\mu Pa^2$/Hz (for maximal noise level). Although control and noise-exposed aquaria were in the same room in the short-term experiments, the SPL of the control aquaria did not change when the underwater speaker was turned on in the noise-exposed aquaria. Due to the 40 dB loss of sound energy

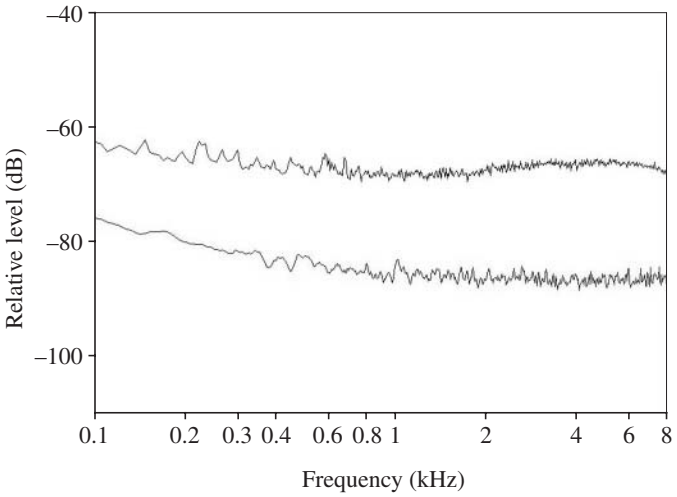


Fig. 1. The power spectra of the 170 dB re 1 µPa white noise used for noise-exposure experiments. The top curve shows the spectrum as recorded directly from the MiniDisc player. The bottom curve shows the spectrum as recorded by a hydrophone placed centrally within the noise-exposure bucket. The spectrum measured within the noise-exposure aquarium is similar to that of the bucket, so it is omitted for clarity.

at the air–water interface (Parvluescu, 1964), very little sound was heard outside of the noise tanks and none of this energy was able to get into the water of the other tanks in the room. Minor differences, however, may have occurred between the short- and long-term experiments because of the smaller volumes of the aquaria and buckets used in the short-term experiment (i.e. closer proximity between the fish and the underwater speaker compared with the large long-term aquaria).

### *Cortisol and glucose assays*

Blood plasma cortisol and glucose concentrations are commonly used as indicators of primary and secondary stress in fishes, with cortisol exhibiting a more rapid, transient response than glucose (Barton et al., 1988; Mazeaud et al., 1977; Mazeaud and Mazeaud, 1981). Preliminary tests were performed prior to noise-exposure experiments as a positive control to evaluate cortisol and glucose levels in response to physiological stress. In these preliminary tests, groups of goldfish ($N$=6) were placed in 10 liters of water in a 19-liter bucket. The control group was left undisturbed for 30 min while the treatment group was exposed to repeated, continuous vibratory stress for 30 min caused by tapping the bucket.

On each of the experimental days (0–21 days) of the long-term noise exposure experiment, five fish were removed and bled from the control aquarium first and then from the noise-exposed aquarium. Blood was collected from the caudal vein using heparinized 1-ml 25G 5/8 tuberculin syringes and placed in centrifuge tubes. Each fish was caught singly in a net and removed slowly in an attempt to minimize capture-induced stress in the caught fish and other fish in the aquarium. The fish was bled immediately after capture and then placed in a bucket of water containing a buffered anesthetic, tricaine methonosulfonate (MS-222). It took approximately 7 min to bleed all five fish. Afterwards, the fish were sacrificed by cervical transection, and their inner ears were removed and placed in 4% paraformaldehyde, 2% glutaraldehyde fixative for future ultrastructure examination using scanning electron microscopy (SEM).

Blood samples were centrifuged for 10 min at 5000 rpm and the plasma was then removed and stored at –70°C until analysis. Plasma cortisol, diluted 1:10 in a 0-cortisol standard in order to fit assay sensitivity, was assayed using an enzyme immunoassay (EIA) kit (DSL-10-2000, Diagnostic Systems Laboratories, Inc., Webster, TX, USA) with a four-parameter curve fit for standard curves. Plasma glucose was assayed using a Sigma Infinity glucose kit (Procedure 17-UV; Sigma Diagnostics, Town, State, Country).

For the short-term noise-exposure experiment, one aquarium was randomly chosen for each exposure duration (0 min, 10 min and 60 min) and all fish ($N$=6) were consecutively removed and bled. Blood plasma was then assayed as described for the long-term noise experiment.

### *Auditory brainstem response (ABR) technique*

Auditory thresholds were measured using the auditory brainstem response (ABR) technique. This is a noninvasive method of measuring the whole brain response to auditory stimuli and is commonly used for measuring hearing in fishes and other vertebrates (Corwin et al., 1982; Kenyon et al., 1998; Higgs et al., 2001; Scholik and Yan, 2001).

Each fish was restrained in a mesh sling and suspended in a 19-liter plastic bucket filled with water. The fish was suspended so that the top of the head was approximately 3 cm below the water surface and 25 cm above a UW-30 underwater speaker. A reference electrode was placed on the dorsal surface of the fish's head along the midline between the anterior portion of the eyes, while a recording electrode was placed on the dorsal midline surface of the fish approximately halfway between the anterior insertion of the dorsal fin and the posterior edge of the operculae, directly over the brainstem. A ground electrode was placed in the water near the body of the fish.

Sound stimuli were presented and ABR waveforms collected using a physiology apparatus using SigGen and BioSig software [Tucker-Davis Technologies (TDT) Inc., Gainesville, FL, USA]. Sounds were computer generated *via* TDT software and passed through a power amplifier connected to the underwater speaker. Tone bursts had a 2 ms rise and fall time, were 10 ms in duration and were gated through a Hanning window – similar to the conditions of other ABR studies (Mann et al., 2001; Higgs et al., 2001). Responses to each tone burst at each SPL were collected using the BioSig software package, with 400 responses averaged for each presentation. The calibration of each frequency used was done using a calibrated Model 902 Interocean Systems, Inc. (Town, State, Country) underwater hydrophone (calibration sensitivity of –195 dB re 1 V/µPa; ±3 dB, 0.02–10 kHz, omnidirectional).

Additional details of this ABR protocol have been previously published (Higgs et al., 2001).

Hearing thresholds of the experimental fish were measured after specified durations of noise exposure. For the long-term experiment, these individuals came from the same aquaria as described for the cortisol and glucose assays, but different individuals were used ($N$=6). Additionally, 21-day-exposed goldfish were allowed to recover in quiet aquaria (<120 dB re 1 μPa), and their hearing thresholds were again measured 7 days and 14 days post-noise exposure.

For the short-term experiment, fish were noise-exposed in a 19-liter bucket. For 10 min exposure durations, fish were held in place by the mesh sling described above and exposed in the same bucket from which ABRs were recorded. For 1–24 h duration exposures, fish were exposed in a separate bucket in which they could swim freely. There was no evidence that the fish sought areas of the lowest SPL (closest to the surface) or avoided the underwater speaker. All ABR recordings were started within a few minutes after noise exposure. Fish noise-exposed for 24 h (short-term experiment) had their hearing measured immediately after noise exposure and then were allowed to recover in quiet aquaria as in the long-term experiment, except that ABR recordings were made 1, 4, 11 and 18 days after noise exposure.

### *Statistical analysis*

Preliminary analyses of variance (ANOVAs) with treatment (control or noise-exposed) and bleeding order as factors showed that bleeding order had a significant effect on the physiological stress response of the fish. To account for this confounding effect on the effects of noise exposure on goldfish plasma cortisol and glucose, analysis of covariance (ANCOVA) was used, with noise-exposure duration as a factor and bleeding order as a covariate. When significant main effects of noise exposure were found, Wilcoxon signed ranks tests were used to make specific pairwise comparisons.

The effects of noise exposure and recovery from the exposure on auditory threshold levels were tested using separate ANOVAs, with duration of exposure or recovery and frequency as factors. Tukey's *post-hoc* test was used to make pairwise comparisons between specific frequencies when significant main effects were found (Zar, 1984). Regression analysis was used to examine the effects of noise exposure duration on temporary threshold shifts (TTS).

## Results

### *Effects of noise on plasma cortisol and glucose*

Preliminary tests using bucket vibration as a stressor showed that post-stress plasma cortisol and glucose were significantly elevated (84–94%) compared with controls ($P$<0.05). Mean (± S.E.M.) control cortisol and glucose levels were 89.7±32.0 ng ml$^{-1}$ and 43.1±4.5 mg dl$^{-1}$, while stressed levels were 165±18.1 ng ml$^{-1}$ and 83.8±6.2 mg dl$^{-1}$, respectively. This confirmed that our assays were appropriate for measuring physiological stress responses in goldfish. In both long- and short-term noise-exposure experiments, bleeding order affected fish plasma cortisol levels ($P$<0.05) but not glucose levels. In general, plasma concentrations of cortisol and glucose increased with bleeding order, suggesting that the fish were exhibiting a stress response due to the netting of previously removed fish.

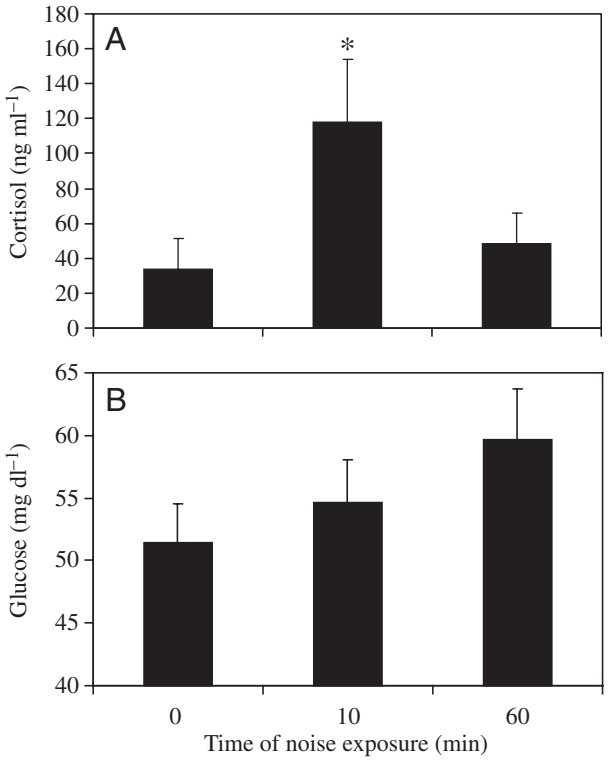


Fig. 2. Mean (+ S.E.M.) goldfish blood plasma cortisol and glucose concentrations after 0 min (control), 10 min or 60 min white noise exposure for the short-term noise-exposure experiment. The asterisk represents a level that is significantly different ($P$=0.01) from the control.

Noise exposure did not significantly affect cortisol or glucose concentrations in the long-term noise experiment (1–28 days exposure; $P$<0.10). In the short-term exposure experiment, noise exposure significantly affected plasma cortisol levels ($P$=0.01) but not glucose levels ($P$=0.27). Specifically, relative to controls, mean cortisol levels tripled after 10 min noise exposure and then decreased back to control levels after 60 min of noise exposure (Fig. 2A). Although there was a trend of increasing mean goldfish glucose concentrations over the 60 min experimental exposure period, this trend was not statistically significant (Fig. 2B).

### *Effects of noise on auditory thresholds*

Goldfish had a bandwidth of auditory sensitivity ranging from 0.1 kHz to 4 kHz, and baseline auditory thresholds ranging between 60 dB re 1 μPa and 120 dB re 1 μPa (Fig. 3). Exposure to the white noise caused an increase in auditory thresholds, referred to as temporary threshold shift (TTS). These threshold shifts are defined as temporary since they

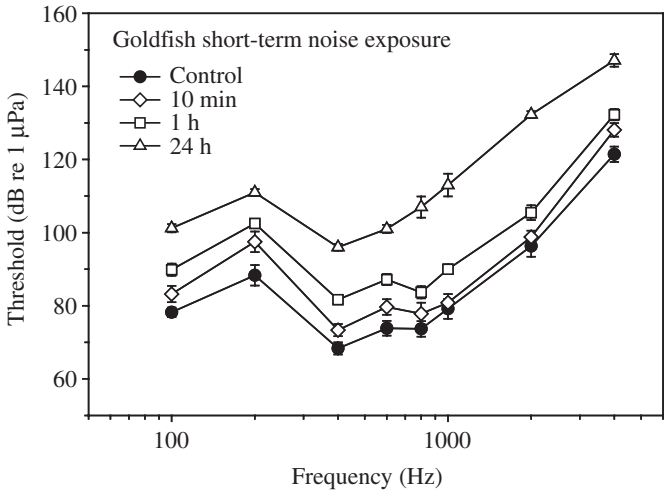


Fig. 3. Mean (± S.E.M.) auditory thresholds of control and noise-exposed goldfish in short-term experiment (10 min, 1 h and 24 h white noise exposures). *N*=6 per data point.

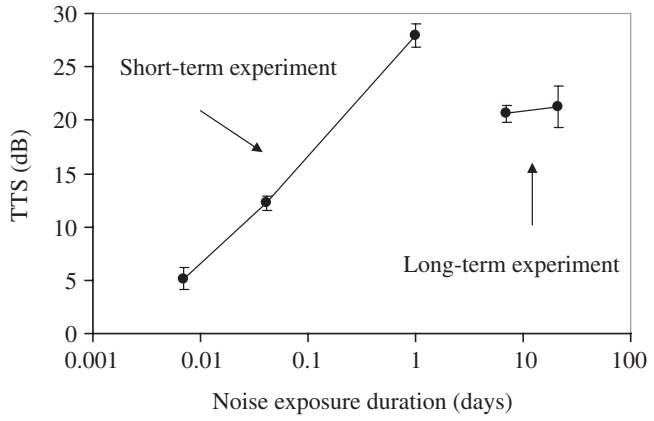


Fig. 4. Mean (± S.E.M.) temporary threshold shifts (TTS; between 0.1 kHz and 2 kHz) of noise-exposed goldfish as a function of exposure duration in the short-term and long-term noise-exposure experiments (see text). *N*=7 per data point (one mean value of six fish for each of seven frequencies).

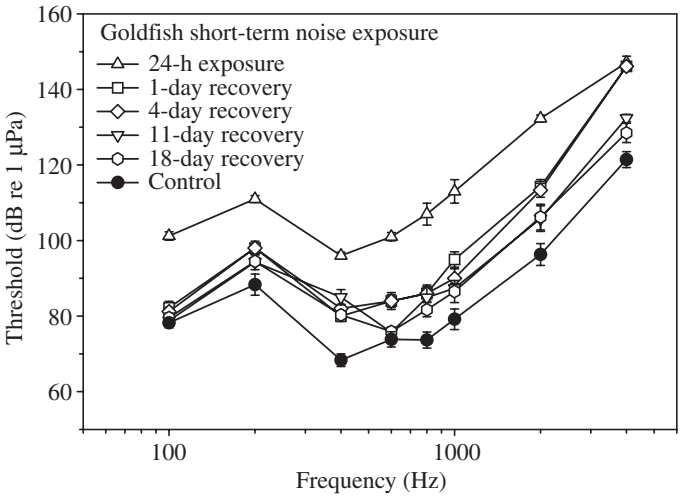


Fig. 5. Mean (± S.E.M.) auditory thresholds of control, 24-h noise-exposed and post-exposure (recovery) goldfish in the short-term experiment. *N*=6 per data point. The same six individual fish were used consecutively for each time point before and after noise exposure.

decreased with time after recovery from noise exposure until thresholds were similar to pre-noise exposure levels. Approximately 5 dB TTS were evident after only 10 min of noise exposure, and TTS increased to approximately 28 dB after 24 h of exposure (Fig. 3). This log-linear increase exhibited in the short-term noise exposure experiment is described by the equation TTS=27.7($\log_{10}D$)+4.63 ($r^2$=0.90, $P$<0.0001), where TTS is the mean temporary threshold shift (between 0.1 kHz and 2 kHz) and $D$ is the duration of noise exposure in days (Fig. 4). Longer durations of the long-term noise experiment (7 days and 21 days) produced threshold shifts similar to that of the 24 h exposure duration, suggesting that an asymptotic threshold shift (AST) is reached within 24 h of noise exposure at the sound levels used in this experiment. In other words, after the duration at which the AST is reached, no greater durations of noise exposure will produce greater TTS. TTS resulting from 7 days and 21 days exposure were statistically less than those of 24 h exposures from the short-term noise experiment ($P$<0.05). After 7 days of noise exposure, goldfish exhibited significant mean threshold shifts that were approximately 20 dB higher than baseline levels. Again, significant TTS occurred at all frequencies examined ($P$<0.05). An additional week of noise exposure (14 days) did not significantly increase the threshold shift. The goldfish audiograms of this long-term noise-exposure experiment are presented elsewhere (Smith et al., in press).

In the short-term noise experiment, goldfish exposed to noise for 24 h had significantly lower thresholds one day after exposure when compared to thresholds determined immediately after noise exposure ($P$<0.0001), but even after 18 days of recovery goldfish exhibited slightly higher thresholds than pre-exposure control levels ($P$<0.0001; Fig. 5).

In the long-term experiment, 21-day-exposed goldfish were allowed to recover from noise exposure. For each fish, TTS was averaged across all frequencies tested (0.1–4 kHz). TTS decreased with duration of recovery (from approximately 18 dB immediately after exposure to 0 dB after 2 weeks). After 7 days of recovery, there was still a significant overall effect of noise exposure compared with controls ($P$=0.003; Fig. 6), although this difference was not significant for any particular frequency. After 14 days of recovery, auditory thresholds between noise-exposed fish and controls were no longer significantly different (Fig. 6).

## Discussion

### *Effects of noise on physiological stress response*

We noted qualitatively that goldfish exhibited an initial startle response to the onset of the white noise, but this

response diminished within a few minutes and the fish did not avoid the area around the underwater speaker nor was there evidence that fish sought areas of the lowest SPL. This startle response started with a rapid burst of erratic swimming followed by general increased swimming activity. Loud sounds are known to induce such behavioral responses (i.e. startle or alarm responses) in fishes. For example, Boussard (1981) found that sound from a high-speed motorboat elicited flight responses in two cyprinid fishes: rudd (*Scardinius erythrophthalmus*) and roach (*Rutilus rutilus*). Pacific herring (*Harengus pallasi*) also exhibited alarm responses in reaction to motorboat noise, particularly when abrupt changes in temporal characteristics of the sound occurred (Schwarz and Greer 1984). The effects of other anthropogenic noises on fishes have also been studied. Pearson et al. (1992) found that sounds from seismic surveys can affect rockfish (*Sebastes* spp.) behavior, and there is evidence for a similar effect on Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*; Engås et al., 1996). While previous studies have shown that relatively loud acoustic stimulation can affect fish behavior, the potentially harmful physiological effects of such stimulation were not examined.

During a stress response, there is an immediate release of catecholamines followed by the activation of the hypothalamic–pituitary–interrenal axis, which stimulates the synthesis and secretion of glucocorticoid hormones (cortisol in teleosts; Schreck et al., 2001). Our results show that noise exposure can elicit this physiological cascade rapidly in goldfish (within 10 min) but that the response is short-lived, with cortisol levels returning to pre-noise exposure levels within 1 h. The magnitude of the cortisol response was similar to that found in other fishes. For example, plasma cortisol levels of rainbow trout, *Onchorynchus mykiss*, increased from 29 ng ml$^{-1}$ to 145 ng ml$^{-1}$ after 4 h confinement (Pankhurst, 1998). Two-hour net confinement resulted in an increase in cortisol levels from <25 ng ml$^{-1}$ to approximately 150 ng ml$^{-1}$ in tilapia (*Oreochromis mossambicus*; Nolan et al., 1999). The effect of stress on plasma glucose levels in fishes is more ambiguous. While Nolan et al. (1999) found a significant increase in tilapia cortisol and glucose levels due to confinement, Waring et al. (1996) only found a cortisol effect in turbot (*Scophthalmus maximus*). Results from the present study indicate a trend towards increasing plasma glucose values with time of noise exposure in goldfish during the short-term experiment (0–60 min); however, no trend in either glucose or cortisol was evident in the long-term experiment.

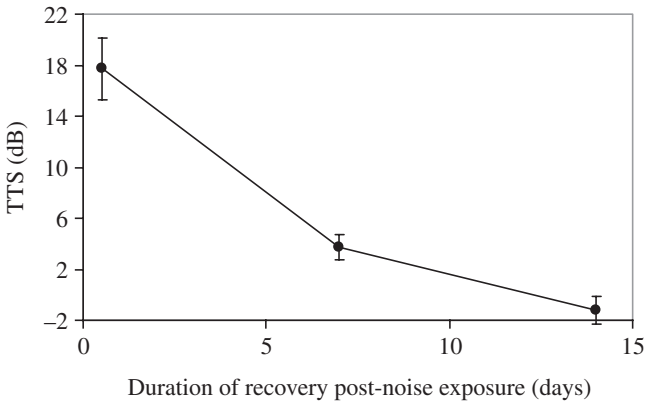


Fig. 6. Mean (± S.E.M.) auditory temporary threshold shifts (TTS) in the long-term experiment as a function of duration of time post-noise exposure (immediately after 21 days exposure = 0 days, 7 days and 14 days). *N*=6 per data point. The same six individual fish were used consecutively for each time point before and after noise exposure. The TTS for each individual was averaged across all frequencies (0.1–4 kHz). The 0-day data point is offset slightly to the right.

Two plausible reasons for this lack of a long-term stress effect are: (1) fish became acclimated to the noise over time and/or (2) noise-induced damage of the inner ear or nerves creates a threshold shift that effectively reduces the level of perceived noise. In support of the first explanation, rapid changes in sound characteristics often stimulate alarm behaviors, and these changes may elicit stress-like responses much more than does continual noise exposure. For instance, startle responses in red drum (*Sciaenops ocellatus*) larvae are elicited by the onset of an acoustic stimulus, not continuous exposure (Fuiman et al., 1999). Physiological adaptation to a continuous stressor is commonly found in fishes (Schreck, 2000). For example, salmonids exposed to stressful social or physical conditions exhibit an initial increase in plasma cortisol but return to pre-stress levels within about a week (Schreck, 1981).

Although the behavioral response of fishes to noise may be transient, the damage to their ears may occur quickly and have a longer-lasting effect. For example, noise-induced damage to the sensory hair cells of codfish (*Gadus morhua*) and oscar (*Astronotus ocellatus*) were noted after only 1 h of continuous exposure to various frequencies (>180 dB re 1 μPa; Enger, 1981; Hastings et al., 1996). Additionally, auditory threshold shifts have been noted after only 1–2 h noise exposure (142 dB re 1 μPa; Scholik and Yan, 2001, 2002a). We noted significant TTS in goldfish after only 10 min of stimulation. Thus, it is possible that after threshold shifts occurred, the perceived level of the noise and the resulting physiological stress level were reduced.

While we did not observe long-term physiological stress associated with continuous noise in goldfish, future studies are needed to examine whether loud intermittent or impulsive sounds produce such a response. Such intermittent sounds may more closely represent loud anthropogenic sounds that fish might experience in the wild (e.g. boat traffic, seismic surveys and sonar).

### *Effects of noise duration and recovery on auditory thresholds*

Our control goldfish audiograms are similar to those previously published in which psychophysical/behavioral methods were utilized (Fay, 1998), except that our audiograms have a slightly higher threshold at 200 Hz compared with 100 Hz. This has been a consistent trend in audiograms obtained using ABR in our lab, and a similar trend has been found in at least one other lab. Scholik and Yan (2001)

published baseline audiograms for *Pimephales promelas* (fathead minnow; a cyprinid-like goldfish) in which the mean thresholds at 500 Hz were higher than those at 300 Hz. One possible explanation of this trend is the ability of fish to use the lateral line to detect lower frequency vibrations, so that a 100 Hz tone may be detected by a goldfish's lateral line as well as the ear, whereas a higher frequency tone would only be detected by the ear (Tavolga and Wodinsky, 1965). Other researchers examining noise-induced damage in fish have not performed ABRs at frequencies below 200 Hz, probably in order to avoid stimulating the lateral line, although in our lab, fishes exposed to cobalt chloride to selectively ablate the lateral line did not have ABR thresholds significantly different from controls (Ramcharitar, 2003). So, despite no clear explanation of why thresholds were higher at 200 Hz compared with 100 Hz for our goldfish, it is important to note that significant noise-induced threshold shifts occur even at low frequencies.

Noise exposure had a considerable impact on threshold shifts (up to 28 dB) at all frequencies in goldfish but with shifts being greater where their hearing sensitivity is best. Popper and Clarke (1976) examined the effects of pure tones on threshold shifts in goldfish and found that SPLs of 149 dB re 1 µPa produced threshold shifts of approximately 7–9 dB and 18–27 dB at 500 Hz and 800 Hz, respectively. Thus, TTS was most dramatic at frequencies where the goldfish is more sensitive. Amoser and Ladich (2003) exposed goldfish to 158 dB re 1 µPa white noise for 24 h and found greatest hearing loss at 800 Hz and 1000 Hz. Similarly, the fathead minnow, another hearing specialist, exhibited approximately 11–20 dB TTS in response to 24 h of 142 dB re 1 µPa white noise (0.3–4 kHz) exposure (Scholik and Yan, 2001) and 8–11 dB TTS in response to 2 h of 142 dB re 1 µPa narrow-bandwidth boat motor noise with a peak frequency near 1.3 kHz (Scholik and Yan, 2002a). These shifts occurred at auditory frequencies where the fathead minnow is most sensitive. Scholik and Yan (2001, 2002a) did not find significant, or as strongly significant, TTS at lower (0.3–0.8 kHz) and higher (2.5–4 kHz) frequencies, while we and Amoser and Ladich (2003) found significant TTS across all frequencies. This may be the result of differences between species or differences in experimental noise SPL and bandwidths.

Significant auditory threshold shifts were evident after only 10 min of noise exposure in goldfish. Thus, loud sounds can have rapid detrimental effects on fish hearing, as well as stress levels. This means that even transient anthropogenic sounds such as boat traffic may affect fishes. Previous studies examining the effect of noise on fish used durations of ≥1 h (Popper and Clarke, 1976; Scholik and Yan, 2001, 2002b; Amoser and Ladich, 2003). When duration of noise exposure was log-transformed, the relationship between TTS and duration was linear for our short-term experiment. This noise duration–TTS relationship is similar to those found in birds and mammals, except that in birds and mammals this relationship is more curvilinear, with the rate of TTS increasing closer to the ATS (Carder and Miller, 1972; Saunders and Dooling, 1974). In goldfish, we noted a maximal threshold shift at 24 h of noise exposure. By contrast, Scholik and Yan (2001a) found that fathead minnows (also hearing specialists) exposed to white noise at 142 dB re 1 µPa reached an ATS after only 2 h of noise exposure. It is possible that goldfish reach an ATS earlier than the initial 24 h observations made in the present study. This is supported by lack of overall threshold differences between goldfish exposed for 12 h and 24 h in Amoser and Ladich's study (Amoser and Ladich, 2003). It is interesting to note that bird and mammal ATS are consistently reached (using various exposure frequencies and SPL) between exposure durations of 8 h and 24 h (Mills et al., 1970; Carder and Miller, 1972; Saunders and Dooling, 1974).

Surprisingly, goldfish TTS observed in the long-term experiment were less than those for the 24 h-exposed fish. This is probably due to differences in container size between the two sets of experiments. Although the underwater speaker output was the same for both experiments, the 19-liter buckets used for the short-term experiment were smaller than the aquaria used in the long-term study. This put the fish in closer proximity to the underwater speaker in the short-term compared with the long-term experiment, which may have led to higher mean SPL and TTS.

Goldfish exposed to noise for 24 h had 10–20 dB decreases in auditory thresholds after only 1 day of recovery. Despite this initial improvement, thresholds did not return to pre-exposure levels even after 18 days of recovery. Similarly, fathead minnows exposed to 142 dB re 1 µPa white noise for 24 h still exhibited significant threshold shifts after 14 days of recovery (Scholik and Yan, 2001). Longer-term recovery experiments are needed to ascertain whether or not these smaller long-term shifts are permanent threshold shifts. No permanent threshold shift has ever been reported for fish. In fact, fathead minnows exposed for only 2 h had thresholds that returned to control levels within 6 days post-exposure (Scholik and Yan, 2001), and goldfish exposed for 12 h or 24 h returned to control levels within 3 days of recovery (Amoser and Ladich, 2003). This earlier recovery, when compared with the current study, may be due to the relatively smaller noise SPL and durations used. In the present study, goldfish exposed to noise for 21 days had auditory thresholds that returned to control levels after 14 days of recovery, with considerable recovery occurring within the first 7 days. As described earlier, the probable higher mean SPL experienced in the short-term compared with the long-term experiment may explain why full recovery occurred in goldfish exposed for 21 days but not for those exposed for only 24 h. Thus, the time course of recovery may be dependent upon noise SPL as well as duration.

Alternatively, since an asymptote of hearing loss was reached within 24 h of noise exposure, it is possible that physiological and cellular repair processes began as soon as noise-induced damage occurred and that the time course of ear repair may be constant, even if noise exposure is continued beyond the asymptote duration. The reason that continued exposure beyond the asymptote duration did not produce greater TTS may be that maximal inner ear hair cell damage

occurs within the first 24 h. When hair cells are damaged, they are extruded to the lumen, and the support cells in the sensory epithelium are triggered to divide and subsequently differentiate into hair cells and supporting cells (Bermingham-McDonogh and Rubel, 2003). This process can take several days. For example, after gentamicin exposure and subsequent hair cell loss, hair cells of the oscar recovered within 10 days (Lombarte et al., 1993). After exposure to intense air-gun signals, pink snapper (*Pagrus auratus*) did not exhibit significant hair cell damage 18 h post-exposure but exhibited significant damage 58 days post-exposure, suggesting that the damage and recovery process can take extended periods of time (McCauley et al., 2003). In the current study, the 24-h-exposed fish did not show recovery after 19 days (1 day exposure + 18 days recovery) of the start of the exposure. The 21-day-exposed fish showed recovery 14 days after 21 days of stimulation, which is 35 days after the start of the noise exposure. Thus, perhaps the bulk of inner ear damage occurred on the first exposure day and then at least 19 days are required for complete repair to take place. Thus, sufficient repair may take 28–35 days, since 21-day-exposed fish did not show control-level thresholds after 7 days of recovery but did recover after 14 days. Although further noise-induced damage to the inner ear sensory epithelium may occur with exposure durations of longer than 24 h, it is also possible that hair cells damaged within the first 24 h undergo programmed cell death followed by extrusion and regeneration (differentiation) from surrounding supporting cells. These newly developing hair cells may effectively be protected from further noise-induced damage until they are completely differentiated.

SPL and duration of noise exposure can affect the magnitude of TTS and the time to recovery as seen in mammals and birds (Carder and Miller, 1972; Saunders and Dooling, 1974; Mills et al., 1979). Despite the differences in the characteristics of sound conduction in air *versus* water and the differences in ear anatomy and hearing mechanisms in terrestrial vertebrates compared with fishes, the process of noise-induced auditory threshold shifts seems to be similar in both groups. Both show fairly linear increases in TTS with noise SPL and duration, followed by an asymptotic maximal threshold. Both show that greater SPLs and longer durations increase the time to recover to normal hearing levels. Thus, it is likely that many of the general principles and relationships discovered in the mammalian hearing literature will be applicable to how loud sounds affect fishes – for example, the relationship between sensory cell loss and hearing thresholds (Hamernik et al., 1989) and the exposure-equivalent principle (Ward et al., 1959).

In summary, our data show that goldfish are susceptible to noise-induced stress and hearing loss. This is probably the result of their hearing sensitivity, since ‘hearing generalists’, or fish with higher baseline hearing thresholds, are less vulnerable to noise-induced hearing loss. For example, bluegill sunfish (*Lepomis machrochirus*) and tilapia did not exhibit threshold shifts in response to intense noise exposure (Scholik and Yan, 2002b; M. E. Smith, A. S. Kane and A. N. Popper, unpublished data). This could be because a certain noise SPL above a fish’s baseline hearing threshold must be reached before a TTS occurs (Hastings et al., 1996; Smith et al., in press). Thus, fish with lower baseline audiograms (hearing specialists) will be more susceptible to noise-induced hearing loss for a given noise level. Despite the dramatic TTS that resulted from noise exposure, goldfish were able to recover to normal hearing levels within two weeks of being exposed to three weeks of noise. This may suggest that fish that have been exposed to intense anthropogenic underwater noise may not have permanent physiological or auditory injury.

The authors wish to thank Dennis Higgs and John Ramcharitar for their assistance with auditory brainstem response protocol, and Dennis Plachta for producing the white noise file used for noise exposure of fish. We thank Audrey Rollo, Cookie Kennedy and Gregory Concepcion for help in maintenance and care of fish used in this experiment. This research was supported by NIH grants T32 DC-00046-08 to the Center for Comparative and Evolutionary Biology of Hearing and F32 DC-05890-01 to M.E.S. and by a program development award from Maryland Sea Grant to A.N.P. and A.S.K.

## References

**Amoser, S. and Ladich, F.** (2003). Diversity in noise-induced temporary hearing loss in otophysine fishes. *J. Acoust. Soc. Am.* **113**, 2170-2179.

**Au, W. W. L. and Nachtigall, P. E.** (1997). Acoustics of echolocating dolphins and small whales. *Mar. Fresh. Behav. Physiol.* **29**, 127-162.

**Banner, A. and Hyatt, M.** (1973). Effects of noise on eggs and larvae of two estuarine fishes. *Trans. Am. Fish. Soc.* **1**, 134-136.

**Bart, A. N., Clark, J., Young, J. and Zohar, Y.** (2001). Underwater ambient noise measurements in aquaculture systems: a survey. *Aquacult. Eng.* **25**, 99-110.

**Barton, B. A., Schreck, C.B. and Fowler, L.G.** (1988) Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile chinook salmon. *Progr. Fish Cult.* **50**, 16-22.

**Bermingham-McDonogh, O. and Rubel, E.** (2003). Hair cell regeneration: winging our way towards a sound future. *Curr. Opin. Neurobiol.* **13**, 119-126.

**Boussard, A.** (1981). The reactions of roach (*Rutilus rutilus*) and rudd (*Scardinius erythropthalmus*) to noises produced by high speed boating. In *Proceedings of 2nd British Freshwater Fisheries Conference* (ed. ????), pp. 188-200. Southampton, UK: Publisher.

**Carder, H. M. and Miller, J. D.** (1972). Temporary threshold shifts from prolonged exposure to noise. *J. Speech Hear. Res.* **15**, 603-623.

**Corwin, J. T., Bullock, T. H. and Schweitzer, J.** (1982). The auditory brainstem response in five vertebrate classes. *Electroencephalogr. Clin. Neurophysiol.* **54**, 629-641.

**Edds-Walton, P. L.** (1997). Acoustic communication signals of mysticete whales. *Bioacoustics* **8**, 47-60.

**Engås, A., Løkkeborg, S., Ona, E. and Soldal, A. V.** (1996). Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). *Can. J. Fish. Aquat. Sci.* **53**, 2238-2249.

**Enger, P. S.** (1981). Frequency discrimination in teleosts – central or peripheral? In *Hearing and Sound Communication in Fishes* (ed. W. N. Tavolga, A. N. Popper and R. R. Fay), pp. 243-255. New York: Springer-Verlag.

**Fay, R. R.** (1998). *Hearing in Vertebrates: a Psychophysics Databook*. Winetka, IL: Hill-Fay.

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

**Fay, R. R. and Popper, A. N.** (2000). Evolution of hearing in vertebrates: the inner ears and processing. *Hear. Res.* **149**, 1-10.

**Fuiman, L. A., Smith, M. E. and Malley, V.** (1999). Ontogeny of routine

swimming speed and startle responses in red drum, with a comparison of responses to acoustic and visual stimuli. *J. Fish Biol.* **55A**, 215-226.

**Hamernik, R. P., Patterson, J. H., Turrentine, G. A. and Ahroon, W. A.** (1989). The quantitative relation between sensory cell loss and hearing thresholds. *Hear. Res.* **38**, 199-212.

**Hastings, M. C., Popper, A. N., Finneran, J. J. and Lanford, P. J.** (1996). Effect of low frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus ocellatus*. *J. Acoust. Soc. Am.* **99**, 1759-1766.

**Hawkins, A. D. and Myrberg, A. A.** (1983). Hearing and sound communication underwater. In *Bioacoustics: A Comparative Approach* (ed. B. Lewis), pp. 347-405. London: Academic Press.

**Higgs, D. M., Souza, M. J., Wilkins, H. R., Presson, J. C. and Popper, A. N.** (2001) Age- and size-related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*). *J. Assoc. Res. Otolaryngol.* **3**, 174-184.

**Kenyon, T. N., Ladich, F. and Yan, H. Y.** (1998). A comparative study of hearing ability in fishes; the auditory brainstem response approach. *J. Comp. Physiol. A* **182**, 307-318.

**Lagardère, J. P.** (1982). Effects of noise on growth and reproduction of *Crangon crangon* in rearing tanks. *Mar. Biol.* **71**, 177-185.

**Lombarte, A., Yan, H. Y., Popper, A. N., Chang, J. S. and Platt, C.** (1993). Damage and regeneration of hair cell ciliary bundles in a fish ear following treatment with gentamicin. *Hear. Res.* **64**, 166-174.

**Mazeaud, M. M. and Mazeaud, F.** (1981). Adrenergic response to stress in fish. In *Stress in Fish* (ed. A. D. Pickering), pp. 49-75. London, Academic Press.

**Mazeaud, M. M., Mazeaud, F. and Donaldson, E. H.** (1977). Stress resulting from handling in fish: primary and secondary effects. *Trans. Am. Fish. Soc.* **106**, 201-212.

**McCauley, R. D., Fewtrell, J., Popper, A. N.** (2003). High intensity anthropogenic sound damages fish ears. *J. Acoust. Soc. Am.* **113**, 1-5.

**Mills, J. H., Gengel, R. W., Watson, C. S. and Miller, J. D.** (1970). Temporary changes of the auditory system due to exposure to noise for one or two days. *J. Acoust. Soc. Am.* **48**, 524-530.

**Mills, J. H., Gilbert, R. M. and Adkins, W. Y.** (1979). Temporary threshold shifts in humans exposed to octave bands of noise for 16 to 24 h. *J. Acoust. Soc. Am.* **65**, 1238-1248.

**Myrberg, A. A., Jr** (1990). The effects of man-made noise on the behavior of marine animals. *Environ. Int.* **16**, 575-586.

**Nolan, D. T., Op't Veld, R. L. J. M., Balm, P. H. M. and Wendelaar Bonga, S. E.** (1999). Ambient salinity modulates the response of tilapia, *Oreochromis mossambicus* (Peters), to net confinement. *Aquaculture* **177**, 297-309.

**NRC** (2000). *Marine Mammals and Low Frequency Sound: Progress Since 1944*. Washington, DC: National Academy Press.

**NRC** (2003). *Ocean Noise and Marine Mammals*. Washington, DC: National Academy Press, in press.

**Pankhurst, N. W.** (1998). Further evidence of the equivocal effects of cortisol on in vitro steroidogenesis by ovarian follicles of rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem.* **19**, 315–323.

**Parvulescu, A.** (1964). Problems of propagation and processing. In *Marine BioAcoustics* (ed. W. N. Tavolga), pp. 87-100. Oxford: Pergamon Press.

**Pearson, W. H., Skalski, J. R. and Malme, C. I.** (1992). Effects of sounds from a geophysical survey device on behavior of captive rockfish (*Sebastes spp.*). *Can. J. Fish. Aquat. Sci.* **49**, 1343-1356.

**Popper, A. N. and Clarke, N. L.** (1976). The auditory system of the goldfish (*Carassius auratus*): effects of intense acoustic stimulation. *Comp. Biochem. Physiol. A* **53**, 11-18.

**Popper, A. N. and Fay, R. R.** (1999). The auditory periphery in fishes. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 43-100. New York: Springer-Verlag.

**Ramcharitar, J.** (2003). Structure-function relations in the inner ears of Western Atlantic Sciaenid fishes. *Ph.D. Dissertation.* University of Maryland, College Park, MD, USA.

**Ross, D.** (1993). *On Ocean Underwater Ambient Noise. Institute of Acoustics Bulletin 18*. Location: Publisher.

**Saunders, J. C. and Dooling, R. J.** (1974). Noise-induced threshold shift in the parakeet (*Melopsittacus undulatus*). *Proc. Natl. Acad. Sci. USA* **71**, 1962-1965.

**Scholik, A. R. and Yan, H. Y.** (2001). Effects of underwater noise on auditory sensitivity of a cyprinid fish. *Hear. Res.* **152**, 17-24.

**Scholik, A. R. and Yan, H. Y.** (2002a). Effects of boat engine noise on the auditory sensitivity of the fathead minnow, *Pimephales promelas*. *Environ. Biol. Fish.* **63**, 203-209.

**Scholik, A. R. and Yan, H. Y.** (2002b). The effects of noise on the auditory sensitivity of the bluegill sunfish, *Lepomis macrochirus*. *Comp. Biochem. Physiol. A* **133**, 43-52.

**Schreck, C. B.** (1981). Stress and compensation in teleostean fishes: response to social and physical factors. In *Stress and Fish* (ed. A. D. Pickering), pp. 295-321. London: Academic Press.

**Schreck, C. B.** (2000). Accumulation and long-term effects of stress in fish. In *The Biology of Animal Stress – Basic Principles and Implications for Animal Welfare* (ed. G. P. Moberg and J. A. Mench), pp. 147-158. New York: CABI Publishing.

**Schreck, C. B., Contreras-Sanchez, W. and Fitzpatrick, M. S.** (2001). Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* **197**, 3-24.

**Schwarz, A. L. and Greer, G. L.** (1984). Responses of Pacific herring, *Clupea harengus pallasi*, to some underwater sounds. *Can. J. Fish. Aquat. Sci.* **41**, 1183-1192.

**Smith, M. E., Kane, A. S. and Popper, A. N.** (in press). Acoustical stress and hearing sensitivity in fishes: does the linear threshold shift hold water? *J. Exp. Biol.*

**Tavolga, W. N. and Wodinsky, J.** (1965). Auditory capacities in fishes: threshold variability in the blue-striped grunt *Haemulon sciurus*. *Anim. Behav.* **13**, 301-311.

**US Department of Transportation** (1999). *An Assessment of The U.S. Marine Transportation System. A Report To Congress.* Location: US Department of Transportation.

**von Frisch, K.** (1938). The sense of hearing in fish. *Nature* **141**, 8-11.

**Ward, W. D., Glorig, A. and Sklar, D. L.** (1959). Temporary threshold shift produced by intermittent exposure to noise. *J. Acoust. Soc. Am.* **31**, 791-794.

**Waring et al.** (1996). Physiological responses to handling in the turbot. *J. Fish Biol.* **48**, 161-173.

**Welch, B. L. and Welch, A.S.** (ed.) (1970). *Physiological Effects of Noise*. New York: Plenum Press.

**Zar, J. H.** (1984). *Biostatistical Analysis*. Second edition. Englewood Cliffs, NJ: Prentice-Hall.

**Zelick, R., Mann, D. and Popper, A. N.** (1999). Acoustic communication in fishes and frogs. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 363-411. New York: Springer-Verlag.