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Acoustical stress and hearing sensitivity in fishes: does the linear threshold shift hypothesis hold water?

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Summary

Mammals exposed to loud aerial sounds exhibit temporary threshold shifts (TTS) that are linearly related to increases of sound pressure above baseline hearing levels. It was unknown if this relationship held true for aquatic ectotherms such as fishes. To test this linear threshold shift hypothesis (LINTS) in fishes, we examined the effects of increased ambient sound on hearing of two species differing in hearing capabilities: goldfish (*Carassius auratus*; a hearing specialist) and tilapia (*Oreochromis niloticus*; a hearing generalist). Fish were exposed to 1–28 days of either quiet (110 dB re 1 μPa) or continuous white noise. First, we examined the effect of noise sound pressure level (SPL; 130, 140, 160 or 170 dB re 1 μPa) on goldfish hearing thresholds after 24 h of noise exposure. Second, in a long-term experiment using 170 dB re 1 μPa white noise, we continuously exposed goldfish and tilapia for either 7 or 21–28 days. In both experiments, we measured alterations in hearing capabilities (using auditory brainstem responses) of noise-exposed fish. While tilapia exposed to noise for 28 days showed little or no hearing loss, goldfish exhibited considerable threshold shifts that reached an asymptote of up to 25 dB after only 24 h of exposure. There was a positive linear relationship between noise-induced TTS and the sound pressure difference between the noise and the baseline hearing thresholds in goldfish but not in tilapia. A similar relationship was found for published noise-induced threshold shifts in birds and mammals, but the slope of the linear relationship was greater in these groups than for fish. The linear threshold shift relationship provides insights into differential susceptibility of hearing specialist and generalist fishes to noise-induced hearing loss for a given SPL and provides a framework for future research on noise-induced threshold shifts in fishes and other animals.

Key words: threshold shift, hearing, fish, noise, LINTS, auditory brainstem response, *Carassius auratus*, *Oreochromis niloticus*.

Introduction

High levels of sound have a significant impact on the auditory system and overall physiology of humans and other animals (Welch and Welch, 1970; Kryter, 1985). Such sounds may result in permanent damage to the auditory system, including deafness. Lower level sounds, over a longer duration, can temporarily or permanently affect hearing. While numerous studies have documented the negative effects of loud sounds on mammals (NRC, 2000), effects of such sounds on fishes remain poorly understood (Myrberg, 1990; Popper, 2003). It is well known that fishes use sound for communication, for detection of predators and prey and for learning about their environment (Popper and Fay, 1999; Zelick et al., 1999; Fay and Popper, 2000; Popper et al., 2003). However, in many areas of their natural environment, as well as in aquaculture facilities, fishes are exposed to higher sound levels as a result of anthropogenic noise that may negatively affect normal behavioral and physiological processes (Bart et al., 2001).

Sounds that are well above those to which an animal is normally exposed are known to cause temporary changes in hearing capabilities of fishes [i.e. temporary threshold shifts (TTS); Popper and Clarke, 1976; Scholik and Yan, 2001]. Even louder sounds, or longer exposure to somewhat quieter sounds, produce damage to the sensory cells of fish ears, as evidenced in the few fish species that have been studied, and this may lead to permanent loss of hearing (i.e. permanent threshold shifts; Enger, 1981; Hastings et al., 1996; McCauley et al., 2003). In addition to causing inner ear damage, high levels of background sound may create physiological and behavioral stress responses in fishes similar to those found in mammals (Smith et al., 2004).

Mammalian models have long been used to understand the

effects of noise on humans. The results of past studies using mammals show that TTS (noise-exposed threshold minus control threshold) increase with duration of noise exposure until an asymptotic threshold shift (ATS) is reached (Clark, 1991). Once the ATS is reached for a given sound pressure level (SPL), further noise exposure no longer increases TTS. The magnitude of the ATS depends upon the SPL of the exposure noise and increases linearly with SPL above a minimal threshold shift (Carder and Miller, 1972).

Although loud sounds were known to induce hearing threshold shifts in fishes (Scholik and Yan, 2001; Amoser and Ladich, 2003; Smith et al., 2004), it was unknown whether fishes exhibit linear threshold shifts with increased SPL, as is found in mammals. Since water is a far more dense medium for sound conduction than air, and since the mechanism of hearing in fishes is very different from that of mammals, it is not intuitive that the relationship between SPL and TTS in fishes would be the same as that for aerial hearing of mammals.

In the present study, we tested the hypothesis that noise-induced threshold shifts in fishes increase linearly with increasing sound pressure differences (SPD) between the exposure noise and baseline hearing thresholds (referred to here as the linear threshold shift hypothesis or LINTS hypothesis). To test this hypothesis, we investigated the effect of intense, continuous white noise exposure on hearing loss in fish utilizing the auditory brainstem response (ABR) technique (Corwin et al., 1982; Kenyon et al., 1998). Two species of fish that differ considerably in hearing sensitivity served as models: goldfish (*Carassius auratus*; a hearing specialist) and tilapia (*Oreochromis niloticus*; a hearing generalist). The goal was to compare alterations in hearing between species to elucidate a potential relationship between hearing sensitivity and susceptibility to acoustic stress.

Although there is a broad continuum in hearing capabilities among various fish taxa, the terms ‘hearing specialist’ and ‘hearing generalist’ (with hearing ‘non-specialist’ used as a synonym) are commonly used to describe the opposite extremes of this continuum. We chose goldfish as a representative hearing specialist because of their excellent hearing sensitivity and the considerable data in the literature about their hearing (see Fay and Popper, 1974; Fay, 1988; Popper et al., 2003). Goldfish are otophysan fishes and therefore possess Weberian ossicles (modified cervical vertebrae that abut the ear; von Frisch, 1938) that allow sound pressure waves impinging upon the swim bladder to be carried directly to the ear, leading to sensitive hearing (wide-frequency range and relatively low thresholds).

Tilapia, a cichlid, have relatively poor hearing. They have no accessory structures connecting the swim bladder to the ear, and sound travels through the ear *via* bone conduction (Fay and Popper, 1975). Hearing sensitivity has previously been characterized for only three other cichlid species – African mouthbreeder (*Tilapia macrocephala*), oscar (*Astronotus ocellatus*) and African cichlid (*Tramitichromis intermedius*). Audiograms for these species show that, compared with goldfish, they hear a smaller bandwidth and at higher thresholds (Tavolga, 1974; Kenyon et al., 1998; Ripley et al., 2002).

Materials and methods

Experimental animals

Goldfish (*Carassius auratus* L.) and tilapia (*Oreochromis niloticus* L.) were obtained from commercial suppliers and then maintained at the Aquatic Pathobiology Laboratory at the University of Maryland, College Park. For the short-term noise exposure experiment (Experiment 1), goldfish were maintained in 38-liter glass aquaria with biological filtration and were exposed to noise in 19-liter buckets. Standard length for goldfish used in Experiment 1 was 4.8±0.1 cm (mean ± S.E.M.). For the long-term experiment (Experiment 2), fish (first tilapia and then goldfish) were maintained in each of two 600-liter all-glass aquaria with biological filtration and 65% water changes three times a week. Each of these aquaria was kept in a separate room. One was a noise-exposure tank with an underwater speaker and the second was a quiet control tank at ambient room-level noise. Standard lengths for goldfish and tilapia used in Experiment 2 were 10.5±0.1 and 11.9±0.1 cm (mean ± S.E.M.), respectively. Experiments and animal care were approved by the Institutional Animal Care and Use Committee of the University of Maryland.

White noise exposure

Fish were exposed to white noise with a bandwidth from 0.1 to 10 kHz. The sound was generated using a Sony MiniDisc player connected through an amplifier (5.2 A monoblock; AudioSource, Portland, OR, USA) to an underwater speaker (UW-30; Underwater Sound, Inc., Oklahoma City, OK, USA) placed centrally on the bottom of the aquarium. White noise, 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 (WaveMetrics, Inc., Lake Oswego, OR, USA). Characteristics of the noise exposure (bandwidth and SPL) were similar in both short- and long-term noise exposure experiments, with transduction in the tanks having little effect on the digitally generated flat, ‘white noise’ spectra (Fig. 1; Smith et al., 2004). For Experiment 1, 24 h noise exposures were presented at overall SPLs of either 110 (ambient control), 130, 140 or 160 dB re 1 μPa to goldfish. These overall SPLs are equivalent to power spectral densities of approximately 80, 90, 97, 118 and 122 dB re 1 μPa^2/Hz, which were measured using a Brüel and Kjar (Nærum, Denmark) 8103 hydrophone and Type 4223 hydrophone calibrator. Additional 24-h exposure data from a previous goldfish study (Smith et al., 2004) that used a SPL of 170 dB re 1 μPa (124 dB re 1 μPa^2/Hz) were compared with the other three SPLs of Experiment 1. For simplicity, in describing the noise to which fish were exposed in the remainder of this paper, SPL will be given in terms of overall dB re 1 μPa, instead of the associated power spectral density.

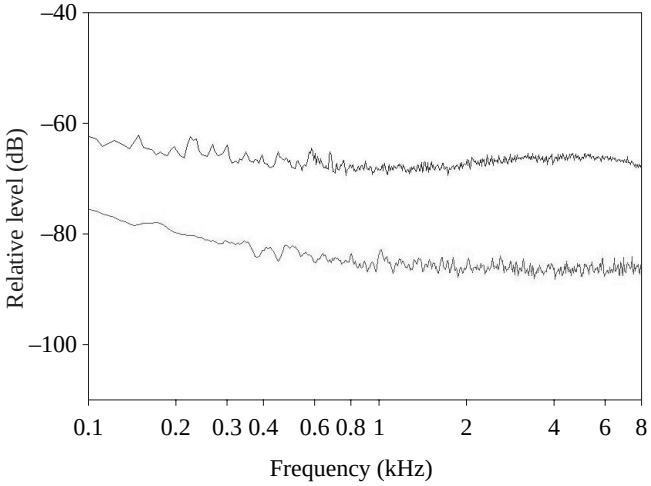

Fig. 1. The power spectra level of the 170 dB re 1 μPa white noise used for noise exposure experiments (from Smith et al., 2004). The top curve shows the spectrum as recorded directly from the MiniDisc player. The bottom curve shows the spectrum as recorded by a hydrophone placed centrally within the noise exposure bucket. The spectrum measured within the noise exposure aquarium is similar to that of the bucket, so it is omitted for clarity.

For Experiment 2, long-term noise exposures of 164–170 dB re 1 μPa were presented to goldfish and tilapia for either 7 days or 21–28 days. Goldfish were exposed for 21 days, while tilapia were exposed for 28 days because goldfish reach an ATS by 1 day (Smith et al., 2004). Once we established that there was no difference in ATS for goldfish between days 1 and 21, we terminated the goldfish exposure early (i.e. no differences in TTS between 21- and 28-day noise-exposed goldfish expected) in order to return the fish to a quiet and less stressful environment.

In 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. The SPL of the noise exposure in the long-term experiments varied slightly within an aquarium, with a maximum (170 dB re 1 μPa) directly above the underwater speaker and minimum (161–168 dB re 1 μPa) near the sides of the aquarium furthest from the speaker. The SPL of the control aquarium ranged from 110 to 125 dB re 1 μPa.

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 at the air–water interface (Parvulescu, 1964), relatively little sound was heard outside of the noise tanks and none of this energy got 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).

Auditory brainstem response (ABR) technique

Hearing thresholds of the experimental fishes were measured on each specified day of noise exposure (N=5–6 for controls and noise-exposed fish for each exposure group) using the auditory brainstem response (ABR). This technique is a non-invasive method of measuring the neural activity of the brainstem in response to auditory stimuli and is commonly used for measuring hearing in fishes and other vertebrates (Corwin et al., 1982; Kenyon et al., 1998). Each fish was restrained in a mesh sling and suspended underwater in a 19-liter plastic vessel. The fish was suspended so that the top of the head was approximately 3 cm below the surface of the water and 25 cm above the underwater speaker.

A reference electrode was inserted subdermally into the medial dorsal surface of the head between the anterior portion of the eyes while a recording electrode was placed into 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 were collected using a TDT physiology apparatus using SigGen and BioSig software (Tucker-Davis Technologies, 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 total duration and were gated through a Hanning window (similar to the conditions of other ABR studies; e.g. 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 SPLs of each presented frequency were confirmed using a calibrated underwater hydrophone (calibration sensitivity of –195 dB re 1 V/μPa; ±3 dB, 0.02–10 kHz, omnidirectional; model 902; Interocean Systems, Inc., San Diego, CA, USA). Auditory thresholds were determined by visual inspection of ABRs, as has been done in previous studies. Additional details of this ABR protocol have been previously published (Higgs et al., 2001).

Statistical analysis

For Experiment 1, the effects of noise exposure SPL on fish auditory threshold levels were tested using analysis of variance (ANOVA) with SPL 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). In Experiment 1, regression analysis was used to test for relationships between noise exposure SPL and the resulting TTS. The threshold shifts were labeled temporary because goldfish exposed to 170 dB re 1 μPa white noise for 21 days recovered to control hearing levels within two weeks post-noise exposure (data presented in Smith et al., 2004). For this analysis, mean TTS for each SPL was averaged across five frequencies (400, 600, 800, 1000 and 2000 Hz), so that each point was calculated using 30 thresholds (N=6 fish × 5 frequencies). While TTS data for SPLs of 130, 140 and 160 dB

Table 1. *Sources of data used in Figs 7, 8, including experimental animals, sound stimulus characteristics used for exposure and sound exposure duration for each study*

Taxa	Species	Reference	Data	Stimulus	Duration
Fish	Bluegill sunfish*	Scholik and Yan (2002b)	Table 1	142 dB white noise	24 h
	Fathead minnow	Scholik and Yan (2001)	Fig. 2; Table 1	142 dB white noise	24 h
	Goldfish	Amoser and Ladich (2003)	Fig. 2; Table 2	158 dB white noise	24 h
	Catfish	Amoser and Ladich (2003)	Fig. 4; Table 4	158 dB white noise	24 h
	Goldfish	Present study	Fig. 6B	170 dB white noise	21 days
	Tilapia*	Present study	Fig. 6A	170 dB white noise	28 days
Birds	Chick	Pugliano et al. (1993)	Fig. 1	120 dB re 0.9 kHz tone	48 h
	Chick	Alder (1993)	Fig. 2	120 dB re 0.9 kHz tone	48 h
	Quail	Ryals et al. (1999)	Fig. 2	112 dB re 2.9 kHz tone	12 h
	Budgerigar	Ryals et al. (1999)	Figs 2, 6	112–120 dB re 2.9 kHz tone or 2–6 kHz BPN	12–24 h
	Canary	Ryals et al. (1999)	Fig. 6	112–120 dB re 2.9 kHz tone or 2–6 kHz BPN	24 h
	Zebrafinch	Ryals et al. (1999)	Fig. 6	112–120 dB re 2.9 kHz tone or 2–6 kHz BPN	24 h
Mammals	Human	Melnick (1976)	Fig. 3	80–85 dB OBN at 4 kHz	24 h
	Human	Mills et al. (1970)	Fig. 1	81.5–92.5 dB OBN at 0.5 kHz	8–30 h
	Human	Ward (1975)	Fig. 1	75–85 dB OBN at 4 kHz	24 h
	Chinchilla	Saunders et al. (1977)	Fig. 11	57–100 dB OBN at 4 kHz	9 days
	Chinchilla	Carder and Miller (1972)	Fig. 5	75–105 dB OBN at 0.5 kHz	2–21 days
	Chinchilla	Campo et al. (1991)	Fig. 3	106 dB OBN at 0.5 kHz	48 h
	Guinea pig	Canlon et al. (1987)	Fig. 1	105 dB re 1 kHz tone	3 days

Decibels (dB) are relative to 1 μPa in fish (underwater) and relative to 20 μPa in birds and mammals (aerial). BPN, band pass noise; OBN, octave band noise; *, hearing generalist fish.

re 1 μPa came from Experiment 1, the raw data for mean TTS at an SPL of 170 dB re 1 μPa are presented elsewhere (Smith et al., 2004).

Regression analysis was also used to test for relationships between SPD (in dB) between the exposure noise and baseline auditory thresholds and TTS in goldfish. Using SPD from baseline auditory thresholds instead of absolute SPL is similar to A-weighting, or measuring perceived sound levels (loudness) in human hearing studies. This relationship between noise SPD from baseline thresholds and TTS is referred to as the LINTS (linear threshold shift) relationship throughout this paper. In these analyses, each data point represents a TTS at a specific frequency, and the variability in the SPD above baseline threshold is due to differences in baseline thresholds across frequencies and not necessarily absolute experimental noise SPL. Analysis of covariance (ANCOVA) was used to examine the effects of frequency on TTS, with SPD above baseline thresholds as the covariate. Before ANCOVA was used, we tested for homogeneity of slopes of the separate regressions for each frequency using ANOVA with SPL, frequency and the interaction between the two factors. An insignificant interaction meant that the assumption of homogeneity of slopes could not be rejected. A similar analysis was used to test for differences in slopes between different SPL.

For Experiment 2, the effects of long-term noise exposure on goldfish and tilapia auditory threshold levels were tested using separate ANOVAs for each exposure duration, with treatment (control or noise exposed) and frequency as factors. Regression analysis was used to test for relationships between SPD from baseline auditory thresholds and TTS using data from Experiment 2 and published data for three other fish species (see Table 1). Separate and pooled regressions were done for hearing generalist and specialist fishes. Regression analysis was also used to examine the LINTS relationship in birds and mammals using published data (Table 1).

Results

Goldfish, but not tilapia, exhibited an initial startle response to the onset of the noise in Experiments 1 and 2. This response diminished rapidly (within a few minutes) and neither goldfish nor tilapia avoided the area around the underwater speaker. In Experiment 1, there was a significant overall effect of noise exposure on goldfish auditory thresholds at each SPL tested ($P<0.001$; Fig. 2). Significant differences between the thresholds of control (110 dB re 1 μPa) and noise-exposed fish occurred from 600 to 4000 Hz for goldfish exposed to 130 dB re 1 μPa ($P<0.05$) and at all frequencies tested for goldfish exposed to 140, 160 and 170 dB re 1 μPa ($P<0.05$).

There was a statistically significant linear relationship ($r^2=0.98$) between mean TTS (averaged across frequencies) and the SPL of the noise exposure (Fig. 3). The mean TTS was approximately 7 dB for a noise level of 130 dB re 1 μPa and 32 dB at a noise level of 170 dB re 1 μPa. When the mean TTS values at each frequency (instead of the values averaged across frequencies as in Fig. 3) were plotted against the SPD between the noise and baseline hearing thresholds, similar linear relationships were evident (Fig. 4A). When a separate linear regression analysis was done with each of the eight

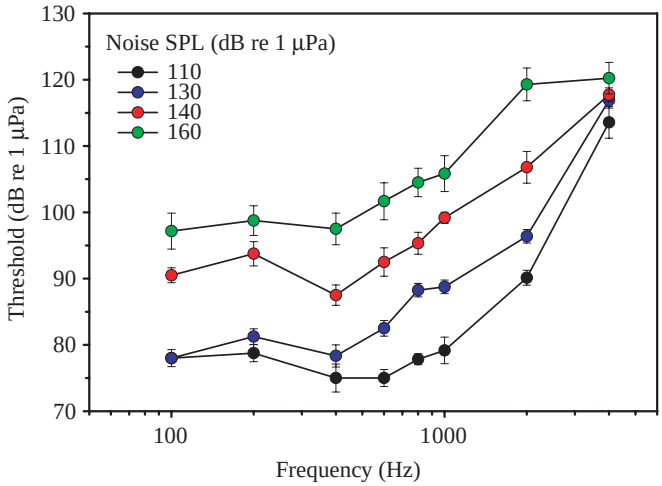

Fig. 2. Mean (± S.E.M.) auditory thresholds of control (110 dB re 1 µPa) and noise-exposed (130–160 dB re 1 µPa) goldfish after 24 h of white noise exposure.

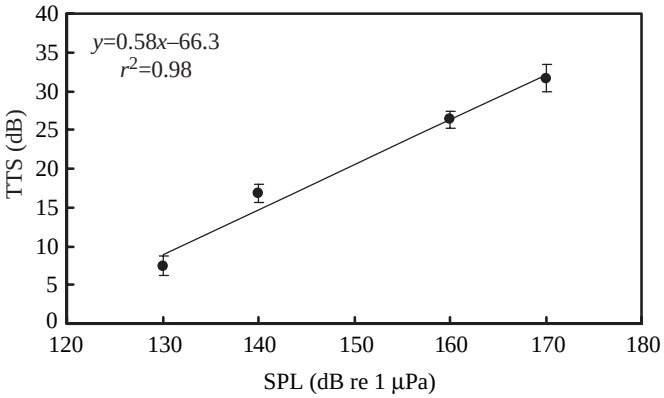

Fig. 3. Temporary threshold shift (TTS) as a function of absolute sound pressure level (SPL) of the exposure noise. Data points represent mean (± S.E.M.) TTS across five frequencies (400–2000 Hz), with *N*=6 fish for each frequency. The line represents the linear regression equation for the data shown.

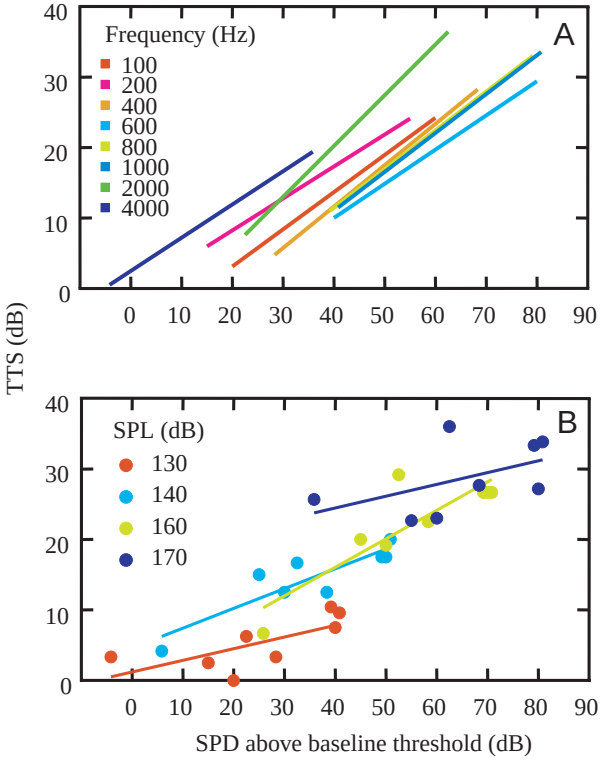

Fig. 4. Temporary threshold shift (TTS) as a function of sound pressure differences (SPD) between the noise exposure sound pressure level (SPL) and goldfish baseline auditory threshold SPL. This relationship is shown at (A) different frequencies tested and (B) different noise SPL used. Colored lines in A represent separate linear regressions for each frequency tested, while in B they represent separate linear regressions for each of the four SPLs (130, 140, 160 or 170 dB re1 µPa) at all frequencies tested. Each data point is the mean TTS for a particular frequency (*N*=6 fish). The same data points are plotted in A and B, but the individual data points are not shown in A for clarity.

frequencies tested, all had a significantly linear relationship ($P<0.0001$) that did not significantly differ in slope from one another ($P=0.39$) but did differ in TTS after accounting for SPD from baseline levels as the covariate ($P<0.0001$; Fig. 4A).

When separate regression analyses were done with each of the four SPLs tested across frequencies, all had a significantly linear relationship ($P<0.02$; Fig. 4B). There were significant differences in the slopes of these relationships, with slopes for 130 and 170 dB being slightly lower than those of 140 and 160 dB ($P<0.01$; Fig. 4B). LINTS relationships were more predictive when separated by frequency (Fig. 4A) than by SPL (Fig. 4B), with r^2 (coefficient of determination) values ranging from 0.50 to 0.82 and 0.14 to 0.48, respectively.

As mentioned above, TTS varied significantly with frequency. When TTS was plotted against frequency and compared with baseline audiograms, an inverse relationship between baseline thresholds and TTS was evident (Fig. 5); i.e. at frequencies at which goldfish had lower thresholds and more sensitive hearing, TTS produced by constant white noise was generally the greatest, so that the audiogram and TTS curves mirror each other. This mirrored image is not a perfect reflection though, with the greatest TTS occurring at 800 and 1000 Hz whereas goldfish are most sensitive at hearing frequencies of 400 and 600 Hz.

In Experiment 2, distinguishable ABRs were detectable from 100 to 800 Hz for tilapia, with auditory thresholds ranging from 90 to 130 dB re 1 µPa (Fig. 6A). Tilapia exposed to white noise for 7 days did not exhibit auditory thresholds that were significantly different from controls. Tilapia exposed for 28 days did exhibit an overall treatment effect, but this effect was only significant at 800 Hz ($P=0.02$), where noise-exposed tilapia had thresholds approximately 10 dB higher than controls.

Goldfish, as expected based upon the literature and other studies in our laboratory, had a much broader bandwidth of auditory sensitivity (as described above), with ABRs detectable up to 4 kHz and baseline auditory thresholds

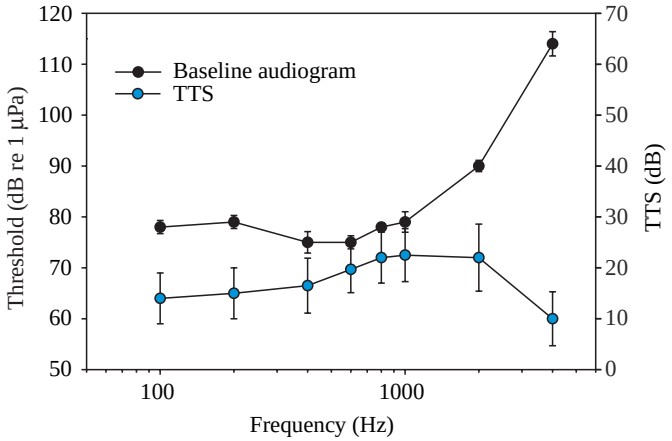

Fig. 5. Mean (± S.E.M.) temporary threshold shift (TTS) for all sound pressure levels (SPL) tested (130, 140, 160, 170 dB re 1 µPa) as a function of frequency (blue circles). The baseline goldfish audiogram (black circles) is presented for comparison to show the relationship between baseline thresholds and TTS.

ranging between 60 and 120 dB re 1 µPa (Fig. 6B). After 7 days of noise exposure, goldfish had significant threshold shifts that were up to 25 dB higher than baseline levels. Temporary threshold shifts (TTS) occurred at all frequencies examined (P<0.05; Fig. 6B). Two additional weeks of noise exposure (21 days) did not significantly increase the threshold shift. Differences in the effects of constant noise on the auditory thresholds between goldfish and tilapia were notable (Fig. 6). While significant differences between 28-day noise-exposed and control tilapia were small and found at only one frequency, goldfish exhibited considerable threshold shifts after only 7 days of noise exposure.

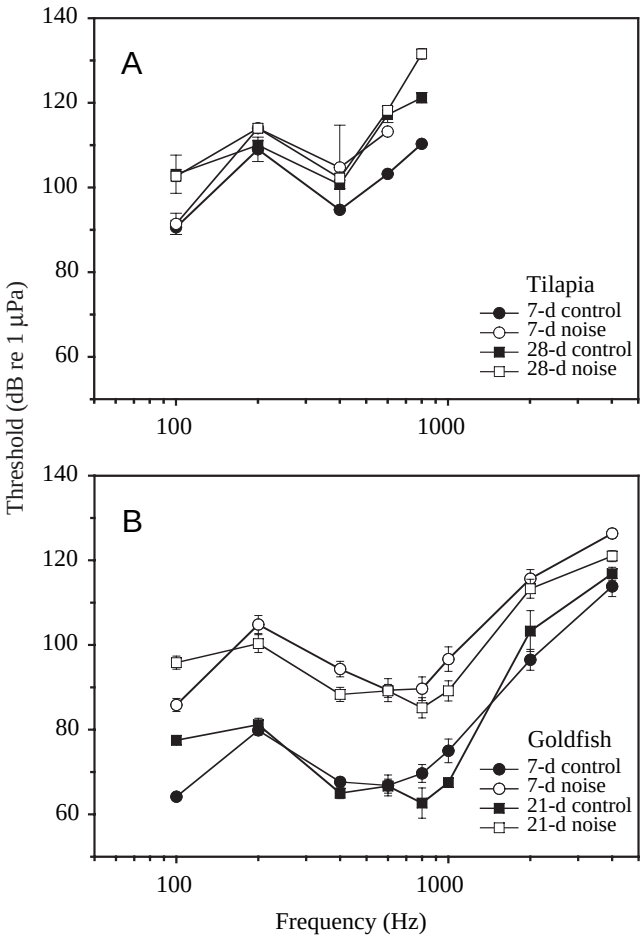

Fig. 6. Mean (± S.E.M.) auditory thresholds of control and noise-exposed (A) tilapia and (B) goldfish after 7 and 21 or 28 days noise exposure. N=5–6.

Discussion

Relationships between SPL, frequency and TTS

The mammalian literature clearly documents that TTS reach an ATS after a specific duration of continuous noise exposure at a given SPL (Mills et al., 1979; Clark, 1991). This ATS increases linearly with SPL above a minimal threshold shift. We asked whether this linear threshold shift relationship is valid for fishes.

Our experiments are the first to examine the effects of multiple SPLs on hearing thresholds in fish, and the data show that there is a predictable relationship between TTS and SPL in goldfish exposed to white noise (Fig. 3). A previous goldfish study showed that this TTS is at ATS after 24 h of noise exposure and that noise exposures of durations greater than 24 h did not show greater TTS (Smith et al., 2004).

In order to account for frequency-specific TTS, we plotted TTS against the difference between the noise sound pressure and baseline hearing thresholds at specific frequencies (the LINTS relationship; Fig. 7), instead of plotting TTS by absolute SPL as in Fig. 3. Thus, it was possible to focus on the relationship between SPL and TTS alone. The linear relationship between SPD between the noise and baseline thresholds and TTS was significant and similar (i.e. homogeneous slopes) for all the frequencies tested (Fig. 4). Thus, this relationship seems robust for all frequencies within the range of fish hearing.

Another advantage of using the LINTS relationship instead of absolute noise exposure SPL is that even though different studies utilize different species and methodologies and stimulate with sounds of various characteristics (e.g. frequency and SPL; Table 1), the LINTS relationship minimizes these differences and fosters species-wise comparisons. For example, subtracting the baseline hearing threshold from the noise exposure SPL for a particular experiment and/or species standardizes the LINTS relationship so that data from different laboratories and experiments can be compared. Since the LINTS relationship plots SPD (for both TTS and SPD above baseline levels), inter-laboratory differences in absolute SPL calibration of acoustic equipment become less important.

The LINTS relationship is robust and is predictive on many different levels. On the level of an individual animal, it predicts that, when stimulated with white noise, the threshold shift will

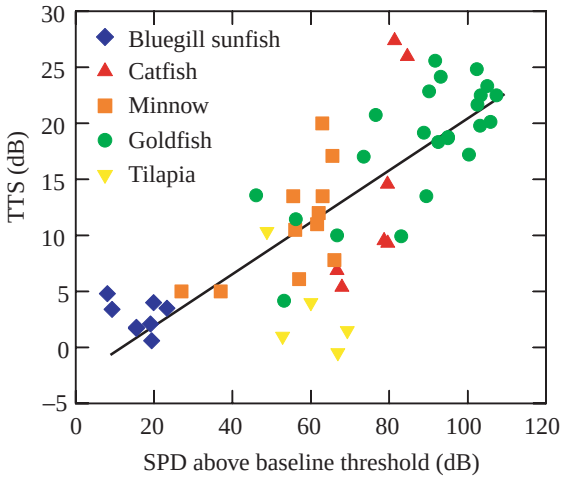

Fig. 7. Temporary threshold shift (TTS) as a function of noise sound pressure differences (SPD) between the noise exposure sound pressure level (SPL) and baseline hearing threshold SPL of five species of teleost. The line shows the linear regression relationship for all the species (TTS=0.23x–2.44, r^2=0.62).

be greatest at frequencies where the baseline hearing threshold is the most sensitive. This relationship for goldfish is shown in Fig. 5, where the lowest TTS was exhibited where the baseline threshold was the highest (i.e. 4000 Hz) and the highest TTS was exhibited at frequencies where the baseline was lowest (i.e. 800 and 1000 Hz). Although Fig. 5 shows that TTS generally mirrors the baseline hearing thresholds of goldfish, it is unclear why this mirrored image is shifted slightly to the right. For example, although baseline hearing thresholds increase considerably from 1 to 2 kHz, a corresponding decrease in TTS does not occur between 1 and 2 kHz, but does between 2 and 4 kHz. One potential explanation for this is the asymmetry of auditory filters. Both psychophysical and physiological tuning curves of goldfish are V-shaped, with steep slopes on the right, higher-frequency side and more gradual slopes on the left, lower-frequency side of the best frequency (Fay et al., 1978; Fay and Ream, 1986). Since these tuning curves are skewed to the right, this asymmetry may produce greater TTS on the right side of a frequency being tested. A similar phenomenon occurs in masking patterns, where, at high intensities of narrow-band noise maskers, levels of masking are greater to the right of the center of the noise band (Egan and Hake, 1950). The frequency specificity of goldfish TTS suggests that fish may have multiple, narrow-band detection channels that are tuned to detect specific frequency bandwidths. In support of this hypothesis, the data from Scholik and Yan (2002a) show that fathead minnows (*Promelas pimephales*) exposed to boat motor noise with a peak frequency at 1.3 kHz had significant hearing threshold shifts only at frequencies near the peak noise frequency (1.0, 1.5 and 2.0 kHz), with the greatest TTS occurring at 1.5 kHz. Similarly, the masking effects of tones in goldfish were greatest at or near the frequency of the tone (Tavolga, 1974).

Fish audiograms are generally U-shaped, with higher thresholds at low and high frequencies and lower thresholds at intermediate frequencies (Fay, 1988). As a result, the SPD between a flat spectrum white noise and a baseline threshold of a fish differs across frequencies and is greatest where hearing sensitivity is the best. This further suggests that the degree of effect of the noise may not be uniform for all frequencies, as seems to be the case, at least for LINTS relationships for hearing specialists. Thus, noise-induced TTS in fish, as well as sound detection, may be mediated by an auditory filter bank with multiple peripheral detection filters (i.e. hypothetical detection channels) operating at each frequency or span of frequencies, with the effects of the background noise varying across filters.

If noise-induced TTS were mediated by a single wideband filter, one would expect TTS to be constant across frequencies, which is not the case for the data presented here. It is interesting to note that in cod and goldfish, calculated effective bandwidths of auditory filters increase with frequency (Fay and Megela Simmons, 1999). In the presence of white noise, larger filter bandwidths would allow more acoustic energy through to the rest of the auditory system. This would presumably produce greater TTS at higher frequencies, which is not what we found, especially at 4000 Hz where TTS was minimal (Fig. 5). While it is beyond the results reported here to suggest specific filter mechanisms, future investigations are needed to examine the relationship between filter characteristics and TTS in fishes.

The issue of auditory filters in the fish auditory system is quite complex since filtering can occur at multiple levels in the auditory pathway, both peripherally and centrally. Although data are available for very few species, it is known that some species of fish can discriminate between frequencies (as little as 3% from a given pitch; Dijkgraaf and Verheijen, 1950; Fay, 1970), although it is still a matter of debate whether frequency discrimination is largely controlled by the peripheral or central auditory system (Enger, 1981). At the most peripheral level, mechanical properties of the otoliths and, in the case of hearing specialists, the swim bladder and Weberian ossicles are likely to be frequency dependent (Sand and Hawkins, 1973; Sand and Michelsen, 1978). At the level of the sensory epithelia, the goldfish saccule is crudely tonotopically organized, with higher center frequency afferents originating from the rostral region, while lower center frequency afferents originate from the caudal region (Furukawa and Ishii, 1967). Similarly, the saccular epithelia of the cod *Gadus morhua* may also be tonotopically organized. When exposed to an intense 350-Hz tone, most of the hair cells damaged occurred in the rostral region of the saccule but, after similar exposure to a 50-Hz tone, most of the damaged hair cells occurred in the caudal region (Enger, 1981).

To date, the only data relating to filters in the primary auditory afferents of fishes suggest very broad tuning, and just a few filters, across the hearing bandwidth (Furukawa and Ishii, 1967; Fay, 1974, 1978, 1981). Fay and Ream (1986) reported four non-overlapping categories of saccular nerve fibers (untuned, low-frequency, mid-frequency and high-frequency)

in the goldfish, with the degree of tuning remaining fairly constant across the goldfish hearing range. Although varying degrees of spontaneous activity and tuning can be found in the goldfish saccular afferents, birds and mammals show a more continuous distribution of fibers with a trend of increased tuning with greater frequencies. This suggests that the overall level of tuning is greater in other vertebrates compared with goldfish.

Using reverse correlation analysis to examine filter shapes of afferent impulse responses, Fay (1997) found that goldfish filter functions could be classified into two groups; low- and high-characteristic frequency filters. Both of these groups had similar characteristics that may reflect hair cell membrane properties, while it was suggested that differences in the groups were due to differences in hair cell bundle stiffness and mode of attachment to the otolithic membrane. It is possible that the characteristics of these two broad filters are responsible for the frequency dependency of noise-induced TTS in goldfish. The low- and high-frequency filters have impulse responses that have roll-offs below 200 Hz and above 1000 Hz, respectively. Similarly, we report TTS that was slightly lower at 100 and 200 Hz and above 2000 Hz, but TTS was similar at intermediate frequencies.

Although it is clear that some broad-frequency selectivity can occur at the level of the auditory periphery (hair cells and their associated primary afferent neurons), higher order central processing, such as phase-locking in auditory medullary units (Feng and Schellart, 1999), is probably necessary to produce the precise frequency discrimination and narrow critical bands evident from psychophysical tuning curves of fishes (Hawkins and Chapman, 1975; Hawkins and Johnstone, 1978). Critical bands are defined as the frequency span of noise that effectively masks a pure tone stimulus (Fletcher, 1940). There is evidence that narrow critical bands are associated with a wider total bandwidth and more acute hearing. For example, steep-sided masking functions are found in goldfish, a hearing specialist (Tavolga, 1974), while broader functions are found in the hearing generalists cod and salmon (Hawkins and Chapman, 1975; Hawkins and Johnstone, 1978). These differences in critical bandwidths between hearing specialists and generalists may be associated with the differential TTS effect of noise exposure that we found between these two groups of fishes.

LINTS hypothesis in relation to different fish species

Our goldfish audiograms were similar to those published in which psychophysical/behavioral methods were utilized (Fay, 1988), with a broad bandwidth (100–4000 Hz) and the most sensitive hearing occurring between 400–800 Hz. *Tilapia* had auditory thresholds that are 30–50 dB higher than those of goldfish. They had a small bandwidth of sensitivity, with ABRs only detectable up to 800 Hz. The absolute auditory thresholds and the 30–50 dB difference between goldfish and tilapia baseline audiograms found in this study are consistent with previous comparisons using psychophysical methods (Tavolga, 1974). Audiograms for two other tilapia species, *T. macrocephala* and *T. intermedius*, were similar to our audiograms for *O. niloticus*, with a small bandwidth (100–800 Hz) and relatively high thresholds (90–135 dB re 1 μPa; Tavolga, 1974; Ripley et al., 2002). The oscar, *Astronotus ocellatus*, also had similarly high thresholds but a broader bandwidth (100–2000 Hz; Kenyon et al., 1998).

Exposure to intense white noise had little effect on tilapia, except that noise-exposed tilapia had significantly higher thresholds at 800 Hz than controls. It is unclear why this threshold shift only occurred at 800 Hz but it is possible that tilapia respond to particle velocity at lower frequencies but are able to detect sound pressure at 800 Hz. Future experiments are needed to examine which components of sound (particle motion or pressure) tilapia are sensitive to over their bandwidth of hearing. Bluegill sunfish (*Lepomis macrochirus*), another generalist hearing fish, exhibited a slight, but not statistically significant, threshold shift after 24 h of white noise exposure (142 dB re 1 μPa; Scholik and Yan, 2002b). By contrast, noise exposure produced considerable threshold shifts (up to 25 dB) in goldfish, but with shifts being greatest where their hearing sensitivity is greatest (400–1000 Hz). Similarly, the fathead minnow (*Pimephales promelas*), another hearing specialist, exhibited approximately 10–15 and 20 dB threshold shifts at its most sensitive auditory frequencies in response to 24 h of white noise exposure and 2 h of boat motor noise with a peak frequency near 1.3 kHz (both 142 dB re 1 μPa), respectively (Scholik and Yan, 2001, 2002a).

The LINTS hypothesis predicts that, for a given intensity of sound, more sensitive species will be more prone to TTS than less sensitive species. The difference in the effects of noise exposure between goldfish and tilapia is probably due to the relationship between the noise SPL and the varying baseline auditory thresholds between the two species. To test this hypothesis, the TTS at each frequency was plotted against the difference between the noise SPL and the SPL of the baseline audiogram for goldfish and tilapia (present study) and for bluegill sunfish and fathead minnow (from Scholik and Yan, 2001, 2002b), goldfish and the catfish *Pimelodus pictus* (Amoser and Ladich, 2003).

With all five species, the resulting linear relationship between TTS and SPD above baseline threshold is TTS=0.23(SPD)–2.44 (r^2=0.62, P<0.0001; Fig. 7). A separate regression was done with hearing specialists only (goldfish, fathead minnows and catfish) since the hearing generalist species (bluegill and tilapia) did not exhibit significant TTS (except for a 10 dB shift at 800 Hz observed in tilapia). This regression was also significantly linear [TTS=0.24(SPL)–3.17; r^2=0.53, P<0.0001]. Mean TTS increased from fathead minnow to catfish to goldfish (all hearing specialists), which also corresponded with increasing experimental noise exposure SPLs of 142, 159 and 170 dB re 1 μPa, respectively (Table 1). All individual hearing specialist species had a significant regression relationship (P<0.05), while generalist species did not and could not be properly evaluated since TTS did not occur.

Experiments with higher noise levels will be needed to

ascertain whether the LINTS relationship is valid for hearing generalists. At 60 dB above the baseline threshold for tilapia, the linear relationship obtained using the other four species predicts that tilapia would exhibit a mean TTS of approximately 11 dB. It is interesting to note, however, that at the one frequency at which a significant TTS occurred (800 Hz) in tilapia, the threshold shift was approximately 10 dB, i.e. near the predicted value (Fig. 7).

A possible reason why tilapia did not exhibit threshold shifts in response to 170 dB re 1 μPa white noise, whereas goldfish did, is that a certain SPD above a baseline threshold must be reached before hearing loss occurs. Because baseline thresholds for tilapia are 20–50 dB higher than those of goldfish, one might predict that a 20–50 dB greater SPL (i.e. 190–220 dB re 1 μPa) will be required to produce the same threshold shifts as found in goldfish exposed to 170 dB re 1 μPa. Anthropogenic sound sources such as some SONARS and seismic air gun arrays produce sound levels of such intensities close to the source (NRC, 2000). Such SPLs would be difficult and dangerous to achieve in the laboratory, but higher SPLs than those tested here are needed to examine whether the LINTS relationship is only valid with hearing specialist fish or whether, given sufficient noise SPL, hearing generalists, or even intermediate-hearing species, will also exhibit a similar TTS.

In the LINTS relationships for fish species shown in Fig. 7, within-species variance in SPD from baseline thresholds is only due to the shape of the audiogram for each species since each was only exposed to one experimental SPL. This supports the view that fish are more prone to hearing loss at frequencies where they are most sensitive. All hearing specialists (goldfish, fathead minnows and catfish) had significant LINTS regressions when plotted individually, suggesting that this relationship is valid, at least for hearing specialists.

The prediction that better hearing species will be more prone to TTS from a specific noise SPL than poor hearing species also holds for birds. Canaries (*Serinus canaria*) and zebrafinches (*Taenopyga guttata*) were less sensitive to noise-induced basilar papillae damage and threshold shifts than more sensitive species such as quail (*Coturnix coturnix japonica*) and budgerigars (*Melopsittacus undulates*; Ryals et al., 1999).

The LINTS hypothesis in relation to different taxa

To compare LINTS relationships between hearing specialist fish and other taxa, regression analysis was also done using published data for birds and mammals (Table 1; Fig. 8). Despite differences in experimental protocols used in the previously published studies, all vertebrate taxa for which there are sufficient hearing studies showed significant linear relationships between the noise SPD above baseline hearing thresholds and the resulting threshold shift (P<0.001). The slope of this relationship was greatest for mammals, intermediate for birds and least for fishes. On this multi-taxon level, the LINTS hypothesis predicts that, for a given noise SPL, taxa with more sensitive hearing will be more likely to exhibit noise-induced threshold shifts than less sensitive taxa.

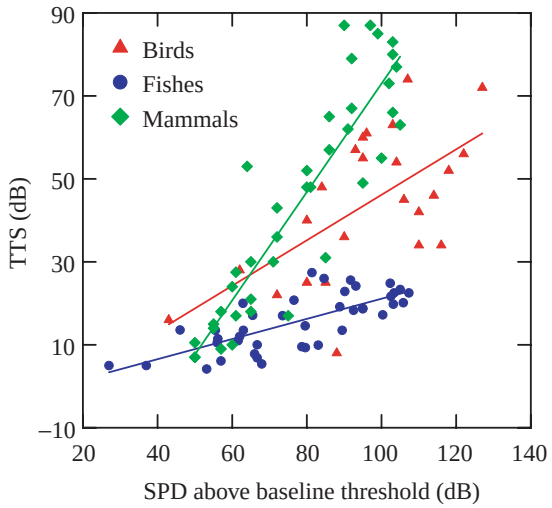

Fig. 8. Temporary threshold shift (TTS) as a function of noise sound pressure differences (SPD) between the noise exposure sound pressure level (SPL) and baseline hearing threshold SPL of birds, fish (hearing specialists only) and mammals. Lines represent significant linear regression relationships (TTS_{birds}=0.55x–8.64, r^2=0.36; TTS_{fish}=0.24x–3.17, r^2=0.54; TTS_{mamm}=0.55x–57.64, r^2=0.81).

This seems to be the case for mammals, birds and fishes, with TTS being greatest for mammals, intermediate for birds and least for fishes, for a specific SPL above baseline thresholds. Thus, a greater SPD between the noise exposure and the baseline hearing threshold is required in fishes to achieve a TTS similar to that found in mammals.

This is probably due to differences in mechanisms of sound detection and/or in the structure of the ear between groups. While the ears of fishes respond directly to the particle motion of a sound field, either through direct stimulation of the otolith end organs or *via* a pressure detecting device such as the swim bladder (Popper and Fay, 1999), birds and mammals possess a tympanic membrane and middle ear bones that amplify sounds impinging the tympanic membrane. Such specializations affect how efficiently the energy from a noise of specific SPL is transferred from the animal periphery to the inner ear.

If the TTS we report for goldfish is directly related to damage of inner ear hair cells, then the difference in slopes of the LINTS relationships between fish, birds and mammals may be due to differences in susceptibility to noise-induced hair cell damage. The LINTS relationship plots TTS as a function of SPL above baseline hearing thresholds. Experimentally, these SPL are usually measured outside of the body of an animal. Such measurements may not accurately reflect the amount of energy actually reaching the inner ear. For example, the resonance of the external ear of humans can increase the SPL at the tympanic membrane by 15–20 dB at 2.5 kHz (Weiner and Ross, 1946). Additionally, the middle ear bones act as an impedance transformer to minimize losses of sound energy associated with transmission from the air to cochlear fluids. According to Nedzelnitsky (1980), the transfer function of the middle ear shows a peak gain of ~30 dB at 1 kHz. Most

importantly, the mammalian cochlea acts as an active amplifier, with a gain of up to 60 dB (Viergever and Diependaal, 1986).

Although hearing specialist fishes such as goldfish detect sound *via* their swim bladder, and swim bladder motion is transmitted to the inner ear through the Weberian ossicles, no data exist on the actual transfer function of the swim bladder and/or Weberian ossicles. Based upon our current knowledge of the morphology of Weberian ossicles, there is no reason to think that potential amplification of sound by the swim bladder and ossicles approximates the magnitude of the peripheral amplification in mammals. Thus, the difference in the LINTS slopes between fish, birds and mammals may simply be a function of efficiency of sound conduction to the ear for a given sound level. An assumption of this hypothesis is that hair cells operate similarly in fish, birds and mammals. This is probably a safe assumption since it is generally believed that all vertebrate hair cells have fundamental characteristics in common and function according to similar principles (Popper and Fay, 1999). For example, the most sensitive inner ear afferents of goldfish can detect otolith particle motion as small as 0.1 nm (Fay, 1984). This displacement sensitivity is similar to the threshold of displacement in the guinea pig (0.2 nm; Allen, 1997), suggesting that the physiological processes of transduction are similar in fish and mammals.

In mammals, there is a relationship between hair cell loss and hearing loss. For example, tuning curves of cat auditory nerve fibers were elevated following noise and kanamycin exposure (Liberman and Dodds, 1984). Differences in the shape of these tuning curves were dependent upon specific damage to the hair cells of the organ of Corti (i.e. whether inner, outer or both hair cell types were damaged). Although there have been reports of fish hair cells being damaged by exposure to sound or ototoxic drugs, no data are yet available on the relationship between hair cell loss and hearing loss in fishes.

Effects of noise duration and recovery

In Experiment 2, goldfish auditory thresholds returned to control levels after 14 days of recovery, with considerable recovery occurring within the first 7 days. It remains to be tested whether the recovery from hearing loss was due to repair of mildly damaged hair cells or replacement of hair cells that were destroyed. Four species of birds exposed to noise showed considerable threshold shifts and hair cell damage immediately following exposure, but over time both threshold shifts and hair cell numbers recovered (Ryals et al., 1999). The difficulty in making a correlation between hearing and hair cell recovery in fishes arises because the only hair cell regeneration data available for fishes come from studies using ototoxic drugs in which hearing was never tested and there have been no comparable studies using acoustic trauma. Hair cell ciliary bundle replacement appeared to be complete 10 days after maximal gentamicin-induced hair cell damage in the oscar (Lombarte et al., 1993). Similarly, mitotic activity suggesting hair cell regeneration was found in adult quail 10 days after noise exposure (Ryals and Rubel, 1988).

After only 2 h of white noise exposure (142 dB re 1 μPa), fathead minnows had thresholds that returned to control levels, but after 24 h of exposure, thresholds were still significantly elevated after 14 days (Scholik and Yan, 2001). Thus, although goldfish and fathead minnows are both cyprinids and hearing specialists, there appear to be species-specific differences in recovery time from acoustic stimulation, although it is impossible at this point to rule out subtle experimental differences as also contributing to the differences in recovery time between species. Species-specific differences in recovery from acoustic trauma have also been reported for birds (Ryals et al., 1999).

Duration of noise exposure (7 or 21/28 days) did not significantly affect thresholds of goldfish in Experiment 2 because the ATS had already occurred. In an additional short-term experiment, goldfish exhibited significant threshold shifts after only 10 min of noise exposure and reached an ATS by 24 h of exposure (Smith et al., 2004). This asymptotic relationship between duration of exposure and hearing threshold shifts is well documented for mammals (Clark, 1991). Duration of exposure can also affect time to recovery in mammals (Mills et al., 1979). While we found that goldfish hearing recovered 14 days after a 21-day noise exposure, further experiments are needed to understand the relationship between exposure time and recovery time. A more thorough examination of the effects of noise-exposure duration on TTS and recovery of goldfish hearing is provided elsewhere (Smith et al., 2004).

Importance

The LINTS hypothesis is valid for underwater noise-induced TTS in some fishes, as it is in aerial noise-induced TTS in land vertebrates. This relationship standardizes TTS data from different studies for comparison. The LINTS relationship is valid across different frequencies and SPLs and multiple fish species and predicts, based on species-specific baseline thresholds, that some species will exhibit TTS in response to a certain SPL of noise exposure, while other species will not. Noise differentially affects species that differ in hearing sensitivity and confirms intuition that a given noise exposure would affect hearing specialists more than hearing generalists. The differential threshold shifts between bluegill sunfish and goldfish can be explained by a linear relationship between TTS and SPD above the fish's baseline threshold, but the data for tilapia do not seem to fit LINTS predictions. This LINTS hypothesis needs to be tested with more teleost species and a broader range of noise SPLs. Such a linear relationship for teleosts is consistent with what is found for birds and mammals, but greater underwater SPLs are required to induce a comparable threshold shift as in birds and mammals in air.

The LINTS relationship has potential utility in attempting to mitigate the effects of anthropogenic underwater noise, although it would also need to be determined for impulsive and repetitive sounds rather than continuous noise, as used in these

experiments and as experienced by fishes in aquaculture and other similar facilities. Once a general LINTS relationship is agreed upon for fishes, if a single relationship exists for all species that exhibit noise-induced hearing loss, the expected TTS of a previously unstudied species for a specified noise exposure (e.g. by an air gun) is only dependent upon the species' audiogram. If unknown, an audiogram can be readily attained using the ABR technique. This type of interpolation would be especially useful for the impacts of extremely loud sounds that are difficult to produce in the laboratory. McCauley et al. (2003) found that fish caged in the vicinity of seismic survey sounds exhibited severe inner ear damage. Similar field experiments using intense sound are needed to examine how such intense sounds affect fish hearing thresholds.

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