Diversity in noise-induced temporary hearing loss in otophysine fishes

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The effects of intense white noise (158 dB re 1 μPa for 12 and 24 h) on the hearing abilities of two otophysine fish species—the nonvocal goldfish Carassius auratus and the vocalizing catfish Pimelodus pictus—were investigated in relation to noise exposure duration. Hearing sensitivity was determined utilizing the auditory brainstem response (ABR) recording technique. Measurements in the frequency range between 0.2 and 4.0 kHz were conducted prior and directly after noise exposure as well as after 3, 7, and 14 days of recovery. Both species showed a significant loss of sensitivity (up to 26 dB in C. auratus and 32 dB in P. pictus) immediately after noise exposure, with the greatest hearing loss in the range of their most sensitive frequencies. Hearing loss differed between both species, and was more pronounced in the catfish. Exposure duration had no influence on hearing loss. Hearing thresholds of C. auratus recovered within three days, whereas those of P. pictus only returned to their initial values within 14 days after exposure in all but one frequency. The results indicate that hearing specialists are affected differently by noise exposure and that acoustic communication might be restricted in noisy habitats. © 2003 Acoustical Society of America.

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I. INTRODUCTION

In the aquatic environment, sound is one of the most important signal carriers, for it is transported five times faster than in air, is not attenuated as fast as light or chemical substances, and is propagated over large distances due to existing sound channels (Hawkins and Myrberg, 1983). There is a natural background noise due to currents, surf, rain, seismic events and sounds of biological origin (e.g., snapping shrimps, vocalizing animals). In the last decades, however, this has been augmented by an ever-increasing amount of anthropogenic noise, such as noise from shipping, hydroelectric power plants, drilling, or seismic surveys. For example, Andrew et al. (2002) compared ocean ambient sound data off the Californian coast from the 1960s with data from the 1990s and found an increase of up to 10 dB between 20 and 400 Hz.

There is growing concern that anthropogenic noise affects aquatic animals, in particular whales. Certain whale species change their vocalizing or migration behavior when exposed to intense low-frequency sonar sounds (Miller et al., 2000) or during acoustic thermometry of ocean climate (ATOC) transmissions (Frankel and Clark, 2000).

Intense noise exposure can impair auditory abilities by causing elevated auditory thresholds or damage to ear organs. In the last decades, the study of sound- or noise-induced hearing loss (NIHL) has concentrated on humans and mammals (humans: e.g., Hamernik et al., 1982; Bauer et al., 1991; mammals: e.g., Clark, 1991; Griffiths et al., 1994). Only a few studies have dealt with other taxa such as birds (Saunders and Dooling, 1975; Dooling et al., 1997).

Fish, as other aquatic animals, are highly dependent on the auditory system. By simply listening to the sounds coming from different sources, they obtain the information relevant for survival, finding mates and prey, or avoiding predators (Hawkins and Myrberg, 1983; Popper and Fay, 1993; Ladich, 1999; Fay and Popper, 2000). Based on current knowledge, all fish are able to perceive low-frequency sounds ("hearing generalists"), whereas some groups have developed accessory hearing structures to enable them to detect high frequencies up to 4 kHz ("hearing specialists"). Hearing enhancement is based on a connection between an air-filled cavity within the body and the inner ear. Within otophysans, vibrations of the swimbladder are transmitted via 1–4 bony ossicles (Weberian apparatus) to the inner ear.

Noise can lead to behavioral changes in fish. This was investigated mainly in commercially important marine fish species (Blaxter and Hoss, 1981; Blaxter et al., 1981; Schwarz and Greer, 1984). Pearson et al. (1992) observed alarm and startle responses of rockfish, Sebastes spp., in the presence of air-gun sounds.

The effects of intense sound on the auditory epithelia have rarely been investigated. Enger (1981) observed damages to the sensory epithelia of the inner ear of the cod Gadus morhua after exposure to intense noise. Hastings et al. (1996) exposed the hearing generalist Astronotus ocellatus to 60- and 300-Hz pure tones at three different sound pressure levels (SPLs) to determine the effects on the sensory epithelia of the inner ear and the lateral line.

The detection of a signal is frequently impaired by the presence of another. Several studies described a decrease in the ability to detect a signal in the presence of a second (masking) sound for the goldfish C. auratus and the cod G. morhua. In both species, the masking effect of a sound (pure...
tonal or noise band) is confined to the frequency region of the signal to be detected (Buerkle, 1968, 1969; Fay, 1974; Hawkins and Chapman, 1975; Fay et al., 1978); threshold shifts occurred during nonsimultaneous masking, as well (Popper and Clarke, 1979).

Another approach is to assess the influence of intense noise on auditory thresholds after exposure. In a behavioral study, Popper and Clarke (1976) stimulated goldfish with intense pure tones (0.3, 0.5, 0.8 and 1.0 kHz) for 4 h and observed temporary threshold shifts (TTS) and complete recovery within 24 h. Scholik and Yan (2001, 2002a) exposed the fathead minnow Pimephales promelas to intense white noise or boat noise for 2 to 24 hours. White noise bands (0.3–4 kHz) increased the auditory thresholds at five out of eight frequencies, whereas boat noise only affected three frequencies. Recovery was dependent on the frequency and exposure duration. In a subsequent study, Scholik and Yan (2002b) could not observe any significant changes in the auditory sensitivity of the bluegill sunfish Lepomis macrochirus, a hearing generalist.

The goal of our present study was threefold: (1) to investigate the immediate effects of unfiltered white noise on the hearing thresholds of two hearing specialists, (2) to assess the course of recovery and (3) to clarify any exposure duration effects. We chose two otophysan species, the goldfish C. auratus and the catfish P. pictus. The latter produces two types of sound: low-frequency drumming sounds by muscular vibrations of the swimbladder and broad-band pulsed sounds by stridulating the pectoral spines (Ladich, 1999, 2001). C. auratus and P. pictus were chosen to compare a nonvocal and a vocal fish species and to assess the influence of the hearing loss on acoustic communication.

II. MATERIAL AND METHODS

A. Animals

Twelve specimens of C. auratus (78–92 mm standard length; 14.5–20.9 g body mass) and 19 of P. pictus (43–79 mm; 2.8–7.1 g) were obtained from local aquarium fish suppliers. Animals were maintained in externally filtered aquaria and a 12:12 h light:dark cycle was maintained. All aquaria were planted, equipped with half flowerpots as hiding places, and a 12:12 h light:dark cycle was maintained. All aquaria were obtained from local aquarium fish suppliers.

B. Noise exposure

The animals were exposed singly to unfiltered white noise at 157.8±1.6 dB (x±S.D.) re 1 μPa for 12 and 24 h in a plastic bucket (20 cm height, 24 cm diameter, 15 cm water depth). Fish could move freely within the bucket. White noise was generated by a noise generator (IVIE Electronics IE 20B), sent to a 24-band equalizer (Alesis MEQ 230) to obtain a flat noise spectrum, and fed to a power amplifier (Brüel & Kjaer 2713) that drove an underwater loudspeaker (University Sound UW 30) situated on the bottom of the bucket.

C. Auditory sensitivity measurements

The auditory sensitivity was measured utilizing the auditory brainstem response (ABR) recording technique, following the ABR recording protocol recently described in Ladich (1999) and Wysocki and Ladich (2001). Therefore, only a brief summary of the basic technique will be given here.

All fish were measured at least 6 days prior to noise exposure to obtain a baseline audiogram. Control tests keeping goldfish (N=6) in the noise exposure bucket without presenting noise revealed that manipulations per se did not lower auditory sensitivity at any frequency. After the end of the 12- or 24-h noise exposure period, hearing thresholds were tested immediately (day 0). This measurement was completed within 3 h. Both species were remeasured 3 and 7 days following the exposure to obtain the recovery hearing thresholds (day 3, day 7). In addition P. pictus was measured after 14 days of recovery (day 14).

1. Experimental setup

Test subjects were secured in a half-bowl shaped 11-l plastic tub (33 cm diameter, 13 cm height, 1 cm layer of fine sand) filled with water and adjusted so that the head was approximately 1 mm above the water surface; a respiration pipette was inserted into the subject’s mouth. Respiration was achieved through a simple temperature-controlled (24 °C), gravity-fed water circulation system. In order to immobilize animals and to reduce the myogenic noise level they were injected with a curariform agent (galamine triethiodide—Flaxedil), with a required dosage of 1–2 μg g⁻¹ for C. auratus and 7–10 μg g⁻¹ for P. pictus. The plastic tub was positioned on an air table (TMC Micro g-63-450), which rested on a vibration-isolated concrete plate. The entire setup was enclosed in a walk-in soundproof room, which was constructed as a Faraday cage (interior dimensions: 3.2×3.2×2.4 m).

The recording of ABRs was performed using silver wire electrodes (0.25 mm diameter), which were pressed firmly against the skin. The portion of the skin above the water surface was covered by a small piece of Kimwipes tissue paper to keep it moist in order to ensure proper contact between the skin and electrodes during the experiment. The recording electrode was placed on the midline of the skull over the region of the medulla. The reference electrode was placed cranially between the nares.

2. ABR-recording apparatus and stimulus presentation

Both sound stimuli presentation and ABR waveform recording were accomplished using a Tucker-Davis Technologies (Gainesville, FL) modular rack-mount system controlled by an optically-linked Pentium PC containing a TDT digital processing board and running TDT Bio-Sig 2.2 Software.

Sound stimuli consisted of tone bursts. The number of cycles in a tone burst (2–8) was adjusted according to the
frequency in order to obtain the best combination of stimulus rise time (shorter rise time = greater efficacy at generating ABRs) and peak frequency bandwidth (longer duration = sharper spectral peak) (Silman and Silverman, 1991). Sound stimuli waveforms were constructed using TDT Sig-Gen software and fed through a DA1 digital-analog converter, a PA4 programmable attenuator, and a power amplifier (Denon PMA 715R). A dual-cone speaker (Tannoy System 600, frequency response 50 Hz to 15 kHz ±3 dB), suspended in air, was mounted 1 m above the test subject. A hydrophone (Brüel & Kjaer 8101, frequency range: 1 Hz to 80 kHz ±2 dB; voltage sensitivity: -184 dB re 1 V/μPa) was placed close to the right side of the animals (2 cm apart) in order to determine absolute stimulus SPLs underwater in close vicinity of the subjects.

For each test condition, 1000 stimuli at opposing polarities (90° and 270°) were presented and averaged by the Bio-Sig software. The ABR traces of signals presented at different polarities do not cancel out each other when averaged. This is contrary to sound pressure waveforms when averaged (see Fig. 3 in Kenyon et al., 1998) and efficiently eliminates artifacts. SPL was reduced in 4-dB steps until the ABR waveform was no longer apparent. The lowest SPL, for which a repeatable ABR trace could be obtained, as determined by overlaying replicate traces, was considered the threshold. This method of visual inspection correlation is the traditional means of determining thresholds in ABR audimetry (Kileny and Shea, 1986; Gorga et al., 1988; Hall, 1992) and proved also to agree well with the correlation coefficient method developed by Yan (1998).

Animals were tested at frequencies of 0.2, 0.3, 0.5, 0.8, 1.0, 2.0, and 4.0 kHz presented in random order.

D. ABR waveform analysis and statistics

ABR waveform characteristics (peak-to-peak amplitude and latency of first negative peak) were analyzed in three to six individuals of each species before and immediately after exposure to white noise at 20 dB above hearing level (20 dB HL, baseline) and tested with an unpaired \( T \)-test.

Hearing thresholds of six individuals of each species were determined at both exposure durations (12 and 24 h). Contrary to goldfish, catfish had to be measured five times due to longer recovery periods. In order to avoid mortality seven catfish were only measured twice, four thrice, four four times and three five times. Threshold values from all individuals of one group as measured at seven different frequencies were compared between pre- and all postexposure measurements by a two-way analysis of variance (ANOVA) using a general linear model where one factor was exposure and the other was frequency. Exposure factor should indicate overall differences between the pre- and postexposure measurements; in combination with the frequency factor, it should indicate if different tendencies exist at different frequencies of the audiograms. This was followed by Scheffe’s multiple comparison procedure. Significant differences between measurements indicate that noise- and recovery effects outweigh interindividual differences.

One-way ANOVAs, followed by Scheffe’s multiple comparison procedure, were applied to evaluate the different effects of noise exposure at different frequencies (\( P \)-value adjusted to 0.007, due to the seven frequencies tested) and to analyze possible exposure duration effects. Baseline auditory thresholds measured prior to noise exposure were pooled because a one-way ANOVA did not reveal any significant differences between the 12 and the 24 h group, either in \( C. auratus \) (\( F_{1,90} = 0.55 \), n.s.) or in \( P. pictus \) (\( F_{1,111} = 3.01 \), n.s.). All statistical tests were run using SPSS version 10.0.

III. RESULTS

A. ABR waveforms

ABR waveforms were obtained from all individuals of the two species, both before and after noise exposure. As an
Noise exposure lowered the maximum ABR amplitudes by 240 to 320 nV in *C. auratus* and by 170 to 740 nV in *P. pictus* (Table I). In contrast, latencies increased after exposure by 0.6 ms to 1.2 ms in *C. auratus* and by 0.3 ms to 2.6 ms in *P. pictus* (Table I). The increase in latencies and the decrease of peak-to-peak amplitude did not differ between both exposure durations in either of the two species.

### B. Auditory thresholds

#### 1. Carassius auratus

The auditory thresholds of *C. auratus* shifted upwards after 12 h and after 24 h exposure (Fig. 2, Table II). Comparison between audiograms revealed significant overall differences between thresholds before and after both exposure durations (two-way ANOVA: 12 h: $F_{3,180}=202.28$, $P<0.001$; 24-h: $F_{3,168}=240.52$, $P<0.001$). A significant interaction between noise exposure and frequencies could only be found after 24-h exposure (two-way ANOVA: $F_{18,168}=3.43$, $P<0.001$), but not after 12-h exposure (two-way ANOVA: $F_{18,180}=1.43$, n.s.), indicating that only for the 24-h exposure were the changes of the auditory sensitivity different at different frequencies. There were no significant differences of overall threshold shift between the two exposure durations (one-way ANOVA: $F_{1,88}=0.001$, n.s.).

Thresholds at all seven frequencies tested were significantly elevated after noise exposure compared to baseline values, tested by a one-way ANOVA for each frequency separately (Fig. 2, Table III). After three days of recovery, auditory thresholds of *C. auratus* were not significantly elevated compared to baseline values (Fig. 3, Table III), suggesting that hearing abilities recovered fully. Similar to day 0, no differences between the two exposure durations could be found after 3 days of recovery (one-way ANOVA: $F_{1,90}=0.27$, n.s.).

#### 2. Pimelodus pictus

In *P. pictus* a significant elevation of the audiogram was observed (Fig. 4, Table IV), tested by a two-way ANOVA, after both 12 h ($F_{4,252}=127.01$, $P<0.001$) and 24-h exposure ($F_{4,245}=113.44$, $P<0.001$). In contrast to *C. auratus* a significant interaction between noise exposure and frequencies was found after both exposure durations (two-way ANOVA: 12 h: $F_{4,252}=10.01$, $P<0.001$; 24 h: $F_{4,245}=7.81$, $P<0.001$). Again, there were no significant differences of overall threshold shift between the two exposure durations (one-way ANOVA: $F_{1,90}=0.83$, n.s.).

Contrary to the goldfish, where all seven frequencies tested were significantly elevated after both exposure durations, this was not the case for *P. pictus*. After 12-h exposure, six out of seven frequencies (except 0.3 kHz) were significantly elevated compared to preexposure values, which was tested with one-way ANOVA for each frequency. After 24-h exposure, only four frequencies (0.8–4.0 kHz) were significantly different from the baseline thresholds (Fig. 4, Table V).

In contrast to *C. auratus* the audiogram of *P. pictus* had not fully recovered at all seven frequencies within three days following exposure (Fig. 5, Table V). On day three, two out of seven frequencies had not returned to the baseline level following 12- and 24-h exposure. Even on day 7, one fre...
IV. DISCUSSION

A. ABR waveforms

The decrease in the maximum ABR amplitude as well as the increase in latency of the first negative peak after noise exposure clearly indicates that the auditory sensitivity of both species diminished. This resulted in higher auditory thresholds.

Scholik and Yan (2001, 2002a) exposed fathead minnows *Pimephales promelas* to white noise (0.3–4.0 kHz) and to boat engine noise (0.3–6.0 kHz with a major peak at 1.3 kHz) and revealed a decrease of ABR amplitudes after exposure. Unfortunately, they did not quantify the amount of amplitude shift or report whether the latencies shifted, so a further comparison with our data was not possible.

In a mammalian study on the effects of intense sounds on hearing, Griffiths et al. (1994) found small latency increases in sheep fetuses exposed to 120 dB (re 20 μPa) broadband noise for 16 h in utero. They also observed recovery of the latencies to preexposure values.

B. Noise effects on auditory thresholds

Exposing the two otophysan species *C. auratus* and *P. pictus* to white noise at 158 dB SPL (re 1 μPa) for 12 and 24 h significantly elevated the auditory thresholds in both species. The amount of threshold shift clearly differed between both species, being more pronounced in the catfish (5–32 dB versus 10–24 dB in the goldfish). In *C. auratus* the greatest threshold shift was observed at 0.8 and 1.0 kHz, while in *P. pictus* the higher frequencies (2.0 and 4.0 kHz) were most affected by noise exposure. For both species the greatest effects of noise exposure were seen in their most sensitive hearing range. The larger effect in the catfish was most likely due to the larger sensitivity at higher frequencies. As reviewed by Ladich and Bass (2003) catfishes in general possess very flat hearing curves and a high frequency hearing sensitivity, which is even unusual among hearing specialists such as cyprinids.

Similar to our data Scholik and Yan (2001) observed in the cyprinid *P. promelas* after exposure to white noise for 1 to 24 h at 142 dB SPL (re 1 μPa), the greatest threshold shift in the fish’s most sensitive frequency range (0.8–2.0 kHz). The amount of threshold shift in *P. promelas* ranged from 11 to 20 dB, which is comparable to the shift observed in our study in *C. auratus*. Contrary to our results, where all frequencies of *C. auratus* were significantly elevated immediately after noise exposure, in *P. promelas* 0.5, 2.5, and 4.0 kHz were not significantly affected. This difference is most likely due to different noise bandwidths applied. We exposed the fish to unfiltered white noise, while Scholik and Yan (2001) used white noise with a bandwidth ranging from 0.3 kHz (4.0 111.75 0.90 125.33 1.09 109.50 1.88 109.67 3.33 4.0 111.75 0.90 125.67 1.36 112.17 1.62 109.67 0.33 0.2 74.75 1.39 81.25 1.27 71.67 2.20 77.33 2.40 1.0 66.08 1.28 81.25 1.27 68.33 1.89 73.33 2.40 2.0 81.25 1.27 99.33 2.20 81.67 2.16 77.33 2.40 4.0 111.75 0.90 125.33 1.36 112.17 1.62 109.67 0.33 0.8 64.67 1.23 81.25 1.27 65.17 1.77 64.00 1.55 1.0 66.08 1.28 81.25 1.27 63.00 1.89 65.33 1.73 2.0 81.25 1.27 99.33 2.20 77.33 2.16 77.33 2.40 4.0 111.75 0.90 125.33 1.36 112.17 1.62 109.67 0.33
TABLE III. Differences of mean hearing thresholds (dB) of C. auratus at the seven frequencies tested calculated by one-way ANOVA for each frequency followed by pair wise multiple comparison procedure (Scheffé).

<table>
<thead>
<tr>
<th>kHz</th>
<th>Baseline—day 0</th>
<th>Baseline—day 3</th>
<th>Baseline—day 7</th>
<th>F_{3,23}</th>
<th>P</th>
</tr>
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<tr>
<td>12-h exposure</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>0.2</td>
<td>14.1*</td>
<td>2.20</td>
<td>-2.6</td>
<td>2.63</td>
<td>-6.1</td>
</tr>
<tr>
<td>0.3</td>
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<td>2.34</td>
<td>2.0</td>
<td>2.47</td>
<td>-0.0</td>
</tr>
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<td>0.5</td>
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<td>1.5</td>
<td>1.83</td>
<td>2.5</td>
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<tr>
<td>0.8</td>
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<td>1.89</td>
<td>1.8</td>
<td>2.14</td>
<td>0.67</td>
</tr>
<tr>
<td>1.0</td>
<td>21.6*</td>
<td>2.40</td>
<td>2.3</td>
<td>2.04</td>
<td>-3.1</td>
</tr>
<tr>
<td>2.0</td>
<td>18.1*</td>
<td>2.37</td>
<td>0.4</td>
<td>2.35</td>
<td>-3.9</td>
</tr>
<tr>
<td>4.0</td>
<td>13.9*</td>
<td>1.59</td>
<td>0.4</td>
<td>1.70</td>
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<table>
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<tr>
<th>kHz</th>
<th>Baseline—day 0</th>
<th>Baseline—day 3</th>
<th>Baseline—day 7</th>
<th>F_{3,23}</th>
<th>P</th>
</tr>
</thead>
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<td>12-h exposure</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>9.9*</td>
<td>2.58</td>
<td>-3.1</td>
<td>2.27</td>
<td>5.4</td>
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<tr>
<td>0.3</td>
<td>13.5*</td>
<td>2.28</td>
<td>0.0</td>
<td>2.36</td>
<td>5.0</td>
</tr>
<tr>
<td>0.5</td>
<td>18.7*</td>
<td>1.78</td>
<td>-1.0</td>
<td>1.83</td>
<td>-0.8</td>
</tr>
<tr>
<td>0.8</td>
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<td>2.11</td>
<td>0.5</td>
<td>1.77</td>
<td>-5.3</td>
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<tr>
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<td>1.91</td>
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<td>2.0</td>
<td>20.8*</td>
<td>2.05</td>
<td>-1.1</td>
<td>2.13</td>
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<tr>
<td>4.0</td>
<td>13.6*</td>
<td>1.49</td>
<td>-2.3</td>
<td>1.82</td>
<td>-2.1</td>
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*Statistically significant differences following adjustment to the number of frequencies tested (P<0.007). Positive threshold differences mean a higher auditory threshold of the second group of a pairing at this particular frequency.

FIG. 3. Amount of threshold shift in C. auratus immediately after exposure (day 0) and after three (day 3) and seven (day 7) days of recovery. The dashed line at zero indicates baseline values.

FIG. 4. Audiograms of P. pictus before (baseline) and after (day 0) white noise exposure. Asterisks indicate statistically significant differences following adjustment to the number of frequencies tested (P<0.007).
to 4.0 kHz. This yields less noise energy at the lower and higher frequencies of the hearing range and thus results in smaller threshold shifts.

In another approach, Popper and Clarke (1976) exposed goldfish to pure tones (0.3, 0.5, 0.8, and 1.0 kHz; SPL of 149 dB re 1 μPa) for 4 h and tested the auditory thresholds at 0.5 and 0.8 kHz behaviorally. They observed shifts ranging from 6 to 26 dB at 0.5 kHz and from 4 to 29 dB at 0.8 kHz. Threshold shifts were similar for 0.3 and 0.5 kHz and for 0.8 and 1.0 kHz, respectively.

A subsequent study using the bluegill sunfish Lepomis macrochirus, a hearing generalist of the family Centrarchidae, revealed no significant changes in auditory sensitivity (Scholik and Yan, 2002b). This could be due to the low noise level in relation to the hearing threshold of the generalist, which was approximately 20 dB above HL. To assess the effects on hearing, noise levels of at least 60 dB above HL need to be applied.

Utilizing a different method, some authors observed elevation of auditory thresholds in the presence of another signal. Buerkle (1968) reported that varying background noise levels resulted in thresholds shifts up to 25 dB in the cod Gadus morhua. Hawkins and Chapman (1975) revealed that thresholds were elevated up to 14 dB in the presence of a broad-band noise (0.03–1.0 kHz; 20–30 dB above ambient sea noise level). The signal and second tone need not be

### TABLE IV. Hearing threshold values (dB re 1 μPa) of P. plicus before (baseline), immediately after (day 0) noise exposure and after 3, 7, and 14 days of recovery (day 3, day 7, day 14).

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Baseline</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>12-h exposure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>91.13</td>
<td>0.55</td>
<td>98.14</td>
<td>1.16</td>
<td>92.00</td>
</tr>
<tr>
<td>0.3</td>
<td>89.88</td>
<td>0.80</td>
<td>95.29</td>
<td>1.55</td>
<td>89.17</td>
</tr>
<tr>
<td>0.5</td>
<td>78.19</td>
<td>1.67</td>
<td>89.29</td>
<td>1.15</td>
<td>79.00</td>
</tr>
<tr>
<td>0.8</td>
<td>79.13</td>
<td>0.87</td>
<td>91.71</td>
<td>0.61</td>
<td>81.50</td>
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<tr>
<td>1.0</td>
<td>78.25</td>
<td>1.40</td>
<td>89.86</td>
<td>0.94</td>
<td>81.00</td>
</tr>
<tr>
<td>2.0</td>
<td>73.19</td>
<td>1.47</td>
<td>101.29</td>
<td>2.03</td>
<td>87.33</td>
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<tr>
<td>4.0</td>
<td>76.44</td>
<td>0.94</td>
<td>108.14</td>
<td>1.55</td>
<td>95.33</td>
</tr>
</tbody>
</table>

| 24-h exposure  |          |        |        |        |        |
| 0.2            | 91.13    | 0.55   | 98.00  | 2.05   | 93.17  | 1.40   | 90.33  | 2.20   | 87.83  | 1.17   |
| 0.3            | 89.88    | 0.80   | 84.50  | 1.18   | 93.83  | 1.14   | 88.67  | 0.92   | 86.33  | 1.23   |
| 0.5            | 78.19    | 1.67   | 87.50  | 1.58   | 80.50  | 1.06   | 78.67  | 1.99   | 78.00  | 0.97   |
| 0.8            | 79.13    | 0.87   | 88.67  | 1.15   | 84.17  | 0.70   | 82.00  | 1.90   | 80.00  | 0.68   |
| 1.0            | 78.25    | 1.40   | 92.83  | 1.35   | 85.83  | 1.56   | 79.50  | 0.99   | 80.67  | 2.32   |
| 2.0            | 73.19    | 1.47   | 99.17  | 1.72   | 92.17  | 0.75   | 80.17  | 1.38   | 76.00  | 1.29   |
| 4.0            | 76.44    | 0.94   | 103.83 | 1.11   | 94.50  | 1.48   | 86.00  | 1.37   | 83.17  | 1.05   |

### TABLE V. Differences of mean hearing thresholds (dB) of P. plicus at the seven frequencies tested calculated by one-way ANOVA for each frequency followed by pair wise multiple comparison procedure (Scheffe).

<table>
<thead>
<tr>
<th>kHz</th>
<th>12-h exposure</th>
<th>24-h exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline—day 0</td>
<td>Baseline—day 3</td>
</tr>
<tr>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td>0.2</td>
<td>7.0* 1.21</td>
<td>0.9 1.07</td>
</tr>
<tr>
<td>0.3</td>
<td>5.4 1.57</td>
<td>−0.7 1.45</td>
</tr>
<tr>
<td>0.5</td>
<td>11.1* 2.66</td>
<td>0.8 3.05</td>
</tr>
<tr>
<td>0.8</td>
<td>12.6* 1.39</td>
<td>2.4 1.72</td>
</tr>
<tr>
<td>1.0</td>
<td>11.6* 2.22</td>
<td>2.8 2.46</td>
</tr>
<tr>
<td>2.0</td>
<td>28.1* 2.60</td>
<td>14.1* 2.80</td>
</tr>
<tr>
<td>4.0</td>
<td>31.7* 1.74</td>
<td>18.9* 2.15</td>
</tr>
</tbody>
</table>

*Statistically significant differences following adjustment to the number of frequencies tested (P<0.007). Positive threshold differences mean a higher auditory threshold of the second group of a pairing at this particular frequency.

presented simultaneously to change hearing thresholds. Popper and Clarke (1979) investigated nonsimultaneous masking in the goldfish and revealed threshold shifts of up to 35 dB for pure tone pulses of different durations (15–50 ms), regardless of whether these were presented before or after a 250 ms white noise pulse. Fay (1974) noted that a 10-dB noise increment produced an approximately 10-dB masking increment, independent of frequency. In natural environments thresholds may often be elevated due to the presence of background noise.

A comparison of the effects of the two noise exposure durations applied in our study did not reveal any significant differences in either species. In contrast, Scholik and Yan (2001) did observe duration-dependent effects on threshold shifts in \( P. \) promelas. Threshold elevation was smaller after 1 h compared to 2–24 h of duration. They termed this asymptotic threshold shift (ATS), which is a well-known phenomenon in mammalian auditory studies. These results indicate that auditory sensitivity diminishes quickly (1–2 h) following noise exposure.

The course of recovery varied between species and seems to depend on exposure duration and amount of threshold shift. In \( C. \) auratus all frequencies returned to baseline values within 3 days and no effect of exposure duration on recovery was observed. In \( P. \) pictus, on the other hand, it took 14 days until thresholds returned to baseline values following 12 h noise exposure. After 24 h exposure, one (4.0 kHz) out of seven frequencies tested had not returned to baseline values even after 14 days. Scholik and Yan (2001) also revealed that longer exposure durations seem to influence recovery: at the highest frequencies the thresholds had not fully recovered to baseline values even 14 days after 24 h exposure, while at the same frequencies following 2-h exposure, recovery was observed within 6 days. This could be because the responses to higher frequencies tend to recover more slowly than those to lower frequencies or because at frequencies where large threshold changes occurred, such as in \( P. \) pictus, recovery of the responses takes more than 2 weeks.

The effects of noise exposure on the microanatomy and physiology of fish hair cells are less clear. Enger (1981) exposed the cod \( G. \) morhua to high-level sounds (50–400 Hz, 180 dB re 1 \( \mu \)Pa) for 1–5 h and observed destroyed regions with missing sensory cilia on the saccular macula. Hastings et al. (1996) found only damage to the sensory hair cells of the oscar \( A. \) ocellatus after stimulation with 300 Hz continuous tones at 180 dB (re 1 \( \mu \)Pa) and allowed to survive for 4 days. Damage was limited to small regions of the utricle and lagena, but not the saccule. Fish are able to replace or regenerate hair cells in the ear after treatment with ototoxic drugs after several days (Lombarte et al., 1993). The recovery of auditory sensitivity in our goldfish within three days indicates that threshold changes were most likely due to hair cell fatigue. The much longer recovery time in our catfish (14 days) might additionally be explained by microanatomical injuries and hair cell replacement. In all cases loss of sensitivity in our animals was temporary and parallels temporary thresholds shifts (TTS) in other animal taxa (Clark, 1991).

C. Behavior and acoustic communication

The observed threshold elevations following noise exposure may be crucial for the survival of the examined species because fish obtain information about predators and prey, competitors and potential mates by listening to their acoustic environment. If the hearing abilities of fishes were impaired by exposure to noise, the distances over which such information could be obtained would decrease, and the quality of the obtained information would deteriorate.

One of the target species, the pimelodid catfish \( P. \) pictus, is known to produce two types of sound: low-frequency drumming sounds and high-pitched stridulatory sounds, which are used during agonistic encounters. Comparison between the audiograms and sound spectra in \( P. \) pictus revealed that, before the exposure to intense noise, drumming sound energies were up to 20 dB above hearing thresholds and stridulatory sound energies were up to 31 dB above hearing thresholds [sound spectra after Ladich (1999)]. Immediately after noise exposure the differences between drumming sound energies and the audiogram were maximally 9 dB (12 h) and 11 dB (24 h), and between stridulatory sound energies and the audiogram maximally 11 dB (12 h) and 6 dB (24 h) (Fig. 6). Hearing impairment could alter a fish’s possibility to

FIG. 5. Threshold shift in \( P. \) pictus immediately after noise exposure (day 0) and after 3, 7, and 14 days of recovery (day 3, day 7, day 14). The dashed line at zero indicates baseline values.
assess the opponent’s quality acoustically and therefore influence the outcome of a fight (Ladich et al., 1992).

Despite the lack of data about effects on acoustic communication, existing data show behavioral changes of fishes exposed to intense sounds. Pearson et al. (1992) observed startle responses of rockfish Sebastes spp. during playback of air-gun-like sounds at 200–205 dB SPL (re 1 μPa), indicating that alarm as well as startle responses could be elicited by sound from actual survey operations. In contrast, Klímy and Beavers (1998) were unable to observe significant behavioral changes of rockfish exposed to ATOC-like signals, but this may be due to the relatively low SPL of 145 dB.

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Differences in hearing loss and recovery between both species, the goldfish C. auratus and the catfish P. pictus, were remarkable: the threshold shift was more pronounced in P. pictus, particularly at higher frequencies (32 vs 14 dB), and hearing thresholds recovered more slowly than in C. auratus. We suggest two possible explanations for this observation: (1) the auditory system of the catfish could be more susceptible to auditory fatigue and therefore may need more time to fully recover or (2) the higher auditory sensitivity in the catfish resulted in a greater threshold shift at higher frequencies which simply needed more time to return to baseline values in comparison to the goldfish.

The shift in auditory thresholds in the vocalizing catfish and a comparison with the spectra of conspecific sounds revealed that acoustic communication is severely impaired when fish live in noisy environments. Although it is assumed that hearing thresholds are always masked under natural conditions, high noise levels such as those applied here would reduce the effective communication distance to a few centimeters at most. Besides impacting intraspecific communication, elevated hearing thresholds would also limit the detection of predators or prey, thus reducing fitness.

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V. CONCLUSIONS

Our experiments show that exposure to intense white noise affects the hearing abilities of two otophysan fishes. This is expressed by a decrease in amplitudes and an increase in latency of the auditory evoked responses and subsequently by an upward shift of hearing thresholds in both species.


