Impacts of broadband sound on silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp hearing thresholds determined using auditory evoked potential audiometry

Andrew C. Nissen · Brooke J. Vetter · Loranzie S. Rogers · Allen F. Mensinger

Abstract Invasive silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp, collectively referred to as bigheaded carps, threaten aquatic ecosystems of the Upper Midwestern USA. Due to the extensive ecological impacts associated with these species, prevention of their further range expansion is the aim for fisheries management. Recent behavioral studies indicate bigheaded carps are deterred by acoustic barriers and exhibit negative phonotaxis in response to anthropogenic sound sources (≥ 150 dB re 1 μPa). However, the impact of long-term exposure to these sounds on the hearing capabilities of bigheaded carps has not been well documented. In this study, the auditory evoked potential (AEP) technique was used to determine auditory thresholds among bigheaded carps before and after exposure to high intensity (155.7 ± 4.7 dB re 1 μPa SPL_{rms}; -8.0 ± 4.7 dB re 1 ms^{-2} PAL_{rms}; mean $\pm$ SD) broadband sound. Fish were exposed to sound for 30 min or 24 h and AEP measurements were taken at three time points: immediately after exposure, 48 h, or 96 h later. Results indicate that silver and bighead carp experience temporary threshold shifts (TTSs) in frequency detection following sound exposure with the magnitude and length of TTS correlated with exposure duration. The findings from this study will be used to increase the long-term efficacy of acoustical deterrent measures aimed at preventing further range expansion of bigheaded carps.

Keywords Auditory evoked potential · Temporary threshold shift · Broadband sound · Silver carp · Bighead carp · Invasive carp

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Introduction

Silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp, collectively referred to as bigheaded carps, are invasive fishes that have negatively impacted aquatic ecosystems in the Mississippi River Drainage Basin and currently threaten the Laurentian Great Lakes. Bigheaded carps were imported from eastern Asia to the Southeastern USA for water quality and plankton control at sewage water treatment and aquaculture facilities in the 1970s. Their subsequent escape into natural waterways led to the establishment of viable populations and an aggressive northward range expansion through the Mississippi River Drainage Basin (Kolar et al. 2007). A recent estimate indicates populations of bigheaded carps in the Upper Mississippi River System, where they are poised to invade the Laurentian Great Lakes, are among the densest in the world (Sass et al. 2010).

Extensive ecological impacts are associated with the bigheaded carp invasion of the Mississippi River Drainage Basin. As filter feeders, bigheaded carps compete with native paddlefish (*Polyodon spathula*), gizzard shad (*Dorosoma cepedianum*), and bigmouth buffalo (*Ictiobus cyprinellus*) (Schrank et al. 2003; Irons et al. 2007; Sampson et al. 2009; Solomon et al. 2016). Additionally, shifts in zooplankton communities associated with bigheaded carps likely impact lower trophic-level organisms and early life-history stages of other fishes (Xie and Yang 2000; Cooke and Hill 2010; Sass et al. 2014). Though the impact of bigheaded carps on Laurentian Great Lakes food webs remains uncertain, bioenergetics and population modeling studies indicate that small founder populations may become established in areas of high productivity within these waters and their tributaries (Cooke and Hill 2010; Cuddington et al. 2014; Anderson et al. 2015; Zhang et al. 2016). Therefore, developing effective non-physical deterrent measures and barriers against further range expansion of bigheaded carps, particularly into the Laurentian Great Lakes, is a management priority.

Acoustic deterrence is a promising control measure for bigheaded carps as they are otophysans, fish characterized by Weberian ossicles that transmit vibrations from the swim bladder to the inner ear. This otophysic connection can increase hearing sensitivity and frequency bandwidth compared with non-otophysan fishes (Popper and Fay 2011). Previous behavioral studies have shown that a broadband sound (~ 150 dB re 1 μPa$_{rms}$) can elicit negative phonotaxis and effectively prevent the passage of bigheaded carps through a narrow (1 m^2) channel (Vetter et al. 2015, 2017; Murchy et al. 2017). Therefore, it is important to determine the effect of anthropogenic sound on bigheaded carp hearing, especially at the sound pressure levels (SPL) being proposed for acoustic deterrent systems.

Auditory temporary threshold shifts (TTSs) among otophysan fishes following exposure to white noise have been reported in a variety of studies (Amoser and Ladich 2003; Smith et al. 2004a, 2004b). Amoser and Ladich (2003) observed auditory TTSs for goldfish (*Carassius auratus*) and catfish (*Primeus pictus*), after sound exposure (~ 158 dB re 1 μPa) and noted that after initial TTSs, goldfish hearing thresholds returned to baseline levels within 3 days, whereas catfish experienced greater and

prolonged TTS that remained above baseline levels for up to 14 days after exposure. These experiments demonstrate the impacts of sound exposure are species-dependent. Therefore, to develop effective acoustic deterrents against bigheaded carps, it is important to understand how sound exposure impacts the hearing abilities of both species.

The auditory evoked potential (AEP) is a minimally invasive technique that records compound potentials from the auditory brainstem via electrodes placed on the skull. Originally developed for mammals (Jewett 1970; Jewett and Williston 1971), the AEP method has been adapted for fish (Corwin et al. 1982) and used in a variety of studies on fish hearing (reviewed in Ladich and Fay 2013). Fish AEPs likely record the microphonic potentials from hair cells of auditory end organs and/or their afferent nerve fibers (Sisneros et al. 2016) and are characterized by a double-frequency response (Kojima et al. 2005; Maruska et al. 2007) that results from the stimulation of opposite-oriented auditory hair cells (Fay 1974). The AEP technique is most effective in determining the range of frequencies a fish can detect, and relative differences in auditory thresholds to given frequencies observed between fish under similar conditions (Sisneros et al. 2016). Therefore, by comparing auditory thresholds determined from AEPs before and after sound exposure, relative shifts in fish hearing thresholds can be monitored.

The goal of this study was to use the AEP technique to measure auditory thresholds in bigheaded carps following short-term (30 min) and long-term (24 h) exposure to a broadband sound, which is being considered for use with acoustic deterrent systems (Vetter et al. 2015, 2017; Murchy et al. 2017). Auditory thresholds measured from fish exposed to sound were compared with baseline thresholds measured from control fish to determine the impacts of sound exposure on auditory abilities. Auditory thresholds were also measured from sound-exposed fish following a recovery period (48 and 96 h) to determine whether threshold shifts were permanent or transient. As silver and bighead carp, like other otophysan fishes, likely detect both sound pressure and particle acceleration, hearing thresholds for both species were determined in relation to SPL and particle acceleration levels (PAL) as suggested by Popper and Fay (2011).

Methods

Animal husbandry

Juvenile silver ($n = 49$; TL = 145.3 mm ± 34.0 mm SD) and bighead ($n = 49$; TL = 161.9 mm ± 53.2 mm SD) carp were obtained from the USGS Upper Midwest Environmental Science Center in La Crosse, WI, and maintained indoors at the University of Minnesota Duluth in Duluth, MN. The two species were held separately in 380 to 1230-L fiberglass tanks with recirculating water systems. Each tank was filled with buffered pond water (1.4 g KCl, 1.1 g NaCl, and 3.3 g CaCl$_2$ per 190 L of deionized water; pH = 7.0) and equipped with mechanical, chemical, and biological filters. Water temperature was between 19 and 22 °C. Both species were fed a liquid algae mixture (~ 1000 mL; 1:1 chlorella and spirulina; Bulk Foods, Toledo, OH) three times weekly. Fishes were maintained in a secured room with restricted access in accordance with the Prohibited Invasive Species Permit (#391) from the Minnesota Department of Natural Resources and an Injurious Wildlife Permit (MA-98346B-0) from the United States Fish and Wildlife Service. All experiments were conducted under protocol 1604-33658A and approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Sound exposure procedure

Fish were exposed to an underwater recording of a 100-hp outboard boat motor (four-stroke) with a broadband frequency range of 0.06–10 kHz (Supplementary Material Fig. 1). The original sound file (Vetter et al. 2015) was reduced to 30-s duration to minimize amplitude modulation and looped continuously during playback. The sound file was played from a Roland 4-channel portable recorder (R-44; Roland Corporation; Hamamatsu, Japan) and amplified with a TOA 60-watt amplifier (CA-160; TOA Corporation; Kobe, Japan) connected to an underwater speaker (LL916; Lubell Labs Inc.; Whitehall, OH). Fish were placed in a Rubbermaid stock tank (380 L, length 90 cm; width 50 cm; depth 48 cm; Rubbermaid Commercial Products; Winchester, VA) that was filled with buffered pond water. The underwater speaker was placed horizontally on the bottom center of the tank. Fishes (silver carp (SVC), $n = 42$; bighead carp (BHC), $n = 42$) were exposed to continuous playback of broadband sound for either 30 min

(BHC, $n = 21$; SVC, $n = 21$) or 24 h (BHC, $n = 21$; SVC, $n = 21$) and then tested immediately 0 h ($n = 7$ for both species and exposure treatments), 48 h ($n = 7$ for both species and exposure treatments), or 96 h ($n = 7$ for both species and exposure treatments) after sound exposure. Control fish (BHC, $n = 7$; SVC, $n = 7$) were not exposed to sound prior to AEP testing. Fish were exposed to sound individually for each treatment so that AEP testing started immediately following the specified recovery periods (0, 48, or 96 h). Though serial testing of individual fish prior to and throughout a recovery duration following sound exposure is possible with the AEP technique, each fish was only tested once and then sacrificed by overdosing in 0.5% MS-222 for future analysis of inner ear morphology.

Sound pressure (SPL) and particle acceleration levels (PAL) were determined in the sound exposure tank for ambient sound conditions and during playback of broadband sound (Supplementary Material Figs. 2 and 3). A Cartesian grid that consisted of 31 equally spaced points was established for the tank and recordings were made at each point at 16, 24, and 32 cm below the water surface. The SPL V_{rms} at each point was recorded using a Brüel and Kjaer hydrophone (8103; Brüel and Kjaer; Naerum, Denmark) connected to a Nexus Conditioning Amplifier (2692-01s; Brüel and Kjaer; Naerum, Denmark). PAL V_{rms} measurements were taken with a triaxial accelerometer (sensitivity, $x = 10.47$ mV/ms^{-2}, $y = 10.35$ mV/ms^{-2}, $z = 10.29$ mV/ms^{-2}; W356A12/NC; PCB Piezotronics, Inc.; Depew, NY) modified to be neutrally buoyant and connected to a signal conditioner (482C15, PCB Piezotronics Inc.). The hydrophone and accelerometer were connected to a PowerLab (SP/4; AD Instruments; Colorado Springs, CO) and data analyzed using LabChart 7 software (AD Instruments; Colorado Springs, CO). To calculate the PAL at each point, the V_{rms} was determined for each axis (x, y, and z) and these measurements were converted to individual magnitude vectors. The following equation was then used to calculate the PAL thresholds:

$$\text{dB re ms}^{-2}{}_{rms} = 20\log\left(\sqrt{x^2 + y^2 + z^2}\right) \qquad (1)$$

(Wysocki et al. 2009; Vasconcelos et al. 2011; Radford et al. 2012; Bhandiwad et al. 2017; Vetter et al. 2018: Vetter et al. 2019).

The mean SPL and PAL were determined for each depth and throughout the exposure tank. Mean ambient
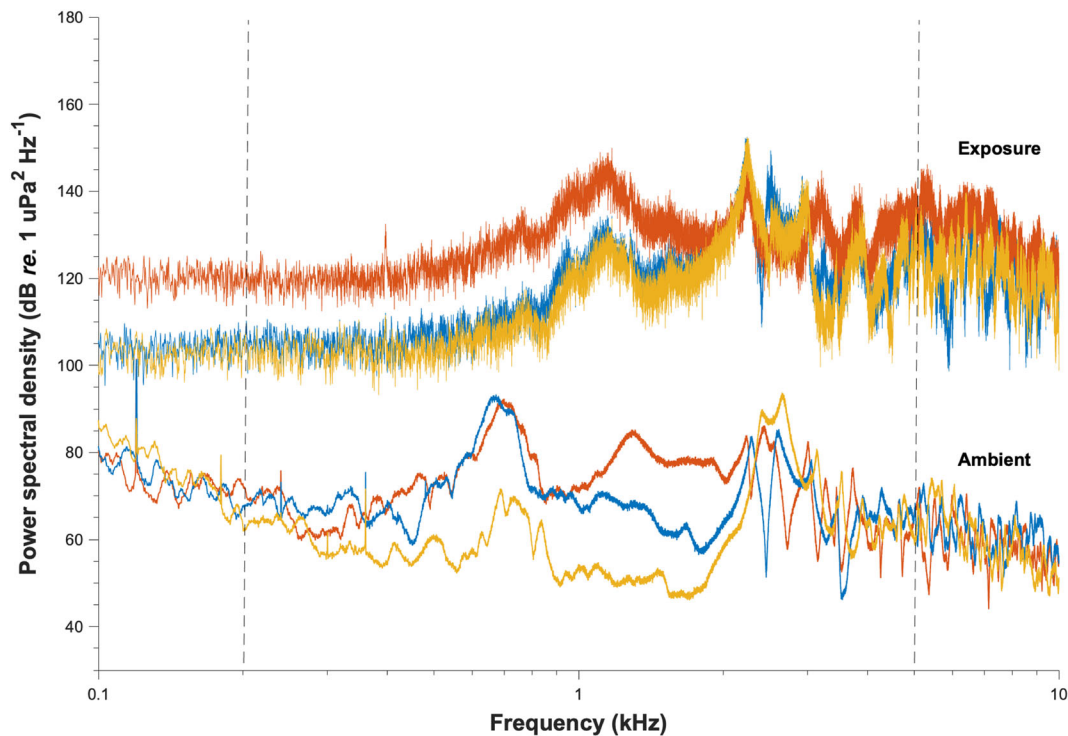
Fig. 1 Power spectral density analysis of ambient and sound exposure recordings taken from within the exposure tank. Dashed reference lines represent the lowest (0.2 kHz) and highest (5.0 kHz) tested frequency. Recordings were taken at 24 cm below the water surface from directly above the speaker (red) and 30 cm to the left (blue) and right (yellow) of the speaker along the median of the greatest length of the oblong tank for both ambient and sound exposure conditions

SPLs within the exposure tank at 16, 24, and 32 cm depths were 114.1 ± 2.4, 114.1 ± 3.1, and 113.3 ± 2.8 dB re 1 μPa SPL_{rms} (mean ± SD), respectively, and increased during sound exposure to 157.8 ± 3.7, 157.8 ± 4.4, and 155.8 ± 5.5 dB re 1 μPa SPL_{rms}, respectively. The average SPL_{rms} for all points at all depths were 113.8 ± 2.8 dB re 1 μPa SPL_{rms} (ambient) and 155.7 ± 4.7 dB re 1 μPa SPL_{rms} (exposure). The mean background particle motion values at 16, 24, and 32 cm depths were -41.6 ± 1.4, -40.9 ± 1.9, and -27.2 ± 4.7 dB re 1 ms^{-2} PAL_{rms}, respectively, and increased during sound exposure to -8.1 ± 2.0, -7.3 ± 2.1, and -8.8 ± 3.7 dB re 1 ms^{-2} PAL_{rms}, respectively. The average PAL for all points at all depths was -36.6 ± 7.2 dB re 1 ms^{-2} PAL_{rms} (ambient) and -8.0 ± 2.8 dB re 1 ms^{-2} PAL_{rms} (exposure). Mean SPL and PAL during broadband sound playback within the exposure tank were 41.9 dB re 1 μPa SPL_{rms} and 28.6 dB re 1 ms^{-2} PAL_{rms} greater than ambient levels, respectively.

To determine the power spectral density of ambient sound and broadband sound playback, SPL recordings were taken using a hydrophone (Sound Trap v 1.7;

Ocean Instruments; New Zealand) from three points along the median of the greater length of the oblong exposure tank at 24 cm below the water surface (Fig. 1). The three points corresponded with points within the Cartesian grid used for SPL and PAL mapping and were directly above and 30 cm to each side of the speaker. Power spectral density analysis of the ambient and sound exposure SPL from 0.1 to 10 kHz was completed using Matlab (v. R2017a; Mathworks; The MathWorks, Inc.).

Auditory evoked potentials

Experiments were conducted within a 350-L cylindrical fiberglass tank (88 cm inside diameter, 62 cm height, 57 cm water depth) and placed on a 1-cm-thick rubber mat on cinderblocks ($41 \times 20 \times 10$ cm) to dampen vibrations. A galvanized angle iron frame ($110 \times 125 \times 182$ cm) surrounded the tank and was covered on the top and three sides with FOAMULAR Insulation Sheathing (2.54 cm thick; Owens Corning; Toledo, OH) to reduce background sound and prevent fish from seeing the

Fig. 2 Silver carp mean auditory SPL (**a**) and PAL (**b**) thresholds (± SD) for control (circle), 30-min (square), and 24-h (triangle) noise-exposed fish. Filled symbols indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds for control and noise-exposed fish. Asterisks indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds for 30-min and 24-h noise-exposed fish
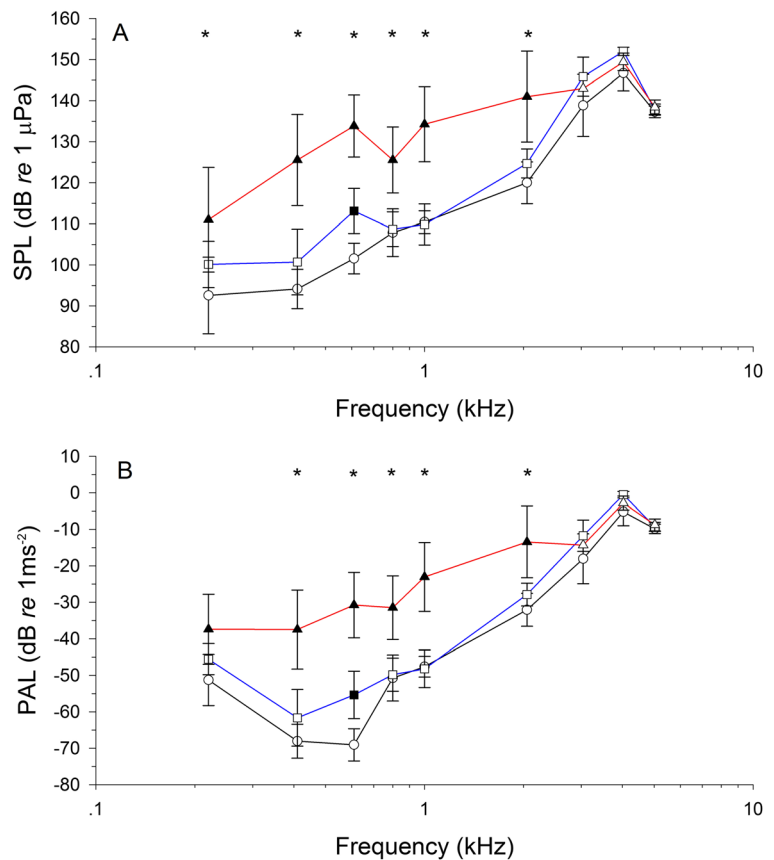
experimenter. Each fish was suspended in a mesh sling with its head 4 cm below the surface and 35 cm above an underwater speaker (UW-30; Lubell Labs Inc.; Whitehall, OH).

Stainless steel electrodes (Rochester Electro-Medical Inc.; Tampa, FL) were insulated with fingernail polish, leaving a 2-mm exposed tip, and implanted subcutaneously. A reference electrode was placed medially between the nares on the dorsal surface of the head. Recording electrodes were positioned above the brainstem and placed medially on the dorsal surface of the head approximately 2 mm posterior to an imaginary line drawn between the anterior margins of the opercula. AEP signals were amplified with a headstage (gain = 10×) connected to an extracellular differential amplifier (gain = 100×; Dagan Corporation; Minneapolis, MN) with 0.02-kHz low-pass and 5.0-kHz high-pass filters.

A Cambridge Electronic Design data acquisition system (micro3 1401; CED; Cambridge, UK) and custom Spike2 (version 8; CED; Cambridge, UK) scripts were used to set sound signal parameters, calibrate SPL attenuation, and digitize incoming AEP signals. A

programmable attenuator (CED 3505; CED; Cambridge, UK) and amplifier (AS-35; Accusonic) controlled the SPL of the presented signals. The attenuator and amplifier were calibrated using a Brüel and Kjaer hydrophone (8103; Brüel and Kjaer; Naerum, Denmark) connected to a Nexus Conditioning Amplifier (2692-01s; Brüel and Kjaer; Naerum, Denmark). Pure tone signals were attenuated in 3 dB re 1 μPa SPL_{rms} steps.

Auditory thresholds to nine frequencies (0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, and 5 kHz) were determined. Auditory evoked potentials were first elicited using a SPL 9 dB re 1 μPa SPL_{rms} above the reported thresholds for each frequency (Vetter et al. 2018). For fish that underwent sound exposure, if AEP responses were not observed at a given frequency at 9 dB re 1 μPa SPL_{rms} above previously reported thresholds, SPL_{rms} was increased until AEPs were detected or the maximum output of the speaker at a given frequency was reached.

For stimulus presentation, pure tone bursts for each frequency were broadcast (50 ms; 500 repetitions; 3-ms delay) and responses were collected and averaged using

a custom Spike2 script. AEPs were determined by observing the characteristic wave above the background noise (e.g., Higgs et al. 2001; Mann et al. 2001; Egner and Mann 2005). Visual AEPs were verified by fast Fourier transform power spectrum analysis (FFT, Hanning window = 1024). Visual AEPs with FFT peaks above the background noise (≥ 0.001 μV) at the second harmonic of the stimulation frequency were considered evoked potentials. The auditory threshold at each tested frequency was defined as the minimum SPL that elicited an observable AEP response and a FFT peak at the second harmonic of the stimulus frequency.

Particle acceleration thresholds

PALs were measured using a triaxial accelerometer (W356A12/NC, PCB Piezotronics Inc., Depew, NY) modified to be neutrally buoyant and connected to a signal conditioner (482C15, PCB Piezotronics Inc.) and positioned within the AEP tank at the location of the fish head. For each frequency, corresponding PAL measurements were made for each SPL throughout the attenuation range. The accelerometer was placed such that its x-axis corresponded to the rostral-caudal, the y-axis to left-right, and the z-axis to dorsal-ventral positions. For each axis, PAL measurements were determined using Eq. 1.

Statistical analysis

Two-way repeated measures ANOVAs with frequency and treatment (control or sound-exposure duration with recovery period) as factors and auditory thresholds as the dependent variable were used to determine the significant ($\alpha = 0.05$) differences between the baseline auditory thresholds measured from controls and those measured from fish that underwent sound exposure (30 min or 24 h) and recovery (0, 48, or 96 h). Data met the ANOVA assumptions of normal distribution and equal variance. Post hoc pairwise Holm-Šidák tests were used to compare auditory thresholds between control and sound-exposed fish for each tested frequency to determine whether the exposure durations (30 min, 24 h) impacted thresholds differently and whether hearing recovery occurred following sound exposure. Statistical analysis was completed using SigmaPlot (version 12.5). Data are reported as mean ± SD.

Results

Auditory thresholds for silver and bighead carp were lowest between 0.2 and 0.6 kHz and increased with higher frequencies up to 4 kHz with slightly lower thresholds to 5 kHz (Figs. 2 and 3). Silver carp only displayed significant (Holm-Šidák, $P < 0.05$) TTS for both sound pressure and particle acceleration at 0.6 kHz after 30-min sound exposure; however, TTSs were observed for frequencies ≥ 0.2 and ≤ 2 kHz following 24-h exposure (Tables 1 and 2; Fig. 2). Significant auditory TTSs (Holm-Šidák, $P < 0.05$) were observed for bighead carp over most frequencies ≥ 0.2 and ≤ 2 kHz, with the exception of 0.8 kHz, immediately following sound exposure (30 min or 24 h), and auditory thresholds to 0.8 kHz were higher following 24-h compared with 30-min exposure (Tables 1 and 2; Fig. 3). Hearing recovery was observed for each species 48 and 96 h after sound exposure (30 min or 24 h; Tables 3 and 4). However, the differences in auditory TTS and hearing recovery between the two species were observed.

For silver carp, an auditory TTS was only noted at 0.6 kHz (SPL) at 48 h following 30-min exposure. However, after 24-h exposure, auditory thresholds were higher than baseline from 0.4 to 1 kHz at 48-h recovery (except 1-kHz PAL), while response to 0.6 kHz remained above the baseline after 96-h recovery (Fig. 4). Auditory thresholds at 2 kHz remained higher than the baseline in bighead carp 48 (SPL) and 96 h (SPL and PAL) after 30 min of sound presentation. Following 24 h of sound presentation, auditory thresholds remained higher than baseline at 0.6, 1, and 2 kHz after 48 h and at 2 kHz after 96 h (Fig. 5).

Silver carp

The 30-min sound exposure had a less dramatic effect on silver carp than on bighead carp. The only significant (Holm-Šidák, $P < 0.05$) TTSs were observed at 0.6 kHz for both SPL and PAL in fish tested immediately after exposure and 48 h following sound exposure (Tables 3 and 4; Fig. 4). By 96 h, the mean thresholds returned to baseline (Holm-Šidák, $P > 0.05$) levels. However, silver carp exposed to sound for 24 h showed TTS of higher magnitude and a wider frequency range compared with those in bighead carp. All frequencies from 0.2 to 2 kHz were significantly (Holm-Šidák, $P < 0.05$) elevated

compared with baseline levels, with shifts ranging from 10 to 30 dB for both SPL and PAL. After 48-h recovery, the 0.2-kHz and 2-kHz SPL and PAL as well as 1.0-kHz PAL thresholds were not significantly different from baseline (Holm-Šidák, $P > 0.05$). Following 96-h of recovery, 0.6-kHz SPL and PAL thresholds remained elevated.

Bighead carp

For bighead carp, SPL and PAL were significantly elevated compared with baseline thresholds by approximately 10 dB for 0.4, 0.6, 1, and 2 kHz immediately following 30-min sound exposure. (Tables 3 and 4; Fig. 5) After 48 and 96 h of recovery, only TTS at 2 kHz remained significantly different (Holm-Šidák, $P < 0.05$). Greater and more prolonged threshold shifts followed exposure to 24 h of sound. Immediate TTSs of up to 20 dB were noted for 0.4 to 2 kHz. Fish still had significantly higher (Holm-Šidák, $P < 0.05$) thresholds at 0.6 kHz, 1, and 2 kHz after 48 h and at 2 kHz after 96 h.
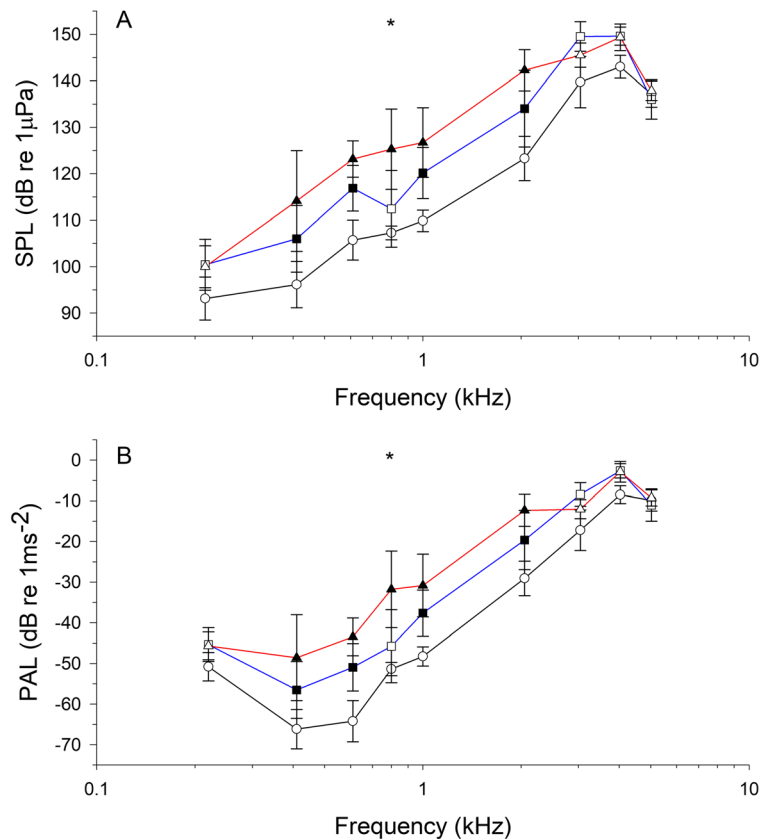
Discussion

Broadband sound exposure resulted in SPL and PAL auditory threshold shifts in silver and bighead carp at frequencies from 0.2 to 2 kHz. For both species, the largest magnitude TTSs were observed between 0.4 and 2 kHz following a 24-h sound exposure with a 0-h recovery. Bighead carps were more greatly impacted by 30-min sound exposure compared with silver carp. However, the greatest auditory TTSs were seen among silver carp immediately following a 24-h exposure and silver carp thresholds returned to baseline after 96 h for more frequencies than did bighead carp following either exposure duration.

In this study, the AEP technique was used to measure the frequency range and extent of auditory TTSs measured from bigheaded carps following broadband sound exposure. Although results from the AEP method have inherent variability brought on by observer subjectivity and varying experimental tank dimensions and sound presentation methods (Sisneros et al. 2016), it is appropriate for meeting the goals of this study, as all fish were

Fig. 3 Bighead carp mean auditory SPL (a) and PAL (b) thresholds (± SD) for control (circle), 30-min (square), and 24-h (triangle) noise-exposed fish. Filled symbols indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds for control and noise-exposed fish. Asterisks indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds for 30-min and 24-h noise-exposed fish

Table 1 Frequencies showing SPL threshold shifts immediately following sound exposure. Statistical analysis completed using two-way repeated measures ANOVA with P values from Holm-Šidák post hoc tests (ns indicates no significant difference). Fishes exposed to 0 (control), 30-min, or 24-h sound presentation

Frequency (kHz)

	0.2	0.4	0.6	0.8	1	2	3	4	5
Bighead carp									
Control vs 30 min	ns	$P=0.038$	$P=0.011$	ns	$P=0.031$	$P=0.018$	ns	ns	ns
Control vs 24 h	ns	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	ns	ns
30 min vs 24 h	ns	ns	ns	$P<0.001$	ns	ns	ns	ns	ns
Silver carp									
Control vs 30 min	ns	ns	$P=0.002$	ns	ns	ns	ns	ns	ns
Control vs 24 h	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	ns	ns
30 min vs 24 h	$P=0.011$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	ns	ns

tested within the same tank under the same acoustic conditions. However, there was a considerable range of thresholds measured for some frequencies, particularly for fish that underwent sound exposure. An examination of hearing recovery within individual fish may provide further insight into TTS variation. However, fish were sacrificed after AEP testing for examination of inner ear morphology. Thus, determining hearing recovery in the same animal was not possible in this study.

Acoustic deterrents are currently being considered to stop the upstream migration of invasive bigheaded carps at strategic locations. Several studies have shown that broadband sound causes repeated and prolonged negative phonotaxis in both silver and bighead carps and has been effective in preventing egress through a small

channel in an outdoor pond (Vetter et al. 2015, 2017; Murchy et al. 2017). Pure tones have been much less effective in altering behavior and therefore the broadband sound that caused negative phonotaxis in the previous studies was used for sound exposure. As acoustic deterrents rely on the target species to continually detect and localize the sound source, SPL and PAL levels must be sufficient to deter movement without causing transient or permanent damage to the auditory sensory hair cells.

The amplitude of 155.7 ± 4.7 dB re 1 μPa SPL_{rms} (-8 ± 7.2 dB re 1 ms^{-2} PAL_{rms}) for sound exposure was chosen for this study as this level has been proven effective in continually deterring carp in large outdoor ponds (Vetter et al. 2015, 2017; Murchy et al. 2017). However, greater amplitudes, especially at the source,

Table 2 Frequencies showing PAL threshold shifts immediately following sound exposure. Statistical analysis completed using two-way repeated measures ANOVA with P values from Holm-Šidák post hoc tests (ns indicates no significant difference). Fishes exposed to 0 (control), 30-min, or 24-h sound presentation

Frequency (kHz)

	0.2	0.4	0.6	0.8	1	2	3	4	5
Bighead carp									
Control vs 30 min	ns	$P=0.033$	$P<0.001$	ns	$P=0.014$	$P=0.039$	ns	ns	ns
Control vs 24 h	ns	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	ns	ns
30 min vs 24 h	ns	ns	ns	$P<0.001$	ns	ns	ns	ns	ns
Silver carp									
Control vs 30 min	ns	ns	$P=0.002$	ns	ns	ns	ns	ns	ns
Control vs 24 h	$P=0.002$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	ns	ns
30 min vs 24 h	ns	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	ns	ns	ns

Table 3 Frequencies showing SPL threshold shifts following sound exposure and recovery. Statistical analysis completed using two-way repeated measures ANOVA with P values from Holm-Šidák post hoc tests (ns indicates no significant difference). Fishes exposed to a 30-min or 24-h sound presentation and given a 48- or 96-h recovery period before AEP testing

Frequency (kHz)

	0.2	0.4	0.6	0.8	1	2	3	4	5
BHC 30 min									
48 h vs control	ns	ns	ns	ns	ns	$P = 0.033$	ns	ns	ns
96 h vs control	ns	ns	ns	ns	ns	$P = 0.017$	ns	ns	ns
BHC 24 h									
48 h vs control	ns	ns	$P = 0.011$	ns	$P = 0.045$	$P = 0.017$	ns	ns	ns
96 h vs control	ns	ns	ns	ns	ns	$P < 0.001$	ns	ns	ns
SVC 30 min									
48 h vs control	ns	ns	$P = 0.027$	ns	ns	ns	ns	ns	ns
96 h vs control	ns	ns	ns	ns	ns	ns	ns	ns	ns
SVC 24 h									
48 h vs control	ns	$P = 0.020$	$P < 0.001$	$P = 0.029$	$P = 0.043$	ns	ns	ns	ns
96 h vs control	ns	ns	$P = 0.002$	ns	ns	ns	ns	ns	ns

are likely necessary for large-scale sound projection at locations where acoustic deterrents may be deployed, such as locks and dams. Therefore, the results should be treated conservatively, with presumably greater damage or TTS incurred by shorter exposures to louder sound sources.

Previous studies on the impacts of sound exposure on the hearing capabilities of otophysan fishes indicate that the magnitude of a TTS for a tested frequency and the time of recovery are dependent upon the intensity and duration of the exposure as well as the baseline hearing thresholds for the test species (Scholik and Yan 2001; Scholik and Hong 2002; Amoser and Ladich 2003; Smith et al. 2004a, 2004b). For example, goldfish (*Carassius auratus*) exposed to white noise (0.1–10 kHz) at 160–170 dB re 1 μPa showed significant

Table 4 Frequencies showing PAL threshold shifts following sound exposure and recovery. Statistical analysis completed using two-way repeated measures ANOVA with P values from Holm-Šidák post hoc tests (ns indicates no significant difference). Fishes exposed to a 30-min or 24-h sound presentation and given a 48- or 96-h recovery period before AEP testing

Frequency (kHz)

|	0.2	0.4	0.6	0.8	1	2	3	4	5
BHC 30 min									
48 h vs control	ns	ns	ns	ns	ns	ns	ns	ns	ns
96 h vs control	ns	ns	ns	ns	ns	$P = 0.039$	ns	ns	ns
BHC 24 h									
48 h vs control	ns	ns	$P < 0.001$	ns	$P = 0.021$	$P = 0.034$	ns	ns	ns
96 h vs control	ns	ns	ns	ns	ns	$P < 0.001$	ns	ns	ns
SVC 30 min									
48 h vs control	ns	ns	ns	ns	ns	ns	ns	ns	ns
96 h vs control	ns	ns	ns	ns	ns	ns	ns	ns	ns
SVC 24 h									
48 h vs control	ns	$P = 0.015$	$P < 0.001$	$P = 0.043$	ns	ns	ns	ns	ns
96 h vs control	ns	ns	$P < 0.001$	ns	ns	ns	ns	ns	ns
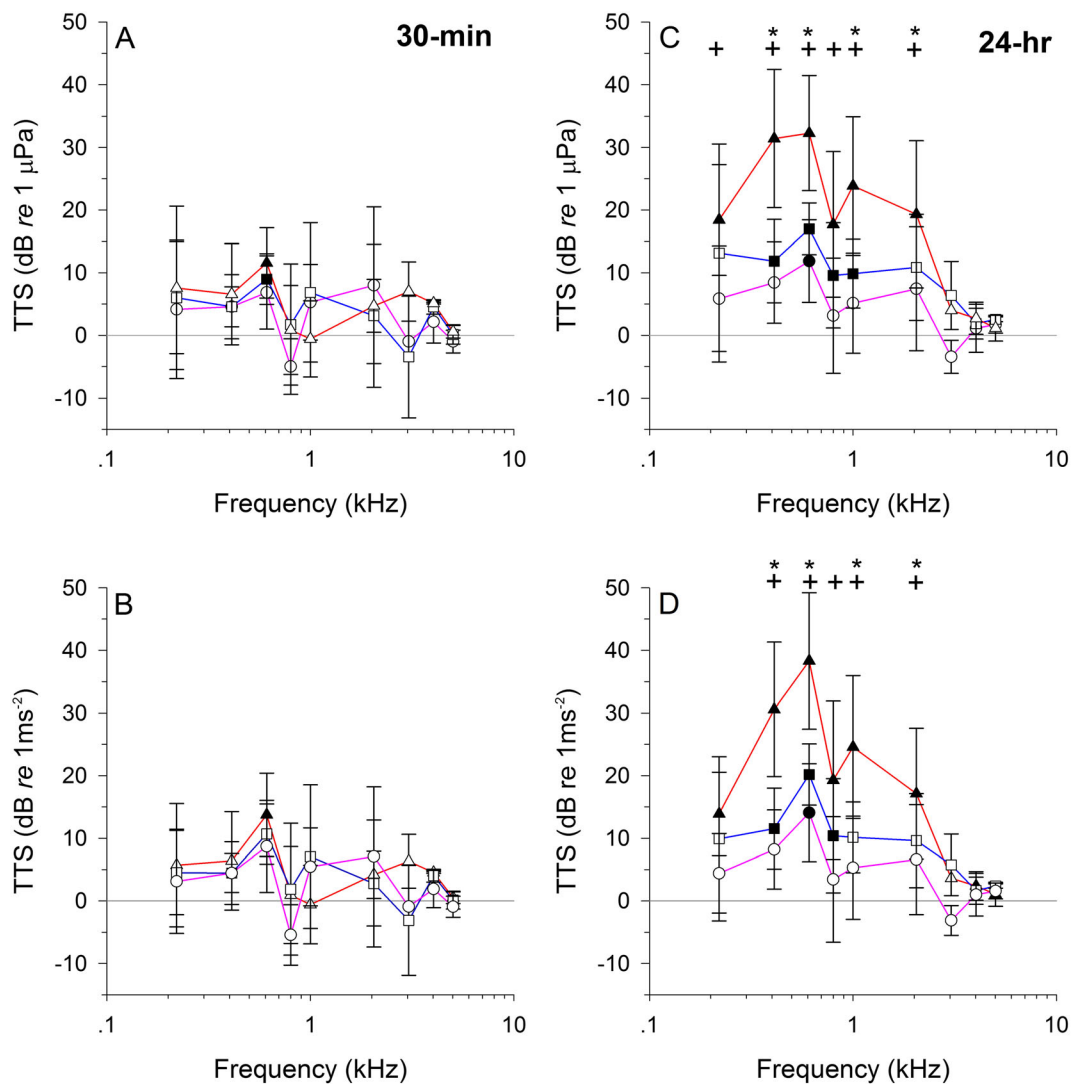
Fig. 4 Silver carp mean auditory SPL (**a**, **c**) and PAL (**b**, **d**) threshold shifts following 30-min (a, b) and 24-h (c, d) noise exposure after 0-h (triangle), 48-h (square), and 96-h (circle) recovery periods. Filled symbols indicate a significant difference (Holm-Šidák, $P < 0.05$) from baseline thresholds (gray reference line). Asterisks indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds following 0-h and 48-h recovery periods. Crosses indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds following 0-h and 96-h recovery periods

threshold shifts following varying sound exposure durations with increased duration resulting in increased TTSs (Smith et al. 2004a). In a similar study, Smith et al. (2004b) found that goldfish exposed to 110, 130, 140, and 160 dB re 1 μPa for 24 h experienced linear TTSs in relation to exposure intensity. In a comparison between catfish (*Primeus pictus*) and goldfish hearing thresholds following exposure to unfiltered white noise at 158.0 dB re 1 μPa, the greatest TTSs were measured in the frequencies each species showed the lowest baseline thresholds to (Amoser and Ladich 2003).

Considering the sound exposure intensity was consistent for all trials and there were differences in TTS between 30-min and 24-h exposed silver and bighead carps, the results from this study are consistent with previous findings.

Hearing recovery differed between species as well. Following a 96-h recovery period, auditory thresholds to 2 kHz remained significantly ($P < 0.05$) shifted among bighead carp exposed to sound for either exposure duration (30 min, 24 h). This is similar to what Scholik and Yan (2001) reported for noise-exposed fathead minnows
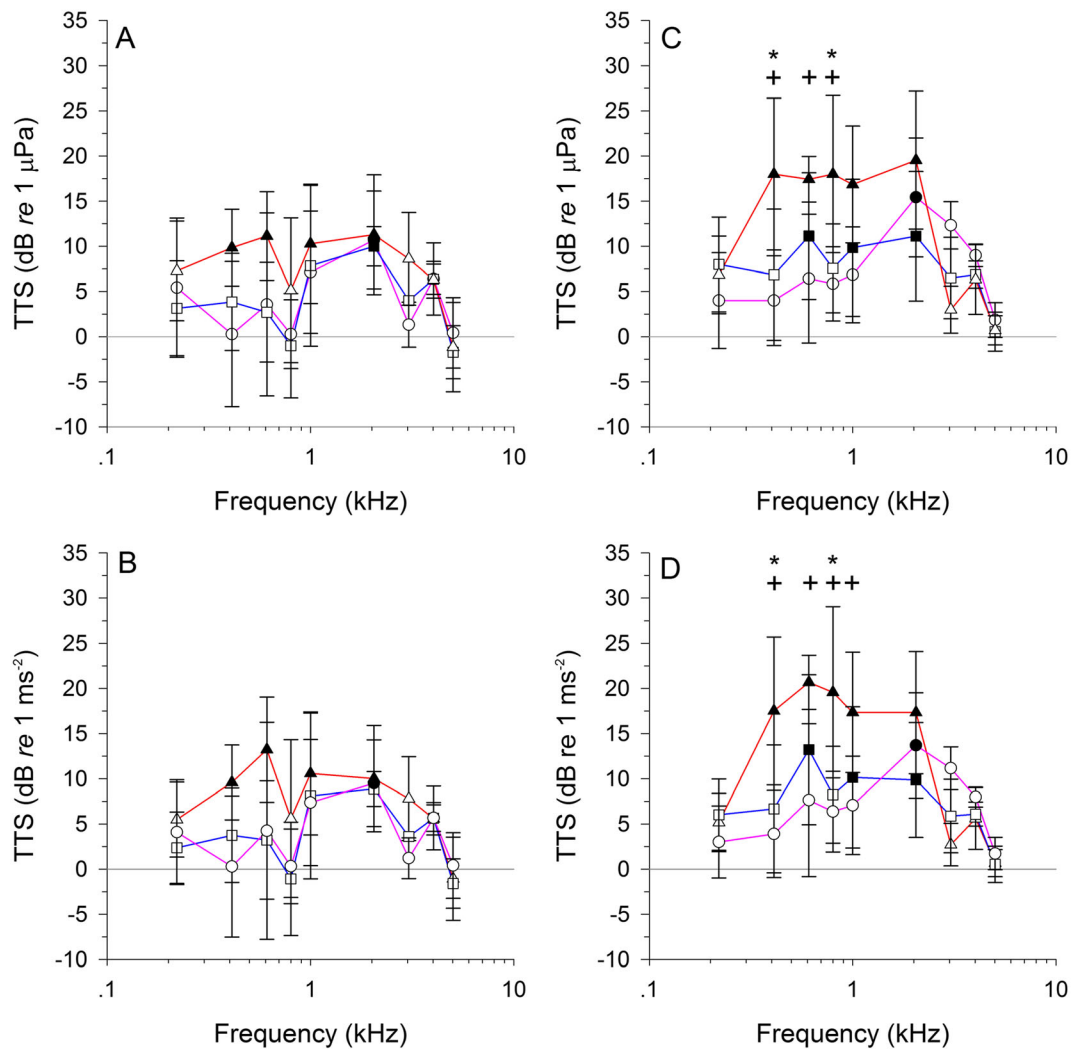
Fig. 5 Bighead carp mean auditory SPL (**a**, **c**) and PAL (**b**, **d**) threshold shifts following 30-min (a, b) and 24-h (c, d) noise exposure measured after 0-h (triangle), 48-h (square), and 96-h (circle) recovery periods. Filled symbols indicate a significant difference (Holm-Šidák, $P < 0.05$) from baseline thresholds (gray reference line). Asterisks indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds following 0-h and 48-h recovery periods. Crosses indicate a significant difference (Holm-Šidák, $P < 0.05$) between thresholds following 0-h and 96-h recovery periods

(*Pimephales promelas*) and may indicate that the morphological features resulting in higher audible frequency range may be more susceptible to damage. In contrast, auditory thresholds among silver carp exposed to sound for 30 min were similar to baseline ($P > 0.05$) thresholds for all tested frequencies following a 96-h recovery. However, silver carp exposed to sound for 24 h did not fully recover their pre-sound exposure hearing ability at 0.6 kHz within 96 h. As this frequency had the lowest baseline PAL thresholds for silver carp, it is possible that hearing recovery may depend upon baseline auditory ability. Furthermore, morphological factors

related to high-frequency (> 1.0 kHz) sound detection, which vary between species, may also play a role in hearing recovery after loud sound exposure.

Whether fish would remain near high-intensity sound fields long enough to cause transient damage to auditory hair cells resulting in TTSs remains to be determined. Site-specific parameters such as water depth, turbidity, flow rate, and ambient noise levels will impact both fish behavior and the acoustic field presented. Therefore, it is imperative that behavioral studies in a field setting be conducted prior to implementing any acoustic deterrent. The results presented here give important guidance as to

the appropriate frequencies, SPL, and exposure durations such studies should examine in order to determine the potential impacts such deterrents may have on the hearing and behavior of targeted species.

The overall findings from this study indicate that high-intensity (≥ 155 dB re 1 µPa SPL_{rms}, ≥ -8 dB re 1 ms^{-2} PAL_{rms}) broadband sound (0.06–10 kHz), or a similar signal that may be used as a deterrent for bigheaded carps, has the potential to temporarily decrease the frequency detection at the auditory periphery of the targeted fish, thus impacting the deterrent's efficacy. In addition, the period necessary for hearing recovery depends upon the exposure duration. This is the first study to examine the effects of short- (30 min) and long-term (24-h) exposure to sound among bigheaded carps. Despite being closely related enough to hybridize, the two species showed differences in hearing impacts following sound exposure, demonstrating the need to evaluate each species individually. Large-scale behavioral studies in the field should now be conducted to further assess the efficacy of broadband sound as an acoustic deterrent for silver and bighead carps and evaluate appropriate SPLs. It is important to consider these relationships when developing acoustic fish deterrents and when defining the short-term and long-term goals associated with their use.

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