Behavioral responses of native and invasive fishes of the Upper Mississippi River to 100 hp boat motor acoustic stimulus

Kelsie A. Murchy1,2,*, Brooke J. Vetter1, Marybeth K. Brey3 and Allen F. Mensinger2

1Current address: Biology Department, University of Victoria, Victoria, British Columbia V8W 3N5, Canada
2Biology Department, University of Minnesota Duluth, Duluth, Minnesota 55812, USA
3Biology Department, University of St. Thomas, St. Paul, MN 55805, USA
4U. S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin 54603, USA

*Corresponding author
E-mail: kmurchy@uvic.ca

Abstract

Acoustic deterrents are currently being considered for deployment at strategic bottlenecks, such as lock and dams of major rivers, to deter upstream movement of invasive carp. Previous studies have demonstrated that bighead and silver carp (Hypophthalmichthys nobilis and H. molitrix, respectively) display negative phonotaxis to playbacks of broadband sound recordings produced from a 100 hp outboard boat motor. However, there is concern that acoustic deterrents may impact the movement of non-target native fishes in the Upper Mississippi River. We evaluated the potential impacts of a broadband underwater acoustic deterrent on native ostariophysans [bigmouth buffalo (Ictiobus cyprinellus), channel catfish (Ictalurus punctatus) and fathead minnow (Pimephales promelas)], invasive ostariophysans [bighead carp, common carp (Cyprinus carpio), grass carp (Ctenopharyngodon idella) and silver carp], and native non-ostariophysans [(American eel (Anguilla rostrata), gizzard shad (Dorosoma cepedianum), hybrid striped bass (M. saxatilis × M. chrysops), lake sturgeon (Acipenser fulvescens) and paddlefish (Polyodon spathula)]. Fish were exposed to playback of the broadband sound (60–10000 Hz), and their behavior was evaluated. Bighead carp showed a strong negative phonotaxis response to the stimulus [12.3 ± 7.5 (SD) mean consecutive reactions], silver carp and grass carp showed moderate responses (4.5 ± 5.2 and 3.8 ± 3.5 reactions), and common carp displayed low responses (1.3 ± 1.9 reactions). Of the native fish, bigmouth buffalo (2.1 ± 2.9 reactions) and hybrid striped bass (0.3 ± 0.5 reactions) were the only species to demonstrate observable response to the acoustic stimulus. Based on this small-scale behavioral screening, acoustic deterrents should have minimal impact on native species; however, larger pond and field trials are necessary to confirm this finding.

Key words: invasive species, sound, behavior, acoustic deterrent

Introduction

Invasive fish species can cause widespread ecological and economic damage in aquatic ecosystems. The common carp (Cyprinus carpio Linnaeus, 1758), a fish native to Europe, was intentionally stocked throughout North American waterways beginning in the late 1800s (Smiley 1886; Barnickol and Starrett 1951). Common carp are now ubiquitous in the continental United States, and this species’ foraging behavior can
quickly degrade water bodies (Weber and Brown 2009). Bighead and silver carp (*Hypophthalmichthys nobilis* Richardson, 1845 and *H. molitrix* Valenciennes, 1884; collectively known as bigheaded carps) along with grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) are native to eastern China and Siberia (Pierce 1983; Kolar et al. 2007) and were imported into the United States for phytoplankton control in eutrophic water bodies (Pierce 1983; Buck et al. 2010). However, these invasive carp species escaped from their containment ponds or were intentionally stocked and have continued to spread throughout the Mississippi River Basin (Jennings 1988; Buck et al. 2010; Mitchell and Kelly 2011). These invasions have caused major ecological and economical damage by decreasing phytoplankton and zooplankton abundance and disrupting native food webs (DeBoer et al. 2018).

A major concern for resource managers is the range expansion of invasive bigheaded carps into the Laurentian Great Lakes (Cudmore et al. 2012; Cudmore et al. 2017). Although a select number of dams provide physical barriers along the Upper Mississippi River, fish can migrate through the adjacent navigation locks (Wilcox 1999; Tripp et al. 2014; Vallazza et al. 2021). Therefore, a variety of non-physical deterrents are being considered for deployment at strategic bottlenecks (such as lock chambers) including carbon dioxide (Cupp et al. 2016; Cupp et al. 2021), bubble curtains (Zielinski et al. 2014), and acoustic stimuli (Putland and Mensinger 2019a), to control invasive carps and deter movement. However, limiting undesirable effects of any deterrent on native or non-target species is highly desirable.

Acoustic deterrents have shown promise in the field in modifying fish behavior. For example, a 20–600 Hz auditory stimulus at 174 dB re: 1 µPa decreased impingement of fish in a power plant by 60% (Maes et al. 2004). Ultrasound at 190 dB re: 1 µPa reduced alewife (*Alosa pseudoharengus* Wilson, 1811) passage in a power plant intake by 87% (Ross et al. 1993), and infrasound (11.8 Hz) reduced abundance of European silver eels (*Anguilla anguilla* Linnaeus, 1758) near the sound by 43% (Sand et al. 2000). Acoustic stimuli have been effective at modifying invasive carp behavior in controlled settings (Vetter et al. 2015, 2017; Murchy et al. 2017; Dennis et al. 2019). Playback of a 100 hp outboard motor sound (60–10,000 Hz, which will be referred to as “broadband sound”) caused a rapid and consistent negative phonotaxis response in bighead and silver carp (Vetter et al. 2015, 2017) and prevented > 90% of bigheaded carps from transiting a one-meter channel in outdoor ponds (Murchy et al. 2017). Similarly, when bighead, common, and silver carp were exposed to a 40 hp boat motor playback (100–10000 Hz at 150 dB re: 1 µPa), all three species displayed negative phonotaxis (Zielinski and Sorensen 2017) and reduced passage through an ensonified flume (Dennis et al. 2019). Recent field studies have evaluated outboard motor stimuli for invasive carp control.
with mixed results depending on the species evaluated (Wamboldt et al. 2019; Dennis and Sorensen 2020). Dennis and Sorensen (2020) reported that common carp quickly habituated to a 40 hp boat sound, while Wamboldt et al. (2019) found silver carp showed a variety of responses to a 100 hp boat motor.

The management of invasive carps using acoustic deterrents may be especially promising because these species are ostariophysans and possess hearing specializations that allow them to detect a broader range of frequencies (100–5000 Hz; Lovell et al. 2006; Vetter et al. 2018) than most native non-ostariophysans (100–1000 Hz; see Putland and Mensinger 2019a for a review). Thus, higher frequency sound, outside the hearing range of non-ostariophysan fish, may limit impact on native species. Available data from native ostariophysans in the Mississippi River Basin suggest a hearing range between 100–4000 Hz (Scholik and Yan 2001; Wysocki et al. 2009), while most native non-ostariophysan fishes are less sensitive and detect lower frequencies. For example, paddlefish and lake sturgeon cannot detect sound frequencies beyond 500 Hz (Lovell et al. 2005). Given the wide range of frequencies that can be detected by native species in the Mississippi River Basin, it is important to evaluate which, if any, native species may detect broadband acoustic signals.

The goal of this study was to examine if any native species would exhibit repeated negative phonotaxis (i.e., move away from the source) to a 100 hp outboard motor broadband stimulus (Vetter et al. 2015, 2017; Murchy et al. 2017). The negative phonotaxis response was chosen because it is an avoidance reaction that has been observed in various species of fish (Kastelein et al. 2008; Wilson et al. 2011; Qin et al. 2020) and is consistently seen in bighead and silver carp in response to the same broadband stimulus examined in this study (Vetter et al. 2015, 2017). Native ostariophysan and non-ostariophysan species that were of high concern or value (i.e., declining populations or commercially/recreationally harvested species) to management agencies and were available were prioritized for testing. These species included bigmouth buffalo (*Ictiobus cyprinellus* Valenciennes, 1844), channel catfish (*Ictalurus punctatus* Rafinesque, 1818), fathead minnow (*Pimephales promelas* Rafinesque, 1820), American eel (*Anguilla rostrata* Lesueur, 1817), gizzard shad [surrogate for skipjack herring (*Alosa chrysochloris*), and also occur in the Mississippi River], hybrid striped bass (*M. saxatilis* × *M. chrysops* Walbaum, 1792; surrogate for white bass, *Morone chrysops*), lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) and paddlefish (*Polyodon spathula* Walbaum, 1792; Table 1). In addition, four species of invasive carp (bighead, silver, common, and grass carp) were tested. We hypothesized that native non-ostariophysans would display different rates of negative phonotaxis than native ostariophysans and invasive carps due to differences in auditory sensitves.
Table 1. Summary of reactions and swim speed for all species.

<table>
<thead>
<tr>
<th>Species (n=trials)</th>
<th>Reported hearing frequency range</th>
<th>Size range TL (cm)</th>
<th>Reactions: Mean ± SD</th>
<th>Reactions: Max</th>
<th>Control Swim Speed (cm/s) (n = individuals)</th>
<th>Reaction Swim Speed (cm/s) (n = individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ostariophysan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver carp¹ (n = 4)</td>
<td>100–5000 Hz, some up to 7000 Hz (Lovell et al. 2006; Vetter et al. 2018)</td>
<td>18–24 cm</td>
<td>4.5 ± 5.2</td>
<td>9</td>
<td>3.9 ± 2.1 (n = 40)</td>
<td>6.4 ± 9.4 (n = 40)</td>
</tr>
<tr>
<td>Bighead carp¹ (n = 4)</td>
<td>100–5000 Hz, some up to 7000 Hz (Lovell et al. 2006; Vetter et al. 2018)</td>
<td>18–24 cm</td>
<td>12.3 ± 7.5</td>
<td>23</td>
<td>17.6 ± 5.0 (n = 40)</td>
<td>39.0 ± 12.0 (n = 40)</td>
</tr>
<tr>
<td>Common carp¹ (n = 4)</td>
<td>100–4000 Hz (Maiditsch and Ladich 2014)</td>
<td>20–25 cm</td>
<td>1.3 ± 1.9</td>
<td>4</td>
<td>7.0 ± 9.0 (n = 21)</td>
<td>13.4 ± 18.4 (n = 20)</td>
</tr>
<tr>
<td>Grass carp¹ (n = 4)</td>
<td>200–4000 Hz (Nissen 2018)</td>
<td>10–25 cm</td>
<td>3.8 ±3.5</td>
<td>8</td>
<td>4.9 ± 3.0 (n = 40)</td>
<td>7.5 ± 6.0 (n = 28)</td>
</tr>
<tr>
<td>Native ostariophysan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Channel catfish² (n = 4)</td>
<td>100–4000 Hz (Wysocki et al. 2009)</td>
<td>9–13 cm</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>1.6 ± 3.1 (n = 2)</td>
<td>NA</td>
</tr>
<tr>
<td>Fathead minnow² (n = 4)</td>
<td>300–4000 Hz (Scholik and Yan 2001)</td>
<td>6–9 cm</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>4.4 ± 3.8 (n = 22)</td>
<td>NA</td>
</tr>
<tr>
<td>Bigmouth buffalo² (n = 8)</td>
<td>100–2500 Hz (Putland unpublished)</td>
<td>19–25 cm</td>
<td>2.1 ± 2.9</td>
<td>8</td>
<td>7.7 ± 11.4 (n = 28)</td>
<td>15.8 ± 17.2 (n = 39)</td>
</tr>
<tr>
<td>Native non-ostariophysan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake sturgeon³ (n = 4)</td>
<td>100–500 Hz (Lovell et al. 2005)</td>
<td>13–20 cm</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>1.0 ± 1.1 (n = 6)</td>
<td>NA</td>
</tr>
<tr>
<td>Paddlefish³ (n = 4)</td>
<td>100–500 Hz (Lovell et al. 2005)</td>
<td>16–30 cm</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>11.5 ± 0.9 (n = 40)</td>
<td>NA</td>
</tr>
<tr>
<td>Gizzard shad⁴ (n = 4)</td>
<td>0.1–100 kHz (Mann et al. 1998)</td>
<td>5–10 cm</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>8.9 ± 4.5 (n = 38)</td>
<td>NA</td>
</tr>
<tr>
<td>Hybrid Striped Bass⁵ (n = 4)</td>
<td>unknown</td>
<td>7–10 cm</td>
<td>0.3 ± 0.5</td>
<td>1</td>
<td>5.9 ± 4.4 (n = 10)</td>
<td>NA</td>
</tr>
<tr>
<td>American Eel⁶ (n = 4)</td>
<td>10–300 Hz (European Eel Anguilla anguilla Linnaeus, 1758: Jerko et al. 1989)</td>
<td>28–40 cm</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>0.2 ± 0.3 (n = 3)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data represent mean ± standard deviation, NA indicates no swim speed data were collected.
¹Invasive carp species.
²Native species with overlap in hearing abilities with invasive carp.
³Threatened Species in the Upper Mississippi River.
⁴Surrogate for threatened skipjack herring.
⁵Surrogate for white bass.

Materials and methods

Animal husbandry

Experiments were conducted at the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin, USA. American eel (28–40 cm total length, TL), gizzard shad (5–10 cm TL), lake sturgeon (13–20 cm TL), paddlefish (16–30 cm TL), bigmouth buffalo (8–13 cm TL), channel catfish (9–13 cm TL), fathead minnow (6–9 cm TL), and hybrid striped bass (7–10 cm TL), were maintained separately in tanks using flowthrough well water and fed a diet of classic pellet feed specific to their size (Skretting, Tooele, UT, USA), except lake sturgeon, which were fed a diet of bloodworms (Brine Shrimp Direct, Ogden, UT, USA). Bighead carp (9–20 cm TL), common carp (5–11 cm TL), grass carp (9–20 cm TL), and silver carp (8–20 cm TL) were maintained in tanks using recirculated water and fed classic fry diet (Skretting, Tooele, UT). All species’ diets were calculated at a rate of 0.5% body weight per day to maintain healthy individuals. Food was withheld for 24 hours prior to transport to the experimental tanks, and fish were not fed during the testing periods.
Experimental tanks

Experiments were conducted in four 69 cm × 292 cm × 30 cm (735 L; water depth = 25 cm) indoor flow-through tanks placed one-meter apart to avoid sound propagating to neighboring tanks. The tanks were provided with flow-through water at 200 mL·s⁻¹ from onsite wells. Hot and cold water were mixed in a head box (two tanks per head box) to maintain a temperature of 18 ± 2 °C in all tanks (mean ± standard deviation hardness = 193.1 ± 4.651 mg·L⁻¹ CaCO₃; alkalinity = 142.6 ± 1.685 µeq·L⁻¹; specific conductance = 405.9 ± 8.87 S·m⁻¹ of well water during the time of this study; J. Louma, US Geological Survey, unpublished data). Mesh netting was placed on top of each tank to ensure fish did not jump from tanks, and black plastic sheets (5 mm) surrounded the four tanks from floor to ceiling to eliminate outside influences on behavior. Overhead fluorescent lights provided lighting and were turned on at least one-hour before trials began. Room access was restricted during the trials, and the single observer was positioned five meters away from the nearest tank.

An underwater speaker (UW-30: frequency response of 0.1–10 kHz, Lubell Labs™ Inc., Whitehall, OH, USA) was placed at each end of the experimental tank at approximately 30 cm from the tank end, and plastic mesh netting was placed directly behind the speakers to prevent fish from swimming behind them (Figure 1). To assess behavioral responses, the tank was divided into zones. On each end, the 50 cm section extending in front of each speaker towards the middle of the tank was defined as the “end zone” (Figure 1). The mid-line of the tank was approximately 120 cm from the front of each speaker.

Three overhead SONY bullet 500 TVL video cameras connected to a computer operating ProGold software (Security Camera World, Cooper City, FL, USA) monitored fish behavior in each tank, with each camera covering approximately 1/3 of the experimental tank. To aid in fish tracking, black grid lines (23 cm × 20.8 cm) were drawn on the bottom of the light blue tank. The observer monitored swimming behavior on video monitors in real time and manually controlled each speaker activation.
Acoustic stimulus and acoustic monitoring

The acoustic stimulus broadcast to the fish was a 36-second repeating loop recorded from a 100 Hp Honda 4-stroke outboard motor moving at 32 km/hr\(^{-1}\) in the Illinois River, hereafter, referred to as the “broadband stimulus.” The broadband stimulus contained a broad spectrum of sound (0.06–10.0 kHz) with maximal energy between 0.06–2.5 kHz (Figure 2). The boat movement resulted in amplitude modulation of the sound with sound intensity increased up to the midpoint of recording (18 seconds) and then declining over the second half of 36-second sound loop.

In each tank, a hydrophone (HTI-96-MIN; High Tech Inc., Long Beach, MS, USA) was positioned approximately 80 cm from the right speaker and was connected to a PowerLab 4SP data acquisition system controlled by LabChart 7 software (AD Instruments, Colorado Springs, CO, USA). Hydrophones in all tanks were monitored to ensure sound was not detected in tanks where the broadband stimulus was not being played. Sound intensity was regulated using an amplifier (UMA-752; Peavey Electronics, Meridian, MS, USA), and speaker activation was controlled separately with a switchbox (MCM Electronics, Centerville, OH, USA). Each tank was sound mapped with a SoundTrap 300 STD digital hydrophone (Ocean Instruments New Zealand, Auckland, NZ; sampling rate 24 kHz; 20–12000 Hz frequency range) placed at a single depth (5 cm above bottom) at 60 locations that were equally divided (23 cm apart in the x-plane and 20.8 cm in the y-plane). Acoustic sampling rate was set to encompass the frequency response range of UW-30 speakers. Prior to mapping each tank, the Soundtrap was calibrated with a 250 Hz tone using a 42AA pistonphone (G.R.A.S. Sound and Vibration, Denmark). Audio files
collected by the hydrophone were uploaded into Matlab R2016b (MathWorks™, Inc., Natick, MA, USA) and sub-sectioned into a four-second time blocks at the peak amplitude of the broadband stimulus for each point. Mean sound pressure levels (SPL_{rms} dB re: 1 μPa) for each point were derived from the root mean squared voltage ($V_{rms}$) for frequencies between 20 Hz and 10000 Hz, such that:

$$SPL_{rms} = 20 \times \log_{10} \left( \frac{V_{rms}}{1 \mu Pa} \right).$$

Frequency components of the acoustic stimulus in the experimental tanks were analyzed using Welch’s power spectral density estimate with a 1024-point Fourier transformation, Hamming window, and 24 kHz sampling rate.

**Behavioral experiments**

Trials were conducted between 0800 and 1800 from September 2015 to March 2017. Most species were tested in mono-specific groups of 10, with the exception of the smaller fathead minnow and gizzard shad, which were tested in schools of 20. All species were acclimated in the experimental tank for at least 24 hours prior to the initiation of experiments, and only one tank was tested at time. The broadband stimulus was not initiated until the majority of fish (≥ 6) were within an end zone (Figure 1). Following the initial stimulus exposure, if most fish (≥ 6) moved into the opposite end zone in < 60 seconds, the original speaker was deactivated, and the opposite speaker was activated within 1 second. The process of alternating speakers back and forth continued until fish failed to respond in 60 seconds or 10 minutes elapsed. A negative phonotaxis response was defined as ≥ 6 fish swimming away from the active speaker end zone (i.e., orienting 90–180° relative to the fish’s original position, and swimming towards the inactive speaker), crossing the midpoint of the tank in < 30 seconds, and entering the opposite endzone in 60 seconds. Non-responses were defined as fish not moving, swimming towards active speaker (positive phonotaxis), or failing to cross the midpoint of tank within the time limit. Each group was tested once, resulting in a total of four trials per species ($N_{individuals} = 40$ or $N_{individuals} = 80$, depending on fish length), except bigmouth buffalo, which required an additional four trials due to inconsistent behavior in the initial set ($N_{individuals} = 80$).

**Data Analysis**

Fish movement was quantified during post-trial video analysis. If a species consistently schooled, the school was treated as a single unit, with the midpoint of the school position tracked (x, y) every five seconds. If a species did not school, individual fish or subgroups were tracked every five seconds by counting the number of fish in each square created by the 23 × 20.8 cm gridlines. Density plots were created to assess distribution within
the tank for a school or individual fish and were created in SigmaPlot version 12.5. Ambient movements, swimming pattern, schooling, and distribution in tank were monitored for 10 minutes before each treatment (to coincide with maximum trial time). Schooling behavior was defined as the majority (≥ 6) of individuals moving together (reviewed in Pavlov and Kasumyan 2000), and mean proportion of schooling during control and sound exposure were compared using paired t-tests. Total number of consecutive negative phonotaxis responses were enumerated during each acoustic stimulus trial for each replicate. Consecutive negative phonotaxis response was analyzed using a zero inflated generalized linear model (GLM) with a Poisson distribution and a Tukey post hoc test to detected significant differences. The control swimming speed for each group was calculated by taking the swimming speed for each fish in the tank that swam over a 40 cm subsection though the middle of the tank (area between the end zones). If individual fish were not moving, no swimming speed was calculated. Reaction swimming speeds were calculated for the first 40 cm of fish movement away from the active speaker. These reaction swimming speeds were only calculated for species that demonstrated more than one negative phonotaxis response to the broadband sound. Mean reaction speeds were compared to control periods using paired t-tests. All statistical tests were completed in RStudio Version 1.2.5019 (R Core Team 2020). Mean consecutive reactions, percent schooling, and swimming speed were expressed as the mean ± one standard deviation.

Results

Acoustic monitoring

Ambient noise in the tanks was 126 dB re: 1 µPa, due to pumps and water flow noise in the room. During sound trials, SPL_{rms} was highest (142 dB re: 1 µPa) directly in front of the speaker and attenuated throughout the tank, declining to 135 dB re: 1 µPa at the opposite end, with a minimum of 128 dB re: 1 µPa along the walls (Figure 3). Frequency composition of the broadband stimulus in the end zones of the tanks ranged from 60–10000 Hz, with the maximum energy peaking from 2000–3000 Hz and a smaller peak from 6500–9000 Hz at 10 cm from the speaker. Frequencies below 2750 Hz diminished by more than 20 dB re: 1 µPa at 50 cm from speaker and continued to decrease with distance away from the speaker, but frequencies above 3000 Hz remained similar throughout the end zone (Figure 4).

Schooling behavior

Fish movement and schooling (≥ 6 fish moving together in same direction) were assessed for all species during control conditions and playback of the broadband stimulus (treatment). The American eel remained in consistent locations, with slow intermittent movements that did not deviate more than
Figure 3. A. Ambient sound map of representative experimental tank, inactive speakers denoted with an O. B. Sound map of representative experimental tank with colors indicating sound intensity level dB re: 1μPa during active broadcast of the underwater speaker marked with an X and inactive speaker marked with an O. Intensity level (dB re: 1μPa) is indicated by color panel.

Figure 4. Power Spectrums of the acoustic stimulus in the end zone (50 cm in front of speaker). Black line is 10 cm from speaker and blue line is 50 cm from speaker at the end of the end zone.

40 cm from beginning location and were not categorized as schooling. Channel catfish also did not school and remained in consistent locations throughout the two different time points (control and treatment). Bigmouth buffalo remained in schools 75 ± 46.3% (Mean ± SD) of the time during control conditions and 60.3 ± 42.0% during the broadband stimulus playbacks. Bigmouth buffalo schools displayed two behaviors: half the groups (Ngroups = 4) remained static, and the others swam continuously around the perimeter of the tank. Fathead minnows swam throughout the tank during both control and treatment conditions and remained in schools 77.5 ± 29.0% (control) and 92.6 ± 13.0% (treatment) of the time. Gizzard shad were distributed throughout the tank in small groupings of four or fewer individuals and continually swam during ambient and
broadband conditions, but they never coalesced into a single school. Hybrid striped bass continuously swam throughout the tank and remained in schools 57.1 ± 30.9% and 77.7 ± 12.6% of the time during control conditions and broadband sound playbacks, respectively. Lake sturgeon and paddlefish did not school at any point. When sound was initiated, lake sturgeon remained in one location. Paddlefish continued to move in the tank, though they never traveled more than 40 cm in either direction from their initial spot (i.e., their location in the tank when a playback began).

Prior to sound exposure, all invasive carp species (bighead carp, common carp, grass carp, and silver carp) swam continuously around the edge of the tank. Silver carp spent the entire time schooling while bighead carp schooled 81.6 ± 12.8% of the time during ambient conditions and 90.7 ± 12.2% during sound playback. Common carp schooled 75 ± 24.4% and 48 ± 44.3% during ambient and broadband conditions, respectively, while grass carp infrequently schooled during control (3.5 ± 7.1%) or treatment conditions (9.4 ± 18.8%). All species tested showed no significant difference in schooling behavior between control and treatment conditions.

**Negative phonotaxis**

The number of negative phonotaxis responses to the broadband stimulus was assessed for each species (Table 1). Native ostariophysans (channel catfish and fathead minnow) and native non-ostariophysans (American eel, gizzard shad, lake sturgeon, and paddlefish) did not display visible change in behavior to the broadband stimulus and behaved the same as during control conditions. The channel catfish and lake sturgeon remained in similar locations compared with ambient conditions and did not leave the end zone during the broadband stimulus (Figures S1 and S2). Although one group of channel catfish did react to sound onset (i.e., startle response), none left the end zone. American eel (Figure S3), gizzard shad (Figure S4), and paddlefish continued to move during playback of the broadband stimulus (Figure 5); however, all groups remained in the end zone during acoustic playback. The fathead minnows also moved during the broadband trials; however, only two groups moved out of the end zone during playback, but at the midline moved back towards active speaker (Figure S5). The bigmouth buffalo (2.1 ± 2.9 responses; mean ± SD: Figure 6) and hybrid striped bass (0.3 ± 0.5 responses) reacted infrequently to the broadband stimulus (Figure S6).

The invasive carp showed variable responses to the sound stimulus. Common carp responded 1.3 ± 1.9 times with a maximum of 4 consecutive responses (Figure S7), while grass carp (Figure S8) and silver carp (Figure S9) displayed moderate responses to the broadband stimulus with mean responses of 3.8 ± 3.5 (maximum 8) and 4.5 ± 5.2 (maximum 9), respectively (Table 1). In contrast, bighead carp showed more responses with a mean of 12.3 ± 7.5 reactions and up to 23 consecutive responses (Figure 7). Bighead carp was
the only species to have significantly greater number of successive responses than all native species tested (GLM; df = 11, p < 0.0001, Tukey post hoc), and for two other invasive carp species tested (GLM; df = 11, Tukey post hoc: grass carp: p = 0.0097; common carp: p < 0.0001). However, consecutive responses of silver carp were not significantly different (GLM; df = 11, p = 0.3097, Tukey post hoc).

Swimming speed

Species that had a mean negative phonotaxis response greater than 1.0 also exhibited elevated swimming speeds during movement away from the sound versus swimming speeds during control trials (Table 1). All carp species increased swimming speeds during sound playback compared to control periods, with a significant increase seen in the bighead carp (two tailed t-test, df = 3, −4.4794, p = 0.0207). Bigmouth buffalo also displayed increased swimming speeds, but this difference was not significant (two tailed t-test, df = 7, t = −2.1845, p = 0.0652).

Discussion

A broad behavioral screening was conducted on 12 species of fish to address relative differences in negative phonotaxis responses between invasive and native fishes to a broadband sound stimulus. Invasive grass, bighead, and silver carp showed greater negative phonotaxis than all native species and common carp tested in this small laboratory study. This broadband sound stimulus, along with others, is currently being used in
experimental studies of underwater acoustic deterrents in the Mississippi River Basin, and this study provides insight on the potential behavioral responses that acoustic deterrents may illicit from native fishes compared to invasive carp species. Our findings indicate that the broadband sound could be an effective acoustic deterrent, as it elicited limited negative phonotaxis responses in non-ostariophysan fishes. This is an initial screening using a wide range of species, and as such, can be used as a starting point for future studies evaluating the impacts of acoustic deterrents on native species.

All four invasive carp species showed negative phonotaxis responses when exposed to the broadband sound stimulus, consistent with prior
Figure 7. Representative tracks of one of the four groups of bighead carp (n = 10) plotted every 5 seconds, demonstrating movement in an experimental tank during sound off (Top) and sound on (Bottom). Solid red lines denote mesh, solid black lines denote locations of speakers along mesh, and dashed red lines denote the midline of the tank. A. Ambient time period (10 minutes) with no stimulus playback: fish tended to swim in circles around the tank periphery. B. Acoustic stimulus playback: swimming pattern had more transects back and forth across the tank, showing that the school of bighead carp swam through the middle tank away from active speaker multiple times in a row and spent less time (fewer tracks) next to the speakers (compared with control swimming pattern shown in the top panel).

research using bighead, common, and silver carp (Zielinski et al. 2014; Vetter et al. 2015, 2017; Murchy et al. 2016, 2017; Zielinski and Sorensen 2016, 2017). However, common carp habituated relatively quickly to the sound, which is consistent with results from a larger, concrete outdoor pond (Murchy et al. 2016). Moderate responses (mean > 3 consecutive negative phonotaxis responses) were observed in silver and grass carp. Bighead carp showed the highest number of reactions and greatest increase in swimming speed during negative phonotaxis.

While both silver and bigheaded carp exhibited consistent responses to the broadband sound, their degree of response was less than observed in previous outdoor pond experiments (Vetter et al. 2015, 2017). This is not unusual, as fish can display different behavior when placed indoors, and small, narrow tanks such as those used for this experiment can create complex acoustic soundscapes that can make it challenging for fish to detect and move away from the sound source (Gray et al. 2016; Rogers et al. 2016). Also, sound is not uniformly distributed throughout the tank, and fish may move to areas of reduced intensity (e.g., along the walls) rather than traveling the full length of the tank. Silver carp displayed behavior similar to bighead carp in response to 100 hp and 40 hp boat
Native and Invasive fish behavior to acoustic stimulus

Motor sounds in larger and deeper tanks (> 1 m: Vetter et al. 2015, 2017; Zielinski and Sorensen 2017). However, silver carp failed to respond consistently in a shallow field environment (< 0.5 m: Wamboldt et al. 2019). Thus, water depth should be considered an important factor. To improve results, future studies could use a large outdoor pond or deeper field sites to avoid the complex acoustic soundscape in small tanks.

Of the seven native Mississippi River fish species and one surrogate tested, only bigmouth buffalo (native ostariophysan) exhibited multiple, but inconsistent, negative phonotaxis reactions. However, even the most active bigmouth buffalo groups reacted fewer times than all bighead carp (maximum reactions = 23) indicating that, if they detect a broadband acoustical deterrent, they will likely not be impacted to the same level as bighead carp or habituate quickly. Bigmouth buffalo do show seasonal migration patterns when spring snow melt increases river discharge (Johnson 1963), and perhaps sound deterrents could be turned off during this time so they would not coincide with bigmouth buffalo migration. Bigmouth buffalo have a hearing range of 100–2500 Hz, but they are less sensitive compared to other members of the Catastomidae family (Putland unpublished). Refining and optimizing the acoustic stimulus for bigheaded carp deterrents to narrower bandwidths may help lessen the effect on the bigmouth buffalo, but additional testing is needed to confirm this assumption.

Surprisingly, some native species with elevated hearing abilities (channel catfish, fathead minnow, and gizzard shad) did not alter their behavior in response to the broadband stimulus. The lack of a negative phonotaxis response seen in native ostariophysans could be due to morphological differences in the Weberian ossicles (Bird and Hernandez 2007) or a reduced swim bladder size (Lechner and Ladich 2008), which could lessen their hearing sensitivity compared to other ostariophysans. For example, gizzard shad are members of the order Clupeiformes. The inner ear of the clupeiform ear has air-filled bulla that are thought to interact with the utricles and afford sensitivity to ultrasound (Nestler et al. 1992; Mann et al. 1998). A closely related species, American shad (Alosa sapidissima), has a hearing range of 0.1–100 kHz, which suggests that gizzard shad and other native species from the family Clupeidae might be able to hear high frequency sounds (> 1000 Hz) and could be impacted by a higher frequency acoustic deterrent. Although channel catfish, fathead minnow, and gizzard shad did not show any negative phonotaxis response during this study, further field research could be conducted to ensure other behavioral changes are not occurring, especially if higher frequencies are being used.

Frequency range and playback sound pressure levels for this study were established based on published auditory evoked potential (AEP) values to ensure the acoustic stimulus was within the hearing range and above minimal sound pressure level to evoke a neurological response (Jerkø et al. 1989; Mann et al. 1998, 2007; Scholik and Yan 2001; Lovell et al. 2005;
Wysocki et al. 2009; Speares et al. 2011; Maiditsch and Ladich 2014). The SPL near the speakers remained above a detectable range of all species (Jerkø et al. 1989; Mann et al. 1998, 2007; Scholik and Yan 2001; Lovell et al. 2005; Wysocki et al. 2009; Speares et al. 2011; Maiditsch and Ladich 2014), yet native fishes (or surrogates) tested did not exhibit an avoidance response (i.e., negative phonotaxis) in response to sound. The observation that native fishes did not exhibit negative phonotaxis suggests minimal impacts to native fishes when using this broadband sound stimulus as an acoustic deterrent. However, auditory evoked potential measurement of auditory sensitivities can be an underestimation of the SPL required to evoke a behavioral response (Kastelein et al. 2008) and therefore, behavioral studies such as this one may provide a more accurate assessment of how fish respond to sound (Popper and Fay 2011; Ladich and Fay 2013; Sisneros et al. 2016).

In addition, the playback SPL in our study was below the noise conditions that have been measured in a lock chamber. Since lock chambers provide a potential site for implementing an acoustic deterrent, replicating the sound levels and gradient could provide insight to management agencies. In a lock chamber during a recreational boat passage, sound levels can increase as high as 35 dB re: 1 μPa above ambient levels and provide a 10 dB re: 1 μPa gradient across the lock chamber (Putland and Mensinger 2019b). Since noise levels in our tanks were elevated (126 dB re: 1 μPa) due to machinery contained in the building, the sound levels in the small tanks could not be raised to the same change (35 dB re: 1 μPa) observed in a lock chamber without causing hearing damage (Smith et al. 2004). However, a 10 dB re: 1 μPa gradient was achieved to match conditions in a lock chamber.

In this study, we exposed native species to an acoustic stimulus to compare their negative phonotaxis response to the consistent negative phonotaxis behavior observed for silver and bighead carp. Our results provide the first comparison of behavioral responses to a 100 hp boat motor stimulus on native and invasive species and suggest that this acoustic stimulus may be effective in targeting silver and bighead carps, while limiting effects on native species. However, the study is not without its limitations. The acoustics in a small tank do not mimic a free field environment as the tank walls and surface interfere with the sound propagation and cause distortions (Parvulescu 1967; Gray et al. 2016; Rogers et al. 2016). Fish may react more to particle motion than sound pressure levels (Popper and Hawkins 2018); however, detailed mapping of particle motion was outside the scope of this study but would ideally be measured in future studies. In the present study, both silver and bighead carp showed fewer responses in indoor tanks compared to similar studies conducted in outdoor ponds (Vetter et al. 2015, 2017). Other studies have documented a reduction in responses with other carp species when
responding to an acoustic stimulus (400 Hz) in an indoor tank compared to an outdoor pool (common carp: Sloan et al. 2013). While our findings suggest that bighead and silver carp are more likely to respond to the outboard motor acoustic stimulus than any of the other species, it is important these studies be scaled up to a more realistic setting where native species and invasive carp can be tested.

Bigheaded carps pose a danger to many naïve ecosystems, and deterrents being tested to stop their upstream movement could have negative effects on native species. Non-structural deterrents are techniques that could be used to prevent invasive carp expansion into the Great Lakes and other habitats (Noatch and Suski 2012; USACE 2014; Grippo et al. 2017). Prior research has suggested that boat motor playback can elicit negative phonotaxis response in bighead and silver carp (Vetter et al. 2015, 2017; Zielinski and Sorensen 2017) and could provide a potential non-structural deterrent (Murchy et al. 2017). Our results suggest that a 100 hp boat motor sound would have minimal negative impacts on native Mississippi River Basin fishes. As this study addressed a broad screening in small tanks, further research in a riverine setting could be conducted on species of concern, specifically ostariophysans or other species with high frequency hearing, to confirm no behavioral changes are occurring. This acoustic stimulus, produced by outboard boat motors, may be an option in managing invasive bighead and silver carp migration until more refined signals can be developed. These results should provide management agencies with additional information regarding the potential impacts to native fish in the Mississippi River, which will aid in making decisions about testing or using acoustic deterrents in the future.

Acknowledgements

We would like to thank the USGS Upper Midwest Environmental Sciences Center (UMESC) staff and interns, especially Blake Sauey and James Wamboldt for their assistance with this project, Brian Gray for statistical consulting, and Todd Johnson for reviewing an earlier draft of this manuscript. We would also like to thank Emily Cardinal for her assistance in analysis and helping to conduct trials. Lastly, we would like to thank the reviewers for their helpful feedback which improved our manuscript.

Funding declaration

Funding was provided by the Minnesota Environment and Natural Resources Trust Fund, the U.S. Geological Survey Ecosystems Mission Area Biological Threats Research Program, and the U.S. Environmental Protection Agency’s Great Lakes Restoration Initiative.

Ethics statement

All fish handling, care, and experimental procedures used were reviewed and approved by the UMESC Institutional Animal Care and Use Committee (IACUC Protocol AEH-12-PPT-AC-01).

Data availability

All data from this study are available on U.S. Geological Survey ScienceBase (Murchy et al. 2018).

Disclaimer

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.
References


Cudmore B, Mandrak NE, Dettmers JM, Chapman DC, Kolar CS (2012) Binational ecological risk assessment of bighead carp (*Hypophthalmichthys spp.*) for the Great Lakes Basin. Department of Fisheries and Oceans (Canada), Canadian Science Advisory Secretariat, pp 1499-3848


Nissen A (2018) Hearing Thresholds and the Impact of Anthropogenic Noise in Four Invasive Fishes: Silver (Hypophthalmichthys molitrix), Bighed (H. nobilis), Black (Mylopharyngodon piceus), and Grass (Ctenopharyngodon idella) Carp. MS Thesis. University of Minnesota, 65 pp


Supplementary material

The following supplementary material is available for this article:

**Figure S1.** Density plot of one of the four groups of Channel catfish (n = 10), demonstrating distribution in experimental tank.

**Figure S2.** Density plot of one of the four groups of Lake Sturgeon (n = 10), demonstrating distribution in experimental tank.

**Figure S3.** Density plot of one of the four groups of American eel (n = 10), demonstrating distribution in experimental tank.

**Figure S4.** Density plot of one of the four groups of gizzard shad (n = 20), demonstrating distribution in experimental tank.

**Figure S5.** Density plot of one of the four groups of Fathead minnow (n = 20), demonstrating distribution in experimental tank.

**Figure S6.** Density plot of one of the four groups of hybrid striped bass (n = 10), demonstrating distribution in experimental tank.

**Figure S7.** Density plot of one of the four groups of Common carp (n = 10), demonstrating distribution in experimental tank.

**Figure S8.** Density plot of one of the four groups of grass carp (n = 10), demonstrating distribution in experimental tank.

**Figure S9.** Density plot of one of the four groups of silver carp (n = 10), demonstrating distribution in experimental tank.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2022/Supplements/MBI_2022_Murchy_SupplementaryMaterial.pdf