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Research Article

Evaluation of an acoustic fish deterrent system in shallow water application at the Emiquon Preserve, Lewistown, IL

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Abstract

Expansion of non-native fish have caused ecological and economic damage and can negatively impact native fish populations. Current research on deterrent technologies for bighead *Hypophthalmichthys nobilis* and silver carp *H. molitrix* have primarily focused on reducing upstream movement in large river lock approaches. However, there is also interest in excluding carp from smaller-scale locations. A water control structure at Emiquon Preserve, Lewistown, Illinois, USA reconnected the Preserve's wetland lakes to the Illinois River, and is a pinch point that site managers seek to deter immigration of non-native fishes without restricting native fish movement. One possible deterrent strategy that was evaluated within the water control structure in 2017 was the use of a 100 hp boat motor acoustic stimulus. Two underwater speakers were installed in each of two culverts to discourage fish movement though the water control structure. Fish passage was monitored using a series of passive integrated transponder (PIT) antennas in a confined study area. A combination of 176 fish consisting of seven different species (native and non-native) were implanted with PIT tags and relocated downstream of the water control structure. Over 2 days of periodic playback of the acoustic stimulus, 29% of tagged silver carp that were detected crossed though the active underwater speaker array. The acoustic treatment did not significantly reduce silver carp or native centrarchid passage through the culverts. However, numerous silver carp were observed jumping out of the acoustically active culvert at the onset of the stimulus. The acoustic stimulus, especially the frequencies to which silver carp are most sensitive to (< 2000 Hz), rapidly attenuated in the water control structure (water depth 0.55–0.38 m). Depth related attenuation observed in and around the water control structure may have reduced the efficacy of the acoustic fish deterrent system at this location.

Key words: Silver carp, acoustic deterrent, underwater sound, Illinois River

Introduction

Bighead carp *Hypophthalmichthys nobilis* and silver carp *H. molitrix*, collectively known as bigheaded carps, are native to the rivers of eastern China and Russia (Kelly et al. 2011). Bigheaded carps filter feed on plankton and were imported to the United States in the early 1970s to

improve water quality in wastewater treatment plants and aquaculture facilities (Kolar et al. 2007). Establishment of large populations following escapements of fish between the 1970s and 1980s has had damaging effects on native fish populations (Schrank et al. 2003; Irons et al. 2007; Sampson et al. 2009; Solomon et al. 2016) and negative impacts on the ecology and economy of invaded regions (Ready et al. 2018). U.S. and Canadian natural resource agencies have focused on preventing establishment of self-sustaining populations of bigheaded carp in new locations (Conover et al. 2007). The primary management effort has been to reduce large-scale range expansion of these fish in the Illinois and Upper Mississippi River systems. Deterrent technologies that are being developed to reduce upstream movement through large river navigation lock approaches may have utility at smaller-scale locations where carp exclusion is also of interest. One possible deterrent strategy currently under investigation is the use of an underwater acoustic deterrent.

Bigheaded carps' auditory system, consisting of a swim bladder coupled to the inner ear by Weberian ossicles, has led researchers to evaluate the use of acoustic stimuli as a potential technique for altering movement and behavior. As ostariophysians, bigheaded carp have increased hearing range (up to 5000 Hz) and sensitivity (Lovell et al. 2006; Vetter et al. 2018) compared to many native gamefish species where acoustic deterrent technologies may be implemented. Additionally, some evidence indicates that grass carp *Ctenopharyngodon idella* and common carp *Cyprinus carpio*, to a lesser extent, may have a similar response to an acoustic stimulus (Willis et al. 2002). However, concern over fish passage and watershed connectivity in large rivers (Roscoe and Hinch 2010; Tripp et al. 2014) also warrants being addressed, and installation of any barrier or general fish deterrent needs to consider the perspectives and needs of many stakeholders. Current development of an acoustic deterrent for bigheaded carp has shown promising results for a species-specific solution to manipulating movement and behavior.

A higher frequency, broadband boat motor sound (60–24,000 Hz) has proven more effective in modifying swimming behavior of bighead (Vetter et al. 2017a) and silver carps (Vetter et al. 2015) compared with pure tones (500 Hz, 1000 Hz, 1500 Hz, and 2000 Hz). The broadband boat motor sound (hereinafter referred to as "acoustic stimulus") is described in Vetter et al. (2015). It was demonstrated that this acoustic stimulus was > 90% effective at deterring bigheaded carps egress through a constructed channel in concrete research ponds (Murchy et al. 2017). Little research has focused on the use of this acoustic stimulus as a deterrent for bigheaded carps in small, shallow water locations where carp exclusion is desired because navigation locks have been prioritized as locations where deterrence strategies would be most effective at reducing range expansion (Upper Mississippi River Asian Carp Partnership 2018; Conover et al. 2007).

The Nature Conservancy (TNC) acquired Emiquon Preserve in 2000 with the goal of restoring its wetlands and lakes by reconnecting the Preserve to the Illinois River near Lewistown, IL. In 2016 a newly constructed water control structure (WCS) became fully operational and effectively reconnected the historical floodplain back to the river. Water levels of lakes and wetlands in the Preserve can be regulated using the WCS. However, this reconnection has raised concerns over the potential for facilitating establishment of invasive fish species within the Preserve (Lemke et al. 2017; Sparks et al. 2016). Populations of bigheaded carps within the Illinois River have seen exponential population growth for decades (Tsehaye et al. 2013) and the river section near Emiquon Preserve has one of the most dense populations of silver carp in the world (Sass et al. 2010). Although bigheaded carp have been detected in Emiquon Preserve since 2013, abundance continues to be low with no reproduction or recruitment detected (Hagen et al. 2017). To prevent movement of non-native species into Emiquon, TNC installed a series of removable metal screens (3.8 × 1.9 cm mesh) in the WCS's two concrete culverts. However, the screens require daily cleaning of vegetation, trapped fish/wildlife, and other debris, which has led site managers to seek an alternative method of carp exclusion that would also not impede native fish passage.

The goal of this study was to investigate the efficacy of an acoustic deterrent system for bigheaded carp deployed in the WCS at Emiquon Preserve in Lewistown, IL. Our first objective was to determine if the acoustic stimulus would alter passage rates of fish species present near the WCS, specifically, bigheaded carp and native sport fish. Our second objective was to describe and map the acoustic properties in and around the WCS during operation of the acoustic fish deterrent system using a previously tested stimulus. The Emiquon Preserve served as a unique location to assess our ability to propagate sound under dynamic field conditions and test an acoustic stimulus, proven to deter bigheaded carp under laboratory conditions, on wild fish in a semi-controlled, shallow-water environment.

Materials and methods

Preliminary data

A preliminary assessment was conducted in November 2016, during which, the underwater speaker array was tested, sound was recorded in and around the WCS, and fish sampled for species composition. Water depth within the culverts was 1.8 m, and two LL916C underwater speakers (200–23,000 Hz frequency response; Lubell Labs Inc., Whitehall, OH) were positioned at midwater depth, off the bottom in each culvert. Underwater recordings of the acoustic stimulus described below were recorded using a Sound Trap 300 STD hydrophone (Ocean Instruments, Auckland, New

Figure 1. Water control structure connecting Illinois River (bottom left) to the Emiquon Preserve (top right) in Lewistown, Illinois. Two identical concrete culverts (37 m long × 2.4 m wide × 2.1 m high; north culvert on the right) allow water through the levee and are controlled by sluice gates and stop logs on either side of the structure. Fish block fence (×) was temporarily installed for duration of study.

Zealand). Fish were collected using a modified fyke net designed specifically for use in the WCS, which allowed for the cod-end to be positioned upstream of the mouth to collect fish moving from the river into the Preserve against the flow. These data were used to design the 2017 study and compare sound attenuation between years.

Study site

With the exception of major floods that overtop sections of the levee, the WCS serves as the only surface-water connection between the Emiquon Preserve and Illinois River and is located 3 km north of Havana, IL (40°20.066′N; 90°3.165′W). It is owned by TNC and is managed by the Thompson Drainage and Levee District. Water level of the lakes and wetlands is regulated by the WCS through two identical 2.1- × 2.4-m concrete culverts with sluice gates and stop logs on either side of the structure (Figure 1; Cupp et al. 2018). The WCS is used to restore natural seasonal variation in hydrology and ecological process within the Preserve through water level management between the Preserve and the Illinois River (Lemke et al. 2017). During passive draining, water depth within the culverts can be between < 0.1–2.4 m. IQ Plus flow meters (SonTek, San Diego, CA) were installed in 2017 to monitor discharge continuously from each culvert.

Two LL916C underwater speakers were installed at midwater depth, 0.3 m off the bottom of each culvert and 3 m from the downstream opening facing the Illinois River (Figure 2). Speaker output was adjusted to 150–155 dB re

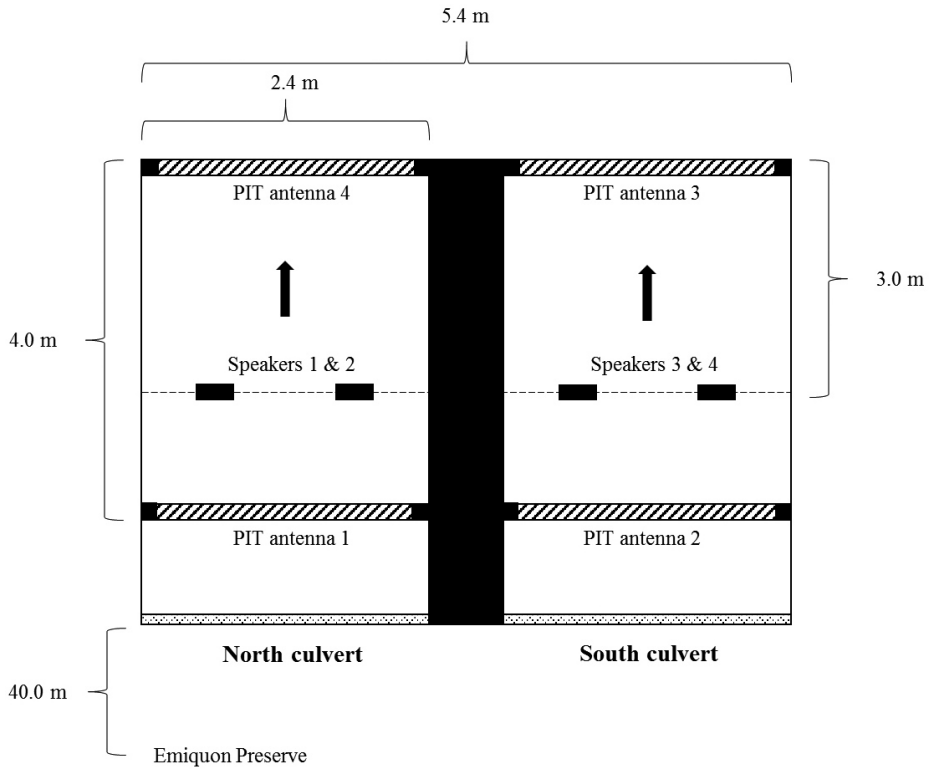

Figure 2. Overhead perspective of PIT antenna and speaker locations in the north and south culverts of the water control structure located at Emiquon Preserve. Arrows indicate direction of water flow towards the Illinois River.

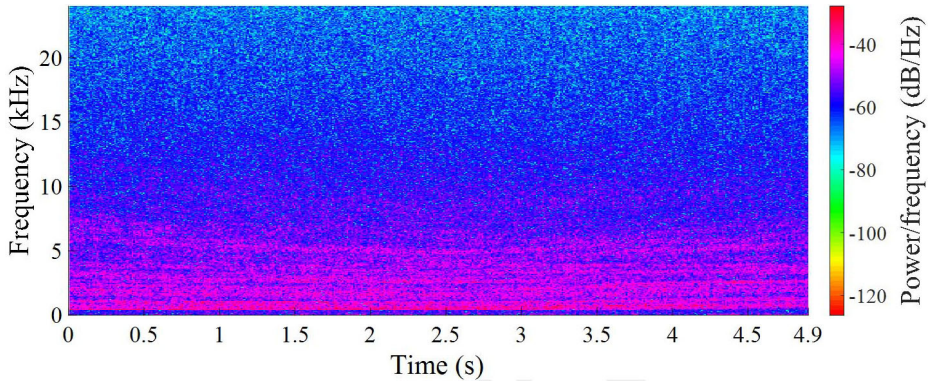

Figure 3. Spectrogram of a 5-s audio clip taken at the peak signal amplitude of the original 30-s boat-motor recording used in previous studies (Vetter et al. 2015, 2017a; Murchy et al. 2017). Spectrogram was generated using MATLAB R2017B using a 1024-point Fourier transformation and Hamming window with a sampling rate of 24,000 Hz.

1 μPa, 1 m from each speaker and controlled with a Peavey PVi4B amplifier (Peavey Electornics, Meridian, MS) connected to a computer equipped with iTunes™ for audio playback. The acoustic stimulus used during active treatments was a 6-h .wav file that looped a 5 second clip of an outboard boat motor recording used in previous research (Vetter et al. 2015, 2017a; Murchy et al. 2017). The audio clip was taken at the peak signal amplitude of the original boat motor recording (Figure 3), tested prior to this study on silver carp in a 69 × 292 × 30-cm (735 L) flow-through tank, and demonstrated to evoke a negative phonotaxis during preliminary testing.

On either side of the underwater speaker array, two pass-through passive integrated transponder (PIT) antennas (2.6 m high × 1.8 m wide) were installed 4 m apart in each culvert (Figure 2). All four PIT antennas were insulated from the concrete by 12.7 cm of treated lumber and monitored with an IS1001 Master Controller (Biomark, Boise, ID). Antenna status was continuously monitored though BioStat with virtual test tags fired hourly to each node. Tag detection distance was < 1 m. The *Unique Delay* function on the master control was set to 1 minute for detecting duplicate tag IDs to reduce repeated detections of an individual fish and minimize tag collisions. Detection range of antennas did not overlap, and a fish could only be detected by one antenna at a time. To prevent fish escapement to the Illinois River, a 1.2 m high welded wire block fence (2.5 × 2.5 cm mesh) was installed across the channel, 30 m downstream of the culvert openings (Figure 1). Fencing extended roughly 0.75 m above the water's surface to block jumping fish. To eliminate the possibility of test fish from entering Emiquon Preserve, two sets of metal screens were installed in each culvert on the far upstream side of the WCS, closest to Emiquon.

Study design

In 2017, a combination of native and invasive fish species was collected from the Illinois River and near the WCS, inside the Preserve, using pulsed-DC electrofishing boats. Minimum targeted number of each species was 30 adult individuals. Invasive fish species targeted for collection were bighead, silver, and grass carps. Native fish species targeted were those known to pass though the WCS from sampling efforts in 2016 and included black crappie *Pomoxis nigromaculatus*, largemouth bass *Micropterus salmoide*, bluegill *Lepomis macrochirus*, buffalo *Ictiobus* spp., gizzard shad *Dorosoma cepedianum*, and white bass *Morone chrysops*. Total length (TL) and weight of each fish was recorded prior to inserting a uniquely coded Biomark HPT-12 PIT tag (12 mm) into the abdominal cavity as described by Prentice et al. (1990). A total of 176 fish consisting of 7 species were tagged and released in the pool below the WCS on the river side of the Preserve. No bighead carp were collected. Fish were immediately released into the pool below the south culvert, along the edge of the river side of the WCS. This location was chosen because an eddy in the current allowed refuge during recovery from handling. Metal screens were temporarily installed in the downstream entrance of each culvert to prevent fish entering while collecting and tagging continued. Resident, non-tagged fish were observed in the study area and included bluegill, largemouth bass, gizzard shad, silver carp, and white bass. Dead fish were removed from the block fence and checked for PIT tags following study completion. Trapped, tagged fish were removed from study sample size.

Table 1. Treatment schedule for acoustic stimulus exposure in the water control structure at the Emiquon Preserve Lewistown, Illinois.

Date	Time	Stimulus	Culvert
9/12/2017	00:15	Off	
9/13/2017	6:15	On	south
9/13/2017	12:15	Off	
9/13/2017	18:15	On	south
9/14/2017	23:15	Off	
9/14/2017	12:15	On	north
9/14/2017	18:15	Off	
9/15/2017	23:15	On*	north
9/15/2017	6:15	End	

* Scheduled acoustic stimulus exposure failed due to an equipment malfunction. Study ended at 00:15 on 15 September 2017 due to this malfunction.

The study was designed to include a 6-h acclimation period and a 6-h pre-sound treatment (12 h total), followed by two 24-h treatment periods that incorporated dawn, dusk, day, and night hours equally between days (Table 1). Treatments included active (acoustic stimulus on) and inactive (acoustic stimulus off) periods in 6-h time blocks. During active treatments, the acoustic stimulus was projected exclusively in one culvert. After the initial 24 hours, the acoustic stimulus was switched from the south culvert to the north culvert and treatments were repeated. Block screens located in the entrance of the culverts were removed from each culvert at 18:15 on 12 September 2017 to initiate the acclimation period. The first exposure to the acoustic stimulus began at 06:15 on 13 September 2017 in the south culvert following a pre-programed automated schedule (Table 1). A malfunction failed to initiate the final acoustic exposure on the last day, and that treatment was never completed. The study ended at 00:15 on 15 September 2017.

Signal detection of an individual fish on any of the four antennas in the two culverts constituted a detection within the culverts. Sequential detection of an individual on the corresponding antenna at the opposite end of culvert was considered a successful culvert crossing. We did not distinguish direction of culvert crossings. Fish that crossed though an active culvert during an acoustic treatment period were considered undeterred by the acoustic stimulus. Individual fish movements were not restricted within the study area. As such, individual fish could account for multiple crossings in one or both culverts during each time block. Raw detection data were summarized by (1) total number of individuals detected within each culvert (2) number of successful crossings observed for each culvert and (3) the proportion of individuals present in each culvert that successfully crossed. Fish had to be detected at least once during the 2-day study to be included for analysis. Analysis of the total number of detections within a time-block and whether a detection event resulted in a crossing were analyzed using generalized linear mixed effect regression models by means of the lme4 package in R (version 3.4.2; Bates et al. 2015). Each species was analyzed separately. Analysis of the total number of detections

included sound (on/off), and time of day (day/night) as fixed effects and tag code as a random effect with Poisson error terms. Whether a detection resulted in a crossing was analyzed with models similar to the total detection models except they were modeled as logistic regressions. Because the sound from the speaker arrays in an active culvert was also present in the adjacent inactive culvert we counted all crossings that occurred in either culvert.

Acoustic Analyses

Following the completion of the study, underwater sound was recorded during stimulus playback and ambient conditions using a Sound Trap 300 STD hydrophone at midwater depth from 37 locations on a gridded plane within the culverts and in the downstream pool. Sound was projected from underwater speakers one culvert at a time (north or south) at each sampling location. Maximum water depth was roughly 0.75 m and recordings were not collected unless the hydrophone was completely submerged. Additional downstream recordings were collected at the center of the channel to determine the distance the acoustic stimulus could be detected over ambient conditions. The hydrophone was calibrated using a 42AA pistonphone (G.R.A.S. Sound and Vibration, Denmark) prior to data collection.

Underwater recordings were clipped to 4 s fragments to include only the acoustic stimulus and processed using MATLAB R2017B (MathWorks, Inc., Natick, MA). Sound was expressed for each sampling location as the root mean square (rms) sound pressure level (SPL) between 20–5000 Hz for each clip (dB re 1 μPa)

$$SPL_{rms} = 20 \times log_{10}\left[\frac{\hat{p}}{p_0}\right]$$

where $\hat{p}$ is the root mean square sound pressure and p_0 is the reference value of the sound pressure (1 μPa). Although the acoustic stimulus included frequencies above 5000 Hz, for this analysis we focused on the known hearing range of bigheaded carp (Lovell et al. 2006; Vetter et al. 2018). Sound maps were generated from SPL_{rms} in SigmaPlot 12.0 (Systat Software Inc.) using the contour map function. Dominant frequencies at each sampling location were determined using a Welch power spectral density estimate in Matlab R2017B. Sound attenuation was described by comparing the frequency content of the projected signal (< 1.0 m from speaker) to that collected at regular intervals (0.75 m, 2.8 m, 7.5 m, and 11.3 m) from the speaker using the magnitude-squared coherence function in Matlab R2017B. Power spectral density and magnitude-squared coherence estimates were calculated using a 1024-point Fourier transformation and Hamming window at a sampling rate of 24,000 Hz for each sampling location. Squared coherence estimates compare the frequency band similarities between two signals, such that a coherence of 1 indicates the two signals are perfectly in

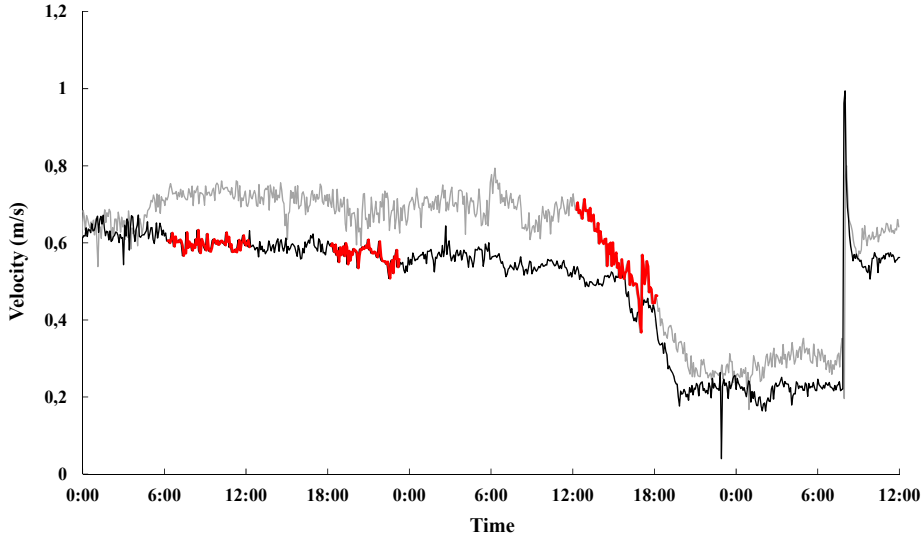

Figure 4. Water velocity (m/s) passing through the north (grey) and south (black) culverts of the water control structure connecting the Illinois River to Emiquon Preserve, Lewison, Illinois from 9/13/2017 to 9/15/2017. Red portions indicate acoustically active culvert.

phase and 0 indicates they are incoherent. A coherence significance test was conducted to express the 1% critical values following the methods in Biltoft and Pardyjak (2009).

Results

The WCS was in the second year of operation in 2017, and it passively drained 11.9 mil m^3 of water during 50 days of operation between 14 August 2017 and 5 October 2017 (Hagen et al. 2018). Initial water depth on 12 September 2017 in both culverts was 0.55 m and decreased to 0.38 m by study completion (15 September 2017). During the trial period, 136,583 m^3 of water passed through the WCS. Mean (± SD) water temperature was 21.3 (1.1) °C. Mean (± SD) water velocity was 0.54 (0.18) m/s and 0.44 (0.16) m/s in the north and sound culverts respectively. Mean water velocity in the north culvert was significantly greater than in the south culvert (ANOVA; $F = 2516$, $p < 0.05$). As vegetation built up on upstream screens in the WCS, water velocity gradually dropped in both culverts at similar rates, until debris was cleared following the final exposure period (Figure 4). Water depth during the 2016 preliminary assessment was 1.8 m. Flow meters were installed after the preliminary assessment.

Two silver carp out of 68 tagged individuals were found dead in the study arena prior to the initial acoustic exposure and were removed from the study. No other silver carp mortality was observed. Only three fish species had enough individuals detected from the total tagged population for analysis (Table 2): bluegill (n = 25; 83%), largemouth bass (n = 26; 87%), and silver carp (n = 28; 42%). One tagged white bass was detected but its movement was not quantified. Of the three species analyzed, there were no significant differences within species between mean TL and weight

Table 2. Mean (± SD) length (mm) and weight (g) of fish released for acoustic deterrent trials in pool on the river side of the water control structure (WCS) at Emiquon Preserve, Lewistown, Illinois. Bluegill (BLG) and largemouth bass (LMB) were collected near the WCS from inside Emiquon Preserve. Gizzard shad (GIZ), grass carp (GRC), smallmouth buffalo (SMB), silver carp (SVC), and white bass (WHB) were collected form the Illinois River, downstream of the WCS. All fish were collected using pulsed-DC electrofishing boats on 9/12/2017.

Species	Length	Weight	No. tagged	No. Detected	Total detections
BLG	188 (19)	156 (57)	30	25	1,586
LMB	400 (74)	3,438 (43)	30	26	668
GIZ	187 (42)	–	15	0	0
GRC	700 (6)	1,078 (324)	2	0	0
SMB	285	214	1	0	0
SVC	521 (84)	1,466 (681)	66	28	436
WHB	173 (28)	75 (57)	30	1	19

of detected individuals when compared to the total number tagged (independent t-test; all $p < 0.05$). High gizzard shad and white bass mortality was observed impinged against the block fence after study completion, but no tagged individuals were recovered. Given the small fish size and swift current, it was assumed that the downstream block fence was the cause of high mortality.

All virtual test tags were detected for each of the four PIT antenna nodes when fired. Three antennas had relatively low noise throughout the study, and in all instances of high noise, node activity returned to normal in less than one minute. Mean (± SD) number of high noise alarms for those three antennas was 26.3 (6.6) throughout the duration of the study. However, antenna 4 had 338 total high noise alarms. Most of high noise alarms for antenna 4 also returned to normal activity in ≤ 1 min. However, during 6 instances, normal node activity of antenna 4 did not return for 4–10 min. During the 2-day trial, there was a total of 42 min in which fish may not have been detected if crossing through antenna 4. Because this antenna was downstream of the acoustic stimulus, a crossing would only have been missed if there was a simultaneous tag collision at the antenna upstream of the speaker array while a non-detected tag crossed antenna 4. Only 18 tag detections were out of sequential antenna series from a total of 2,891 tag detections, indicating that tag collisions were rare. Most tag collisions were caused by a nonmobile bluegill near an antenna for extended periods of time.

Fish Movement

Not all fish detected by a PIT antenna at the entrance of a culvert were subsequently detected by the upstream antenna. Given the 1-minute delay setting for the detection of duplicate tag IDs at a PIT antenna, a single detection could indicate that a fish swam past the entrance of a culvert or entered the culvert and turned around. Additionally, many individuals (primarily largemouth bass and silver carp) were detected by both PIT antennas in each culvert during a given time block. These incidences allowed for an individual fish to be counted in both culverts and potentially be included as a crossing in one or both culverts.

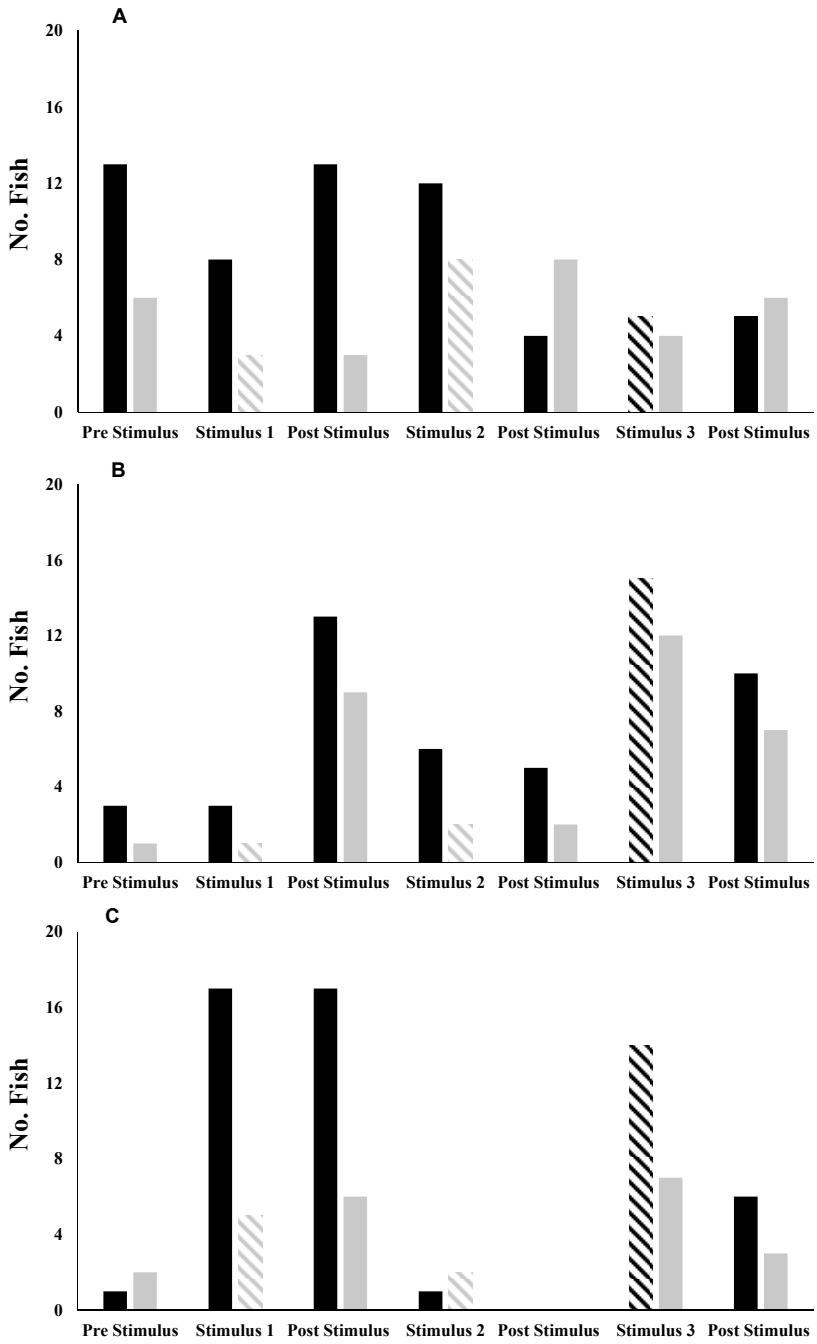

Figure 5. Number of (a) silver carp, (b) bluegill, and (c) largemouth bass detected in the north (black) and south (grey) culverts of the water control structure located at Emiquon Preserve, Lewistown, Illinois during 6-h treatment blocks. Broken bars indicate acoustically active culvert.

Prior to the initial acoustic exposure, 16 silver carp were detected in the culverts during the 6 h (pre-stimulus) time block (Figure 5). Those fish crossed through (both directions) the speaker array a total 24 (n = 12) times in the north culvert and 7 (n = 4) times in the south culvert (n = number of individual fish). The proportion of detected silver carp that crossed though the inactive speaker arrays was 46% (north) and 11% (south) throughout this pre-exposure period. During this time, 4 bluegill and 3 largemouth bass were detected, none of which crossed through either speaker array.

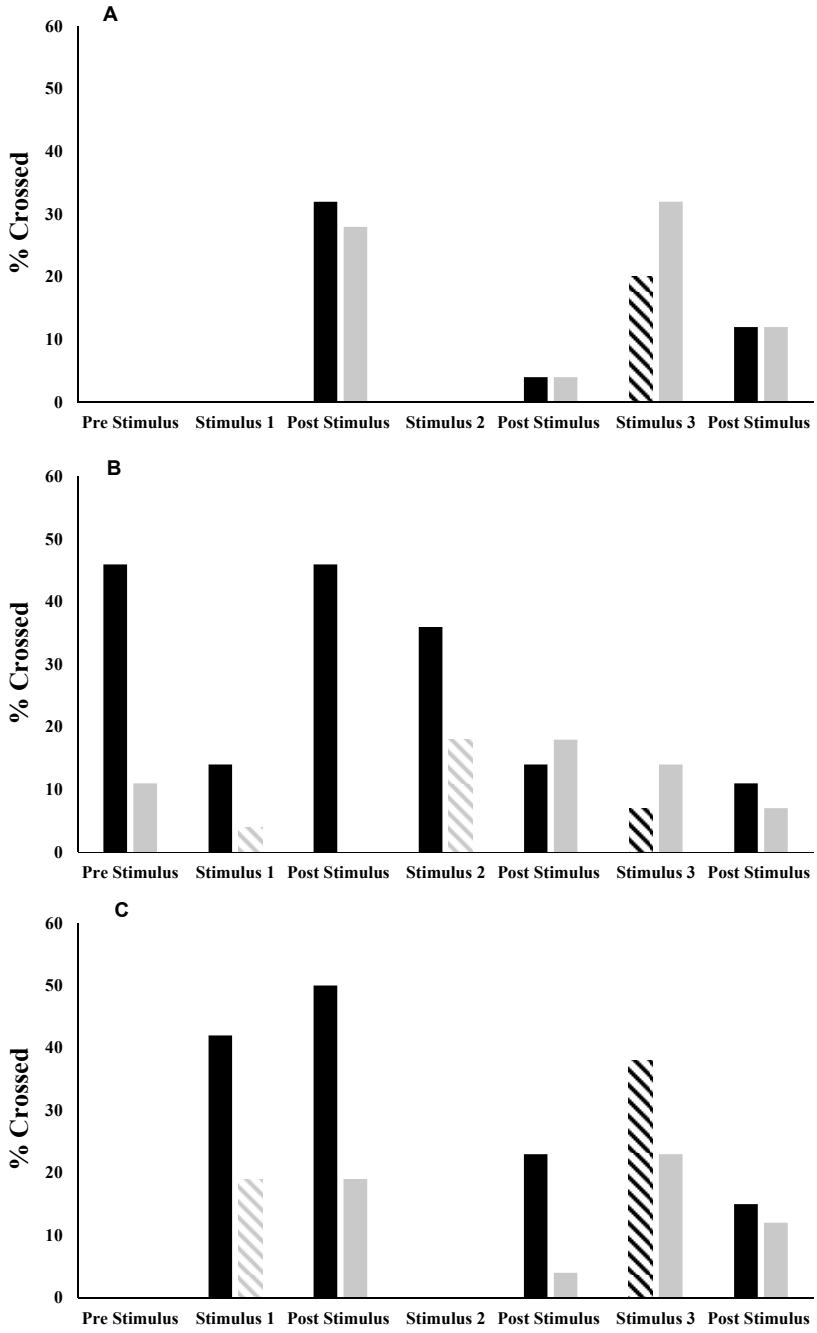

Figure 6. Proportion of detected (a) silver carp, (b) bluegill, and (c) largemouth bass that crossed through the speaker array in the north (black) and south (grey) culverts of the water control structure located at Emiquon Preserve, Lewistown, Illinois during 6-h treatment blocks. Broken bars indicate acoustically active culvert.

During the first acoustic exposure period (south culvert active), 9 silver carp were detected in the culverts. Those fish crossed through the speaker array a total of 12 (n = 5) times in the north (inactive) culvert and 3 (n = 2) times in the south (active) culvert. The proportion of PIT-tagged silver carp detected during a given timepoint that also crossed through the speaker array (n crossed / n detected) from the river side was 14% (inactive) and 4% (active; Figure 6). One silver carp entered the active culvert 305 min after the onset of the first acoustic exposure and subsequently swam back

out (2 crossings). The second silver carp that crossed through the active speaker array exited Emiquon Preserve after the onset of the acoustic stimulus. During this time, 4 bluegill and 18 largemouth bass were detected, with 0 (n = 0) and 8 (n = 5) crossings respectively though the active speaker array.

During the second acoustic exposure period (south culvert active), 12 silver carp were detected in the culverts. Those fish crossed through the speaker array 29 (n = 12) times in the north (inactive) culvert and 6 (n = 5) times in the south (active) culvert. The proportion of PIT-tagged silver carp that crossed through the speaker array from the river side was 43% (inactive) and 18% (active). All of the silver carp that crossed through the active culvert did so between 195–305 min after the onset of the acoustic stimulus. While the south culvert was active, 11 bluegill and 3 largemouth bass were detected, none of which crossed though the active speaker array.

During the final acoustic exposure period (north culvert active), 7 silver carp were detected in the culverts. Those fish crossed through the speaker array a total 3 (n = 3) times in the north (active) culvert and 5 (n = 4) times in the south (inactive) culvert. The proportion of PIT-tagged silver carp that crossed through the speaker array from the river side was 7% (active) and 14% (inactive). The two silver carp that crossed through the active culvert did so between 117–260 min after the onset of acoustic stimulus. During this final exposure period, 21 bluegill were detected, with 10 (n = 7) crossings in the active culvert and 20 (n = 11) crossings in the inactive culvert. Similarly, 18 largemouth bass were detected, with 67 (n = 14) crossings in the active culvert and 26 (n = 7) crossings in the inactive culvert.

Time of day and active acoustic treatments both affected the total number of bluegill detections ($z = -13.48$; $p < 0.001$ and $z = -8.36$; $p < 0.001$ respectively), while only time of day affected the total number of largemouth bass and silver carp detections ($z = 6.32$; $p < 0.001$ and $z = -5.94$; $p < 0.001$ respectively). Daytime positively influenced whether bluegill crossed the culverts ($z = 3.78$; $p < 0.001$). Largemouth bass crossing probability was positively influenced by the sound being on ($z = 3.72$; $p < 0.001$), with no significant effect of time of day or sound treatment on silver carp crossing in either culvert ($p > 0.05$). Throughout the full duration of the study, 8 individual silver carp (29%) crossed through an active speaker array; only 1 of which crossed through during multiple active treatment periods. Conversely, 22 silver carp (79%) crossed though an inactive culvert while the acoustic stimulus was being projected from the neighboring culvert. All silver carp that were detected crossed though one of the culverts at any given time at least once throughout the duration of the study.

Observations

Shortly after the onset of the first acoustic exposure, three silver carp were observed jumping around the downstream block fence. No fish were observed

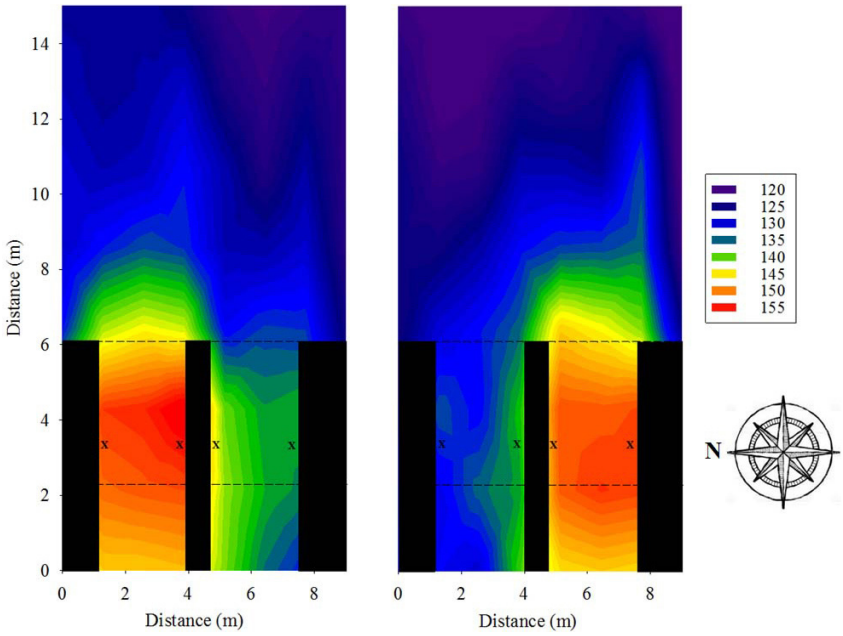

Figure 7. Overhead perspective of the water control structure located at Emiquon Preserve, with the Illinois River 50 m to the east and the Preserve 40 m to the west. Color gradient displays mean sound pressure level (dB re 1 µPa) while underwater speakers (X) were active in the north (left) and south (right) culverts. Sound pressure level was calculated in MATLAB R2017B from a 5-s playback of the boat motor acoustic stimulus described previously (Vetter et al. 2015, 2017a; Murchy et al. 2017) and filtered 20–5000 Hz. Fish movement was monitored using four PIT tag antennas (dashed lines) installed on either side of the speaker arrays.

jumping prior to that period. Escapement from the study area was possible if fish breached the water, rebounded off the shoreline and landed back in the river, downstream of the block fence. However, no fish were observed escaping the study area following the initial onset of the first acoustic exposure period. Eleven seconds after the onset of the second exposure period (south culvert active), 32 silver carp were filmed jumping out of the south culvert (Wamboldt et al. 2018). Similarly, 2 silver carp were filmed jumping out of the active north culvert at the onset of the third exposure period (north culvert active). PIT antennas did not detect silver carp in the culverts during either of these times. Throughout the duration of the third acoustic exposure, a school of 25–30 silver carp were photographed downstream of the south culvert in a zone of relatively low SPL (125–130 dB re 1 µPa). No fish were observed jumping in an inactive culvert during any period. Using seven still-frame photos taken from the video collected during the second acoustic exposure and a measured reference point, mean (± SD) fish TL was estimated to be 347 (96) mm. Surveillance cameras were not in use during the study; all observational fish behavior was made by researchers onsite during daylight hours.

Acoustic Stimulus

Although the concrete wall (66 cm thick) between the two culverts blocked some of the acoustic signal from the neighboring culvert, the stimulus was still detectable in the inactive culvert (Figure 7). Maximum SPL within the

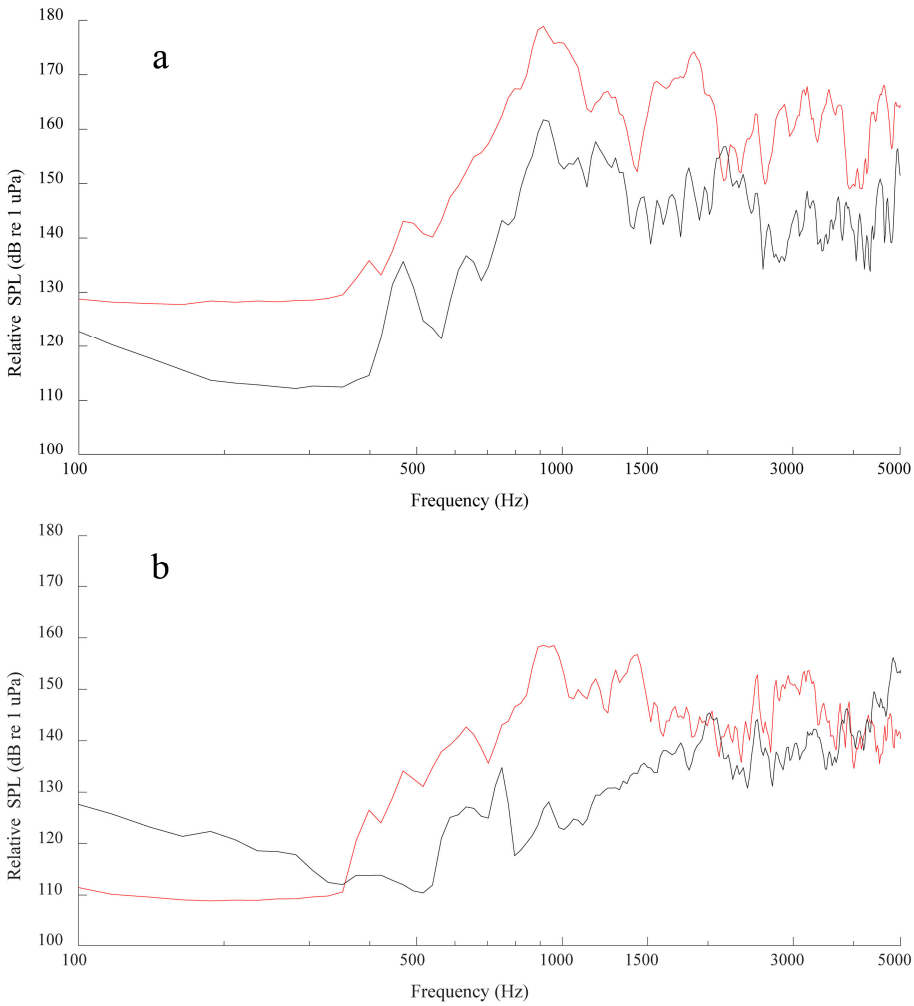

Figure 8. (a) Power spectrum of the boat motor acoustic stimulus projected from the north culvert and collected at the center of the north (red) and south (black) culverts of the water control structure located at Emiquon Preserve, Lewistown, IL. Each collection point was 0.75 m from the center of the speaker array in respective culvert. (b) Power spectrum of the boat motor acoustic stimulus projected from the north culvert collected 7.5 m downstream of the active culvert in 2016 (red) and 2017(black). Analysis was conducted in MATLAB R2017B using a 1024-point Fourier transformation and Hamming window at a sampling rate of 24,000 Hz.

active and inactive culverts were 159 and 141 dB re 1 μPa, respectively. Most of the inactive culvert remained below 135 dB re 1 μPa, with a minimum SPL of 132 dB re 1 μPa when speakers in the adjoining culvert were active. The signal detected in the inactive culvert had a similar frequency composition to that of the signal in the active culvert, but with a reduced relative SPL (Figure 8a). Ambient SPL within both culverts ranged from 92–108 dB re 1 μPa. Within the pool downstream of the WCS, SPLs ranged from 124–139 dB re 1 μPa when speakers were active. Ambient SPL in the pool ranged from 86–113 dB re 1 μPa. Ambient conditions masked the projected signal in the downstream pool between 9–20 m from the speakers.

Frequency composition of the acoustic stimulus changed with distance from active speakers (Figure 9). The acoustic stimulus at 0.75 m from the active speakers was characterized as a broad peak from 500–1500 Hz, with the greatest amplitude at a frequency of 960 Hz, and a second smaller peak

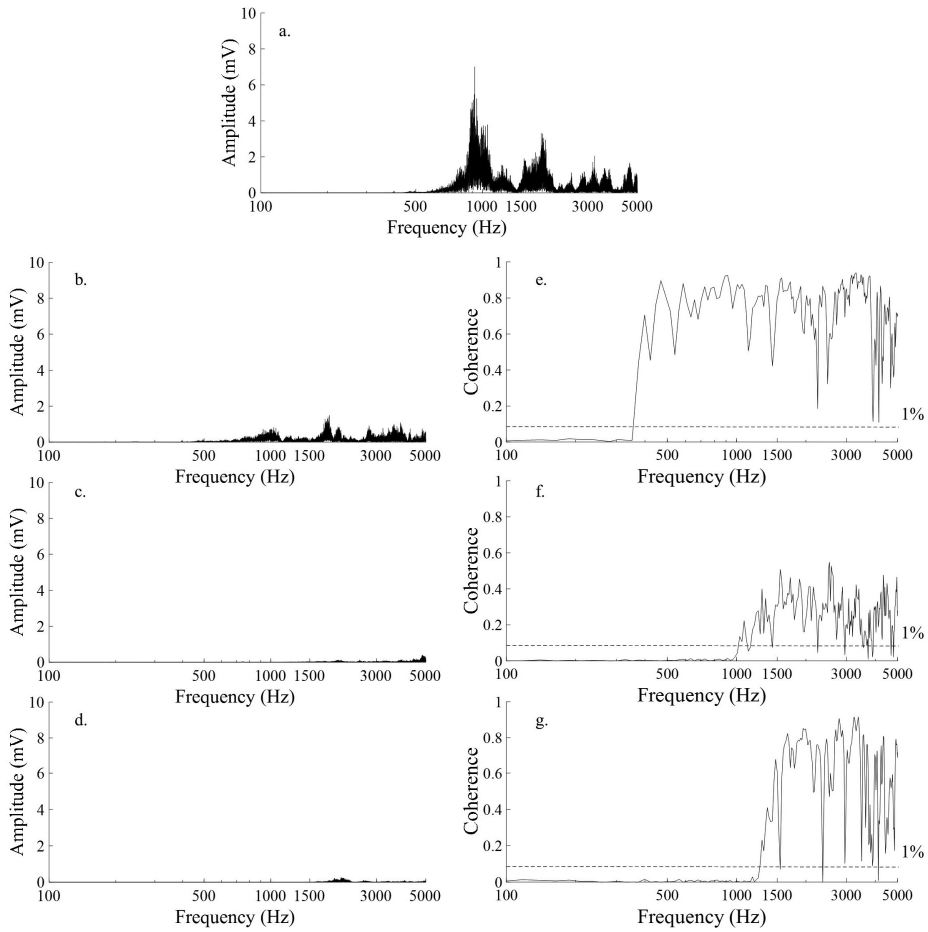

Figure 9. Fast Fourier transformation of the boat motor acoustic stimulus (a) 0.75 m, (b) 2.8 m, (c) 7.5 m, and (d) 11.6 m from active speaker array in the north culvert of the water control structure located at Emiquon Preserve, Lewistown, IL. Magnitude squared coherence (e, f, and g) of the acoustic stimulus (a) compared to each sampling location (b, c, and d). Dashed lines indicate the 1% critical value of coherence (Biltoft and Pardyjak 2009). Analysis was conducted in MATLAB R2017B using a 1024-point Fourier transformation and Hamming window at a sampling rate of 24,000 Hz.

from 1700–2500 Hz. Downstream of the culvert, lower frequencies (100–1500 Hz) attenuated rapidly. Frequencies between 400 and 1500 Hz at 7.5 m compared to 0.75 m downstream of the active speaker had a mean (± SD) percent reduction of −53.5 (15.8)%; with a maximum percent reduction of −82.3% at 850 Hz. Frequencies above 1500 Hz had a mean (± SD) percent reduction of −23.0 (7.4)% and a maximum percent reduction of −38.4% at 3090 Hz. Shallower water in 2017 attenuated the signal more rapid as it projected from the speakers than in 2016. Frequency composition of the acoustic stimulus was different (Figure 8b) at similar sampling distances from active speakers between sampling years, due to water depth. The spectral density curve of the acoustic stimulus at 7.5 m during high water conditions in 2016 more resembled that of the close range (0.75 m) curve from 2017 (Figure 8). Additionally, lower frequencies (300–1800 Hz) were much less prevalent in 2017 from roughly the same collection points in high water conditions.

Magnitude squared coherence tests confirmed a significant loss in lower frequencies (< 1300 Hz) with increased distance from the speaker array (Figure 9). At 2.8 m from the speaker, frequencies less than 375 Hz were below the 1% critical value (n = 112,640; nfft = 1024; ε = 110; $F_{0.95}$ = 3.09; $F_{0.99}$ = 4.82; $X_{0.05}$ = 0.054; $X_{0.01}$ = 0.082), indicating strong incoherence. Frequencies under the 1% critical value expanded to everything less than 1000 Hz at a distance of 7.5 m and all frequencies lower than 1300 Hz at 11.6 m from the speaker array. Rejecting the null hypothesis at the 1% critical value allows a 1/100 chance that a coherence peak being > 0 was random chance (Biltoft and Pardyjak 2009).

Discussion

Unlike other studies that have investigated the response of bigheaded carp to an acoustic stimulus, this experiment was designed to represent a practical, shallow-water application that exposed wild fish for relatively long periods of time. All prior study designs exposed bigheaded carp to an acoustic stimulus for short durations and activated the stimulus as fish approached the nearfield range of a speaker—requiring real-time fish observation and stimulus activation that is not available during field testing (Vetter et al. 2015, 2017a; Murchy et al. 2017; Zielinski and Sorensen 2017). Previous work has shown negative phonotaxis to an acoustic stimulus, but there has been little work published on long-term efficacy. In this study we documented a strong initial response to the onset of the acoustic stimulus as fish jumped away from activated speakers but found no evidence of long-term effectiveness. It is unclear whether the lack of response seen in the WCS was caused by local conditions, habituation as previously suggested (Neo et al. 2015; Vetter et al. 2015, 2017a; Murchy et al. 2017; Zielinski and Sorensen 2017), or differences in study designs between a real-world application and a laboratory setting in which the acoustic stimulus could be activated as fish approached an underwater speaker. Further research is needed to determine how long a negative phonotaxic response can be elicited on wild fish in a practical application.

At the onset of the second acoustic exposure, 32 silver carp were filmed jumping out of the active culvert (Wamboldt et al. 2018), but no fish detections occurred from either PIT antenna in that culvert, and tag collisions were also not observed. It is possible that some of the fish observed jumping out of the culvert were resident, non-tagged individuals because dead silver carp that did not have a PIT tag or any signs of a puncture wound were collected from block fence. However, Aymes and Rives (2009) demonstrated that the greatest detection error was associated with fast swim speeds (1.35–2.55 m/s) and tags passing through an antenna at a parallel orientation. Silver carp have been documented swimming at speeds greater than 3 m/s (Hoover et al. 2017), and airborne fish would

likely not be optimally orientated to PIT antennas. To our knowledge, this is the first documented occurrence of numerous silver carp jumping away from playback of the boat motor acoustic stimulus in the wild, and its utility for moving fish out of an area for very short periods of time warrants further investigation.

The strong behavioral response documented at onset of the acoustic stimulus was similar, but more dramatic, than previously seen in the laboratory and outdoor pond setting (Vetter et al. 2015, 2017a; Murchy et al. 2017). Furthermore, this response indicates that the jumping behavior can be elicited with sound alone and turbulence from a boat wake is not necessary (Vetter et al. 2017b). Although the acoustic system was not a barrier to silver carp movement though the culverts, fewer detections and crossings occurred in the active culvert compared to the inactive culvert. Observation of a large school of silver carp located in areas of relatively low SPL (< 130 dB re 1 μPa) and greatest attenuation of lower frequencies indicates a preference for the quietest regions, likely due to their sensitivity to frequencies below 2000 Hz (Vetter et al. 2018). Sound attenuation during sound mapping under high water conditions (1.8 m) in 2016 was substantially less, especially at lower frequencies (< 1500 Hz) compared to the attenuation observed contingent with fish testing in 2017. Had water been deeper, it is likely that attenuation of the acoustic stimulus would have been less dramatic; possibly increasing the effective range of the deterrent in 2017. Unlike large-scale applications, fish in this experiment did not have access to quiet ambient locations, which may have increased the likelihood of crossing the speaker array in search of refuge.

Diel cycles appeared to affect bluegill and largemouth bass behavior more than acoustic treatments (Baumann and Kitchell 1974; Cooke et al. 2002; Reebs 2002). Peak crossings of both species occurred between 12:15–18:15 on both days (post stimulus 1 and stimulus 3 time periods) with the fewest crossings detected after 18:00. No bluegill were documented crossing through either culvert between 5:00–12:00 either day. Furthermore, time blocks that had the least number of bluegill crossings had relatively high detections of individual fish from a single PIT antenna, indicating that fish were often not mobile. Relatively few bluegill and largemouth bass detections and crossing prior to the initial acoustic treatment also made it difficult to establish normal fish behavior in the WCS. The small aggregate of describable behaviors from all species via PIT antenna data during all treatment blocks make quantitative assessment difficult and require a qualitative evaluation of behavior. Unfortunately, highly stochastic water conditions in the WCS make a long-term study to assess a fish deterrent system at this site impracticable, and the confined, shallow-water environment make the use of acoustic telemetry not feasible.

Water discharge, water depth, and draining period are all subject to highly changing environmental conditions combined with managed

hydrologic regimes over a large geographic region. During periods of passive draining, water depth and velocity in the WCS are affected by the height of the Preserve and Illinois River, which can fluctuate frequently (Koel and Sparks 2002). Even with these challenges, we and others (Mendenhall et al. 2017; Cupp et al. 2018) have been able to document numerous fish traversing the culverts and occupying the study area. Numerous species have been documented emigrating and immigrating through the WCS as water is passively drained (Hagen et al. 2018), yet our dependence on PIT antennae to track movement limited our ability to monitor fish behavior outside the culverts. It is unclear why the north culvert had greater flow than the south did during this study. However, it is possible elevated water velocity in the north culvert influenced preference by largemouth bass and silver carp (Liao 2007). The steady decline of water velocity seen in both culverts was the result of vegetation and wildlife fowling on upstream block screens and reflects the need for a more reliable method of deterring non-native fish passage into the Preserve.

The major constraint of the acoustic deterrent system installed in the WCS at Emiquon Preserve was water depth. Rapid attenuation of frequencies below 1500 Hz in the pool resulted from very shallow water (0.38–0.55 m) and silty substrate around the WCS (Rogers and Cox 1988; Jensen et al. 2011). Once the signal reached > 7.5 m downstream of the active speakers (4.8 m from the culvert opening) the frequency composition and amplitude were substantially different from what was being projected at the source. The concrete culverts in which speakers were positioned were also highly reflective surfaces and caused a great deal of reflection and refraction of the sound, further reducing propagation of the acoustic stimulus and degrading the coherency from the original signal (Popper and Hawkins 2018). The relatively small area in which sound propagated within the WCS likely reduced the potential effective range of the acoustic deterrent. Furthermore, high sound reflection would greatly reduce a fish's ability to localize the source of the acoustic stimulus (Rogers and Cox 1988), which could account for fish passages though the active speaker array.

The projected frequency range of the acoustic stimulus was within the hearing range of all species tested (Scholik and Yan 2002; Holt and Johnston 2011; Vetter et al. 2018). Bluegill have a reported hearing range of 300–2000 Hz and are believed to be primarily sensitive to high-intensity sound exposure (142 dB re. 1μPa for 24 hr), yielding minimal shifts in hearing thresholds compared to other species (Scholik and Yan 2002). Hearing range has not been reported for largemouth bass but, unpublished data in Holt and Johnson (2011) indicated it is within the range for fish of the same genus (*Micropterus* spp.; 100–2000 Hz). In comparison, silver carp have a broader hearing range extending from 100–5000 Hz and are more sensitive to frequencies below 2000 Hz (Lovell et al. 2006; Vetter et al. 2018) compared to bluegill and largemouth bass (120 dB re. μPa; Scholik

and Yan 2002; Holt and Johnston 2011). Therefore, we would expect a greater response from silver carp compared to bluegill and largemouth bass. Relatively few bluegill and largemouth bass detections during the first two acoustic exposures and inactive time periods made it unclear if the acoustic stimulus influenced their behavior. However, some of the highest crossing rates for bluegill and largemouth bass occurred during the final exposure period, indicating that the acoustic deterrent may not alter passage of these native fish species. Long-term research is needed to investigate the response of wild native fish species given the constraints of diel movement patterns.

It is not known why PIT-tagged gizzard shad, grass carp, smallmouth buffalo, and white bass were not detected in the culverts. The lack of detections limits our ability to infer the impact of the acoustic stimulus on behavioral response in these species. Deterrence from the acoustic stimulus is possible because gizzard shad, grass carp, and smallmouth buffalo all have broad hearing ranges (Dijkgraaf 1960; Popper et al. 2004; Popper and Schilt 2008). Little is known about the hearing sensitivity of *Morone* spp. The small sample size of smallmouth buffalo and grass carp may account for the lack of detections. The large number of small dead gizzard shad and white bass trapped on the block fence may account for the lack of detections. The only white bass detected was the largest tagged individual and likely was able to avoid being swept downstream in the current. Further research is needed to examine the negative effects that an acoustic deterrent may have on native ostariophysans and other hearing specialists.

One important aspect of the acoustic stimulus that was not explored at Emiquon Preserve was the directional propagation of sound and the effect that particle motion had on fish behavior. Because we did not measure particle motion within and around the WCS, it is unclear if we are missing an important component of the boat motor stimulus that could potentially have affected the deterrent system's efficacy. Given the importance that particle motion has on a fish's directional sensitivity (Popper and Hawkins 2018), determining the distance that fish in the WCS could detect particle motion from the signals source could be valuable in understanding why silver carp eventually crossed though the active acoustic array. Because this shallow-water location embodies many of the same acoustic constraints as small research tanks (Duncan et al. 2016; Gray et al. 2016; Rogers et al. 2016; Popper and Hawkins 2018), and it has many acoustic boundaries that frequently change with water depth, measurements of particle motion and SPL would likely vary greatly from year to year (or day to day); making the feasibility of optimizing an acoustic stimulus for fish deterrence extremely difficult.

The jumping response that was documented at the onset of acoustic exposure periods without spatial avoidance to areas of high SPL for extended periods was similar to that described for zebrafish by Neo et al.

(2015). Additional variation to stimulus playback could potentially extend the time a negative phonotaxic response can be elicited. Altering the areal extent of the maximum stimulus, especially in shallow water environments, may impact the magnitude of the response for fast swimming fish, including perhaps exceeding the distance/time that a fish is willing to spend within the area of maximum stimulus. It is possible that the addition of more speakers, installed longitudinally down the culverts, would be more effective at reducing bigheaded carp passage by increasing the size of the deterrence zone in this shallow water environment. Further research is needed to investigate optimal deterrent zone size using additional speakers and to determine the efficacy of alterations to the acoustic stimulus. Regardless, the use of an acoustic deterrent would have the greatest utility in deeper water environments where sound can propagate more effectively.

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