M.L. 2014 Project Abstract
For the Period Ending June 30, 2018

PROJECT TITLE: Bioacoustics to Detect, Deter, and eliminate Silver Carp
PROJECT MANAGER: Allen F. Mensinger
AFFILIATION: University of Minnesota Duluth
MAILING ADDRESS: Biology Department, 1035 Kirby Dr
CITY/STATE/ZIP: Duluth, MN 55812
PHONE: 218 726 7259
E-MAIL: amensing@d.umn.edu
WEBSITE: www.d.umn.edu/biology
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 04b

APPROPRIATION AMOUNT: $262,000
AMOUNT SPENT: $259,692
AMOUNT REMAINING: $ 2,308

Overall Project Outcome and Results

The project examined various emerging technologies to detect and deter the upstream migration of invasive bigheaded carp into Minnesota. Both silver and bighead carp were found to have an aversion to broad band sound and the project focused on how to exploit this weakness. An early detection buoy was developed that stimulates silver carp jumping with sound to allow managers to locate fish. The hearing sensitivities of the fishes were examined and found to have higher frequency hearing than previously reported. Broadband sound was successful in deterring fish and also preventing them from entering a small channel. Fish were successfully herded by broadband sound in the wild, suggesting that sound could be used to increase capture rates. We have also noted that long sound exposure may cause transient hearing loses in fishes so the sound deterrence must be balanced against potential hearing loss. In summary, broadband sound induces aversive behavior in silver and bighead carp however further study is needed to address the duration of its effectiveness.

Project Results Use and Dissemination

Presentations have been made at state, regional and national scientific meeting to disseminate the data and five publications were produced.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2014 Work Plan Final Report

Date of Report: 8/16/2018

Final Report

Date of Work Plan Approval: 6/4/2014

Project Completion Date: 6/30/2018

PROJECT TITLE: Bioacoustics to Detect, Deter, and eliminate Silver Carp

Project Manager: Allen F. Mensinger

Organization: University of Minnesota Duluth

Mailing Address: Biology Department, 1035 Kirby Drive

City/State/Zip Code: Duluth, MN 55812

Telephone Number: (218) 726-7259

Email Address: amensing@d.umn.edu

Web Address: www.d.umn.edu/biology

Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$262,000**
	Amount Spent:	**$259,692**
	Balance:	**$2,308**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 04b; *appropriation extended to June 30, 2018 in M.L. 2017* Ch. 96, Sec. 2 Subd. 18(a)(2),

Appropriation Language:
$262,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota-Duluth to develop bioacoustic technology for detection and early warning systems, capture and elimination methods, and deterrent systems for silver carp. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

Carryforward; Extension (a) The availability of the appropriations for the following projects are extended to June 30, 2018: (2) Laws 2014, chapter 226, section 2, subdivision 4, paragraph (b), Bioacoustics to Detect, Deter, and Eliminate Silver Carp

I. PROJECT TITLE: Bioacoustics to detect, deter and eliminate flying carp

II. PROJECT STATEMENT:

The Asian silver carp, one of four invasive carp species, is migrating north via the Mississippi River and threatening native fish in Minnesota rivers and lakes by outcompeting them for food supplies. Additionally, its unique jumping ability places recreational boaters in danger of being injured during collisions with airborne fish. However, this jumping ability is a weakness that can be exploited to detect, manage and control fish populations. The goals of this project are:

1) use the sound that stimulates jumping to develop early warning and detection systems

2) develop management techniques using sound to exhaust the fish on the surface or to herd the fish into shallow waters for capture and removal

3) use sound to deter or repel fish from moving through strategic waterways

In the previous year, we have made two significant findings: 1) determined the sound that initiates jumping in wild silver carp in the Illinois River; 2) successfully used this sound to repel carp in experimental outdoor ponds at the USGS Upper Midwest Environmental Science Center (UMESC) in LaCrosse, Wisconsin. This proposal would allow us to develop bioacoustic (sound) technology to combat the silver carp. The most effective sound that influences carp behavior is of relatively high frequency and is outside the hearing range of most native and game fishes. Our first goal is to develop remotely operated buoys with underwater speakers and above water video cameras to stimulate carp jumping to ascertain if an early detection or identification system can be developed. Our second goal is to develop a mobile sound system to stimulate continuous jumping for exhausting the fish on the surface and/or use sound to herd the fish into shallow water or nets for easy capture. Finally, we will test the efficacy of using sound to repel carp from specific areas. All the proposed studies will take place in large, secured (caged) outdoor experimental ponds at the USGS UMESC in LaCrosse, Wisconsin or on populations of wild carp in the Illinois River near Havana, IL. Both sites provide access to fish in outdoor locations where they behave naturally, allow large scale trials that cannot be replicated in indoor facilities and pose no danger of silver carp being released in MN waters.

III. PROJECT STATUS UPDATES:

Project Status as of 1/15/2015:

An early warning and detection buoy was fabricated and tested both in tanks and on the Illinois River. The buoy was remotely operated and equipped with an underwater speaker. Preliminary trials indicated that the buoy was effective in dispersing carp from an approximately 25 to 50 m radius both up and downstream. This was verified by electrofishing following acoustic testing. In several incidents, the carp was stimulated to jump however this behavior was inconsistently noted. The speaker was affixed to a boat to determine if fish could be "herded" up or down stream. The boat allowed a larger amplifier to be used and consistent movement away from the speaker as well as jumping was noted. Fish dispersion was approximately 50 meters up and down stream.

Barrier trials were attempted in both small outdoor tanks and a large outdoor pond at the USGS facility. Sound was effective in inhibiting both silver and bighead carp movement through a small opening in a barrier. This was confirmed by video analysis. Sound tests were also conducted on telemetry tagged fish in the large pond and are currently being analyzed.

Project Status as of 7/15/2015*:*

Activity 1: Due to spring flooding on the Illinois river, spring trials were not conducted with the buoy. A larger amplifier and speaker were acquired and the buoy modified for Fall trials pending lower water levels in Havana, IL

Activity 2: We have made significant progress on assessing the reaction of bighead carp and native fish to sound. Bighead carp were shown to be as responsive as silver carp to being moved across the ponds with sound. We determined the optimal sound pressure levels that resulted in repulsion. Native fish including walleye and sunfish were not responsive the sound indicating the sound used as a carp deterrent at the current decibels, while not effect native species. Trails with bigmouth buffalo however were very mixed and fish behavior was not the same as with the silver and big head carp indicating that these fish would be a poor surrogate to test acoustic behavior. We also examined the effect of temperature on silver and bighead behavior and saw significant increase in deterrents with increased behavior which may allow us to model when acoustic deterrents will be most effective.

Activity 3: We continued to concurrently examine acoustic deterrents under controlled conditions at the UMESC facility in LaCrosse WI and in the field in the Illinois River. At UMESC, outdoor concrete ponds were partitioned in half and speakers placed near the small channel in the barrier. Complex sound was very effective in deterring fish from swimming through the channel. The number of crossing with bighead, silver and mixed schools was significantly decreased when sound was broadcast.

Project Status as of 1/15/2016:

We continue to make excellent progress towards all three activity goals. We continue to be the only laboratory in Minnesota combining controlled, large outdoor pond experiments with field studies in areas containing invasive carp. We have been working on a wide range of fish sizes, from 3 cm to 1 meter long fish. We continue to learn more about the effects on sound on both bighead and silver carp as well as native fishes. Our field studies have shown that sound can be an effective tool in dispersing carp from a given area as well as herding them both up and down stream. Our results are being disseminated in the literature and reports to government agencies including the Army Core of Engineers, the USGS, the US Fish and Wildlife, regional agencies and various state DNRs.

Activity 1: Based on situ sound recordings and behavioral observations, it appears the buoy will be more effective with a larger amplifier and speaker. The work is ongoing to modify the hardware on the buoy to optimize its performance. This involves concurrent modification of the battery and charge storage devices to augment the solar panels. Nick Frohnauer of Minnesota Department of Natural Resources was given a tour of the lab and expressed interest in the buoy’s development and application

Activity 2: We continued examining the effect of sound on bigheaded carp and native fish. Additionally, we examined the effects of temperature on fish behavior. We established a new collaboration with the USGS in Columbia Missouri and they hosted us for a week in Missouri. They were anxious to try herding fish and we conducted trial runs on a small tributary of the Missouri River. This was done at no cost to the current grant. They also joined us on the Spoon River in Havana Illinois and provided sonar equipment to allow us to track the underwater movement of the fish with above water jumping. Preliminary analysis indicates that sound will cause the majority of carp to leave the area with subsequent passes causing many of the remaining carp to also leave. The sonar indicated that the remaining carp seek woody debris as refuge and targeting these areas would result in greater removal. However, on straight stretches of the river within debris, which would more closely mimic a lock and dam chamber, the sound was much more effective in moving fish as there was little refuge. This data is important for trying to herd the carp out of the area.

Activity 3. The acoustic deterrent work in the small concrete ponds continued will be providing the foundation for summer 2016 studies especially to further investigate habituation to sound. Broadband sound continued to be an excellent deterrent to bigheaded carps while having little effect on native fishes with approximately 10 species of native tested with plans for several more. A large scale field study was conducted with the USGS in Morris, IL in a backwater of the river. Large fish were captured, immediately tagged and placed in a large netted enclosure with a narrow channel separating the two halfs of the enclosure. Despite non optimal conditions, sound proved effective at both discouraging crossing and concentrating fish into small areas.

Amendment approved by LCCMR 2-10-2016

We respectfully request the transfer of the Professional/Technical/Service Contracts funds that were originally targeted for boat rental and fuel expenses from the Illinois Natural History Survey (INHS)to be moved to equipment to support activity 2 and 3. The INHS has been very supportive of the project and has decided that they prefer to provide boats and personnel as in-kind service.

The funds will be transferred to activity 2 and 3 to support the acquisition of additional acoustical hardware such as hydrophones and speakers. Our recent results have indicated two important pieces of information. The first is that we can drive the fish much further than anticipated and need additional hydrophones so we can understand the sound field at these longer distances. Secondly, we have noticed gaps is our sound field coverage that can be remedied by additional speakers

The Professional/Technical/Service Contracts were for $9000 and equally divided between activities 1,2,and 3. We request permission to move these funds into the Equipment/Tools/Supplies divided evenly between activity 2 outcome 3 and activity 3 outcome 3.

We also request to change the location in activity 3 outcome 3 from the Sand pit to Sand/pit open waters trials. This has no impact on the science being proposed however due to flooding issues, the USGS may not return to the Morris sandpit in 2016 and is investigating other potential sites such as the Brandon Road or Starved Rocks dam. This change will simply provide flexibility to conduct the trials at a slightly different location when it is finally chosen.

Project Status as of 7/15/2016:

We continue to make excellent progress towards all three activity goals. We continue to be the only laboratory in Minnesota combining controlled, large outdoor pond experiments with field studies in areas containing invasive carp. Our results continue to be disseminated in the literature and reports to government agencies including the Army Core of Engineers, the USGS, the US Fish and Wildlife, regional agencies and various state DNRs. We are encouraged by the reaction of the fish to sound stimulus in controlled tank conditions, large outdoor ponds and in the field. Spring weather and river conditions precluded many of the experiments so efforts were focused on building large barrier/channels in 1/10 and ½ acre ponds at the USGS facility in LaCrosse. Previous barrier trails had been conducted in small outdoor concrete tanks and these new ponds will provide a more realistic aspect of carp behavior.

The PI and his students presented at multiple meetings and submitted or had published several manuscripts on the bioacoustics and sound. The PI also entered into a new collaboration with the Nature Conservancy at the Emiquon field station just North of the Havana IL field site that will allows testing of the technology in large culverts with no additional cost to the grant. The laboratory presented their results at a sound deterrent workshop hosted by the USGS in LaCrosse, WI and received interest from a number of management agencies

Project Status as of 1/15/2017:

We continue to make excellent progress towards all three activity goals. We continue to be the only laboratory in Minnesota combining controlled, large outdoor pond experiments with field studies in areas containing invasive carp. Our results continue to be disseminated in the literature and reports to government agencies including the Army Core of Engineers, the USGS, the US Fish and Wildlife, regional agencies and various state DNRs. We attempted to concentrate on habituation to sound and field trails during the summer and fall. We established 3 1/10 acre ponds with barriers and attempted to determine how long the sound will be effective without fish habituating. Unfortunately, this cooperative effort with the USGS was beset by technical problems with the acoustic tags and it is uncertain if the data can be extracted from the study or the experiments need to be repeated this summer. Spring, summer and fall river water levels were at very high levels that precluded many of experiments. Two late fall experiments were conducted. An acoustic barrier was established at Emiquon and achieved operational status. Its efficacy will be assessed this spring. Herding trials continued in the Spoon river and an additional small outreach of the Illinois river. Herding/driving the fish was very effective however many of the silver jumped over the nets and eluded capture. These trials will be conducted again in the spring with nets designed to decrease the jumping. Many publications were submitted or published this fall to disseminate the results.

Project Status 7/15/2017

Habituation trails were restarted at the USGS in LaCrosse, WI to determine the duration that the sound deterrent is effective. Three 1/10 acre ponds and four smaller outdoor tanks were established and sound was played for extensive periods of time to determine how long the sound was effective. Fish were tagged with acoustic telemetry tags and fish position was continuously monitored. The 2016 issues with the tags were resolved with the manufacturer and all tags appeared to work throughout the summer. Data is currently being processed and the results will be reported during the next update.

We continue to participate in herding/driving fish. We were invited to join a USGS and other researchers to use the Unified Method to capture carp. This involved commercial fisherman, electroshocking boats and our sound boats to drive fish in Morris, IL to shallow waters for capture. This was a five day effort and results are still being analyzed.

We also started to evaluate the hearing ability of the silver and bighead carp to sound stimulus using Auditory Evoked Potentials in the laboratory.

Project Status 3/1/2018

We continue to work with our collaborators to examine carp hearing sensitivity and possible damage caused by refine to allow us to refine the optimal sound that will repel the fish without causing hearing damage. A collaborative agreement with the USGS has allowed us to use Auditory Evoked Potentials (AEPs) to examine carp hearing. This is a non invasive technique equivalent to an EKG that measures the electrical activities of the brain and inner ear when the fish is exposed to sound. It has allowed us to determine that carp hearing can detect higher frequencies sound than previously reported and that prolonged sound can damage carp hearing structures making acoustic deterrents less effective. These experiments are key to refining the sound in activities 2 and 3 to make sound deterrent optimal.

We also have been examining the underwater propagation of sound from our underwater speakers and will be modeling the sound patterns to better understand how speakers should be situated. Preliminary data shows that structures associated with the buoy or speakers may deflect some of the sound and we are investigating better ways of deploying/attaching to speakers to maximize sound output.

Presentations were made at several meetings and a manuscript is under final review at PlosOne that reports on the current activities.

Overall Project Outcomes and Results:

The project examined various emerging technologies to detect and deter the upstream migration of invasive bigheaded carp into Minnesota. Both silver and bighead carp were found to have an aversion to broad band sound and the project focused on how to exploit this weakness. An early detection buoy was developed that stimulates silver carp jumping with sound to allow managers to locate fish. The hearing sensitivities of the fishes were examined and found to have higher frequency hearing than previously reported. Broadband sound was successful in deterring fish and also preventing them from entering a small channel. Fish were successfully herded by broadband sound in the wild, suggesting that sound could be used to increase capture rates. We have also noted that long sound exposure may cause transient hearing loses in fishes so the sound deterrence must be balanced against potential hearing loss. In summary, broadband sound induces aversive behavior in silver and bighead carp however further study is needed to address the duration of its effectiveness.

IV. PROJECT ACTIVITIES AND OUTCOMES:

Activity 1: Early warning and detection system development
Description:

One of the challenges in assessing the silver carp invasion is to accurately census the population and to identify the vanguard of new invasion fronts. Carp have been documented to avoid traps and nets which make using traditional fisheries census techniques challenging. Although environmental DNA analysis can confirm the presence of carp DNA at low concentrations in the water, it cannot pinpoint the source of the DNA (ie live carp v vs bird fecal material) or the number and age of the carp. The silver's carp unique jumping ability could be used to develop early warning systems by stimulating the carp to jump and determine the number and composition of the population in the area. Preliminary trials have indicated that individual carp can be stimulated to jump and they do not have to occur in high densities to exhibit this behavior. Therefore, detection systems could be used both to census established populations as well as early warning systems when small numbers of carp first enter an area.

Silver carp of different size and age classes will be maintained in large, secured outdoor tanks at the UMESC facility in Lacrosse, WI which has a captive silver carp population. The carp are viewed remotely with overhead cameras to monitor their normal swimming patterns and their response to sound and/or vibrational stimulus. Underwater speakers will be mounted throughout the tanks and the carp response to complex sound, primarily underwater recordings of boat motor sound will be played through the speakers. The swimming and jumping behavior of the carp will be observed in response to sound stimulus. Preliminary trials have shown that silver carp will rapidly swim away from this type of sound. Other fish behavior such as jumping and schooling will be noted. Various sound frequencies and intensities will be tested to determine the optimal sound that causes the fish to move away from the sound source. As fish behavior is related to age (size), density and temperature, the sound will be tested on both juvenile and adult fish at different temperatures and densities.

We will develop a remotely operated, early warning buoy equipped with video cameras, underwater speakers, vibrational stimulus and hydrophones for the field deployment. This buoy will be designed to stimulate carp jumping behavior in the field. It will be programmed or remotely operated to play sound stimulus at random times during the day and the number of fish jumping will be recorded by the video cameras.

To test the buoy, prior to field deployment, trials will be conducted in a ½ acre pond to create more natural conditions and determine the stimulus range. The pond is equipped with fish tracking systems and underwater cameras which will allow us to monitor fish position and behavior underwater. We will the optimal stimulus that was developed in the smaller tanks to stimulate carp jumping which will be recorded by the video cameras on the buoy. The number of carp jumping and the range of the stimulus will be determined

We will then travel to Havana, IL to test the system on wild populations of carp in the Illinois River. The buoy will be floated into areas of varying carp concentrations and remotely operated to trigger various stimuli to detect the carp. Prior to or after buoy deployment, we will determine the carp concentration in the area by passing through with motor boats and/or electrofishing boats. We will compare its effectiveness in areas of high and low carp concentration to determine its effectiveness as a detection system.

Summary Budget Information for Activity 1:

ENRTF Budget:	**$93,334**
Amount Spent:	**$92,448**
Balance:	**$846**

Activity Completion Date: 6/30/2017

Outcome	Completion Date	Budget
1. optimal stimulus, small outdoor tank trials	12/31/14	$32,334
2. buoy construction, ½ pond trials	12/31/15	$32,000
3. buoy testing, Illinois River	12/31/16	$29,000

Activity Status as of 1/15/2015

The buoy was purchased and modified during the summer of 2014. The buoy was equipped with solar panels to power a 20 W amp. Two battery operated Go-Pro cameras were mounted on the buoy to record above water fish activity. An underwater speaker was mounted below the water line. A MP3 player that continuously looped complex sound stimuli was used as the sound stimulus. All equipment was remotely operated so the buoy could be deployed by boat and allowed to drift downstream.

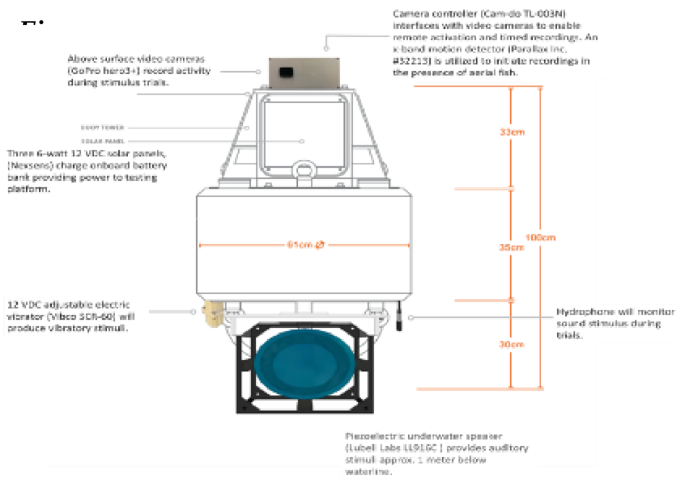


Figure 1. Schematic of buoy

Figure 2. Buoy being readied for preliminary trials on Illinois River


Figure 3. Buoy deployed in Illinois River

Preliminary trials were conducted on the Illinois River. The buoy was deployed and allowed to drift downstream from the boat. The sound stimulus was initiated for approximately one minute and then the boat approached the area and conducted electrofishing to determine the presence or absence of fish.

Jumping was not observed in the main channel however the area within 25 to 50 m of the buoy was devoid of carp. Electrofishing verified that carp had moved both up and downstream. The buoy was then relocated to a side channel that was approximately 30 m wide. The buoy appeared more effective in the narrower channel as carp was displaced at least 50 m up and downstream.

The buoy seems an effective tool to move carp both down and upstream away from a selected area. Jumping was rare however only a small amplifier was used. Please see activity 2 for information on using larger amplifier. Several adjustments will be made to the buoy including a counterweight to help balance the float and incorporating a vibrating probe to determine the effects of vibration without sound.

Activity Status as of 7/15/2015

Due to spring flooding on the Illinois river, spring trials were not conducted with the buoy. A larger amplifier and speaker were acquired and the buoy modified for Fall trials pending lower water levels in Havana, IL

Activity Status as of 1/15/2016

The spring and summer flooding precluded additional buoy field trials as efforts were concentrated on activities 2 and 3 due to the compressed season. The larger speaker was fitted to the buoy but power output delivered by the solar panels remained below the levels for sustained deployment. Efforts are underway this winter to add additional battery capacity to the buoy. These batteries combined with the solar input will allow longer deployments during the next deployment window. The information obtained from sound mapping activities in activity 2 and 3 also allowed better understanding of the sound created by the speakers. There are some gaps in the original sound field that fish can exploit and the buoy will be further modified to reduce these problems

Activity Status as of 7/15/2016

Modifications continue to be done with the buoy to stabilize its position. The three point anchor system is being developed to stabilize the buoy. The larger speaker caused unanticipated rotation either due to the physical stimulus or creating more drag in the current. A mooring/anchor has been develop to maintain the position of the buoy in the stream as rotation confounds sound directionality.   Spring flood conditions again prevented deployment so field trials are postponed to fall.

Activity Status as of 1/15/2017

Fall water conditions and woody debris precluded the use of the buoy. If water conditions continue to challenge its deployment, then ½ acre pond trials will be attempted to be conducted this spring/summer.

Activity Status as of 7/15/2017

River conditions continued to be inconsistent with either high flow or low water conditions. Additional evaluations of the overall technology suggests that the buoy can be highly effective in near shore or banks heavy debris. For channel blockage, multiple buoys will need to be deploy to increase acoustic deterrence.

Activity Status as of 3/1/2018. We are currently collaborating with the MN DNR to evaluate Lock and Dam 5 as potential site for acoustic deterrents. We have been given permission from the Army Core of Engineers to access the site and deployed hydrophones in the lock and dam in late fall to record sounds. The site provides a more accessible environment than the rivers we had been working in and should not be impacted as much by flooding. If the opportunity presents itself in the spring (water levels and river conditions) we plan to test the buoy downstream from the channel to see how it operates in larger river environments.

Additionally, we are testing the directional sound component of the speakers (which is also related to Activity 2) and preliminary finding has found some asymmetries in the sound propagation of the speakers that may either be related to the speakers, supporting structures or the buoy. We are testing a new speaker harness that will help us isolate any issue and allow optimal sound projection.

Final Report Summary:

The buoy proved effective in eliciting silver carp to jump and could certainly be further developed as an early warning system. Based on deployment experience and data from the other experiments in this report, the buoy would be more effective if two, larger underwater speakers were mounted perpendicular to each other underneath the buoy. This would allow a more uniform sound distribution and high intensity sound which would increase the effective area of the underwater sound field and significantly increase the probability that any silver carp in the area will jump.

ACTIVITY 2: Bioacoustical movement of carp

Description: We have determined that carp will swim away from complex sounds such as underwater recordings of outboard boat motors. Commercial fishermen already use crude sound stimulus (banging on the sides of their boats) to concentrate fish and herd them into nets.   It is anticipated that multiple arrays of underwater speakers could herd and/or concentrate the fish into shallow water for capture. We will develop underwater speaker arrays drive and/or herd fish into specific areas of the tanks or use the speakers in the field to drive the fish into nets. As both species of carp exhibit sound aversion, we will employ this technology on both silver and bighead carp. The goal is reduce or eliminate already established populations by concentrating the fish for easy capture and removal

Additional, we plan to take advantage of the silver carp's unique jumping ability and use this behavior against the fish. Aerial jumps are energetically expensive for fish, and even salmon that migrate hundreds of miles upstream, need to rest before jumping successive water falls. If carp are stimulated to jump repeatedly, it may be possible to exhaust them to the point where they will float on the surface and can be easily netted and removed.

The initial trials will be conducted in large, outdoor concrete ponds on the campus of the USGS facility in LaCrosse, WI. Silver carp of different size and age classes will be maintained in large, secured outdoor tanks at the UMESC facility in Lacrosse, WI which has a captive silver carp population. The carp will be viewed remotely with overhead cameras to monitor their normal swimming patterns and their response to sound and/or vibrational stimulus. Underwater speakers will be mounted throughout the tanks and the carp response to complex sound, primarily underwater recordings of boat motor sound will be played through the speakers.

The swimming behavior of the carp will be observed in response to sound stimulus. Preliminary trials have shown that silver carp will rapidly swim away from this type of sound. Different sound frequencies, vibrations and intensities will be tested with the underwater speakers. Preliminary trails indicated playbacks of underwater boat noise is an effective stimulus to displace and move carp. In contrast to Activity 1, multiple speakers (4 to 5) will be placed strategically in the tank to herd the fish into designated areas. As the fish will be viewed remotely with the overhead cameras, different speakers or combinations of speaker can be activated to drive the carp into designated areas of the tank or stimulate jumping to the point of exhaustion.

Trials will move then to the ½ acre pond described in activity 1 to create more natural conditions. Speaker arrays consisting of multiple speakers will be suspended from boats and used to herd the carp into specific areas of the tank. To concentrate them in one area, it is anticipated that two or three arrays will be operating simultaneously. Fish position will be monitored either by jumping or underwater cameras. Trials will be conducted with 25 to 50 fish and the accuracy to technique evaluated based on the number of fish that can concentrated into the designated areas. Small boats with outboard motors may also be used in the pond to move or herd carp into designated areas.

Once the methodology has been optimized, field trials will be conducted on the Illinois River. The field trials will use underwater speakers and/or motor boats to drive the carp into nets or shallow water for capture. All trials may be augmented by electroshocking as electric current has been noted to produce herding behavior similar to sound in field trials. Additionally, before and after carp movement and capture, electroshocking can used to census the river population to test the efficacy of the procedures.

Summary Budget Information for Activity 2: **ENRTF Budget: $ 88,083**
Amount Spent: $ 88,083
Balance: $ 0

Activity Completion Date: 6/30/2017

Outcome	**Completion Date**	**Budget**
1.Bioacoustic movement, outdoor tank trials	12/31/14	$ 29,583
2. develop speaker array, bioacoustic outdoor pond trials	12/31/15	$ 29,000
3. Field trials, Illinois River	06/30/17	$ 31,500

Activity Status as of 1/15/2015

The sensory biology and phonotaxic response of silver carp were investigated using controlled experiments in outdoor concrete ponds (10 x 4.9 x 1.2 m). Pure tones (500-2000 Hz) and complex tones (field recordings of

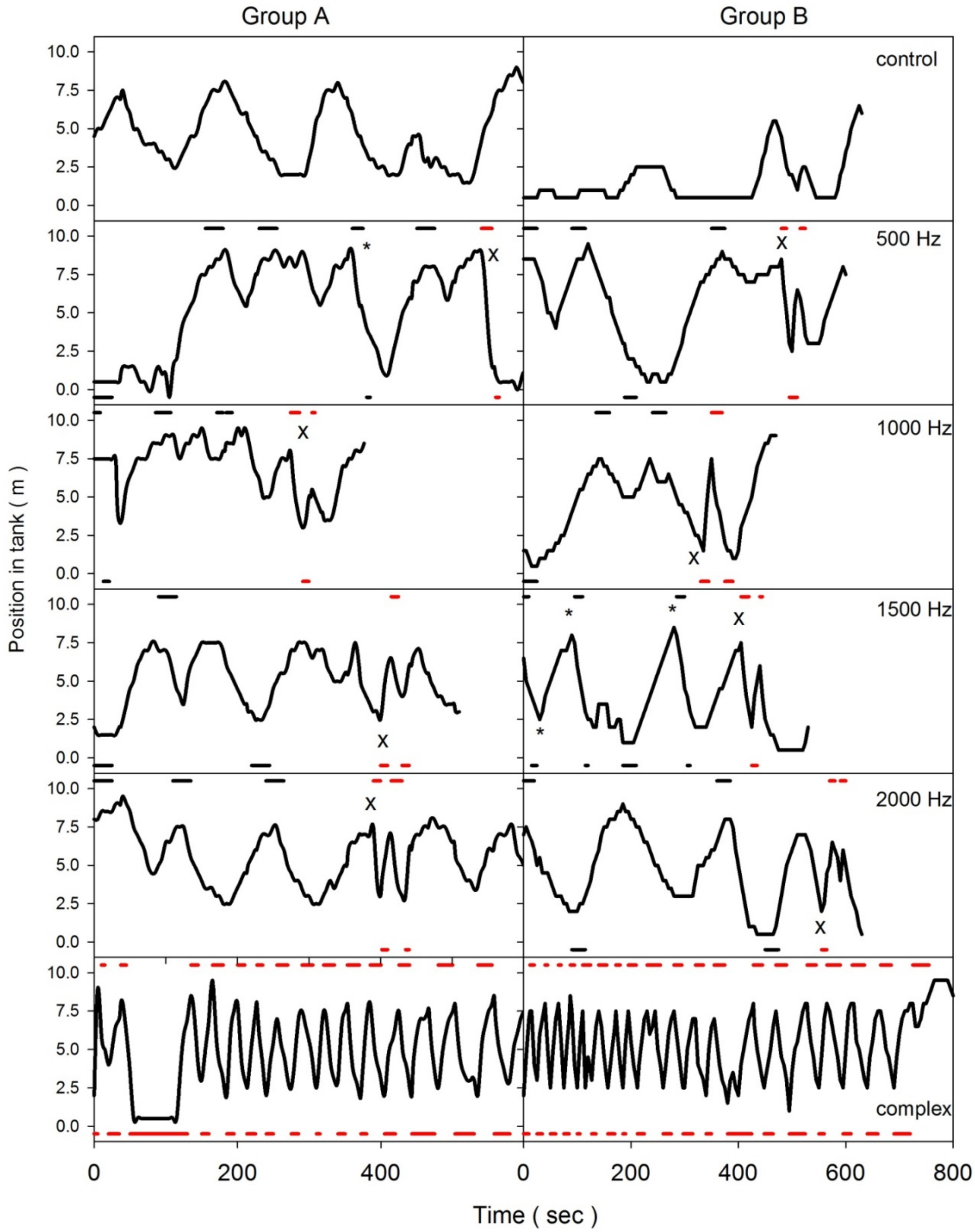


Figure 4. Silver carp movement in response to pure tones and complex sound

outboard motors) were broadcast using underwater speakers. Silver carp exhibited consistent negative phonotaxis to outboard motor sounds, however they habituated quickly to pure tones (after 1-2 trials). By alternating active speakers, silver carp movement was regularly directed away from the sound source to the opposite end of the pond. This research suggests that sound can be used to alter the behavior of silver carp with implications for deterrent barriers or potential control measures (e.g., herding fish into nets).

Trials were also conducted on bighead carp and native fish. The bighead head also were more responsive to complex sound stimulus although the fish did display greater sensitivity to higher frequency pure tones than silver carp. None of the native species tested exhibited response to the sound. Both of these data sets are currently being analyzed and will be available for the next progress report.

Preliminary tests were conducted on telemetry tagged silver and bighead carp in the large outdoor pond. The telemetry system allowed fish position to be continuously tracked. Two large speakers were suspended from floating platforms and complex sound broadcast. The large data sets are currently being analyzed by the USGS. The first trial was inconclusive due to heavy algal fouling of the pond. A follow up trial was more effective. Preliminary analysis indicates that the width of the pond will need at least a third speaker and/or offset speaker pairs to provide better coverage.

Figure 5. Position of the floating platforms that suspended the speakers in the 1/2 acre pond

The underwater speaker was transferred from the buoy to the electrofishing boat and used to drive fish away from designated areas. Fish were monitored using electroshock before and after sound stimulus. A 60 w amplifier was used instead of the 20 W amplifier. Sound was very effective in "herding" or moving fish away from areas. There were several occasions where all the fish in the immediate area jumped. As this did not transpire with the buoy, we believe that this behavior is driven by sound intensity. This is the first example where sound alone was sufficient to elicit jumping behavior. As only the single speaker was available, it is anticipated that using speaker pairs that can be directed could help focus the sound and herd the fish into specific spots.

Activity Status as of 7/15/2015

The sensory biology and phonotaxic response of bigheadcarp and native fishes were investigated using controlled experiments in outdoor concrete ponds (10 x 4.9 x 1.2 m). Pure tones (500-2000 Hz) and complex tones (field recordings of outboard motors) were broadcast using underwater speakers. Bighead carp exhibited consistent negative phonotaxis to outboard motor sounds (figure 6), however they habituated quickly to pure tones (after 1-3 trials) (Figure 7). By alternating active speakers, bighead carp movement was regularly directed away from the sound source to the opposite end of the pond. This research suggests that sound can be used to alter the behavior of silver carp with implications for deterrent barriers or potential control measures (e.g., herding fish into nets).

Native fish including walleye and sunfish show no reaction to the sound. Bigmouth buffalo behavior was quite different than the invasive carp and very inconsistent when exposed to sound, alternating between going away from the speakers and going towards them. The use of bigmouth buffalo as a surrogate to investigate invasive carp is not recommended, under at least these conditions.

The sound pressure levels were relatively modest which in encourages as large speakers that broadcast high amplitude sound will more than likely harm both carp and native fish. Native fish were not affected by the sound used to drive the bighead and silver carp and therefore use of this sound in the field should not affect native fish.

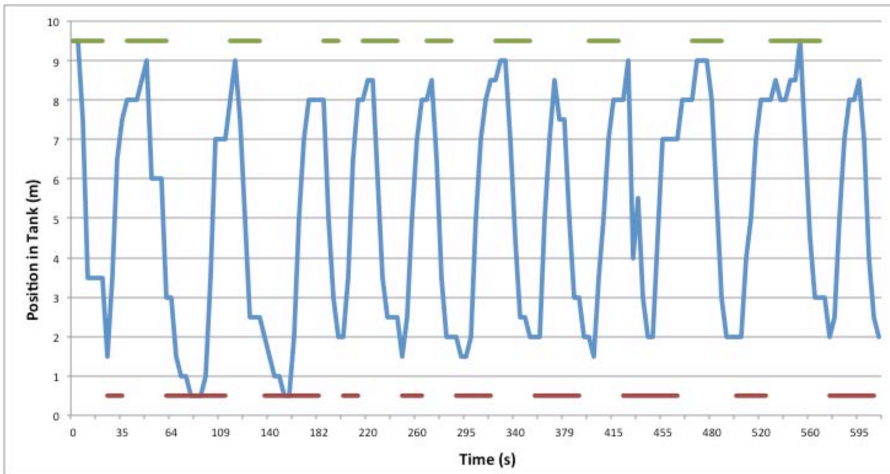


Figure 6. Movement of bighead carp in response to complex sound. The longitudinal position (m) of the center of the school is plotted versus time (s) with fish position mapped every 5 seconds. Solid lines above and below each fish position trace indicate the location and duration of the sound stimulus.

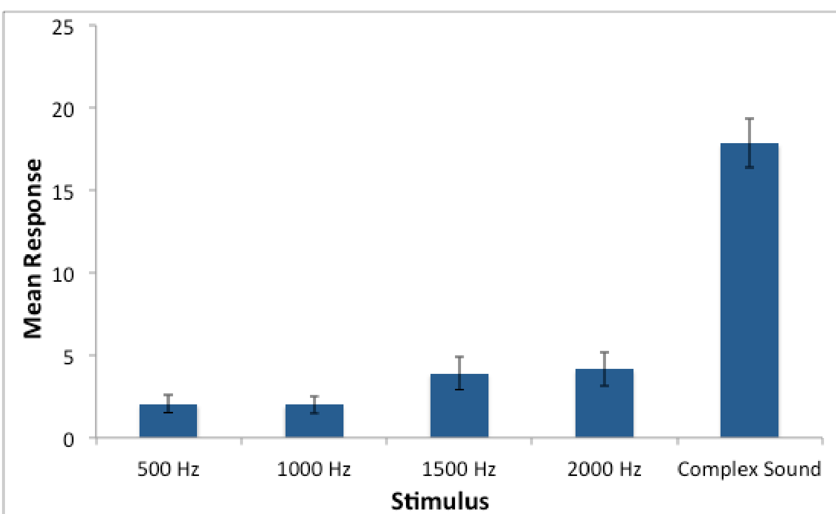


Figure 7 Bighead carp response to pure tones and complex sound. The number of consecutive movements is plotted for each sound.

Activity Status as of 1/15/2016

We continue to receive inquires on the effect of the sound on native fishes. Therefore we repeated the previous experiments on native fishes as well as two additional species on carp.

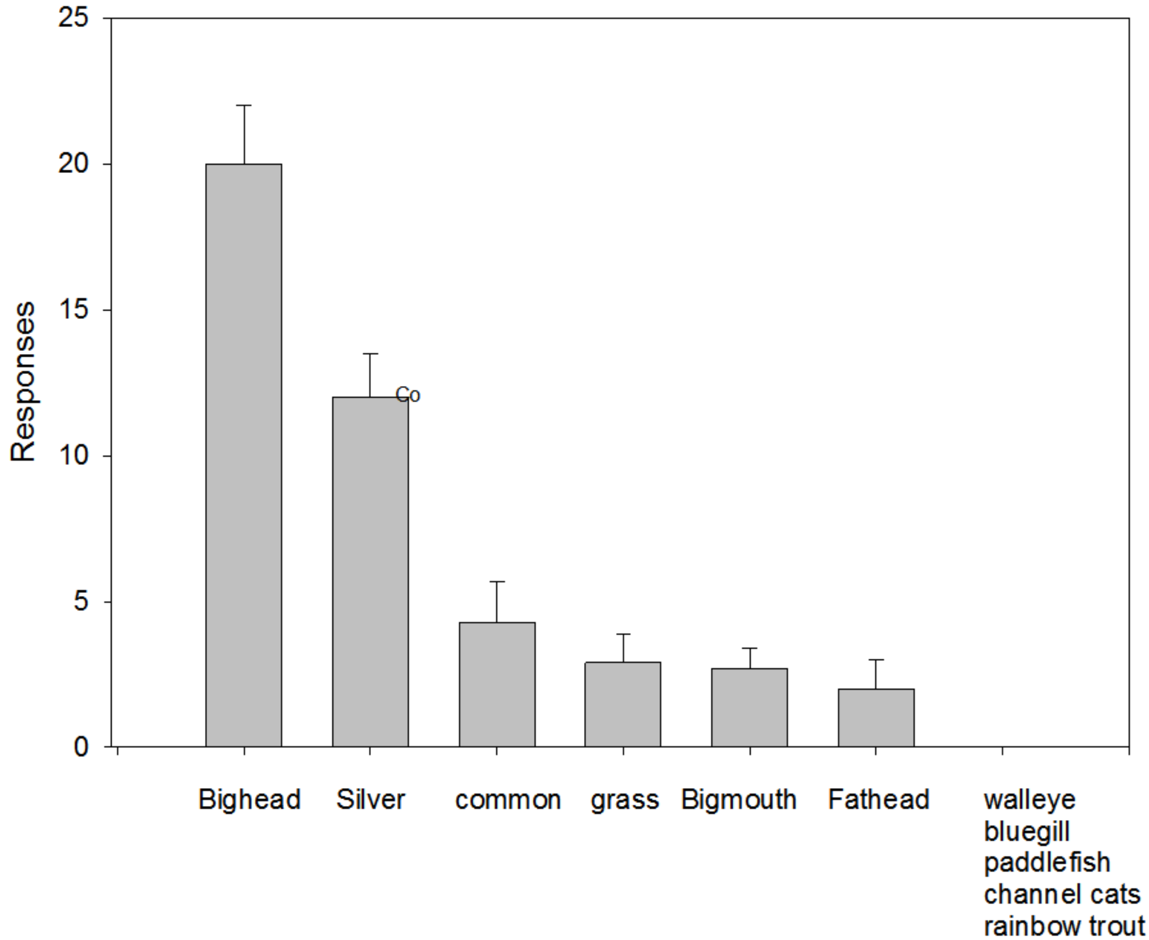


Figure 8. The average number of consecutive responses to broadband sound is plotted versus fish species

The above figure showed that broadband sound is extremely effective in changing the behavior of bighead and silver carp with most schools still responsive to sound after 10 minutes of sound presentation. Any habituation prior to this time period appears to be directly due to swimming fatigue and non habituation. Two other invasive carp species, common carp and grass carp, despite having similar hearing structures, were much less responsive to the sound. Native species such as bigmouth buffalo and fathead minnows showed mixed reactions with most schools non responsive. Other native fish such as walleye, bluegill, paddlefish, channel catfish and rainbow trout displayed no reaction to the sound. This confirms our preliminary hypothesis that silver and bighead would be most responsive to the sound and that other closely related species such as common carp and bigmouth buffalo are not suitable models for acoustic deterrents. Also, most native fishes tested have little or no response to the frequencies and stimuli deployed.

Our outdoor observations noted that the bigheaded carp behavior was influenced by water temperature. To understand the role of water temperature on sound sensitivity, we initiated indoor experiments under controlled water temperature conditions ranging from 12°C to 32°C to study the effect of temperature on bighead and silver carp behavior. Bighead carp displayed high negative phonotaxis at the warmer temperatures but activity was reduced at 12°C. However, swimming speed and overall activity was also reduced at these temperatures. Therefore it is uncertain if the lower temperatures would reduce the effectiveness of the acoustic deterrent as the carp may not have the motivation to challenge the sound.

Herding studies were initiated on the Spoon River in Havana IL and on a tributary of the Missouri in Columbia, MO. The experiments were enhanced by underwater sonar provided by the USGS CERC office. In the Illinois study, a single boat with equipped with two underwater speakers slowly transited 200 m stretches of the river while broadband sound was broadcast. Controls consisted of boat movement with the speakers silent. Initial passes often stimulated > 100 fish to jump with subsequent passes detecting fewer jumpers. Boat movement when the speakers were silent rarely stimulated the carp to jump indicating that sound was the primary stimulus Underwater sonar confirmed that most carp left the area with the remaining carp “hiding” in woody debris. These area were targeted on subsequent passes and were successful in decreasing the number of fish remaining. This suggests that the sound will be most effective if areas, such as entry channels to locks are cleared regularly of debris. Sonar analysis is ongoing and will correlated with the video that has been analyzed. This will allow us to assess the distribution of bighead carps also as they do not jump and therefore their behavior is not observable from the boat.

Two boats were used to herd fish towards nets placed across the tributary of the Missouri river. Sonar confirmed that fish were concentrated in front of the boats for long stretches of water however as fish neared the net, they reversed course and bisected the two boats where there was a gap in the sound field. It is hypothesized that an additional boat or speakers placed on booms on the existing boats would have created a more uniform sound field and drove the fish into the nets. Although the herding concept was proven successful, further refinement will be needed to increase capture.

Mensinger also spent a day with a commercial fisherman that was targeting carp. He observed how the fisherman optimizes catch by using sound and will attempt to incorporate this information in the next herding experiment.

Activity Status as of 7/15/2016

The field studies conducted in the Spoon River were analyzed and the results reported at the Aquatic Invasive Species and the Effects of Noise on Aquatic Animals conference.

We have learned that carp can be induced to jump by both boat movement and sound alone. The jumping patterns are very distinct at least in relation to the boat. During jumping in response to moving watercraft, almost all the fish jump after the boat has passed in very specific arcs defined by water movements associated with the water displacement. The behavior of wild silver carp responding to moving (16, 24, 32, and 40 km/hr) 6 m aluminum boats equipped with 4-stroke outboard motors (100 or 150 hp) was quantified. Experiments were conducted at three sites on the Illinois River near Havana, IL and most boat (57.9%) transits stimulated five or more fish to jump. The frequency of jumping (fish/min) was independent of speed and motor type and the vast majority of fish (> 90.0%) jumped after the boat had passed their position but avoided the area directly astern (< 4.0 m). Furthermore, 79.8% of fish vectored away from the moving watercraft. The results suggest that jumping direction is not random and fish can localize the stimulus source. The “delayed” jumping until after the boat had transited the area indicates that the trigger may be turbulence and/or higher sound pressure levels.

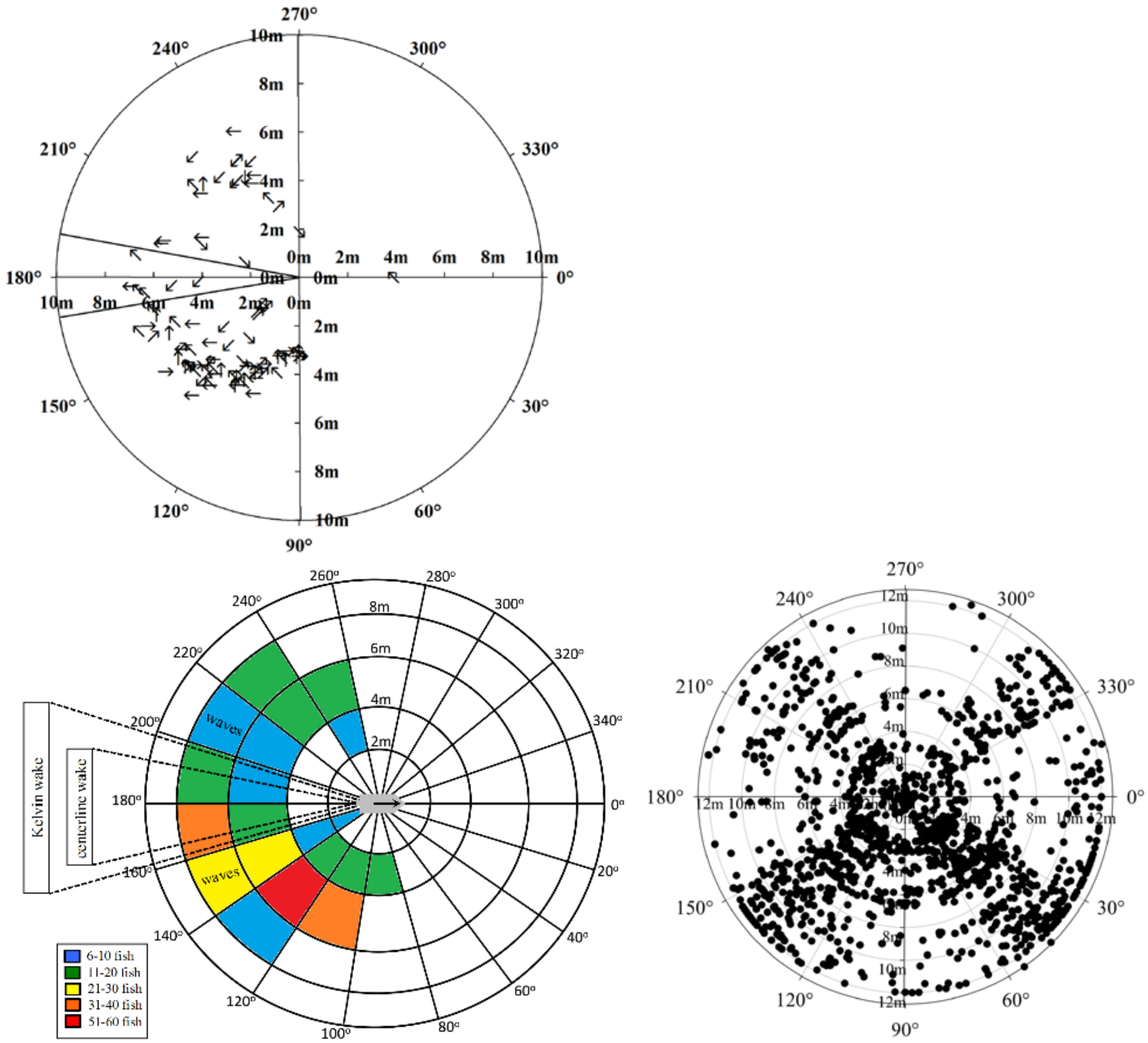


Figure 9. Jumping origin and vector in silver carp responding to fast moving boats (16 – 40 km/hr) equipped with a 100 or 150 hp. A) Arrows indicate individual carp jump origin and direction. B) Jumping number by quadrant. Jumping was not randomly distributed, with the majority of jumps occurring after the boat passed the fish and most jumps were away from the boat. C) Two speakers at front of boat were activated and polar plots show jumping origin and pattern. (Rao's $P < 0.05$).

We also demonstrated that sound alone in the absence of moving watercraft can cause carp to jump at much greater range. Additionally, the jumping pattern is strikingly different with fish jumping all around the boat compared to the fish jumping behind the boat in response to the moving watercraft. This indicates that static or boat mounted speakers can effectively cause carp to jump or perhaps herd them in the absence of boat movement. We are currently modeling the jumping to determine if we can predict the origin and end of the jumps in order to increase capture methods.

Activity Status as of 1/15/2017

Herding activities were conducted in conjunction with the USGS and the Illinois Natural History Survey in Havana IL in the Fall.

The first trials strung a net across the spoon River and herded the fish with two speakers submerged from motor boat. The sonar indicated high success in getting the fish to move in front of the boat and to the net. However, many fish either jumped over the net or moved into the woody debris lining the river banks and moved upstream past the boat. The water levels were not optimal for this herding however, it was the first week that it they were low enough to even attempt the experiments. Lower water levels would reduce the amount of debris submerged and force more fish into the net. These was preliminary trials and we learned a great deal from the net deployment. We believe that the nets can be effective however we will change the area in which we drive fish to a straighter section of the river that contains much less woody debris. We also may place speakers in the woody debris to drive the fish out of these sections. The addition of the sonar units to the boats was extremely valuable and we are still analyzing the data to learn more about fish behavior and how to optimize the sound.

We also tried herding fish in a shallow bay by the Coal docks in Havana. We were extremely successfully in driving fish towards the net with several hundred fish observed to jump over the net however only a few were entangled. Several net modifications could solve this problem and there will be attempted next time. 1) a Double row of nets so after fish jump the first net, they land in the second; 2) a tilapia net that has an extension that floats on the water and prevents fish from jumping 3) raising the net above water so the fish land in the aerial portion of the net.

We also used this area to accurately sound map the sound field that is being generated by the boat. We found that the sound does project several hundred meters in front the boat and maintains high sound pressure levels for 50 to 100 m in front of the boat. This is encouraging as the water was reasonably shallow and this experiment gives further insight into the sound field and its effective range.

We spent the Fall months planning with the US Fish and Wildlife, the Illinois Department of Natural Resources and the USGS to conduct unified field methods the herd and capture carp in a large gravel pit in Morris Illinois. We have designed and contributed to the sound part of the experimental design and we will be following the fisherman as they drive the carp using their own methodology to hopefully increase the capture yields. This experiments will be conducted in March of 2017.

Activity Status as of 7/15/2017

A large scale, multi agency (US Fish and Wildlife, the Illinois Department of Natural Resources and the USGS) carp herding experiment was conducted over the course of five days in a large gravel pit in Morris Illinois. This was derivation of the unified method approach that is used in China to herd fish for capturing. Commercial fisherman set nets daily to herd the fish through designated channels. The commercial fisherman then moved their boats while banging on the hulls. This was followed by electrofishing boats and finally by sound boats playing the acoustic deterrent. Fish positioned was monitored by underwater sonar. On the final day, after the fish were herded into a small area, fish were netted and removed. This results of the study are still being processed and will be reported in future updates.

We contributed by providing equipment and personnel for the sound boats. We also recorded the sounds from the commercial fisherman boats to understand the difference in the sound that these boats generate and the sounds that we have identified being used for the acoustic deterrents. We are currently analyzing the sounds made by the fishing boats and will compare them to the sounds of the acoustic deterrents.

Activity Status as of 3/1/2018

We attended a meeting at the USGS in Columbia, MO to plan further strategies for carp herding and the Creve Couer Lake carp herding that was scheduled for February, 2018. We continue to try to optimize sound and deterrence. In cooperation with the USGS in Columbia, we have extend the trials of native species and found very few native species react to the sound that drive the carp away. The data concerning the native species is currently being prepared for submission to a journal.

Final Report Summary:

Broadband sound proved to be effective deterrent both in outdoor ponds and in small tributaries. Silver and bighead carp displayed consistent negative phonotaxis to the sound.

Trials in outdoor ponds:

This study found that complex broadband sound (0-10 kHz) is effective in altering the behavior of Silver Carp with implications for deterrent barriers or potential control measures (e.g., herding fish into nets). The phonotaxic response of Silver Carp was investigated using controlled experiments in outdoor concrete ponds (10 x 4.9 x 1.2 m). Pure tones (500-2000 Hz) and complex sound (underwater field recordings of outboard motors) were broadcast using underwater speakers. Silver Carp always reacted to the complex sounds by exhibiting negative phonotaxis to the sound source and by alternating speaker location, Silver Carp could be directed consistently, up to 37 consecutive times, to opposite ends of the large outdoor pond. However, fish habituated quickly to pure tones, reacting to only approximately 5 % of these presentations and never showed more than two consecutive responses. Previous studies have demonstrated the success of sound barriers in preventing Silver Carp movement using pure tones and this research suggests that a complex sound stimulus would be an even more effective deterrent.

Recent studies have shown the potential of acoustic deterrents against invasive silver carp (Hypophthalmichthys molitrix). This study examined the phonotaxic response of the bighead carp (H. nobilis) to pure tones (500-2000 Hz) and playbacks of broadband sound from an underwater recording of a 100 hp outboard motor (0.06-10 kHz) in an outdoor concrete pond (10 x 5 x 1.2 m) at the U.S. Geological Survey Upper Midwest Environmental Science Center in La Crosse, WI. The number of consecutive times the fish reacted to sound from alternating locations at each end of the pond was assessed. Bighead carp were relatively indifferent to the pure tones with median consecutive responses ranging from 0 to 2 reactions away from the sound source. However, fish consistently exhibited significantly ($P < 0.001$) greater negative phonotaxis to the broadband sound (outboard motor recording) with an overall median response of 20 consecutive reactions during the 10 min trials. In over 50% of broadband sound tests, carp were still reacting to the stimulus at the end of the trial, implying that fish were not habituating to the sound. This study suggests that broadband sound may be an effective deterrent to bighead carp and provides a basis for conducting studies with wild fish.

Field studies

Invasive silver carp (dominate large regions of the Mississippi River drainage, outcompete native species, and are notorious for their prolific and unusual jumping behavior. High densities of juvenile and adult (similar to 25 kg) carp are known to jump up to 3 m above the water surface in response to moving watercraft. Broadband sound recorded from an outboard motor (100 hp at 32 km/hr) can modulate their behavior in captivity; however, the response of wild silver carp to broadband sound has yet to be determined. In this experiment, broadband sound (0.06-10 kHz) elicited jumping behavior from silver carp in the Spoon River near Havana, IL independent of boat movement, indicating acoustic stimulus alone is sufficient to induce jumping. Furthermore, the number of jumping fish decreased with subsequent sound exposures. Understanding silver carp jumping is not only important from a behavioral standpoint, it is also critical to determine effective techniques for controlling this harmful species, such as herding fish into a net for removal.

Silver carp, an invasive planktivorous fish species in North America, pose a threat to aquatic ecosystems throughout the Mississippi River Drainage. These fish are well known for their airborne leaps in response to passing watercraft, but the trigger for, and functional significance of jumping remains unknown. The behavior of wild silver carp responding to moving (16, 24, 32, and 40 km/hr) 6 m aluminum boats equipped with 4-stroke outboard motors (100 or 150 hp) was quantified. Experiments were conducted at three sites on the Illinois River near Havana, IL and most boat transits (57.9%) stimulated five or more fish to jump. The frequency of jumping (fish/min) was independent of speed and motor type and the vast majority of fish (> 90.0%) jumped after the boat had passed their position but avoided the area directly astern (< 4.0 m). Furthermore, 79.8% of fish vectored away from the moving watercraft. The results suggest that jumping direction is not random and fish can localize the stimulus source. The "delayed" jumping until after the boat had transited the area indicates that the trigger may be turbulence and/or higher sound pressure levels. This is the first study to model silver carp jumping in response to motorized watercraft and can aid fisheries managers in predicting the direction and location of airborne fish to develop effective herding and capture methods.

ACTIVITY 3: Carp deterrence

Description: One of the key strategies for integrated invasive species management is to deter fish from entering areas in which they have been eliminated or from invading new areas. Permanent barriers are expensive to maintain and interfere with commercial ship traffic and native fish movement. The aversion of carp to complex sounds has the potential to provide an environmentally friendly barrier that will not impact ship or native fish movement. We have determined that pure tones (same sound frequency) that normally are used for fish behavior and/or deterrent barriers is ineffective in deterring carp movement, however silver carp will readily swim away from complex sounds (playbacks of outboard motor noise). These preliminary experiments indicate that sound either alone or as part of a combined light and bubble barrier, may provide a cost effective and environmentally friendly barrier to silver or bighead carp migration or repel them from breeding areas.

Carp have specific tank locations either associated with three dimensional structures, sunlight or feeding location at which they prefer to reside. The first series of experiments in outdoor concrete tanks will identify the tank locations and use underwater speakers to deter the carp from these locations. Sound intensity, frequency, duration will be varied and the length of time that fish stay away from the location will be monitored to gain an understanding of how effective sound is as a deterrent.

We will then divide the tank into two sections with an expandable barrier/divider that will allow us to regulate the opening between the two sections of the tank. Three dimensional structures (i. e. milk crates) will be placed in one half, shade cloth erected over the same area to minimize light levels and all feeding will transpire in this section which will make this portion the “preferred” section for the carp to inhabit. We will then use sound from the underwater speakers submerged in the tank to drive the fish from the preferred section to the other half of the tank. Additional speakers will be positioned near the opening in the barrier to repel fish that attempt to return to the preferred half. The width of the barrier opening will be gradually expanded to determine how effective sound can be in larger passageways.

The same experiments will be performed in the large ½ acre pond described for previous activities. Again, a preferred location will be established and then sound used to displace the carp out of this area. Additional speakers will be place at the barrier openings and use to repel fish that try to return to the original location. The opening in the barrier will be gradually expanded to determine the effective width of the deterrent system.

Field trials will be conducted in a large sand pit (5 mile length) that parallels the Illinois River or appropriate alternative open water site. Carp will be herded or netted to the narrow end of the sand pit and a barrier placed across the pit. An opening will be made in the barrier and underwater speakers placed to at the opening to enable use to use sound to deter the carp from leaving the terminal end of the sand pit and migrating back into the main channel. The barrier opening will be gradually widened to determine the effectiveness of the underwater sound in deterring carp from entering large channels.

Summary Budget Information for Activity 3:	**ENRTF Budget:**	**$ 80,583**
	Amount Spent:	**$ 79,121**
	Balance:	**$ 1,462**

Activity Completion Date: 6/30/2017

Outcome	Completion Date	Budget
1. Outdoor tank deterrent trials	12/31/14	$ 27,083
2. Outdoor pond deterrent trials	12/31/15	$ 26,000
3. Sandpit/ open water trials	12/31/16	$ 27,500

Activity Status as of 1/15/2015

Preliminary outdoor tank trials were conducted with silver or bighead carp at the USGS facility. The tank was divided in half with cinder blocks with an approximately 1 meter opening left in the middle of the barrier to allow egress into half of the tank. Two speakers were place in each half of the tank and when fish approached the barrier opening, complex sound was initiated to prevent fish crossing the opening.

Trials were delayed until late summer and early Fall due to funding and weather conditions. Behavior appeared temperature dependent as during low temperatures, carp were not affected by the sound. However, during warmer conditions, sound was very effective at preventing crossing during the initial experiment and reduced the number of crossing in subsequent experiments. The trials were initiated using smaller speakers that the ones that had the greatest success in the field and it is anticipated using the larger speakers would be more effective. This is the first controlled trial to indicate that water temperature may be needed to factored when trying to move or displace fish.

Activity Status as of 7/15/2015

The experiments evaluated the effectiveness of an acoustic barrier to prevent the movement of the invasive silver (*Hypophthalmichthys molitrix)* and bighead (*H. noblis*) carp. Controlled experiments were conducted in outdoor ponds (10 x 5 x 2 m) that were divided into equal halves by a concrete-block barrier (0.4 x 2 x 2 m) with a small channel (1 m across) allowing access to each side of the pond. Underwater speaker pairs were placed on each side of the opening and underwater outboard motor noise (1 to 10 KHz) was used to repel carp that approached to within 1 m of the channel. The complex sound was effective in stopping schools of silver and bighead carp and a combined school of each species. Repulsion rates were 81.6%, 94.4% and 90.5% for silver carp, bighead carp, and mixed species respectfully. This study demonstrates that complex sound is effective in deterring fish movement and could be used to deter carp from entering strategic waterways.

The fish swam slowly through the pond in loose schools and transited readily from the north and south end in the absence of sound (Fig. 8) crossing the barrier approximately every three to five minutes. However, when confronted with sound after entering the reaction zone, the majority of fish turned away and did not cross the barrier (Fig. 8).

Silver carp averaged significantly (Kruskal-Wallis, P = 0.002) fewer attempted crossings per minute during each of the five periods compared to bighead carp or the mixed schools (0.30; 0.20, 0.42). However, there was no significant difference for attempted crossings per minute during the five intervals within any of three groups (Kruskal-Wallis: silver, P=0.66; bighead, P= 0.62; mixed, P= 0.11).

Sound playbacks significantly decreased the number of successful crossing for each group. Figure 9 shows the number of successful crossings per minute during the control (non sound) and sound intervals. All groups showed a significant decrease in the number of successful crossing attempts when challenged with sound (Mann-Whitney, P<0.001). For silver carp, successful crossings decreased significantly (Mann-Whitney, P< 0.001). Bighead carp also showed a significant decline (Mann-Whitney, P< 0.001). The mixed schools also were significantly inhibited from crossing (Mann-Whitney, P< 0.001).

Sound playbacks were successful in stopping fish transiting through the barrier in all three groups with 81.6%, 94.4% and 90.5% repulsion rates for silver carp, bighead carp, and mixed species, respectfully. The first trials were the most successful with sound stopping the silver carp during all 13 attempts during the first sound period for the three groups. Success rates dropped slightly during subsequent trials before rebounding to 91% during trial 5. Bighead carp were less likely to cross the sound barrier with five of the six trials achieving 100% repulsion with 85 out of a total of 90 attempts repulsed. The mixed school also displayed sound avoidance behavior with high (> 90%) repulsion rates observed until the last trial.

The fishes reacted relatively quickly to the sound onset. During successful repels, silver carp exited the reaction zone in a median time of 5.0 sec (3.0, 11.3) while bighead and mixed schools were significantly faster Mann Whitney P < 0.001)with the same median times of 3.0 (2.0, 4.0) sec with less variability.

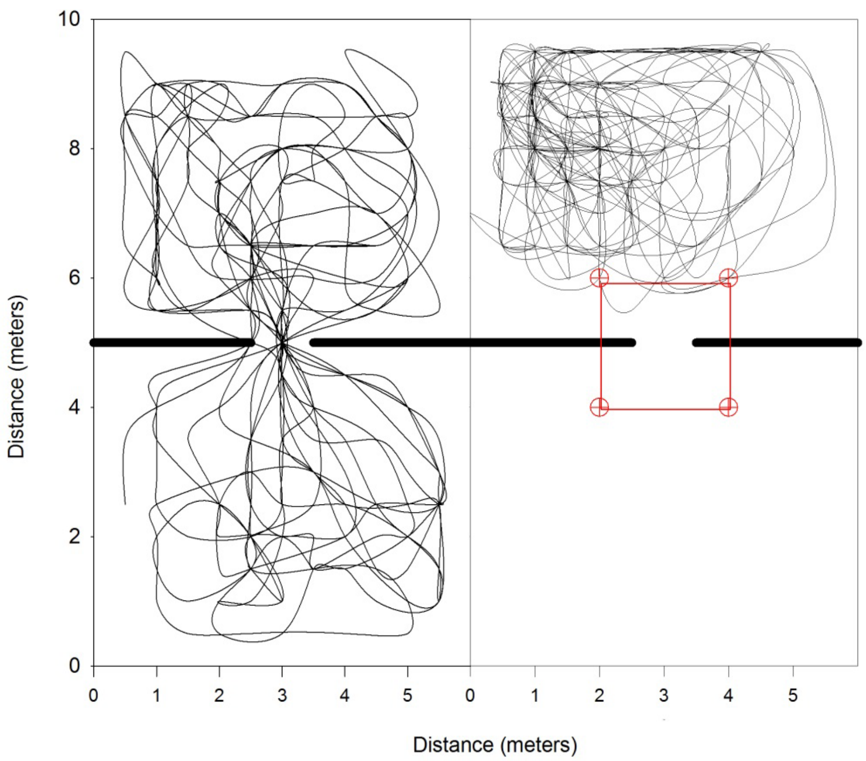


Figure 10. The effect of complex sound on fish movement. The position of a mixed school of bighead and silver carp is plotted in the horizontal axis while inside a partitioned concrete tank. Fish position was recorded every 5 seconds. During the control period (left), speakers were inactive and fish swam throughout the tank. During the experimental trials (right), complex sound was broadcast every time the fish entered the reaction zone (red box).

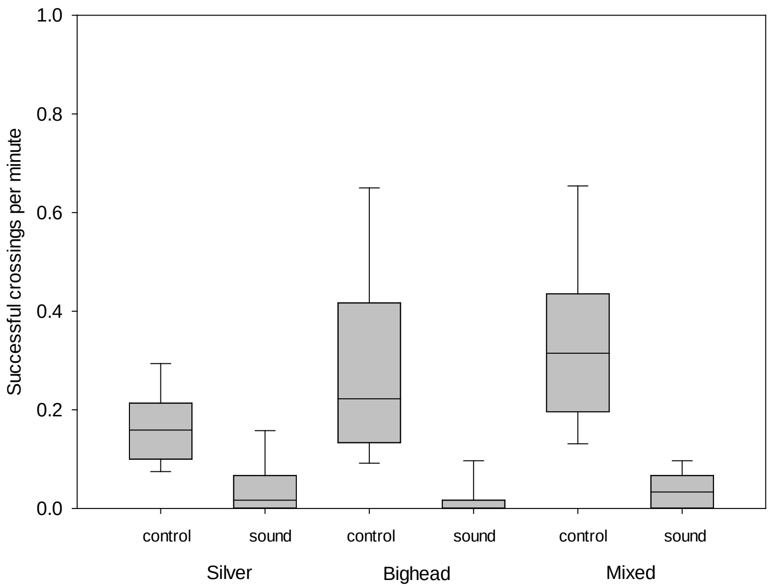


Figure 11. The average number of crossing per minute is plotted during control (no sound) and sound trials for silver, bighead and mixed schools.

Field trials were initiated in Morris Illinois this summer. Both silver and bighead carp were tagged and placed in a partitioned sand pit. A small channel was made with nets in the middle of the waterway and six speakers were suspended from floats. Complex sound was broadcast continually from the speakers and the tagged carp placed on either side of the channel. Controls consisted of an additional group of tagged fish placed under the same conditions with the speakers turned off. Three sets of both experimental and control fish were tested. The fish movements were monitored for 48 hrs. The fish tracking movements are still being processed however preliminary indications suggest that fewer fish remained in the area when the speakers were active.

Activity Status as of 1/15/2016

The field trials were further analyzed from Morris Illinois. There were several reasons why experiment was not conducted under optimal settings. Previous trials investigating water guns observed fish almost immediately escaping the enclosure. Therefore, fish were tagged and immediately subjected to the sound instead of being acclimated for two days. Additionally, the sound field was increased to cover the far reaches of the enclosure and this resulted in very high sound pressure fields throughout the enclosure and few “quiet” areas for the fish to escape the noise. Despite these issues, the trials showed promise with preliminary analysis indicating fewer fish crossing the barrier and movement limited when the sound was broadcast. The fish appeared to find the quietist area of the enclosure and remain there throughout the experiment. Control studies showed the fish more evenly distributed and crossed the inactive barrier more often. Preliminary results also indicated that fish crossing during sound was in different areas then during non sound events with subsequent sound mapping suggesting that the few fish that crossed the barrier were exploiting low sound pressure areas. It is hypothesized that a more uniform sound field will increase success rates. We are currently designing our speaker array to provide a more uniform field.

We are incorporating the information learned from the field trial in Morris to scale up to larger ponds in LaCrosse. We are expanding into 1/10 and ½ acres ponds and attempting to create “lock and dam” like structures to understand how the sound works with hard substrates and if the sound is as effective in the large ponds as it is in the smaller ones. We will also be testing the sound habituation. Additionally, the Morris field site may not be available this in the summer of 2016 and we are working with the USGS to test other open water sites such as Starved Rock Lock and Dam or Brandon Roads Lock and Dam on the Illinois River

Activity Status as of 7/15/2016

Figure 12: Closeup of barrier in ½ acre pond showing channel with speakers

We have set up 3 1/10 acre and 1 ½ acre pond at the USGS center in LaCrosse, WI. Both silver and bighead carp are tagged and placed in the ponds. There is a hydroacoustic array to determine the position of the fish. The ponds are divided into two sections with a channel equipped with speakers connecting the two halves. Sound is played via the speakers and the behavior and the position of the carp is monitored. Sound is played throughout the 24 hr periods. We are conducting long duration trials to determine if the fish habituate to the sound and varying the sound pressure levels to determine what is the most effective stimulus. Trials will continue throughout the fall until weather conditions force pond closure. Sound mapping has been completed and we are currently putting together the sound map

The initial trials in the 1/10 acre ponds have just been completed and we are currently analyzing the data. In the first pond, there were many crossing when the sound was off but very limited number of crossing when the sound was on. Preliminary data is shown for trials through day 4 that were observed with video camera. All the trials will be analyzed with positional tag information however the figure shows the preliminary results from the cameras. Two ponds were analyzed through day 4. There were 40 crossing when sound was off and only 4 crossing (all during the same trial) when sound was on. The sound appears to be effective at preventing crossings at least during the daylight hours and fish are not habituating to the sound.

Sound mapping is starting in the ½ acre ponds with trials set to start in early August. The speakers will project different pressure levels than in the 1/10 acre ponds and habituation trials are set to run for longer periods of time.   Both silver and bighead behavior will be monitored in response to difference frequencies, durations and intensities

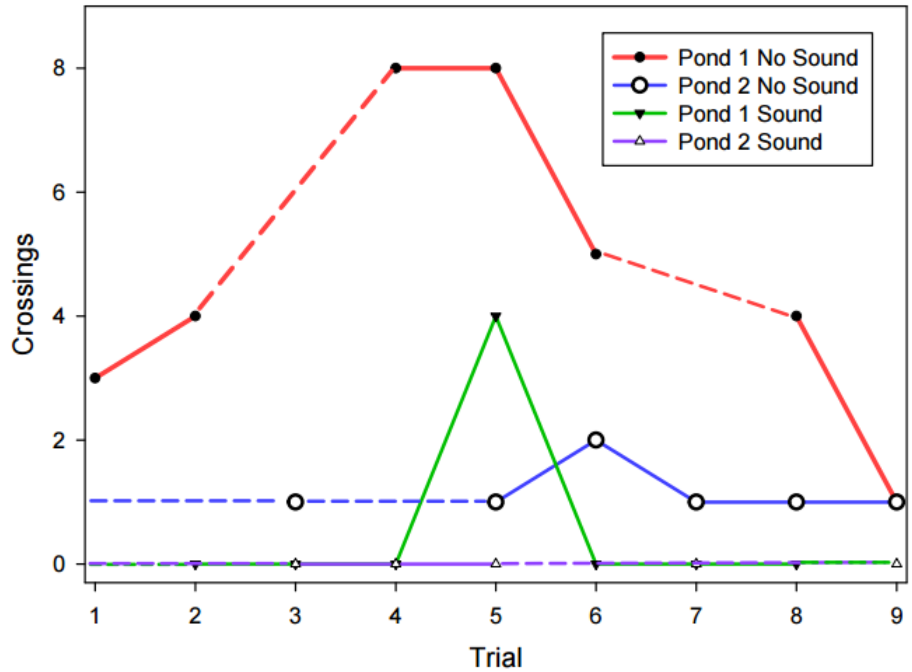


Figure 13. Preliminary data from the 1/10 acre ponds. Trials were conducted over 4 days. Only during one trial did carp cross when sound was on.

Figure 14. Barrier at Emiquon preserve

We have also been invited at no additional cost to the project, to consult with using sound at a barrier connecting the Emiquon reserve with the Illinois River. The Nature Conservancy would like to determine if sound can repel carp from entering from the river into the reserve when the water is flowing out. The Conservancy has purchased their own equipment and Mensinger is providing consultation. The site is just a few miles away from the Spoon River where the fall field studies will be conducted. The Conservancy hosted Mensinger for a site visit and he has designed an experiment where speakers will be placed in the structure shown in Figure 13 to assess whether they are effective in preventing carp egress. The structure contains two parallel channels and sound will be played in one channel with the other acting as a control.

The equipment has been purchased this summer and will be installed with Mensinger serving as a consultant in August. Water flow in and out of the reserve is dependent on river levels so the earliest tests will be Fall of 2016 or spring of 2017

Activity Status as of 1/15/2017

Three 1/10 acre ponds were equipped with barriers with a small channel connecting the two halves. Underwater speakers were set close to each barrier. The acoustically deterrent was cycled on and off similar to a lock/dam opening closure schedule of 30 min on (open) 90 min off (closed). All fish were fitted with acoustical tags. Preliminary results use underwater video cameras showed clear repulsion of the carp during the first 24 hrs. The acoustic tags had issues with battery life and many of them failed during the experiment. The USGS is currently analyzing the data to determine if any additional information can be obtained from the tags.

The barrier at the Emiquon became fully operational this fall. Speakers have been installed in both raceways and preliminary studies showed them to be operational. Water was released from the Emiquon into the Illinois River and sound was played during this time to deter fish from the river swimming into the Emiquon. Additional work will be needed to deploy the proper sonar (USGS) in the area to more accurately count the fish however few in any silver or bighead carp were positively identified in the area during the sound playback. Additional trials are planned in 2017 when water again is released from the Emiquon.

Activity Status as of 7/15/2017

In conjunction with the USGS, we initiated several month long trials on their LaCrosse campus. The 2016 trails were compromised by battery issues with the tags so the trials were repeated.

Three 1/10 acre ponds were equipped with barriers with a small channel connecting the two halves. Underwater speakers were set close to each barrier. The acoustically deterrent was cycled on and off similar to a lock/dam opening closure schedule of 30 min on (open) 90 min off (closed). All fish were fitted with acoustical tags. The preliminary results showed that fish were dispersed to areas away from the sound. Data analysis of exact fish position is currently being performed by the USGS.

We also attempted to determine the effect of sound exposure on fish reaction to the acoustic deterrent. We exposed fish to either 30 mins or 24 hours of sound and repeated the “ping-pong” experiments where we tried to move both control fish and experimental back and forth across the tank using sound. While the first weeks of experiments indicated that the sound exposed fish were less responsive, the results were compromised by lack of fish movement in both the controls and the experimental fish during the rest of summer. This was surprising as most of the time the fish would continuously school in the tank and change behavior when confronted with sound. It is hypothesized that as the control fish did not show steady swimming, they were sick and therefore we were unable to show a difference between experimental and control fish.

However, to optimize the acoustic deterrents, we initiated an experiment using Auditory Evoked Potentials that determined the hearing range of the silver and bigheaded carp. We found that the fish can detect higher frequencies than previously reported and we will be adjusting our acoustic deterrent to maximize the power in these frequencies to optimize the acoustic deterrent.

Activity Status as of 3/01/2018

We have continued to use Auditory Evoked Potentials to understand carp hearing abilities and optimize sound for deterrents in partnership with the USGS. These experiments have been performed at no additional cost of the grant however will allow us to better optimize sound deterrents.

The Auditory Evoked Pontentail or AEP is an external, minimally invasive method to measure hearing. Electrodes are inserted on the fish's head and the gross electrical potential of the inner ear and brain is measured in response to sound. This is somewhat analogous to the EKG that monitors electrical activity of the heart. We have used AEPs to determine the sensitivity of the carp auditory system to sound as well as examine the effect of sound exposure on hearing threshold.

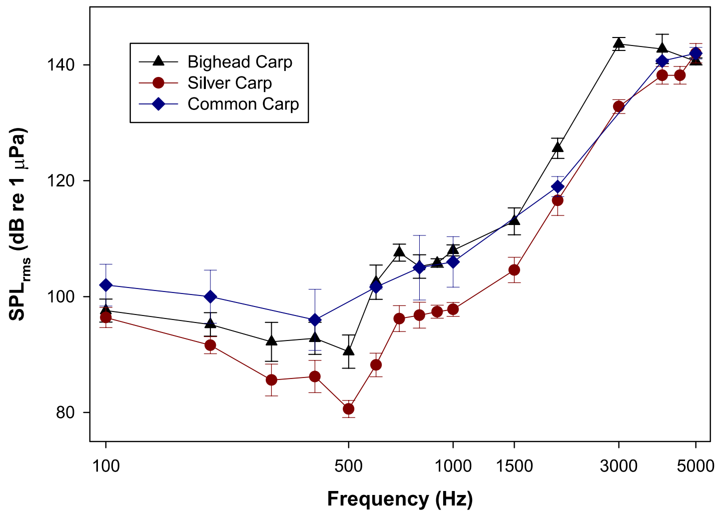


Figure 14

Figure 14 shows the hearing sensitivity of bigheaded carp. Previous work had indicated that hearing threshold only extended to 3 kHz however the AEP show that the hearing range extends to at least 5Khz. This has allowed us to modify our deterrent to produce greater energies in over a small frequency range.

We also investigated the effects of prolonged sound exposure on the bigheaded carp. There needs to be a balance between the intensity and duration that causes repulsion but not hearing loss. Reductions in hearing sensitivity will make the acoustic deterrents less effective. Figure 15 shows that even 30 minutes of sound exposure reduces hearing sensitivity in the carp while 24 hr exposure causes prolonged hearing loss. As this deterrents may work best at a Lock and Dam structure, we are examining the opening and closing of the lock chambers to outline a plan of sound duration that will optimize deterrence while minimizing hearing damage.

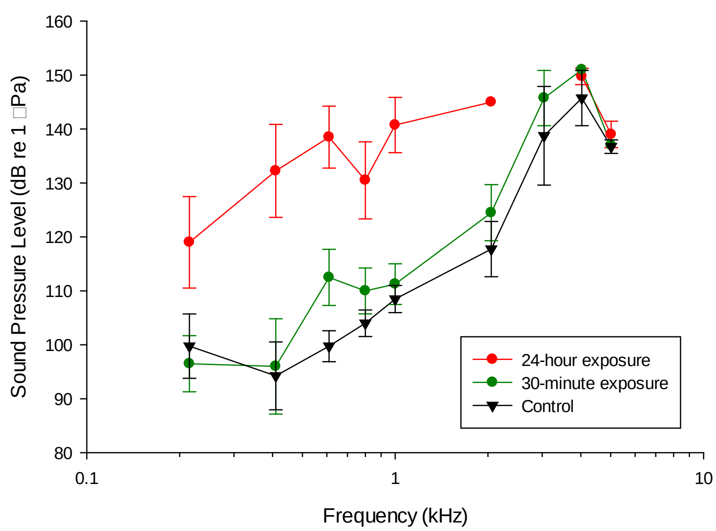


Figure 15

Final Report Summary: Broadband sound was effective in deterring carp from entering a small channel in a concrete pond. Additional experiments characterized the hearing frequency of the bigheaded carp to allow future acoustic deterrents to be optimized against the fish. Preliminary studies show the effect of extended sound exposure on hearing thresholds and indicated that the intensity of sound needs to be balanced against potential hearing damage.

Acoustic barrier:

The effectiveness of an acoustic barrier to deter the movement of silver carp and bighead carp, was evaluated. A pond (10mx5mx1.2m) was divided in half by a concrete-block barrier with a channel (1m across) allowing fish access to each side. Underwater speakers were placed on each side of the barrier opening, and an outboard motor noise (broadband sound; 0.06-10kHz) was broadcast to repel carp that approached within 1m of the channel. Broadband sound was effective at reducing the number of successful crossings in schools of silver carp, bighead carp and a combined school. Repulsion rates were 82.5% (silver carp), 93.7% (bighead carp) and 90.5% (combined). This study demonstrates that broadband sound is effective in deterring carp and could be used as a deterrent in an integrated pest management system.

Bigheaded carp hearing:

Controlling bigheaded carp is a priority of fisheries managers and one area of focus involves developing acoustic deterrents to prevent upstream migration. For an acoustic deterrent to be effective however, the hearing ability of bigheaded carp must be characterized. A previous study showed that bigheaded carp detected sound up to 3 kHz but this range is narrower than what has been reported for other ostariophysans. Therefore, silver and bighead carp frequency detection was evaluated in response to 100 Hz to 9 kHz using auditory evoked potentials (AEPs). AEPs were recorded from 100 Hz to 5 kHz. The lowest thresholds were at 500 Hz for both species (silver carp threshold: 80.6 +/- 3.29 dB re 1 mu Pa SPL_{rms}, bighead carp threshold: 90.5 +/- 5.75 dB re 1 mu Pa SPL_{rms}; mean +/- SD). These results provide fisheries managers with better insight on effective acoustic stimuli for deterrent systems, however, to fully determine bigheaded carp hearing abilities, these results need to be compared with behavioral assessments.

V. DISSEMINATION:

Description: All results of the study will be published in peer reviewed publications. Mensinger and the graduate student will present the results at the appropriate state, regional and national meetings. Mensinger will develop a web page that will contain information, pictures and video of the experiments and results to provide wide dissemination. The USGS will also place information and material about the project on their web site. Mensinger also will be available to consult (at no charge) for the appropriate end users of this technology such as local, state and federal agencies including the MN DNR for the duration of the grant. Mensinger will develop a web page that will have video of carp jumping behavior and the sound deterrent experiments. The web page will also provide updates on the progress of the experiments and incorporate appropriate tables and graphs.

Status as of 1/15/2015

A manuscript is currently under review in the journal Biological Invasions entitled "Acoustical Deterrence of Silver Carp (*Hypophthalmichthys molitrix*)"

An oral presentation was delivered by Brooke Vetter at the American Fisheries Society meeting in Quebec in August describing acoustic control of carp movement.

Status as of 7/15/2015

A manuscript currently under review in the journal Biological Invasions entitled "Acoustical Deterrence of Silver Carp (*Hypophthalmichthys molitrix*)" has received provisional acceptance

A manuscript tentatively entitled "Bioacoustic deterrence of silver and bighead carp" is currently undergoing internal review at USGS with plans for a Fall 2015 submission to a peer reviewed journal

Status as of 1/15/2016

The following manuscript has been published. This was before LCCMR support however it provided the basis for subsequent LCCMR funded experiments:

Acoustical deterrence of Silver Carp (*Hypophthalmichthys molitrix*) Vetter, Brooke J.; Cupp, Aaron R.; Fredricks, Kim T.; Gaikowski, Mark; Mensinger, Allen F. (2015) BIOLOGICAL INVASIONS 17:3383-3392

The following manuscript "The effect of broadband sound on the movement of bighead carp (*Hypophthalmichthys nobilis*)" has been submitted, reviewed and is currently under revision for the Journal PLOS ONE

The manuscript entitled "Potential implications of acoustic stimuli as a non-physical barrier to silver (*Hypophthalmichthys molitrix*) and bighead carp (*H. noblis*)" by Murchy, Kelsie; Cupp, Aaron; Amberg, Jon; Vetter, Brooke; Fredricks, Kim; Gaikowski, Mark; Mensinger, Allen, has been successfully submitted and is presently being given full consideration for publication in Fisheries Management and Ecology.

Allen Mensinger gave an oral presentation, " Grounding the flying carp: Applied neuroethology" and the Society of Integrative and Comparative Biology in Portland Oregon. December 2015

Graduate student Brooke Vetter gave an oral presentation, "Silver Carp *Hypophthalmichthys molitrix* behavior and bioacoustics" and graduate student Kelsie Murchy gave an oral presentation "The effect of temperature on acoustical deterrence of bighead *Hypophthalmichthys nobilis* and Silver Carp *H. molitrix"* at the Midwest Fish and Wildlife Conference in Grand Rapids, MI January 2016.

Status as of 7/15/2016

The following talks were presented at the Invasive Aquatic Species conference

Bigheaded Carp Behavior and Bioacoustics: Brooke J. Vetter, University of Minnesota Duluth
The Effect of Temperature on Acoustical Deterrence of Bighead (*Hypophthalmichthys nobilis*) and Silver Carp (*H. molitrix*): Kelsie A. Murchy, University of Minnesota Duluth
In situ Observations of Silver Carp Behavior when Presented with Broadband Sound: Allen F. Mensinger, University of Minnesota Duluth

The following talks were presented at the Effects of Noise on Aquatic Life conference

Effect of Outboard Motor Sound on Invasive Silver Carp (Hypophthalmichthys molitrix) Jumping Behavior: Brooke J. Vetter

Effects of Anthropogenic Sound on Native and Invasive Fish Behavior in the Upper Mississippi River: Kelsie A. Murchy

The Mensinger lab presented on the results of the project to date at the Sound Deterrent workshop hosted by the USGS in LaCrosse, WI.

The manuscript entitled "Potential implications of acoustic stimuli as a non-physical barrier to silver (Hypophthalmichthys molitrix) and bighead carp (H. noblis)" by Murchy, Kelsie; Cupp, Aaron; Amberg, Jon; Vetter, Brooke; Fredricks, Kim; Gaikowski, Mark; Mensinger, Allen, is under review in Fisheries Management and Ecology.

The manuscript "The effect of broadband sound on the movement of bighead carp (Hypophthalmichthys nobilis) is currently under review at the Journal of Great Lakes Research

Status as of 1/15/2017

The results of our study were broadly disseminated with talks at national and international meetings. The Minnesota Environmental Trust Fund was acknowledged in each paper and presentation. Five full publications including one in press and a published abstract were published during this time period. Presentations were made at the Effect of Noise on Aquatic Life Conference and the Society of Integrative and Comparative Biology. Many of the figure and graphs had been already included in previous reports.

Mensinger, A. F. (2016). Grounding the flying carp: Applied neuroethology. *Integrative and Comparative Biology* 56, E147-E147.

Vetter, B.J., Caspar, A.F. and Mensinger, A.F. (2017). Characterization and management implications of silver carp (*Hypophthalmichthys molitrix*) jumping behavior in response to motorized watercraft *Management of Biological Invasions* 8:113-124.

Vetter, B., Murchy, K., Cupp, A., Amberg, J., Gaikowski, M. and Mensinger, A. (2017a). Acoustic deterrence of bighead carp (H*ypophthalmichthys nobilis)* to a broadband sound stimulus. *Journal of Great Lakes Research* 43.

Murchy, K.A., Cupp, A. R., Amberg, J.J., Vetter, B.J., Fredricks, K.T. Gaikowski, M.P. and Mensinger, A. F. Potential implications of acoustic stimuli as a non-physical barrier to silver carp and bighead carp (2017) *Fisheries Management and Ecology*. In press

Vetter, B.J. and Mensinger A.F. (2016). Broadband sound can induce jumping behavior in invasive silver carp (Hypophthalmichthys molitrix) Proceedings of Meetings on Acoustics 27, 010021 (2016); http://doi.org/10.1121/2.0000279

Murchy, K.A., Cupp, A. R., Amberg, J.J., Vetter, B.J., Fredricks, K.T. Gaikowski, M.P. and Mensinger, A. F. (2017). Not all carp are created equal: Impacts of broadband sound on common carp swimming behavior Proceedings of Meetings on Acoustics 27, 010032 (2016); http://doi.org/10.1121/2.0000314

Status as of 7/15/2017

Vetter, Brooke J.; Calfee, Robin D.; Mensinger, Allen F. (2017) Management implications of broadband sound in modulating wild silver carp (Hypophthalmichthys molitrix) behavior. MANAGEMENT OF BIOLOGICAL INVASIONS Volume: 8 Issue: 3 Special Issue: SI Pages: 371-376 Published: SEP 2017

Status as of 3/1/2018

The carp hearing work was presented at the MN AFS meeting in February by graduate student Andy Nissen.

Dr. Brooke Vetter (former graduate student in the lab) presented her carp work at the Society for Integrative Biology meeting in San Francisco in January.

Dr. Vetter also submitted a paper entitled "Reexamining the frequency range of hearing in silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp" that is currently undergoing its second revision.

Allen Mensinger attended the Carp Workshop in Bloomington, MN in the fall and summarized the acoustic studies.

Final Report Summary:

Current (7/18) list of publications pertinent to the study

Murchy, K. A., Cupp, A. R., Amberg, J. J., Vetter, B. J., Fredricks, K. T., Gaikowski, M. P. and Mensinger, A. F. (2017). Potential implications of acoustic stimuli as a non-physical barrier to silver carp and bighead carp. *Fisheries Management and Ecology* **24**, 208-216.

Vetter, B., Murchy, K., Cupp, A., Amberg, J., Gaikowski, M. and Mensinger, A. (2017b). Acoustic deterrence of bighead carp (H*ypophthalmichthys nobilis)* to a broadband sound stimulus. *Journal of Great Lakes Research* **43**.

Vetter, B. J., Brey, M. K. and Mensinger, A. F. (2018). Reexamining the frequency range of hearing in silver (Hypophthalmichthys molitrix) and bighead (H. nobilis) carp. *Plos One* **13**.

Vetter, B. J., Calfee, R. D. and Mensinger, A. F. (2017c). Management implications of broadband sound in modulating wild silver carp (Hypophthalmichthys molitrix) behavior. *Management of Biological Invasions* **8**, 371-376.

Vetter, B. J., Casper, A. F. and Mensinger, A. F. (2017d). Characterization and management implications of silver carp (Hypophthalmichthys molitrix) jumping behavior in response to motorized watercraft. *Management of Biological Invasions* **8**, 113-124.

Vetter, B. J., Murchy, K. A., Cupp, A. R., Amberg, J. J., Gaikowski, M. P. and Mensinger, A. F. (2017e). Acoustic deterrence of bighead carp (Hypophthalmichthys nobilis) to a broadband sound stimulus. *Journal of Great Lakes Research* **43**, 163-171.

Vetter, B. J., Cupp, A. R., Fredricks, K. T., Gaikowski, M. P. and Mensinger, A. F. (2015). Acoustical deterrence of Silver Carp (Hypophthalmichthys molitrix). *Biological Invasions* **17**, 3383-3392.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 195,400	Salary is budgeted for the Principal investigator (0.55 FTE total for three years) , two graduate students (3.25 FTE total for three years) and two undergraduate students (0.49 FTE total for three years) for the project

Equipment/Tools/Supplies:	$34,500	Funds are budgeted to build the early warning system and the sound arrays for carp movement and deterrence
Other: Travel to LaCrosse WI and Havana, IL	$32,100	Out of state travel is necessary to combat the invasive carp before they become established in MN. The outdoor tank and pond studies will take place in LaCrosse, WI and the field trials will take place in Havana, IL. Rates are based on University of MN travel plan rates
TOTAL ENRTF BUDGET:	**$262,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:
4.29

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
Integrated Biological Sciences graduate program (cash)	$ 9,516	$9,516	The Integrated Biological Sciences graduate program will provide summer salary match for the graduate student budgeted in the project (pending)
UMD (cash)	8,400	4,500	Undergraduate research opportunities grants from UMD to further support undergraduate research in this proposal (pending)
UMD (cash)	5,500	5,500	Pilot grants were obtained from UMD to support preliminary data collection
Mensinger Salary (In-kind)	79,324	79,324	Two months of academic year salary will be provided as in kind support for the proposal (secured)
USGS (In-kind)	10,000	10,0000	Access to silver and big head carp, outdoor tank and pond use (secured)
INHS (In-kind)	9,000	9,000	Boats and personnel for field studies on the Spoon River
TOTAL OTHER FUNDS:	**$126,650**	**$114,340**	

VII. PROJECT STRATEGY:

A. Project Partners:

1) Professor Allen Mensinger of the University of Minnesota Duluth will supervise all aspects of the project. He and UMD will receive $253,000 from the appropriation

2) Mark Gaikowski, USGS, Lacrosse Wisconsin. The PI will work closely with Mark Gaikowski throughout the project. The USGS is providing the outdoor tanks and ponds, fish and support personnel at no cost to the grant. Mensinger and Gaikowski will develope the experimental protocols, train the students, analyze the data and be responsible for dissemination of the work. Mr. Gaikowski will not receive any funds from the appropriation.

3) Illinois Natural History Survey. The INHS will provide boats and personnel for the field trials planned in Havana, IL. This assistance has been changed to an in-kind contribution.

B. Project Impact and Long-term Strategy:

Since their introduction in the southeastern US, silver and bighead head carp have migrated north into the upper Mississippi Valley and pose severe ecological consequences to native Minnesota fish. Currently, the only barriers to carp are large dams or expensive electrical barriers. Based on the carp's avoidance or jumping to boat motors, we propose to use bioacoustics to 1) develop early warning systems 2) herd carp for capture and 3) develop acoustical deterents. As the sound stimulus is well above the hearing threshold of most native fish, it is unlikely to harm the native population. Bioacoustical deterrence is inexpensive, environmentally friendly and portable and can be used both in small streams and larger lakes.

The strategy is to develop bioacoustic early warning and deterent systems and perform controlled tests in outdoor tanks and ponds to develop the optimal sound intensities and frequencies for carp management. The equipment will be then field tested on wild carp population in the Illinois River. The technology will be made available to interested management agencies as part of an integrated pest management strategy for controlling carp.

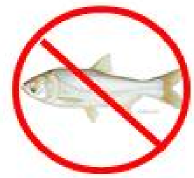
C. Spending History: M.L. 2010 Chp. 362, Sec. 2, Subd 6d. – Bioacoustic traps for Management of Round Goby. This project was related to sound work on invasive species and much of the hardware will be used for the carp study.

Funding Source	**M.L. 2008 or FY09**	**M.L. 2009 or FY10**	**M.L. 2010 or FY11**	**M.L. 2011 or FY12-13**	**M.L. 2013 or FY14**
LCCMR			175,000		

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S):

Bioacoustics to detect, deter and eliminate flying carp



1) Sound makes carp jump

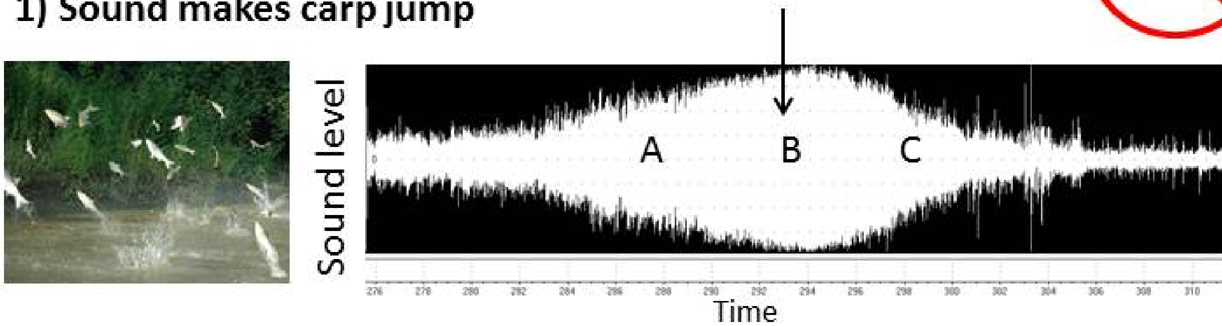


Figure 1. Field recording of underwater outboard motor noise motor boat noise that stimulated carp to jump in the Illinois River as boat passed by designated recording area. Arrow indicates when boat was in center of area. A) initiation of jumping; B) peak jumping; C) cessation of jumping.

2) Different sounds and boat speeds will effect carp jumping

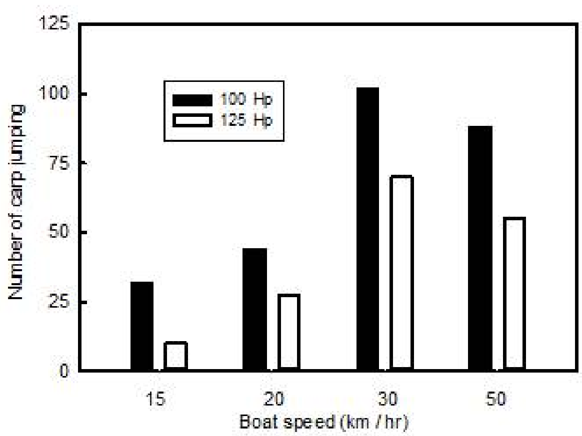


Figure 2. Number of carp jumping vs boat speed (km / hr) and engine size (hp).

3) Sound will repel carp

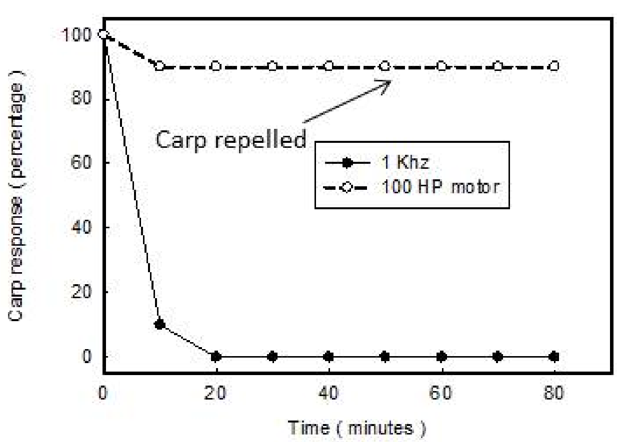


Figure 3. Percentage of carp (N=10) repelled by a 1 KHz pure tone sound and 100 hp outboard motor sounds versus time (min).

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: N/A

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 15, 2015; July 15, 2015; January 15 , 2016; July 15, 2016; and January, 15 2017. A final report and associated products will be submitted between June 30 and August 15, 2018.

Environment and Natural Resources Trust Fund
M.L. 2014 Project Budget

Project Title: Bioacoustics to detect, deter and eliminate flying carp
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 04b
Project Manager: Allen F. Mensinger
Organization: University of Minnesota Duluth
M.L. 2014 ENRTF Appropriation: $ *262,000*
Project Length and Completion Date: 4 Years, June 30, 2018
Date of Report: 8/20/2018

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget revised	Amount Spent	Activity 1 Balance	Budget revised 2-10-	Amount Spent	Activity 2 Balance	Budget revised	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	*Early warning*			*Bioacoustical monitoring*			*Carp deterence*				
Personnel: **PI Allen F. Mensinger PhD.** The PI has a 9 month position at the University of Minnesota Duluth. A total of 3 months of summer salary (1 month /yr) is budgeted. The PI will be on sabatical during the 2014-15 academic year which is a 50% salary appointment. Two months of salary is budgeted for this period to work on the project. Total salary reflects 5 months total over 3 yrs and reflects 74.8% salary and 25.2% fringe (.55 FTE). Estimated total ($67,218) **Graduate student** - support is budgeted for 30 months of support for one graduate student. Total reflects 57.3% salary and 42.7% fringe (2.5 FTE). Estimated salary ($103,471). **Graduate student summer salary**. 50% summer salary is budgeted for an additional graduate student for 3 summers (total 4.5 months) 80.6% salary and 19.4% fringe (.75 FTE). Estimated salary ($12,711). **Undergraduate student summer salary:** 2 months summer salary is budgeted for two undergraduate students each summer (total 12 months) 93.1% salary and 6.9% fringe (.49 FTE). Estimated salary ($12,000).	$65,134	$64,288	$846	$65,133	$65,133	$0	$65,133	$63,671	$1,462	$195,400	$2,308
Equipment/Tools/Supplies: Bouy or floating platform for early warning system plus floats, mooring lines, cables and materials	$7,500	$7,500	$0							$7,500	$0
Equipment/Tools/Supplies: Two amplifiers for underwater speakers arrays @$1000	$2,000	$2,000	$0	$0	$0	$0				$2,000	$0
Equipment/Tools/Supplies: Wireless video cameras, digital video recorders and DC power supplies for filming carp jumping from bouy or boats	$2,500	$2,500	$0	$2,500	$2,500	$0				$5,000	$0
Equipment/Tools/Supplies: Electronics supplies including cables, wireless routers, camera and underwater speaker and control units for remote operation of early warning system, sound exhaustion and deterent **systems**	$5,000	$5,000	$0	$9,500	$9,500	$0	$4,500	$4,500	$0	$19,000	$0
Equipment/Tools/Supplies: Fish food and water testing kits for carp in captivity	$500	$500	$0	$250	$250	$0	$250	$250	$0	$1,000	$0

Other: Out of state travel: Travel to the Illinois Biological Research Station in Havanna, IL. This out of state travel is essential to the project as it allows us to test the equipment and strategies in carp infested water. We will travel in spring, summer and fall for one week each. Car ($620 per trip based on 1100 miles RT @ $0.565 per mile), lodging ($77 per night) and meals ($46 per day) based on Univeristy of Minnesota travel plan rates = $861 per person per week. 9 weeks total for grant with two people each week.	$7,000	$7,000	$0	$7,000	$7,000	$0	$7,000	$7,000	$0	$21,000	$0
Other: Out of State Travel: Travel is requested to the USGS facility in Lacrosse, WI to monitor carp behavior in outdoor ponds and test equipment. This out of state travel is essential for the project as these are the only large and outdoor secure ponds that house silver carp that are available for this research. The graduate student will spend approximately one month in residence at the facility each year to compelete the experiments. Car ($283 per trip based on 500 miles RT). Lodging and meals are $861 per week and 4 weeks are anticipated each year. All rates are based on University of Minnesota travel plan rates.	$3,700	$3,700	$0	$3,700	$3,700	$0	$3,700	$3,700	$0	$11,100	$0
COLUMN TOTAL	**$93,334**	**$92,488**	**$846**	**$88,083**	**$88,083**	**$0**	**$80,583**	**$79,121**	**$1,462**	**$262,000**	**$2,308**

DOI: 10.1111/fme.12220

ORIGINAL ARTICLE

WILEY Fisheries Management and Ecology

Potential implications of acoustic stimuli as a non-physical barrier to silver carp and bighead carp

K. A. Murchy[1,2] | A. R. Cupp[2] | J. J. Amberg[2] | B. J. Vetter[1] | K. T. Fredricks[2] | M. P. Gaikowski[2] | A. F. Mensinger[1]

[1]Biology Department, University of Minnesota Duluth, Duluth, MN, USA

[2]U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI, USA

Correspondence
Allen Mensinger, Biology Department, University of Minnesota Duluth, Duluth, MN, USA.
Email: amensing@d.umn.edu

Funding information
USGS Ecosystem Mission Area Invasive Species Program; Minnesota Environment and Natural Resources Trust Fund; US Environmental Protection Agency Great Lakes Restoration Initiative

Abstract

The effectiveness of an acoustic barrier to deter the movement of silver carp, *Hypophthalmichthys molitrix* (Valenciennes) and bighead carp, *H. nobilis* (Richardson) was evaluated. A pond (10 m × 5 m × 1.2 m) was divided in half by a concrete-block barrier with a channel (1 m across) allowing fish access to each side. Underwater speakers were placed on each side of the barrier opening, and an outboard motor noise (broadband sound; 0.06–10 kHz) was broadcast to repel carp that approached within 1 m of the channel. Broadband sound was effective at reducing the number of successful crossings in schools of silver carp, bighead carp and a combined school. Repulsion rates were 82.5% (silver carp), 93.7% (bighead carp) and 90.5% (combined). This study demonstrates that broadband sound is effective in deterring carp and could be used as a deterrent in an integrated pest management system.

KEYWORDS

behaviour, *Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis*, invasive species, management, sound

1 | INTRODUCTION

Silver carp, *Hypothalmichthys molitrix* (Valenciennes) and bighead carp, *H. nobilis* (Richardson; collectively known as bigheaded carps) were originally imported to the southern United States in the 1970s from eastern Asia to control algal growth in sewage treatment and fish farming facilities (Kolar et al., 2007). Their escape into the wild has resulted in detrimental environmental effects. The species' filter feeding ability, fast growth and high fecundity has allowed them to negatively impact adults of native fishes such as paddlefish, *Polyodon spathula* (Walbaum; Schrank, Guy & Fairchild, 2003), gizzard shad, *Dorosoma cepedianum* (Lesueur; Sampson, Chick & Pegg, 2009) and bigmouth buffalo, *Ictiobus cyprinellus* (Valenciennes; Irons, Sass, McClelland & Stafford, 2007) and the early life stages of most fishes. Furthermore, the resulting decline in the density of lower trophic level organisms or community shifts in zooplankton populations with increasing bigheaded carps populations has likely affected additional native aquatic species (Cooke, Hill & Meyer, 2009; Xie & Chen, 2001). There is an urgent need to create barriers and deterrents to prevent further bigheaded carps range expansion and protect the ecosystems in which carp are not present.

Non-physical barriers to deter or control fish movement were originally developed to reduce entrance into hydroelectric dams or power plants. These barriers target fish sensory (auditory, vision, olfactory or lateral line) or locomotion systems to deter passage through a defined area, and can consist of lights, bubbles, acoustic stimuli or electric fields (Noatch & Suski, 2012; Popper & Carlson, 1998). Unlike physical barriers, such as dams, non-physical barriers have minimal impacts on water flow or navigation and have been proposed to combat the movement of invasive fish (Noatch & Suski, 2012). Other than the electric barrier in the Chicago Sanitary and Ship Canal (near Lake Michigan, USA) and a constructed berm in the Eagle Marsh wetland near Fort Wayne, Indiana, USA, solid structure gravity dams (high head dams) are currently the only barriers slowing the upstream expansion of bigheaded carps and their potential colonisation of the Laurentian Great Lakes (Moy, Polls & Dettmers, 2011; Sass et al., 2010). To limit bigheaded carps range expansion, management agencies are evaluating the efficacy of non-physical barriers to deter invasive carp (Kelly, Engle, Armstrong, Freeze & Mitchell, 2011), with the idea that an integrated pest management system might provide the best approach.

Perhaps the most well-known non-physical barrier is the electric Aquatic Nuisance Species Dispersal Barrier in the Chicago Sanitary and Shipping Canal near Romeoville, Illinois, USA. The barrier was originally installed in 2002 to slow the downstream movement of round gobie, *Neogobius melanostomus* (Pallas), from the Great Lakes into the Illinois River (Moy et al., 2011; Sparks, Barkley, Creque, Dettmers & Stainbrook, 2010), but later improvements to the barrier were made with the goal of blocking the upstream expansion of bigheaded carps into Lake Michigan (Sparks et al., 2010). The electric field targets the neuromuscular junctions, causing temporary paralysis or death and can block fish movement (Lamarque, 1967, 1990). The electric dispersal barrier has been effective in a number of ways, including incapacitating 97%–100% of fish that attempted to pass and has limited the upstream movement of multiple species of fish (Parker et al., 2015; Sparks et al., 2010). However, it also has weaknesses, such as cost, need for continual power, danger to non-target species (including humans), potential ineffectiveness against small fish and disruption by metal-hulled barges (Dettmers, Boisvert, Barkley & Sparks, 2005; Moy et al., 2011; Noatch & Suski, 2012; Parker et al., 2015). During times of power disruption or maintenance, alternative systems are needed to block fish movement (Clarkson, 2004). These shortcomings preclude electric barrier installation in many waterways.

Studies have evaluated other non-physical barriers, such as light (Hamel, Brown & Chipps, 2008), sound (Taylor, Pegg & Chick, 2005; Vetter, Cupp, Fredricks, Gaikowski & Mensinger, 2015) and bubbles (Zielinski et al., 2014), to combat invasive fish species, with the understanding that combinations may be more effective than a single modality (Popper & Carlson, 1998; Welton, Beaumont & Clarke, 2002). For example, Atlantic menhaden, *Brevoortia tyrannus* (Latrobe), spot, *Leiostomus xanthurus* (Lacepède) and white perch, *Morone americana* (Gmelin) demonstrated greater avoidance of strobe lights combined with bubbles compared to either stimulus alone (McIninch & Hocutt, 1987). Patrick, Christie, Sager, Hocutt and Stauffer (1985) found that strobe lighting was more effective in deterring alewife, *Alosa pseudoharengus* (Wilson), smelt, *Osmerus mordax* (Mitchill) and gizzard shad than constant illumination; and a combined strobe light/bubble barrier maximised avoidance behaviour. Finally, bubble curtain barriers that generate 200 Hz sound reduced common carp, *Cyprinus carpio* (Linnaeus) crossing attempts (Zielinski et al., 2014).

In past studies, acoustic stimuli have been used to deter fish from approaching power plants or hydropower dams (Burner & Moore, 1953; Schilt, 2007). Frequencies ranging from 20 to 600 Hz reduced fish approaching a power plant (Maes et al., 2004), and ultrasound deterred (87% repels) alewife from entering a dam intake (Ross et al., 1993). More recently, sound is being examined as a barrier to fish movement (Lovell, Findlay, Moate, Nedwell & Pegg, 2005; Noatch & Suski, 2012; Popper & Carlson, 1998; Vetter et al., 2015).

To use sound as a non-physical barrier, silver carp and bighead carp need to perceive the sound, localise its origin and alter behaviour to avoid the sound. Grass carp, *Ctenopharyngodon idella* (Valenciennes; 600–1000 Hz; Willis, Hoyer, Canfield & Lindberg, 2002) and common carp (400 Hz; Sloan, Cordo & Mensinger, 2013) were classically conditioned to associate sound with feeding, which suggests that close relatives of bigheaded carps can localise sound. Silver carp and bighead carp have demonstrated the ability to detect and alter their behaviour due to sound. Pegg and Chick (2004) found that sound stimuli between 20 and 2000 Hz were more effective at preventing bigheaded carps from crossing an electric and sound barrier compared to frequencies between 20 and 500 Hz. Also, a combination of sound (20–2000 Hz) and bubbles repelled 95% of bighead carp in a shallow, narrow raceway (Taylor et al., 2005). Vetter et al. (2015) showed that complex or broadband sounds (0.06–10 kHz) were more effective than pure tones (500–2000 Hz) in repelling silver carp. Field tests combining sound (500–2000 Hz) with bubbles and strobe lights in a tributary of the Illinois River showed some promise, due to low recapture of bigheaded carps on the opposite side of the barrier, but many of the tagged fish moved out of the area and it is uncertain how many challenged the barrier (Ruebush, Sass, Chick & Stafford, 2012).

For field application, locks represent a key point for management of invasive species moving up or down the Mississippi River and could aid in preventing movement into new habitats. The goal of this study was to examine whether a complex, broadband sound (0.06–10 kHz) could block the movement of silver carp and bighead carp through a barrier, so that the potential for field application, specifically in locks, could be assessed.

2 | MATERIAL AND METHODS

2.1 | Animal husbandry

All experiments were conducted at the U S Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin, USA. Silver carp and bighead carp (18–24 cm TL) were maintained in 1500-L flow-through indoor rearing tanks and fed trout starter diet (Skretting, Tooele, UT, USA) at a rate of 0.5% body weight per day. Each experimental fish was tagged with a passive integrated transponder (PIT) tag (Biomark Inc, Boise, ID, USA) at least 1 week prior to experimentation. Prior to tagging, fish were sedated with 100 mg/L AQUI-S® 20E (10 mg/L eugenol, AQUI-S New Zealand Ltd., Lower Hutt, NZ) in the rearing tank. Fish were hand netted and placed in 300 mg/L AQUI-S® 20E (30 mg/L eugenol) until the fish lost equilibrium and did not move in response to a caudal peduncle pinch. A 1% iodine solution was applied to the injection sites, and a passive integrated transponder (PIT) tag was inserted into the abdomen about 2 cm anterior to the vent. Fish were placed in fresh flowing water to recover and segregated from non-tagged fish. To facilitate transport to the outdoor pond, fish were lightly sedated with 50 mg/L AQUI-S® 20E (5 mg/L eugenol) to minimise jumping, stress and potential injury. Food was withheld for 24 hr prior to transport and fish were not fed while in the outdoor pond (<7 days) to avoid food conditioning. Each group (n = 10) was allowed to acclimate in the pond for at least 48 hr prior to the initiation of experiments. This acclimation period allowed the fish to recover from the transport process and sedation. Previous studies suggest that 48 hr is more than enough time for fish to metabolise eugenol, as the compound was not detected in tissues and normal swimming behaviour resumed in <30 min in fish exposed to greater quantities for longer periods of time (Cupp et al., in press; Hikasa, Takase, Ogasawara & Ogasawara, 1986; Meinertz, Schreier, Porcher, Smerud & Gaikowski, 2014). Two- or three-day trials were conducted from July through August 2014. 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).

Experiments were conducted in a 10 m × 5 m × 1.2 m (55 m^3 at 1.1 m water depth) outdoor concrete flow-through pond. Water was pumped into the pond directly from UMESC wells, and the flow rate was adjusted to allow the water temperature to be 17°C ± 4°C. A 2-m wire fence enclosed each pond vertically with anti-bird netting draped across the top. Pond access was restricted via a door that remained locked throughout the experiment. The north side of the pond was partially shaded during the morning hours.

Two walls were constructed out of concrete blocks (0.4 × 2 × 1.2 m) and divided the pond into north and south halves. The concrete blocks extended perpendicular to the long axis of the pond with a 1-m gap in the middle of the barrier to allow passage. Water depth was maintained at 1.1 m, and the height of the barrier was 0.1 m above the water level. The pond was located outdoors, and trials were conducted in July and August to maintain water temperature within 17°C ± 4°C.

2.2 | Sound stimuli

Sound was delivered via one of two pairs of underwater speakers (UW-30, Lubell Labs Inc., Whitehall, OH, USA) that were placed 1 m from each end of the barrier opening, approximately 2 m from the nearest side wall. One HTI-96-MIN (High Tech Inc., Long Beach, MS, USA) hydrophone was placed in the middle of each end of the pond, 2 m from the end wall. The hydrophones monitored the sound stimulus, which was recorded using a PowerLab 4SP data acquisition system and LabChart 7 software (AD Instruments, Colorado Springs, CO, USA). Acoustic stimuli consisted of a 30-s broadband sound recorded underwater using a stationary hydrophone from a moving 6 m aluminium boat equipped with a 100 horsepower 4-stroke outboard motor (Yamaha, Fukuroi City, Japan) in the Illinois River at Havana, IL. The sound file was recorded during the boat's transit past the hydrophone and therefore was amplitude modulated. The broadband sound ranged from 60-10,000 Hz, with maximal energy contained in two broad peaks, the first between 500 and 2,000 Hz and the second peaking at 7,500 Hz (Figure 1).

An UMA-752 amplifier (Peavey Electronics, Meridian, MS, USA) regulated sound intensity, and each speaker pair was controlled manually with a switchbox (MCM Electronics, Centerville, OH, USA). The acoustic properties of the speakers and pond were mapped using the HTI 96-MIN hydrophone at 60 points evenly distributed throughout the experimental pond. Sound recordings for both ambient and the broadband sound broadcast were collected at each site. Sound pressure levels (SPL) were calculated by measuring the root-mean-square (rms) voltage of the ambient and broadband readings, which was then converted into SPL (dB re 1μPa) using Avisoft-SASLab Pro version 5.2.07. The frequency components and power spectrum of the sound were calculated with a 1,024-point fast Fourier transform (Hamming window) and sampling rate of 40 kHz.

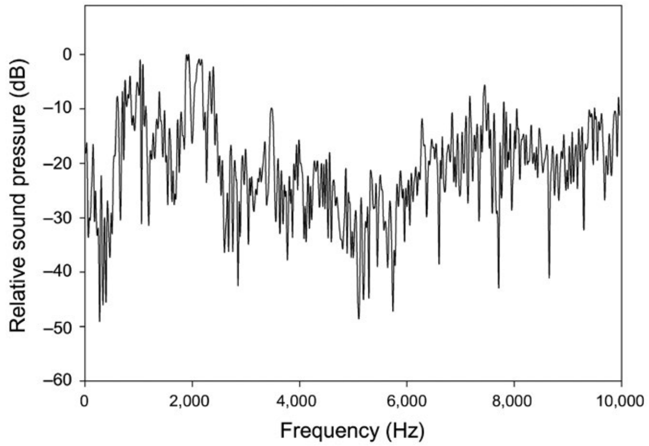


FIGURE 1 The power spectrum in relative dB of the broadband sound stimulus at frequencies of 60-10,000 Hz

2.3 | Behavioural experiments

Behaviour was monitored remotely by an observer who was situated in a shelter approximately 50 m from the test pond using eight overhead SONY bullet 500 TVL video cameras connected to a computer equipped with ProGold software (Security Camera World, Cooper City, FL, USA). The computer viewed four cameras at a time (half of the pond) and could easily be switched to the other four cameras. The cameras continuously monitored the fish and provided full coverage of the pond.

2.4 | Experimental design

One trial consisted of five consecutive periods: (1) pre-sound (120 min); (2) sound playback 1 (30 min); (3) inter-sound (60–270 min); (4) sound playback 2 (30 min) and (5) post-sound (120 min). During the pre-, inter- and post-sound periods, fish were free to swim throughout the pond and the speakers were inactive. All fish remained within 1–2 body lengths of each other in an elliptical-shaped school (diameter ~1 m), in both mono- and hetero-specific groupings; therefore, the fish in each trial were treated as a single unit with position determined as the approximate centre of the school. During the two experimental periods (sound playbacks 1 and 2), the initial location (i.e. north vs south) of fish was randomly chosen, and sound playbacks (i.e. sound stimuli) were not initiated until the school was positioned within the designated end of the pond, opposite the active speakers. Then, the speaker pair on the side of the barrier opposite to the fish was activated whenever at least two fish from the leading edge of the school entered the “reaction zone,” or the area within the rectangle formed by the four speakers, which measured approximately 2 m^2 on each side of the barrier (Figure 2). The sound was terminated (within

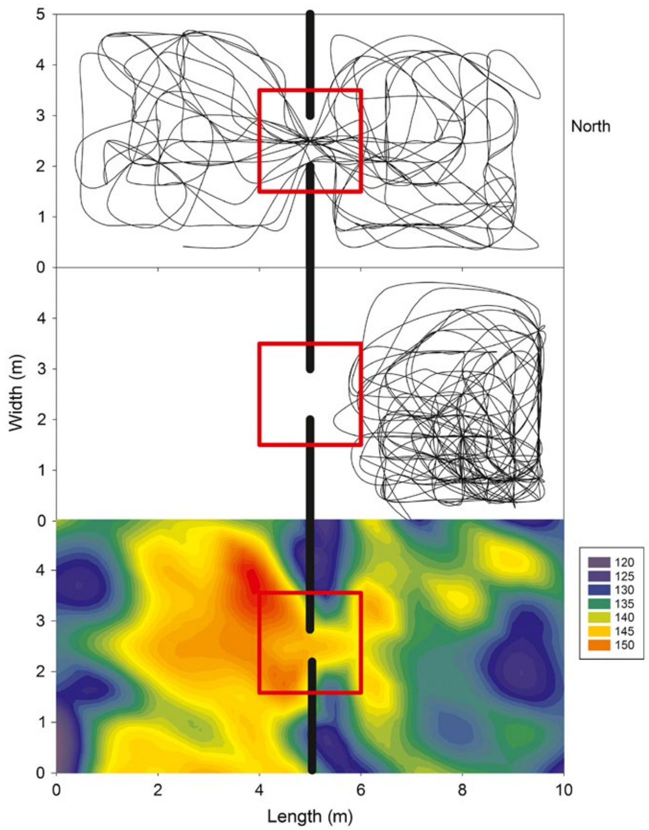


FIGURE 2 Overhead schematic of the experimental pond. The thick black lines indicate the barrier that divided the pond. The length and width of the pond are indicated in metres. The red box indicates the reaction zone with the corners of the box representing speaker locations. The location of the fish school was determined every 5 s and the *x*, *y* coordinates plotted and connected with spline lines. Each trace represents 30 min of swimming for one mixed school with speakers inactive (Top) and speakers activated (Middle). Bottom panel is a sound map of pond with pseudocolour indicating sound intensity level dB re 1µPa @ 1 m during active broadcast of the two underwater speakers on the south side of the barrier. Sound intensity level is indicated by colour panel. [Colour figure can be viewed at wileyonlinelibrary.com]

approximately 20 s of sound initiation) when the fish departed the reaction zone to avoid habituation to sound and fish were not subjected to constant sound during sound playback time periods.

Swimming behaviour was monitored during the pre-sound, inter-sound and post-sound intervals and the 30-min sound playback periods, with fish position recorded every 5 s. The number of attempted and successful crossings and residence time in each side was documented for each trial. The fish position in the reaction zone when sound was first initiated was monitored. Reaction time was defined as the time from sound onset to the point at which the leading edge of the school exited the reaction zone during successful repels.

Silver carp and bighead carp were tested for sound avoidance in both mono-specific and mixed schools. Therefore, three silver carp ($n = 10$), two bighead carp ($n = 10$) and two silver carp and bighead carp equally mixed schools ($n = 20$) were tested. Each school was exposed to between four and six sound playbacks (variation due to weather curtailing playback number) with the overall number of sound playbacks 16, 11 and 10 for silver carp, bighead carp and mixed schools, respectfully.

2.5 | Data analysis

All video and data analysis were performed at the conclusion of the trials. A crossing attempt was defined as at least two fish from the leading edge of the school entering the reaction zone. A successful crossing was scored if the entire school swam through the barrier opening into the other half of the pond. To account for differences in time for the pre-, inter-, post- and sound playback intervals, all attempted or successful crossings were converted to attempted or successful crossings per minute. Conversely, a repulsion was scored if two or more fish entered the reaction zone and did not cross into the other end of the pond following sound initiation. Repulsion rates were calculated by dividing the number of repulsions by the number of times the groups entered the reaction zone. Sound was broadcast from speakers as long as the fish remained in the reaction zone. If the fish breached the barrier despite the sound, they were allowed to cross back to the original side of the pond unimpeded by acoustic stimulus. Two to three trials were conducted for each school, with trials completed over 2–3 days.

Barrier crossings per minute (attempted and successful), percent successful repels, residence time and time to exit the reaction zone were tested for normality using Shapiro–Wilk tests. None of these data sets were normally distributed, and therefore, non-parametric Mann–Whitney rank *t* tests and Kruskal–Wallis ANOVAs with Dunn's post hoc tests were used for analysis. All statistical tests were performed with Sigmaplot, version 12.5. The median and lower and upper quartiles are reported using the following format (median; 1st quartile, 3rd quartile).

3 | RESULTS

The fish swam slowly through the pond in loose schools, 1–2 body lengths apart, and transited readily from the north and south end in the absence of sound (Figure 2: Top), crossing the barrier approximately every 3–5 min. However, when confronted with sound after entering the reaction zone, the majority (255 of 286) of schools turned away and did not cross the barrier (Figure 2: Middle). Fish maintained school formation through sound playbacks, with only one instance of a single fish departing from the school and crossing the barrier without the rest of the school.

Each pair of speakers created a non-uniform sound field throughout the pond, with sound reflected off the barrier, resulting in greater sound pressure level on the same side as the active speakers and reaching a maximum level of 155 dB re 1µPa. The sound stimulus projected through the barrier and reached 146 dB re 1µPa at the barrier midpoint and then attenuated throughout the other half of the pond (Figure 2: Bottom). Sound pressure levels were asymmetrical in each pond half, and fish had a tendency to remain in the area of lowest sound pressure (i.e. north-eastern edge in Figure 2: Bottom) during sound trials.

For each species, the fish continued to challenge the barrier throughout the trials. Attempted crossings per minute did not differ among the five periods within any of three groups (Figure 3: Kruskal–Wallis: silver carp, $p = .662$, $H = 2.403$, $df = 4$; bighead carp, $p = .062$, $H = 8.980$, $df = 4$; mixed, $p = .106$, $H = 7.644$, $df = 4$).

All groups showed a significant decrease in the number of successful crossing attempts when challenged with sound (Mann–Whitney, $p < .001$). For silver carp, successful crossings decreased significantly (Mann–Whitney, $p < .001$, $U = 36.5$, $df = 1$) from 0.16 (0.10, 0.21) to 0.02 (0.00, 0.07) crossings per minute (Figure 4). Bighead carp showed a significant decline (Mann–Whitney, $p < .001$, $U = 4.5$, $df = 1$) from 0.22 (0.13, 0.42) to 0.00 (0.00, 0.02) crossings per minute. The mixed schools also had a significant reduction in successful crossings (Mann–Whitney, $p < 0.001$, $U = 0.0$, $df = 1$), from 0.32 (0.20, 0.44) to 0.03 (0.00, 0.07) crossings per minute.

Sound playbacks were successful in decreasing fish transiting through the barrier in all three groups, with 82.5%, 93.7% and 90.5% repulsion rates for the combined trials of silver carp, bighead carp and mixed species, respectfully. The initial sound playback for each group of silver carp was the most successful, with sound stopping the fish during all 12 attempts. Success rates dropped during subsequent playbacks before rebounding to 91% during sound playback 5 and then falling to 57% during the final playback (Figure 5). Bighead carp were less likely to cross the sound barrier, with four of the six sound playbacks achieving 100% repulsion and 89 of 95 attempts repelled. The mixed school also displayed sound avoidance behaviour as >90% repulsion rates were observed until the last playback.

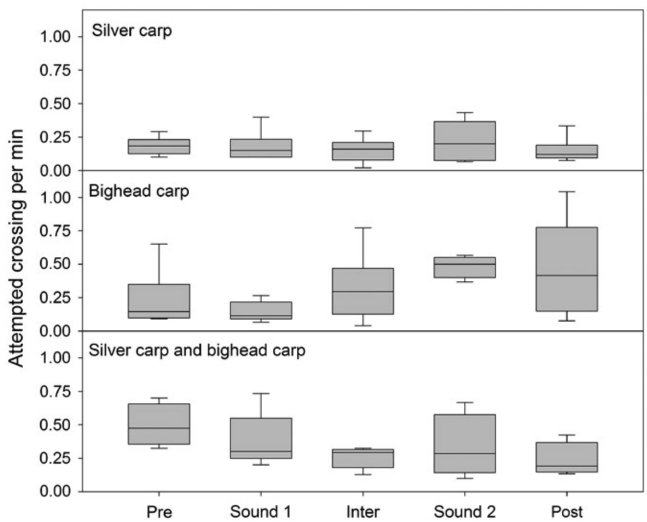


FIGURE 3 Attempted crossings (the number of times fish entered the reaction zone) per minute for silver carp, bighead carp and mixed silver carp and bighead carp schools for the five different intervals (pre—pre-sound; sound 1—sound play back 1; inter—inter-sound interval between the two sound playbacks; sound 2—sound playback 2; post—post-sound interval after playback 2) within a trial. The horizontal line in each box shows the median value, the bottom and top of the box indicate 1st and 3rd quartiles, respectively, and the whiskers are the 10th and 90th percentiles

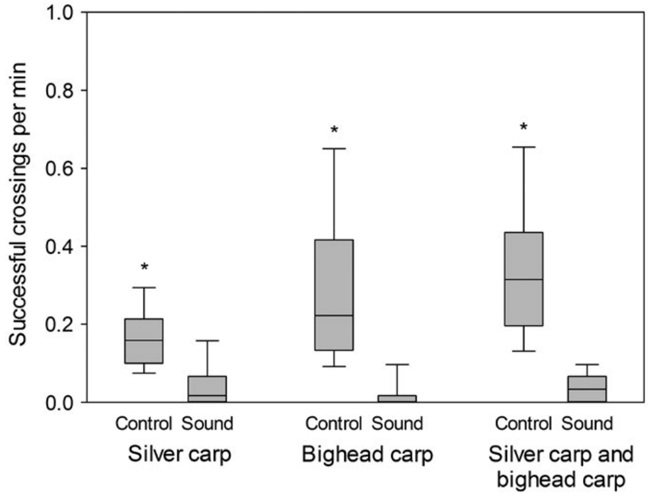


FIGURE 4 Barrier crossings per minute for silver carp, bighead carp and mixed silver carp and bighead carp schools during the control (sound off) and sound activation intervals. The horizontal line in each box shows the median value, the bottom and top of the box indicate 1st and 3rd quartiles, respectively, whiskers are the 10th and 90th percentiles. Asterisks denote significant difference between control and sound interval (Mann–Whitney, $p < .001$) for each species group

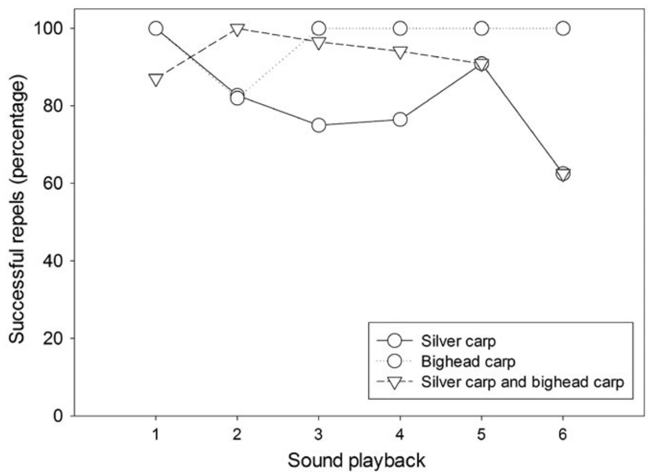


FIGURE 5 The percentage of successful repels (unsuccessful crossing/attempted crossing) for each sound playback for silver carp, bighead carp and mixed silver carp and bighead carp schools. Each data point represents the percentage of all the attempts during successive sound playbacks

The time spent in each half of the pond during the 120-min pre-sound interval was not significantly different for either the silver carp or bighead carp (Figure 6). Silver carp averaged slightly more time in the north end (4,380 s; 3,674 s, 4,869 s) than south (2,796 s; 2,399 s, 3,571 s); however, the results were not significantly different (Mann–Whitney, $p = .12$, $U = 13.0$, $df = 1$). In contrast, bighead carp spent more time in the south (4,483 s; 1,503 s, 5,353 s) than in the north end (2,716 s; 2,104 s, 5,771 s); however, there was no significant difference (Mann–Whitney, $p = .94$, $U = 17.0$, $df = 1$). The mixed schools preferred (Mann–Whitney, $p < .05$, $U = 1.0$, $df = 1$) the north

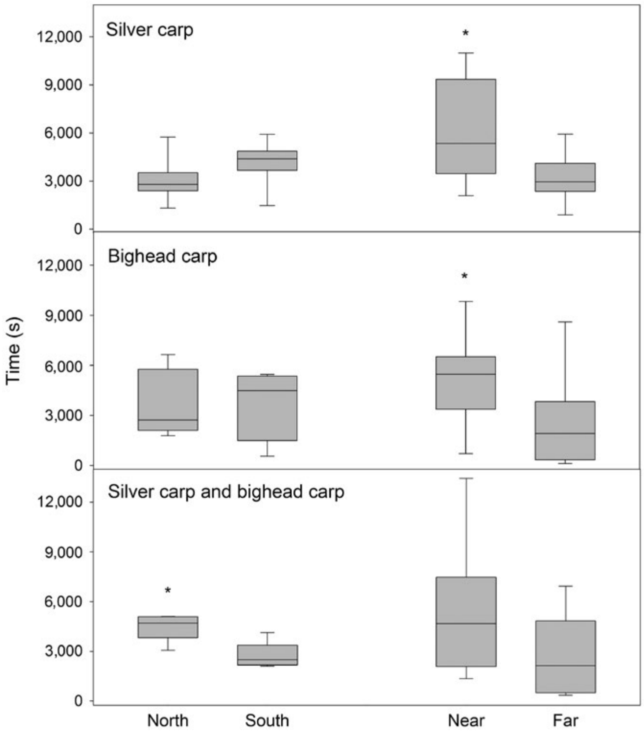


FIGURE 6 Residence times for each side of the pond for the pre-sound interval (north or south) and the inter- and post-sound interval (near or far) for silver carp, bighead carp and mixed silver carp and bighead carp schools. The near side represents the side away from the active speakers of the preceding sound intervals. Each box shows the median value, the bottom and top of the box indicate 1st and 3rd quartiles, respectively, and the whiskers indicate the 10th and 90th percentiles. Asterisks denote significant difference in time intervals (Mann–Whitney, $p < .001$)

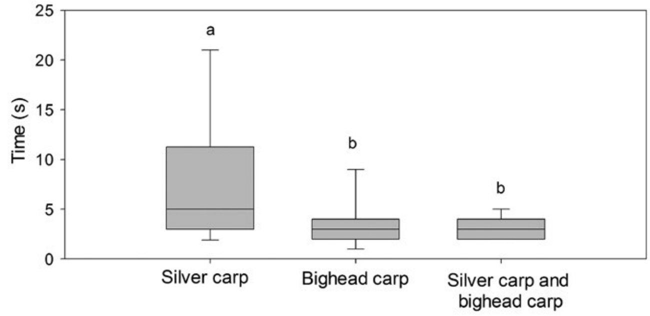


FIGURE 7 Median reaction times following sound onset for fish to exit the reaction zone following successful repels for silver carp, bighead carp and mixed silver carp and bighead carp schools. Each box shows the median value, the bottom and top of the box indicate 1st and 3rd quartiles, respectively, and the whiskers indicate the 10th and 90th percentiles. Letter indicates significantly different means (Mann–Whitney, $p < .001$)

end (5,083 s; 3,410 s, 6,858 s) over the south end (2,103 s; 1,269 s, 2,744 s). Following active sound periods, all fish favoured the side furthest from the previously active speakers (i.e. they did not cross the barrier) during the inter- and post-sound intervals. Silver carp resided significantly longer (64%, Mann–Whitney, $p = .014$, $U = 44.0$, $df = 1$) in the near side (5,344 s; 3,467 s, 9,349 s) vs the far side (2,951 s; 2,365 s, 4,107 s). Bighead carp spent significantly more time (74%, Mann–Whitney, $p = .036$, $U = 28.0$, $df = 1$) away from the speakers (5,462 s; 3,367 s, 6,514 s) vs close to the speakers (1,918 s; 344 s, 3,826 s). The mixed school also spent the majority of the time (69%) in the near side (4,671 s; 2,085 s, 7,467 s) compared with the far side (2,145 s; 498 s, 4,831 s) although the difference was not significant (Mann–Whitney, $p = .085$, $U = 27.0$, $df = 1$).

The carp reacted relatively quickly to the sound onset. During repels, silver carp exited the reaction zone in a median time of 5.0 s (3.0 s, 11.3 s), and bighead carp and mixed schools were significantly faster (Kruskal–Wallis, $p < .001$, $H = 24.2$, $df = 2$), with identical median times of 3.0 s (2.0 s, 4.0 s); (Figure 7). Very few schools showed aversive behaviour to the stimulus upon entering the reaction zone with the sound off; therefore, it was not possible to directly compare time to exit the zone with controls. However, in the absence of sound, 75% of the silver carp, 85% of the bighead carp and 75% of the mixed schools continued through the barrier after entering the reaction zone during control intervals.

4 | DISCUSSION

Playback of underwater sound recorded from motorboats was effective at restricting silver carp and bighead carp passage through a 1-m wide channel, suggesting the potential for acoustic stimuli as a non-physical barrier. The sound was most effective during initial trials; however, repulsion levels remained high (>80%) throughout the study. The broadband sound stimulus also influenced bigheaded carps distribution in the pond, with fish residing for longer periods of time in the section opposite the active speakers. The results are encouraging in that the repulsion rate remained high throughout multiple trials over several days.

Silver carp and bighead carp are ostariophysans and have relatively higher hearing sensitivity than non-ostariophysan fish, and previous work has demonstrated that both carp species can detect frequencies up to at least 3 kHz (Lovell, Findlay, Nedwell & Pegg, 2006). Studies have established that silver carp (Vetter et al., 2015) and bighead carp (Vetter et al., in press) had significantly greater movement away from broadband (0.06–10 kHz) sound stimuli compared to pure tones (500–2000 Hz). Therefore, the underwater recording of an outboard motor was used as the deterrent. The sound pressure levels (145–155 dB re 1μPa) were well above the bigheaded carps' reported hearing threshold, 104 dB re 1μPa (Lovell et al., 2006), and the bigheaded carps remained responsive throughout the study, indicating that the sound pressure levels were not impacting hearing sensitivity. Although increased sound intensity may increase success of a barrier, care must be taken not to generate such high noise that hair cells are damaged and acoustic barriers rendered ineffective (Smith, Kane & Popper, 2004).

Acoustic particle motion may be a better parameter to measure than sound pressure levels and could be the force driving the bigheaded carps' response (Zeddies et al., 2012). However, the purpose of these experiments was to determine whether sound can act as a deterrent to bigheaded carps. It is more important from an integrated pest management approach to first determine whether sound is a deterrent and then to examine what portion of the sound field that is most effective in causing repulsion. Additionally, the practical aspects of deterrents will be deployed in much larger passages where the acoustic environment will be radically different. The future goal is to measure accurately both particle motion and sound pressure under field conditions.

It was predicted that attempted crossings would decline over time because the fish would start to associate the barrier opening with the sound; however, bigheaded carps continuously challenged the barrier during the 7-hr trials. The fish actively swam throughout all five periods and would constantly circle in the near half (side opposite active speakers) of the pond during sound playback periods and invariably challenge the barrier, presumably due to the relatively small swimming area. Their constant movement through the channel during the silent periods indicated that they did not favour one side of the pond over the other and that the sound was restricting movement independent of other variables (e.g. shade). The only exception was the preference for the north side of the pond by the mixed schools during the pre-sound intervals, which had partial shade in the early morning. However, these tests were conducted during warmer days with minimal cloud cover, and the behaviour was consistent with fish preference in shallow water for shaded areas (Gibson & Power, 1975). Sound was only initiated when fish entered the reaction zone and was not on a consistent and predictable time schedule. Bigheaded carps' distribution during sound playback was dependent on sound origination rather than the presence of shady areas, indicating that even when fish favoured a section of the pond, the sound barrier could override this preference. As the fish used were captive in a controlled environment, it is important that an assessment of wild bigheaded carps' behaviour in response to broadband sound is conducted before installing speaker systems in a lock or river setting. This study provides a foundation for conducting such field experiments on wild fish.

Although the pond size provided sufficient opportunity for the bigheaded carps to challenge the barrier, their movements were circuitous and it was not always clear when they would challenge the barrier. To avoid false alarms, a small reaction zone was created close to the barrier opening, based on observations that most schools would cross through this area before entering the channel. However, the small reaction zone only provided a brief period to manually activate the sound before fish would cross the barrier. As fish swim speed fluctuated, the observer needed to visually confirm fish location and manually activate the trigger; therefore, the time needed to activate the speakers was variable. Any observer delay in sound activation could have resulted in further penetration of the carp into the reaction zone before encountering the noise, reducing the distance that the fish needed to swim through the higher sound levels. Therefore, it is likely that the results presented here are a conservative assessment of the efficacy of broadband sound in deterring the experimental fish. Furthermore, the speakers were offset from the opening to reduce any impediment to swimming; therefore, the sound source was never >2 m from the front of the school entering the reaction zone and could be breached in seconds by carp swimming in a direct line. A longer channel would allow a more defined sound gradient and would discourage fish from swimming towards increasing sound pressure levels. Also, an automated detector could provide a more consistent sound trigger.

In the current study, silver carp responded to the sound in approximately 5 s and bighead carp and mixed groups responded in 3 s. Sharp, quick movements indicative of a startle response were rare, suggesting that the fish were not "startled" by the noise onset, but would change their swimming patterns to avoid it. Additionally, the pond had minimal water circulation or directional flow. Under field conditions, downstream flow could slow upstream swimming speeds (Jones, 1963), resulting in greater exposure time to the sound barrier, which could result in higher repulsion rates.

The results demonstrated consistent sound aversion; but, longer observation periods could further refine the behaviour and address potential hearing damage or habituation to the acoustic stimuli. Variability was observed with the silver carp and mixed schools during later trials, but, weather curtailed several day three trials, resulting in lower sample numbers. Future trials will examine fish reactions over a prolonged period to determine when and if habituation to sound will transpire, and it is imperative that this be determined prior to field implementation.

To avoid acoustic interference from concurrent trials, a single concrete pond was used, which reduced the sample size. Temperature has been observed to affect swimming behaviour in fish (Brett, 1967; Brett & Glass, 1973; Jones, Jong & Ellerby, 2008); so, the trials were limited to the period when ambient temperature was sufficient to maintain the outdoor pond above 13°C. Silver carp were tested first and a cold front combined with heavy rainfall resulted in lower water temperatures at the start of these trials (13°C), which could have elicited lower responses to the sound than were observed in succeeding groups. Water temperature was warmer for the bighead carp and mixed trials, and these schools exhibited higher repulsion percentages. Further research is required to fully understand the impact of water temperature on sound aversion behaviour.

The pond was selected as its modest size allowed fish to frequently pass through the channel while providing a small area to swim away from the sound source. Considering the limitations of the small, shallow pond, the results are encouraging for the use of acoustic deterrents as part of an integrated pest management system. In the small concrete pond, echoes were produced from interactions of the sound with the pond's bottom and side, end and barrier walls in addition to the water surface, creating a difficult environment for the fish to localise the sound source. These acoustic challenges would not be as pronounced in a riverine system or even a lock chamber. Also, the experimental fish were constrained to a 25 m^2 area, whereas wild fish would have the opportunity to leave the area in response to a sound stimulus. However, field settings will likely have their own acoustical challenges (e.g. bathymetry, background noise) that will require further analysis for each site before an acoustical deterrent could be deployed.

State and federal agencies are currently developing an integrated pest management approach for bigheaded carps. To create an effective approach, multiple ecological (e.g. risk of invader, prioritising resources) and biological concepts (e.g. life history, response to stimuli) must be combined into one harmonious management plan (Hobbs & Humphries, 1995). Monitoring movements and habitat selection along with control methods like containment and potential deterrents are also considered to develop the best management techniques for bigheaded carps.

The most effective deterrent locations may be at dams with sound used to remove fish from a lock chamber or deter fish from entering the locks with vessel traffic. The pond mimicked the configuration and construction materials of a lock chamber and, despite the study's limitations, the broadband sound elicited consistent and sustained repulsion of the bigheaded carps. Furthermore, the observer was able to monitor fish position in real time and manually operate the stimulus as opposed to broadcasting the sound for continual periods and risking the fish acclimating to the sound. While manually operating the stimulus might not be applicable to a field setting, these results provide support for a deterrent that is not broadcasting constant sound. For example, sound could be initiated prior to opening lock gates as a means to remove fish from the area and prevent ingress. Then, to prevent passage as the lock gates are open, sound could be remain on until the vessel is in the lock with the gates shut. Field studies in a lock chamber are necessary to determine the impact of broadband sound on wild fish.

Sound barriers present advantages over other non-physical barriers. The speakers are relatively inexpensive and require a modest power supply compared to electrical barriers. Small backup generators or batteries could be used to power the speakers in the event of a power failure, and the low cost could allow two independent speaker arrays to be installed providing redundancy in the case of damage to one array. Sound barriers using higher frequencies provide minimal impact on fish that do not possess Weberian ossicles, using acoustic stimuli above their hearing range (Lovell et al., 2006), but their effects on other species with similar hearing ranges remain to be determined.

The current experiments deployed only sound to mediate bigheaded carps behaviour and achieved relatively high success rates compared to multi-stimuli combination studies such as a bubble and sound barrier (Zielinski et al., 2014), a sound and electric barrier (Pegg & Chick, 2004) and a strobe light and bubble barrier (McIninch & Hocutt, 1987). Further work is warranted to evaluate broadband sound combined with other deterrent methods to increase the effectiveness of the deterrent. Also, the high repulsion rates noted in this study may be sufficient to reduce passage of bigheaded carps at locks such that commercial fishermen could substantially decrease local populations. It also remains unclear what specific subset of this acoustical stimulus causes repulsion and further refinement of the broadband sound may lead to greater repulsion rates.

The results suggest that an acoustic deterrent could be an effective means to slow upstream migration of both bighead carp and silver carp. While physical and electric barriers are expensive and not always practical, an acoustic deterrent has a wide range of applications. For instance, speakers playing a broadband sound stimulus could be used to move bighead carp and silver carp towards a net or shore, clear fish out of a lock, as a part of a bubble or strobe light barrier in a river channel or as backup system at in an electric barrier, especially during routine maintenance. This study indicates that because bighead carp and silver carp are responsive to broadband sound, acoustic stimuli may be an important management tool that could be effective either on its own or integrated with other deterrent technology.

ACKNOWLEDGMENTS

We would like to thank the USGS Upper Midwest Environmental Sciences Center (UMESC) staff and interns, especially Riley Buley, for their assistance with this project. We thank Marybeth Brey for comments on the manuscript. Funding was provided by the Minnesota Environment and Natural Resources Trust Fund and resources for the USGS component of the research were provided through the USGS Ecosystem Mission Area Invasive Species Program and from the US Environmental Protection Agency's Great Lakes Restoration Initiative. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

REFERENCES

Brett, J. (1967). Swimming performance of sockeye salmon (*Oncorhynchus nerka*) in relation to fatigue time and temperature. *Journal of the Fisheries Board of Canada*, *24*, 1731–1741.

Brett, J., & Glass, N. (1973). Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *Journal of the Fisheries Board of Canada*, *30*, 379–387.

Burner, C., Moore, H. (1953). *Attempts to guide small fish with underwater sound*. (U. S. Fish and Wildlife Service Special Scientific Report No. 111). Washington, DC: U.S. Fish and Wildlife Service.

Clarkson, R. W. (2004). Effectiveness of electrical fish barriers associated with the Central Arizona Project. *North American Journal of Fisheries Management*, *24*, 94–105.

Cooke, S. L., Hill, W. R., & Meyer, K. P. (2009). Feeding at different plankton densities alters invasive bighead carp (*Hypopthalmichthys nobilis*) growth and zooplankton species composition. *Hydrobiologia*, *625*, 185–193.

Cupp, A. R., Fredricks, K. T., Porcher, S. T., Smerud, J. R., Hartleb, C. F., & Gaikowski, M. P. (in press). Survival and behavioural responses of cool and warm water fish sedated with AQUI-S® 20E (10% eugenol) at high loading densities. *Aquaculture Research*. doi: 10.1111/are.12992

Dettmers, J. M., Boisvert, B. A., Barkley, T., & Sparks, R. E. (2005). *Potential impact of steel-hulled barges on movement of fish across an electric barrier to prevent the entry of invasive carp into Lake Michigan. (Completion Report for U.S. Fish and Wildlife Service, Aquatic Ecology Technical Report 2005/19)*. Urbana, IL: Illinois State Natural History Survey.

Gibson, R. J., & Power, G. (1975). Selection by brook trout (*Salvelinus fontinalis*) and juvenile Atlantic salmon (*Salmo salar*) of shade related to water depth. *Journal of the Fisheries Research Board of Canada*, *32*, 1652–1656.

Hamel, M. J., Brown, M. L., & Chipps, S. R. (2008). Behavioral responses of rainbow smelt to *in situ* strobe lights. *North American Journal of Fisheries Management*, *28*, 394–401.

Hikasa, Y., Takase, K., Ogasawara, T., & Ogasawara, S. (1986). Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. *Japanese Journal of Veterinary Science*, *48*, 341–351.

Hobbs, R. J., & Humphries, S. E. (1995). An integrated approach to the ecology and management of plant invasions. *Conservation Biology*, *9*, 761–770.

Irons, K. S., Sass, G. G., McClelland, M. A., & Stafford, J. D. (2007). Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA Is this evidence for competition and well reduced fitness? *Journal of Fish Biology*, *71*, 258–273.

Jones, F. R. H. (1963). The reaction of fish to moving backgrounds. *Journal of Experimental Biology*, *40*, 437–446.

Jones, E. A., Jong, A. S., & Ellerby, D. J. (2008). The effects of acute temperature change on swimming performance in bluegill sunfish *Lepomis macrochirus*. *Journal of Experimental Biology*, *211*, 1386–1393.

Kelly, A. M., Engle, C. R., Armstrong, M. L., Freeze, M., & Mitchell, A. J. (2011). History of introductions and governmental involvement in promoting the use of grass, silver, and bighead carps. *Invasive Asian Carps in North America, American Fisheries Society Special Symposium*, *74*, 163–174.

Kolar, C. S., Chapman, D. C., Courtenay, W. R. Jr, Housel, C. M., Williams, J. D., & Jennings, D. P. (2007). *Bigheaded carps: A biological synopsis and environmental risk assessment*. Bethesda, MD: American Fisheries Society.

Lamarque, P. (1967). Electrophysiology of fish subject to the action of an electric field. In R. Vibert (Ed.), *Fishing with electricity: Its application to biology and management* (pp. 65–92). London, UK: Fishing News Books.

Lamarque, P. (1990). Electrophysiology of fish in electric fields. In I. G. Cowx, & P. Lamarque (Eds.), *Fishing with electricity: Applications in freshwater fisheries management* (pp. 4–33). Oxford, UK: Fishing News Books.

Lovell, J. M., Findlay, M. M., Moate, R. M., Nedwell, J. R., & Pegg, M. A. (2005). The inner ear morphology and hearing abilities of the paddlefish (*Polyodon spathula*) and the lake sturgeon (*Acipenser fulvescens*). *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, *142*, 286–296.

Lovell, J. M., Findlay, M. M., Nedwell, J. R., & Pegg, M. A. (2006). The hearing abilities of the silver carp (*Hypopthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, *143*, 286–291.

Maes, J., Turnpenny, A. W. H., Lambert, D. R., Nedwell, J. R., Parmentier, A., & Ollevier, F. (2004). Field evaluation of a sound system to reduce estuarine fish intake rates at a power plant cooling water inlet. *Journal of Fish Biology*, *64*, 938–946.

McIninch, S. P., & Hocutt, C. H. (1987). Effects of turbidity on estuarine fish response to strobe lights. *Journal of Applied Ichthyology*, *3*, 97–105.

Meinertz, J. R., Schreier, T. M., Porcher, S. T., Smerud, J. R., & Gaikowski, M. P. (2014). Depletion of eugenol residues from the skin-on fillet tissue of rainbow trout exposed to 14 C-labeled eugenol. *Aquaculture*, *430*, 74–78.

Moy, P. B., Polls, I., & Dettmers, J. M. (2011). The Chicago Sanitary and Ship Canal aquatic nuisance species dispersal barrier. *American Fisheries Society Symposium*, *74*, 121–137.

Noatch, M. R., & Suski, C. D. (2012). Non-physical barriers to deter fish movements. *Environmental Reviews*, *20*, 71–82.

Parker, A. D., Glover, D. C., Finney, S. T., Rogers, P. B., Stewart, J. G., & Simmonds, R. L. Jr (2015). Direct observations of fish incapacitation rates at a large electrical fish barrier in the Chicago Sanitary and Ship Canal. *Journal of Great Lakes Research*, *41*, 396–404.

Patrick, P. H., Christie, A. E., Sager, D., Hocutt, C., & Stauffer, J. (1985). Responses of fish to a strobe light air-bubble barrier. *Fisheries Research*, *3*, 157–172.

Pegg, M. A., & Chick, J. H. (2004). *Aquatic nuisance species: An evaluation of barriers for preventing the spread of Bighead and Silver carp to the Great Lakes. (Final report for the Illinois-Indiana Sea Grant A/SE (ANS)-01-01)*. Urbana, IL; Illinois-Indiana Sea Grant.

Popper, A. N., & Carlson, T. J. (1998). Application of sound and other stimuli to control fish behavior. *Transactions of the American Fisheries Society*, *127*, 673–707.

Ross, Q. E., Dunning, D. J., Thorne, R., Menezes, J. K., Tiller, G. W., & Watson, J. K. (1993). Response of alewives to high-frequency sound at a power plant intake on Lake Ontario. *North American Journal of Fisheries Management*, *13*, 291–303.

Ruebush, B. C., Sass, G. G., Chick, J. H., & Stafford, J. D. (2012). In-situ tests of sound-bubble-strobe light barrier technologies to prevent range expansions of Asian carp. *Aquatic Invasions*, *7*, 37–48.

Sampson, S. J., Chick, J. H., & Pegg, M. A. (2009). Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. *Biological Invasions*, *11*, 483–496.

Sass, G. G., Cook, T. R., Irons, K. S., McClelland, M. A., Michaels, N. N., O'Hara, T. M., & Stroub, M. R. (2010). A mark-recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. *Biological Invasions*, *12*, 433–436.

Schilt, C. R. (2007). Developing fish passage and protection at hydropower dams. *Applied Animal Behaviour Science*, *104*, 295–325.

Schrank, S. J., Guy, C. S., & Fairchild, J. F. (2003). Competitive interactions between age-0 bighead carp and paddlefish. *Transactions of the American Fisheries Society*, *132*, 1222–1228.

Sloan, J. L., Cordo, E. B., & Mensinger, A. F. (2013). Acoustical conditioning and retention in the common carp (*Cyprinus carpio*). *Journal of Great Lakes Research*, *39*, 507–512.

Smith, M. E., Kane, A. S., & Popper, A. N. (2004). Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). *Journal of Experimental Biology*, *207*, 427–435.

Sparks, R. E., Barkley, T. L., Creque, S. M., Dettmers, J. M., & Stainbrook, K. M. (2010). Evaluation of an electric fish dispersal barrier in the Chicago Sanitary and Ship Canal. *Invasive Asian Carps in North America, American Fisheries Society*, *74*, 139–161.

Taylor, R. M., Pegg, M. A., & Chick, J. H. (2005). Response of bighead carp to a bioacoustic behavioural fish guidance system. *Fisheries Management and Ecology*, *12*, 283–286.

Vetter, B. J., Cupp, A. R., Fredricks, K. T., Gaikowski, M. P., & Mensinger, A. F. (2015). Acoustical deterrence of silver carp (*Hypophthalmichthys molitrix*). *Biological Invasions*, *17*, 3383–3392.

Vetter, B. J., Murchy, K. A., Cupp, A. R., Amberg, J. J., Gaikowski, M. P., & Mensinger, A. F. (in press). Acoustic deterrence of bighead carp (*Hypophthalmichthys nobilis*) to a broadband sound stimulus. *Journal of Great Lakes Research*, *43*, 163–171. [Correction added on 29 August 2017, after first online publication: The title of the article in this reference was previously incorrect and has been corrected in this version]

Welton, J. S., Beaumont, W. R. C., & Clarke, R. T. (2002). The efficacy of air, sound and acoustic bubble screens in deflecting atlantic salmon, *Salmo salar* L., smolts in the River Frome UK. *Fisheries Management and Ecology*, *9*, 11–18.

Willis, D. J., Hoyer, M. V., Canfield, D. E., & Lindberg, W. J. (2002). Training grass carp to respond to sound for potential lake management uses. *North American Journal of Fisheries Management*, *22*, 208–212.

Xie, P., & Chen, Y. (2001). Invasive carp in China's plateau lakes. *Science*, *294*, 999–1000.

Zeddies, D. G., Fay, R. R., Gray, M. D., Alderks, P. W., Acob, A., & Sisneros, J. A. (2012). Local acoustic particle motion guides sound-source localization behavior in the plainfin midshipman fish, *Porichthys notatus*. *The Journal of Experimental Biology*, *215*, 152–160.

Zielinski, D. P., Voller, V. R., Svendsen, J. C., Hondzo, M., Mensinger, A. F., & Sorensen, P. (2014). Laboratory experiments demonstrate that bubble curtains can effectively inhibit movement of common carp. *Ecological Engineering*, *67*, 95–103.

How to cite this article: Murchy KA, Cupp AR, Amberg JJ, et al. Potential implications of acoustic stimuli as a non-physical barrier to silver carp and bighead carp. *Fish Manag Ecol*. 2017;24:208–216. https://doi.org/10.1111/fme.12220

Management of Biological Invasions (2017) Volume 8, Issue 3: 371–376
DOI: https://doi.org/10.3391/mbi.2017.8.3.10

Open Access

Special Issue: Management of Invasive Species in Inland Waters

Research Article

Management implications of broadband sound in modulating wild silver carp (*Hypophthalmichthys molitrix*) behavior

Brooke J. Vetter[1,2,]*, Robin D. Calfee[2] and Allen F. Mensinger[1]

[1]*Biology Department, University of Minnesota Duluth, 1035 Kirby Drive, Duluth, MN 55812, USA*
[2]*U.S. Geological Survey, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, MO 65201, USA*

**Corresponding author*
E-mail: vett0114@d.umn.edu

Received: 30 September 2016 / Accepted: 8 May 2017 / Published online: 24 June 2017

Handling editor: Frank Collas

Editor's note:
This study was first presented at the 19th International Conference on Aquatic Invasive Species held in Winnipeg, Canada, April 10–14, 2016 (http://www.icais.org/html/previous19.html). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Abstract

Invasive silver carp (*Hypophthalmichthys molitrix*) dominate large regions of the Mississippi River drainage, outcompete native species, and are notorious for their prolific and unusual jumping behavior. High densities of juvenile and adult (~25 kg) carp are known to jump up to 3 m above the water surface in response to moving watercraft. Broadband sound recorded from an outboard motor (100 hp at 32 km/hr) can modulate their behavior in captivity; however, the response of wild silver carp to broadband sound has yet to be determined. In this experiment, broadband sound (0.06–10 kHz) elicited jumping behavior from silver carp in the Spoon River near Havana, IL independent of boat movement, indicating acoustic stimulus alone is sufficient to induce jumping. Furthermore, the number of jumping fish decreased with subsequent sound exposures. Understanding silver carp jumping is not only important from a behavioral standpoint, it is also critical to determine effective techniques for controlling this harmful species, such as herding fish into a net for removal.

Key words: bioacoustics, jumping, herding fish

Introduction

Since their accidental introduction to the southern region of the United States in the 1970's, silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844) have colonized much of the Mississippi River drainage and now threaten the Laurentian Great Lakes through the Chicago Ship and Sanitary Canal on Lake Michigan. These fish are out competing native species such as gizzard shad (*Dorosoma cepedianum* Lesueur, 1818; Sampson et al. 2009) and bigmouth buffalo (*Ictiobus cyprinellus* Valenciennes, 1844; Irons et al. 2007) because of their prolific spawning and rapid growth rates. They also opportunistically feed on both phytoplankton and zooplankton, impacting other fish populations within this trophic level (Kolar et al. 2007; Sass et al. 2014). In addition to these negative ecological impacts, silver carp also affect humans' recreational activities on affected waterways because they jump in response to motorized watercraft and this presents a hazard, as airborne fish could injure boaters (Kolar et al. 2007).

This jumping behavior also appears to be detrimental to the fish, as they often collide with boat hulls or partially submerged logs and branches. Despite extensive coverage in news and social media outlets, the trigger for and functional significance of

Figure 1. Location of each site on the Spoon River. The Illinois River and the highway bridge on the upstream end of Site 5 are also visible.

jumping remains unknown. When reacting to moving boats (16–40 km/hr), silver carp primarily jumped behind the boat with the pattern of jumping influenced by the boat wake (Vetter et al. 2017). It is also possible that jumping is related to the sound emitted by the outboard motor. Captive silver carp demonstrated consistent negative phonotaxis to a broadband (0.06–10 kHz) outboard motor recording (100 hp at 32 km/hr) and this sound was also >90% effective in deterring silver carp from crossing a narrow (2 m) channel (Vetter et al. 2015; Murchy et al. 2017). However these studies were conducted on captive fish in an artificial environment and the behavioral response of wild silver carp to broadband sound needs to be assessed.

Physical barriers are not always a practical option to prevent aquatic species range expansion, as they can impact shipping and interfere with native species migration and spawning. Therefore broadband sound has been proposed for use as an acoustic deterrent. For instance, broadband sound could be implemented in a lock chamber to manage fish prior to boat passage. However, it is imperative that the behavioral response of silver carp to broadband sound be evaluated in the field. A better understanding of silver carp's jumping behavior, which is likely energetically costly and potentially detrimental to the fish, is also needed. To examine the relationship between broadband sound and silver carp jumping behavior, a field study exposing wild silver carp to broadband sound was conducted on the Spoon River near Havana, IL. Furthermore, this experiment assessed the impact of multiple sound exposures on silver carp behavior with implications for management.

Methods

Study site

The Spoon River is a 237 km tributary of the Illinois River, originating south of Kewanee, IL and meeting the main channel near Havana, IL. Approximately 3.25 km from its terminus, a collapsed bridge had blocked upstream access. Five sites (Figure 1) were selected downstream from the barrier to assess silver carp behavior in response to broadband sound. Sites were chosen because they were far enough apart so the sound stimulus in one site could not influence any of the others (verified with a hydrophone; SoundTrap 202, Ocean Instruments, Auckland, NZ; Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government). Site 1 (100 m long) was located at the mouth of the river and had an average depth of 2.5 m. The second site was approximately 300 m upstream (all distances from river mouth) was 100 m long and an average of 1.8 m deep. Site 3 was located at a large bend approximately 650 m upstream. It was 140 m in length and had an average depth of 2.5 m, except for a deep hole (~ 4.5 m) located half way through the site at the apex of the river bend. The fourth site was 1.1 km upstream, had a length of 100 m, and an average depth of 1.5 m. The final site was 1.5 km upstream and 100 m long with an average depth of 2.3 m. All sites had an approximate water surface area (estimated using Google Earth) of 4,500 m^2 except for Site 3 (6,300 m^2) and turbidity readings ranged between 28 and 90 FTU (USGS 2016).

Sound stimulus

The broadband sound stimulus was recorded in the Illinois River from a 6 m aluminium boat, equipped with a 100 hp motor (4-stroke; Yamaha, Kennesaw, GA), traveling 32 km/hr by a hydrophone (HTI-96-MIN High Tech, Inc., Long Beach, MS) positioned 10 m from the boat's path at a depth of 1 m. In this study, a 30 second clip of this recording was repeatedly broadcast from two underwater speakers (LL916C-025; Lubell Labs, Columbus, OH; frequency response: 200 Hz–23 kHz). Both speakers were housed in cages and the top of each cage was attached to 3 m aluminum poles, which were mounted 1 m apart on the bow railing of a 6 m aluminum boat also equipped with a 100 hp motor. The speakers were submerged such that the tops were approximately 0.15 m under the surface and oriented to project the sound along the longitudinal axis of the boat. A SoundTrap 202 hydrophone (Ocean Instruments, Auckland, NZ) was used to characterize the frequency output of the broadband sound stimulus and measure the sound pressure levels. Acoustic recordings were made in 20 m increments away from the bow of the boat in a direct line from the front of the speakers to 100 m. These measurements were taken after the field experiment in a straight section of the river and at each recording site; the hydrophone was positioned 1.5 m below the water surface and at the midline between the two speakers.

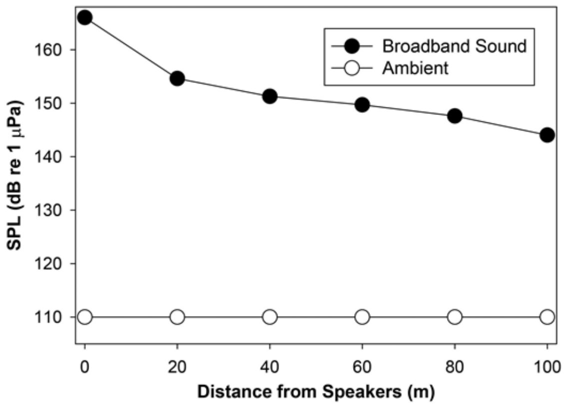


Figure 2. Sound pressure from the stimulus origin and up to 100 m from the speakers.

Behavioral observations

Silver carp jumping behavior was recorded using four cameras (GoPro Hero3; San Mateo, CA) during underwater playback of broadband sound in the Spoon River near Havana, IL. One camera was mounted to the bow, port, stern, and starboard sides of the boat and positioned to prevent overlap between each camera's field of view (recording quality: 1080 pixels; 30 frames/second). At each site, the boat moved downstream through the site before making a slow 180° turn and returning to the origin. This was referred to as a "run". The transits favored the starboard side riverbank, so the complete circuit resembled an elongated ellipse, rather than a straight line bisecting the middle of the channel. Each trial consisted of three complete runs with the sound (experimental) and one complete run without sound (control). The order of control and experimental runs was randomized for each trial. In experimental runs, sound was broadcast during the entire transit, with boat speed maintained between 3–6 km/hr. A 10 minute recovery period was allowed between each run, which were 4–6 minutes in duration. An entire trial took 45–50 minutes, and was conducted once at each site on September 15, 2015.

Data analysis

The sound pressure at the source and up to 100 m from the speakers was analyzed using MatLab (R2016b) and the frequency components and power spectrum of both the sound emitted at the speakers and the original outboard motor stimulus (100 hp motor traveling 32 km/hr on the Illinois River) were assessed using Audacity (version 2.0.5).

Prior to video analysis, the distortion from the GoPro fisheye lens was removed using GoPro Studio software (version 2.5.4, San Mateo, CA). The number of jumping in fish each run was quantified by viewing each frame of video (30 frames per second) using Adobe Photoshop CS6 (version 13; San Jose, CA). To ensure a jumping fish was counted once, only jumps that were initiated in a camera's field of view were quantified. The proportion of jumping fish in each run to the total number of jumping fish in a trial (site) was then determined. Runs 1, 2, and 3 and the control at each site were compared against each other using a parametric ANOVA (Shapiro-Wilk test for normality: $P = 0.672$) with a Holm-Sidack pairwise comparison procedure in SigmaPlot (version 10). Number of jumping fish is reported as mean ± SD.

The fish from Site 3 were further examined using the same method as Vetter et al. (2017) to map the jumping pattern. First, the angle of the jump origin (the point at which the fish broke the water's surface) in relation to the boat's position (bow = 0°; starboard = 90°; stern = 180°; port = 270°) was determined. Next, to estimate the jumping distance from the boat, 2 m PVC pipes marked in 0.25 m increments, were mounted beneath each camera. The number of pixels in each 0.25 m segment was determined in Adobe Photoshop and a linear regression formula for each camera was used to extrapolate jumping distance from the boat.

Results

Sound stimulus

The sound pressure ranged from 166 dB re 1 μPa at the source to 144 dB re 1 μPa 100 m from the speakers while the ambient sound was 110 dB re 1 μPa in this region (Figure 2). The frequency range of the sound emitted from the motorboat was 60 Hz–10 kHz (Figure 3A) while the sound broadcast from the

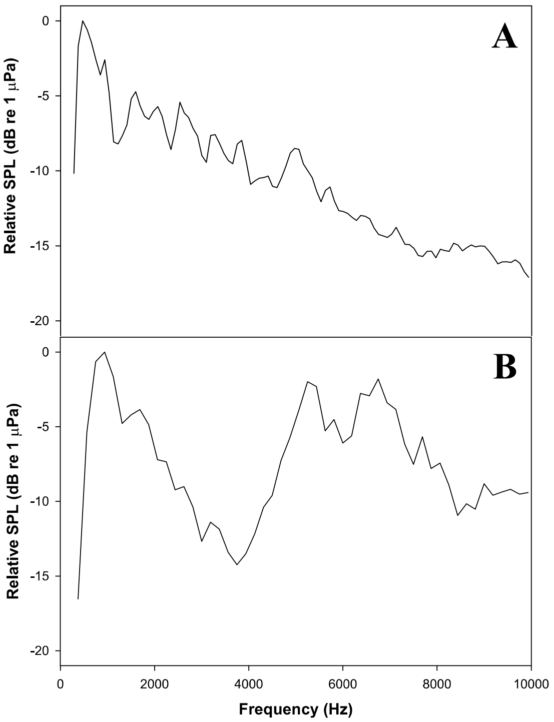


Figure 3. Power spectrum for the (**A**) original outboard motor recording (100 hp motor at 32 km/hr) recorded in the Illinois River and the (**B**) sound stimulus played back to the silver carp on the Spoon River.

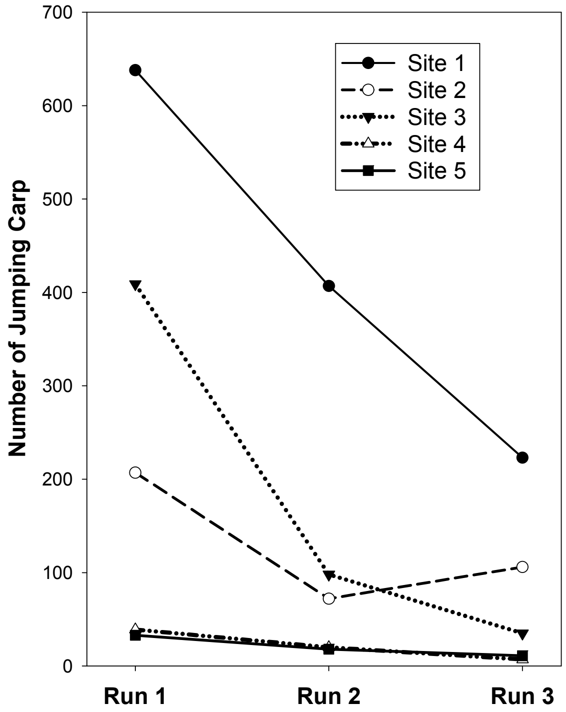


Figure 4. Total number of jumping fish during each run at all five sites.

LL916 speakers ranged from 200 Hz–10 kHz but had two broad peaks between 200 Hz–2 kHz and 6–10 kHz (Figure 3B).

Jumping behavior

The number of jumping carp varied greatly among the three sites, however, the initial run stimulated the most fish at all five sites (Figure 4). Site 1 had the highest total jumping fish (n = 1268), with 638, 407, and 223 jumping during the respective runs (Figure 4). The fewest carp jumped in Sites 4 (n = 66) and 5 (n = 62) (Figure 4). For all five sites, the highest proportion (0.572 ± 0.076) of the total jumps (Figure 5) occurred during the first run ($P < 0.05$) The second runs had the second highest proportion of jumps (0.253 ± 0.071; $P < 0.05$) while the third runs had the lowest proportion of jumping fish of the sound treatments (0.159 ± 0.082; $P < 0.05$). Only control runs in Sites 1 (n = 4) and 3 (n = 46) elicited jumping from the carp and the control runs had the lowest proportion of jumping fish (0.016 ± 0.035; $P < 0.05$). Fish jumped all around the boat during each pass at Site 3 (Figure 6) and a similar pattern was observed at the other sites.

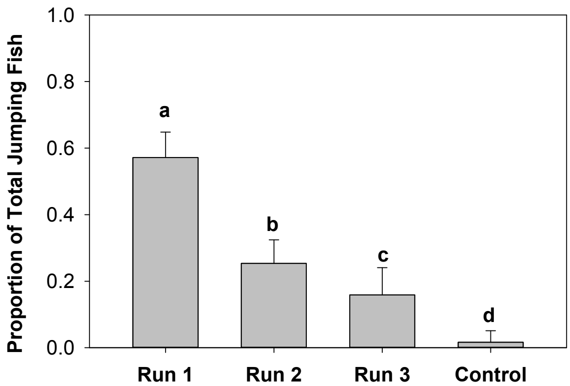


Figure 5. For each run, the mean proportion of total jumping fish is shown. Letters indicate significantly different groups ($P < 0.05$). Error bars represent ± 1 SD.

Discussion

Silver carp jumped in all runs during which broadband sound was played, while only two of the control runs elicited jumping. These results indicate that broadband sound can trigger the jumping response from silver carp. In all trials, there was a decrease in the number of jumping fish from the first to the third run. Finally, fish jumped all around the boat and did not favor the region astern, as has been observed in fish responding to moving boats (Vetter et al. 2017).

Silver carp are ostariophysans and possess a bony connection (Weberian ossicles) between the swim bladder and their inner ear, which allows them to detect higher frequencies than many non-ostariophysans (Fay and Popper 1999). Lovell et al. (2006) reported sensitivity up to 3 kHz for silver carp, however, since the researchers did not test past this frequency, silver carp may be able to hear beyond 3 kHz, as observed in other ostariophysans (see Ladich and Fay 2013 for a review). Therefore, the carp should have been able to detect at least part of the broadband sound stimulus (0.2–10 kHz).

Silver carp have been observed jumping in the wake created by a moving boat (16–40 km/hr; Vetter et al. 2017), however, in this study it appears that sound was the primary stimulus to elicit jumping, as the boat generated little wake and minimal jumping was observed during boat movement with the speakers inactive. Furthermore, the fish reacting to the sound stimulus jumped 360° around the boat, compared with mostly stern concentrated wake jumping in response to fast moving boats. It is still unclear whether or not the fish were responding to the sound pressure or particle motion from the broadband stimulus, therefore both the sound pressure and particle motion fields for the speaker configuration used in this experiment need to be fully characterized to further correlate jumping and broadband sound.

The decline in fish jumping with subsequent trials could have been attributed to fatigue, habituation, or moving out of the area. Jumping is energetically costly (Rome 1998) and the carp could have remained but become exhausted, which could explain the decrease in jumping during the second and third runs. Alternatively, the carp might have habituated to the sound stimulus. However, broadband sound was effective in directing captive silver carp movement with little evidence of habituation during 2–3 day testing periods (Vetter et al. 2015; Murchy et al. 2017). The negative phonotaxis exhibited by captive carp to broadband sound suggests that this stimulus may have caused the fish in the present study to swim away from the site, however, underwater swimming behavior cannot be inferred from the results of the present study. Therefore, it is imperative that long-term sound exposure experiments on both captive and wild silver carp are conducted to better understand the potential for habituation or exhaustion. An additional field study using a sonar system to monitor fish presence and behavior underwater would aid in determining whether or not fish were exiting the area.

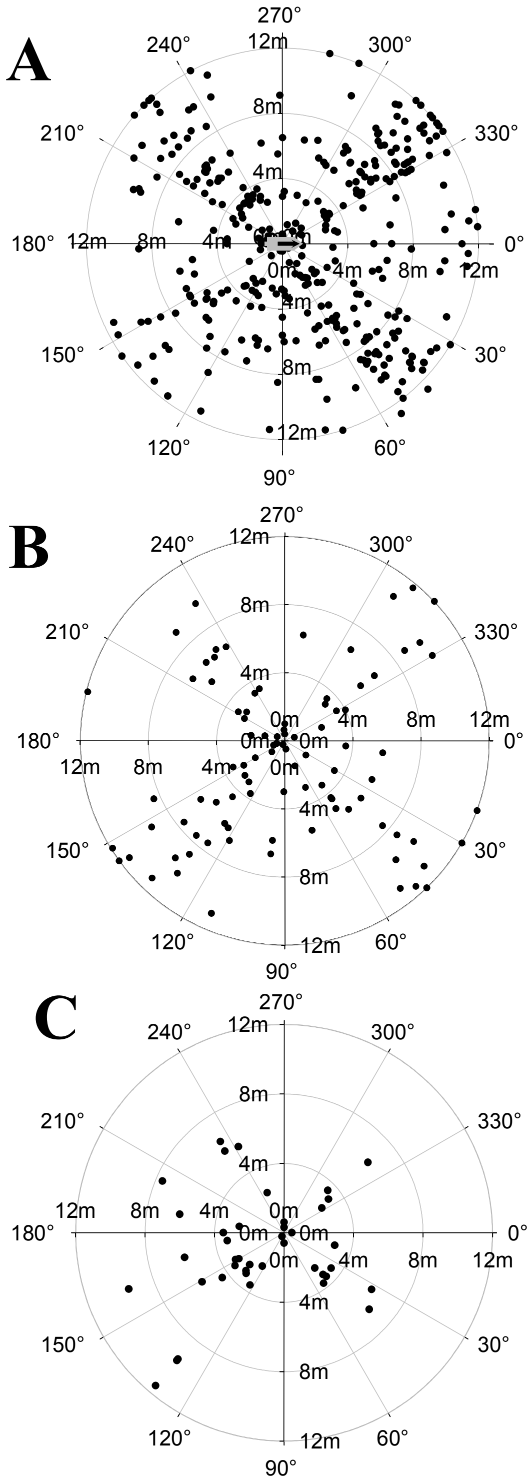


Figure 6. Summary of jump origin angles for airborne fish from an example trial (Site 3) during the (**A**) first, (**B**) second, and (**C**) third runs. Each black circle indicates one fish and 0° represents the boat bow and direction of movement (boat position schematic in (**A**) is not to scale).

The variation in fish jumping between sites may be related to the presence of woody detritus, as areas with partially submerged logs or branches, appeared to have higher densities of jumping fish. Whether fish naturally congregate in submerged structures or retreated to these areas to escape the sound needs further examination. The relationship between presence of partially submerged woody debris and silver carp jumping should be further explored using a sonar system that would allow for accurate census of submerged fish in the regions with and without debris present.

Researchers are currently evaluating the efficacy of acoustical deterrents to prevent further range expansion of silver carp. This study not only implies that sound is capable of eliciting jumping behavior from carp; it also supports the use of broadband sound as a management tool. The decrease in jumping fish with subsequent sound exposures suggests fish could be exhausted or driven from an area using broadband sound however; underwater behavior cannot be determined from the current findings. Therefore, it is imperative that field trials using sonar be conducted to further evaluate fish behavior in response to broadband sound.

Acknowledgements

We would like to thank Andrew Casper, Levi Solomon, and the staff and interns at the Illinois River Biological Station (IRBS) in Havana, IL and their sponsors, Sportfish Restoration Fund project F-101-R, Illinois DNR Great Lakes Restoration Initiative Asian Carp Removal Program, and Upper Mississippi River Restoration Long-term Resource Monitoring Element, for providing resources. Additionally, we wish to acknowledge Samantha Phegley and Gregory Thompson from the U.S. Geological Survey Columbia Environmental Research Center for assistance with fieldwork and analysis. Funding was provided by the Minnesota Environment and Natural Resources Trust Fund and Illinois Natural History Survey. Additional funding and resources were provided through the USGS Ecosystem Mission Area Invasive Species Program and from the US Environmental Protection Agency's Great Lakes Restoration Initiative.

References

Fay RR, Popper AN (1999) The auditory periphery in fishes. In: Fay RR, Popper AN (eds), Comparative Hearing: Fish and Amphibians. Springer Verlag, New York, pp 43–100, https://doi.org/10.1007/978-1-4612-0533-3

Irons KS, Sass GG, McClelland MA, Stafford JD (2007) Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA - Is this evidence for competition and reduced fitness? *Journal of Fish Biology* 71: 258–273, https://doi.org/10.1111/j.1095-8649.2007.01670.x

Kolar KS, Chapman DC, Courtenay WR, Housel CM, Williams JD, Jennings DP (2007) Bigheaded carps: a biological synopsis and environmental risk assessment. American Fisheries Society Special Publication 33. Bethesda, 204 pp

Ladich F, Fay RF (2013) Auditory evoked potential audiometry in fish. *Reviews in Fish Biology and Fisheries* 23: 317–364, https://doi.org/10.1007/s11160-012-9297-z

Lovell JM, Findlay MM, Nedwell JR, Pegg MA (2006) The hearing abilities of the silver carp (*Hypopthalmichthys molitrix*) and bighead carp (*Hypopthalmichthys nobilis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 143: 286–291, https://doi.org/10.1016/j.cbpa.2005.11.015

Murchy KA, Cupp AR, Amberg JJ, Vetter BJ, Fredriks KT, Gaikowski MG, Mensinger AF (2017) Potential implications of acoustic stimuli as a non-physical barrier to silver carp and bighead carp. *Fisheries Management and Ecology* 24: 208–216, https://doi.org/10.1111/fme.12220

Rome LC (1998) Some advances in integrative muscle physiology. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 120: 51–72, https://doi.org/10.1016/S0305-0491(98)00023-6

Sampson SJ, Chick JH, Pegg MA (2009) Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi Rivers. *Biological Invasions* 11: 483–496, https://doi.org/10.1007/s10530-008-9265-7

Sass GG, Hinz C, Erikson AC, McClelland NN, McClelland MA, Epifanio JM (2014) Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. *Journal of Great Lakes Research* 40: 911–921, https://doi.org/10.1016/j.jglr.2014.08.010

Vetter BJ, Cupp AR, Fredriks KT, Gaikowski MG, Mensinger AF (2015) Acoustical deterrence of silver carp (*Hypophthalmichthys molitrix*). *Biological Invasions* 17: 3383–3392, https://doi.org/10.1007/s10530-015-0964-6

Vetter BJ, Casper AF, Mensinger AF (2017) Characterization and management implications of silver carp (*Hypophthalmichthys molitrix*) jumping behavior in response to motorized watercraft. *Management of Biological Invasions* 8: 113–124, https://doi.org/10.3391/mbi.2017.8.1.11

Management of Biological Invasions (2017) Volume 8, Issue 1: 113–124
DOI: https://doi.org/10.3391/mbi.2017.8.1.11

Open Access

Research Article

Characterization and management implications of silver carp (*Hypophthalmichthys molitrix*) jumping behavior in response to motorized watercraft

Brooke J. Vetter[1,]*, Andrew F. Casper[2] and Allen F. Mensinger[1]

[1]*Biology Department, University of Minnesota Duluth, 1035 Kirby Drive, Duluth, MN 55812, USA*
[2]*Illinois River Biological Station, Illinois Natural History Survey, University of Illinois, 704 N. Schrader, Havana, IL 62644, USA*
**Corresponding author*
E-mail: vett0114@d.umn.edu

Received: 22 July 2016 / Accepted: 29 November 2016 / Published online: 9 February 2017

Handling editor: Katherine Dafforn

Abstract

Silver carp (*Hypophthalmichthys molitrix*), an invasive planktivorous fish species in North America, pose a threat to aquatic ecosystems throughout the Mississippi River Drainage. These fish are well known for their airborne leaps in response to passing watercraft, but the trigger for, and functional significance of jumping remains unknown. The behavior of wild silver carp responding to moving (16, 24, 32, and 40 km/hr) 6 m aluminum boats equipped with 4-stroke outboard motors (100 or 150 hp) was quantified. Experiments were conducted at three sites on the Illinois River near Havana, IL and most boat transits (57.9%) stimulated five or more fish to jump. The frequency of jumping (fish/min) was independent of speed and motor type and the vast majority of fish (> 90.0%) jumped after the boat had passed their position but avoided the area directly astern (< 4.0 m). Furthermore, 79.8% of fish vectored away from the moving watercraft. The results suggest that jumping direction is not random and fish can localize the stimulus source. The "delayed" jumping until after the boat had transited the area indicates that the trigger may be turbulence and/or higher sound pressure levels. This is the first study to model silver carp jumping in response to motorized watercraft and can aid fisheries managers in predicting the direction and location of airborne fish to develop effective herding and capture methods.

Key words: invasive species, management, bioacoustics

Introduction

Silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844) are an invasive fish species that escaped from captivity in the southern part of the United States in the 1980's and have since moved northward, colonizing much of the Mississippi River Drainage (Kolar et al. 2005; Kolar et al. 2007). In areas where carp are abundant, these planktivorous fish have drastically altered the composition of the lowest trophic levels (Kolar et al. 2005; Sass et al. 2014). Furthermore, they compete with native filter feeders such as such as bigmouth buffalo (*Ictiobus cyprinellus* Valenciennes, 1844; Irons et al. 2007) and gizzard shad (*Dorosoma cepedianum* Lesueur, 1818; Sampson et al. 2009). Silver carp are abundant in the northern reaches of the Illinois River where they threaten to expand into the Laurentian Great Lakes, which would expose the entire system to ecological disruption (Sass et al. 2010; Moy et al. 2011; Murphy and Jackson 2013).

An additional reason these fish have gained notoriety is their jumping behavior. Both juvenile and adult silver carp jump in response to moving watercraft, with reports of airborne fish injuring boaters (Kolar et al. 2007). Jumping in freshwater fish has been associated with upstream migration, circumventing barriers, or escaping predators (Aronson 1971; Bayliss 1982; Bierman 2013). Smallscale archer fish (*Toxotes microlepis* Günther, 1860; Shih and Techet 2010) can jump up to 2.5 body lengths out of the water to catch prey and salmonid species, such as sockeye salmon (*Oncorhynchus nerka* Walbaum, 1792; Lauritzen et al. 2010) and brook trout (*Salvelinus fontinalis* Mitchill, 1814; Kondratieff and Myrick 2006), will leap several body lengths while negotiating boulders or waterfalls during spawning migrations. Many fish, including the African butterfly fish (*Pantodon buchholzi* Peters, 1877; Saidel et al. 2004) and the hatchet fish (*Carnegiella strigata* Günther, 1864; Wiest 1995), leap as an avoidance response when startled by predators. However, the silver carp's jumping behavior is unusual for cyprinids. Jumping may help larval and juvenile carp evade predation, but mature animals have few, if any, natural predators in North America. Although silver carp jumping has been well documented in the popular literature and numerous social media outlets, the trigger and functional significance of this behavior remains unclear.

This study evaluated wild silver carp responding to different outboard motors and speeds to characterize and better understand the jumping behavior. Jumping is both energetically costly and can have deleterious consequences for carp, such as self-stranding (into boats or on shore) or hard impacts with boat hulls or woody debris that often cause injury. From a management view, it could be the carp's "Achilles' fin" if the behavior can be controlled or directed. For instance, if there is a predictable pattern to jumping, nets could be designed to target jumping fish. A better understanding of this behavior could prove useful to fisheries managers working to control the current silver carp populations and prevent further range expansion.

Methods

Behavioral observations

Three 200 m sections of the Illinois River near Havana, IL were delineated with buoys and served as the testing sites (Figure 1A). Sites 1 and 2 were located 200 m apart in a narrow (180 m width) side channel of the river, which was separated from the navigation channel by a large island. Site 3 was situated in the main channel of the river (370 m width) approximately 1 km upstream from Site 2. Water depth ranged from 6–9 m throughout the sites.

The jumping behavior of wild silver carp was assessed using a 6 m aluminum boat, equipped with either a 100 or 150 hp Yamaha (Kennesaw, GA) 4-stroke outboard motor, and operated at four different speeds (16, 24, 32, or 40 km/hr). Four GoPro Hero3 (San Mateo, CA) cameras (recording quality: 1080 pixels; 30 frames/second) were attached to the bow, stern, port, and starboard sides providing 360° coverage around the boat, to record fish jumping behavior. A 2 m PVC pipe demarcated into 0.25 m sections was mounted below each camera for distance reference (Figure 1B, C).

A trial consisted of the boat, with either the 100 or 150 hp outboard motor, transiting the three sites at one of the four speeds (16, 24, 32, or 40 km/hr). For every trial, the boat started downstream of Site 1, attained the randomly selected speed before entering the first site, and maintained the speed through all sites. Immediately after exiting Site 3, the boat turned 180° to port, moved downstream paralleling the western side of Site 3 (Figure 1A), and continued west of the island that separated the main channel from Sites 1 and 2, to a waiting point downstream of Site 1. After at least a one hour recovery period, the sequence was repeated at a different speed. One session consisted of either four morning or afternoon trials, with the order of speeds randomized for each session and the motor type alternated between each session. All tests were conducted on August 8th and 9th, 2013 and October 7th, 2013.

Underwater sound generated by the boat motor was recorded using two hydrophones (HTI-96-MIN, High Tech Inc., Long Beach, MS) connected to a Zoom H4n Handy Recorder (Ronkonkoma, NY). The hydrophones were placed on the western edge of the sites, either between Sites 1 and 2 or at the halfway point (100 m) of Site 3 (Figure 1A), and were situated in the middle of the water column (3–4 m deep) about 10 m from the transit area. Sound pressure levels (SPL) for both outboard motor types were calculated by measuring the root mean square (rms) voltage and converting to SPL in dB re 1µPa (Avisoft-SASLab Pro version 5.2.07). The frequency components and power spectrum of the sound emitted by both the 100 and 150 hp motor were analyzed using Audacity (version 2.0.5).

Data analysis

<u>Number of Jumping Fish:</u> The GoPro video files (mp4 format) from all four cameras were analyzed using frame-by-frame analysis (30 frames per second) in Adobe Photoshop CS6 (version 13; San Jose, CA). Boat transit time through each site varied with speed, therefore jumping frequency (number of jumping fish/second) was used for analysis.

<u>Jumping Angles:</u> The jump's origin was defined as the point where the fish head broke through the water surface and its position and movement vector relative to the center point of the boat (bow = 0°; starboard = 90°; stern = 180°; port = 270°) (Figure 1C, Angle α), were determined. The jumping vector was defined as the angle of the fish's trajectory from jump

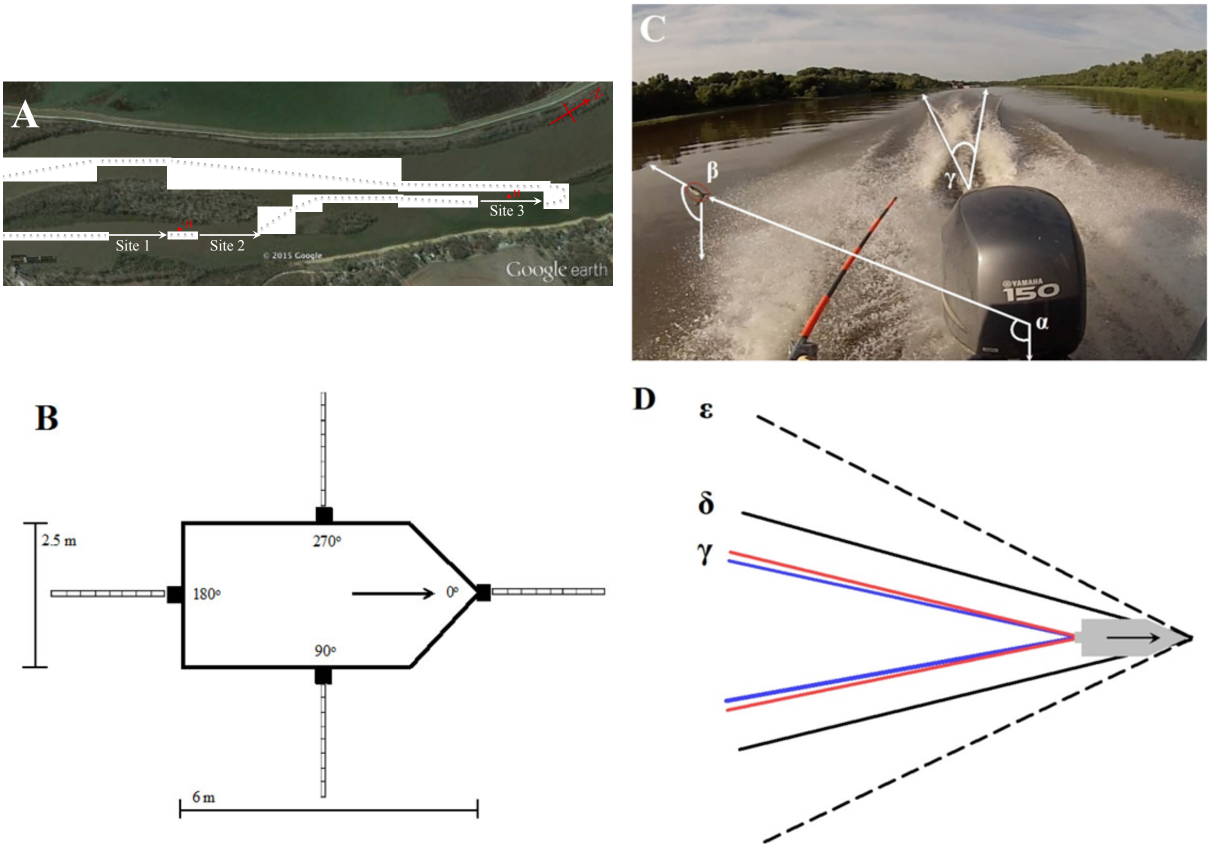


Figure 1. A) Aerial view of the three testing sites near Havana, IL on the Illinois River. Each site is marked with an arrow indicating the direction of boat travel. The dashed line represents the boat path through all three testing sites. Red dots marked with an "H" indicate the location of hydrophones. **B)** Schematic showing the position of the cameras (black boxes) and 2 m PVC pipes used to estimate distance. The boat length and widths are indicated; also the direction of movement and bow = 0° (360°); starboard = 90°; stern = 180°; and port = 270°. Figure is not to scale. **C)** Screen shot from the stern camera taken while using Adobe Photoshop CS6, version 13. The 2 m PVC rod used to estimate distance jumped from boat is visible. The red circle indicates a jumping carp from which the jump initiation angle (α) and jumping vector (β) were measured. The angle of the wake (γ) is also specified. **D)** The entire boat wake, including the average boundary lines of the centerline wake (γ) from the 100 hp (blue) and 150 hp (red) motors, the Kelvin wake (δ), and the waves generated from the Kelvin wake (ε) (Partially adapted from Reed and Milgram 2002).

initiation to reentry (Figure 1C, Angle β) in relation to the center point of the boat at the time of jump initiation. Jumps also were categorized as either "towards" or "away" from the boat. For example, bow camera trajectories > 90° and < 270° were considered towards the boat.

Jumping distance from the boat was determined by calculating the number of pixels for each 0.25 m segment of the 2 m PVC pipe, plotting a linear regression, and extrapolating these measurements for jumps originating beyond the marker. To account for parallax, the pixel measurements were taken using a straight line originating at the bottom of the frame. This measurement technique was verified using objects placed at known distances from the camera but positioned at different angles.

Boat Wake: This study assessed fish jumping in relation to the components that constitute the boat's full wake, including the centerline wake, Kelvin wake, and Kelvin wake-associated surface waves that serve as the boat wake border (Reed and Milgram 2002). The centerline wake, which includes the propeller downwash and viscous wake, lies within the Kelvin wake and is created by the outboard motor. The centerline wake was determined from the stern camera for each of the motor/speed combinations. The angle was measured with the middle of the outboard motor serving as the center point (Figure 1C, D; Angle γ). The extent of the Kelvin wake, which forms a 39° angle starting at the boat bow (Reed and Milgram 2002), was verified using the stern, port, and starboard cameras (Figure 1D;

Angle δ). Finally, footage from the stern camera showed the presence of waves that are typically generated by the Kelvin wake (Figure 1D; Angle ε) and span approximately 15° on either side of the Kelvin wake's boundaries (Reed and Milgram 2002).

Statistical analyses

Sites 1 and 2 were not considered to be independent, as they were located only 200 m apart, and therefore, the number of jumping fish in these sites was pooled. Statistical tests comparing the number of jumping fish at each site, jumping frequency, and jump distance failed the Shapiro-Wilk test of normality ($P < 0.05$), and therefore non-parametric statistics (Mann-Whitney Rank Sum Test or Kruskal-Wallis ANOVA with a post-hoc Tukey Test) were used. The effect of motor and speed on jumping frequency was examined for fish jumping only in Sites 1 and 2, as more fish were observed jumping in these areas than in Site 3. For each motor, jumping frequency and distances were compared between the 16, 24, 32, and 40 km/hr speeds (Kruskal-Wallis ANOVAs) and then by motor type. The median and quartiles (25th and 75th) were reported using the following formats (median; 1st Q, 3rd Q) or median (1st Q, 3rd Q). All analyses were conducted using SigmaPlot for Windows (version 12.5; SYSTAT Software; San Jose, CA). The jump origin and vector were analyzed using Oriana (version 4; Kovach Computing Systems; Wales, UK) for motor type and speed. Rao's Spacing Test was used to determine if the angular data (jump initiation angles and vectors) was randomly distributed around the boat.

Jumping direction (towards vs. away from the boat) was normally distributed among the eight motor/speed combinations, therefore a t-test was performed and the jump totals are represented as mean ± SE. The centerline wake angle measurements were also parametric allowing a one-way ANOVA to compare the four speeds for both motor types (all means reported as mean ± SE).

Results

The maximum sound pressure levels were similar between the two motors and ranged from 130.0–136.0 dB re 1μPa for the 100 hp motor and 131.8–137.2 dB re 1μPa for the 150 hp motor. The power spectrum between the two boats was similar, with the 100 hp peaking at 1.5 kHz and the 150 peaking at 2.0 kHz (Figure 2).

The number of fish jumping at each site during each transit (N = 66) ranged from 0 to 75 and during 57.9% of the transits, at least five fish jumped. In Sites 1 and 2, the boats stimulated jumping from silver carp during 95% of the transits (N = 44). Furthermore, significantly more fish jumped in Sites 1 and 2 (6.0; 3.0, 9.0) than in Site 3 (2.0; 0.0, 5.0) (Mann-Whitney $P < 0.001$) (Figure 3).

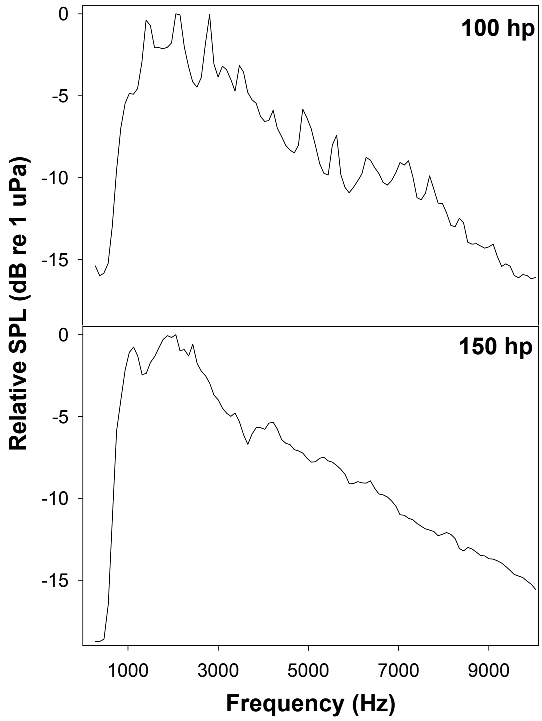


Figure 2. The power spectrum in relative dB of the 100 and 150 hp outboard motors is plotted versus frequency (Hz).

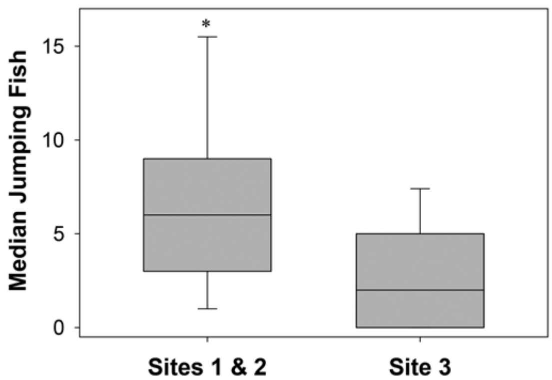


Figure 3. Median jumping silver carp in pooled Sites 1 and 2 versus Site 3. Each box represents the 25th (bottom of box) and 75th (top of box) quartiles with the median marked by the line within the box. Whiskers (error bars) above and below the box indicate the 10th and 90th percentiles. * indicates significantly different group (Mann-Whitney $P < 0.001$).

Table 1. Summary of the percentage of all airborne fish that jumped in the centerline wake, the Kelvin wake (excluding the centerline wake area), the waves generated by the Kelvin wake, and the full boat wake, which is bordered by the waves generated from the Kelvin wake. N represents the total number of jumping fish for each motor and speed combination.

Motor (hp) @ Speed (km/hr)	N	Centerline Wake (%)	Kelvin Wake (%)	Kelvin Waves (%)	Full Wake (%)
100 @ 16	41	22.0	2.4	19.5	43.9
100 @ 24	32	3.1	6.3	12.5	21.9
100 @ 32	17	0	5.9	17.6	23.5
100 @ 40	27	3.7	22.3	18.4	44.4
150 @ 16	63	12.9	13.1	9.5	35.5
150 @ 24	52	30.8	17.3	9.6	57.7
150 @ 32	70	8.6	5.7	30.0	44.3
150 @ 40	96	6.3	9.3	20.9	36.5

The median jumping frequency varied from 0.11 fish/second (0.05, 0.22; 16 km/hr) to 0.31 fish/second (0.10, 0.46; 40 km/hr) for the 100 hp motor and 0.13 fish/second (0.11, 0.51; 32 km/hr) to 0.39 fish/second (0.13, 1.6; 40 km/hr) for the 150 hp motor (Figure 4). There was no significant difference in jumping frequency between the four speeds for either the 100 hp or the 150 hp (Kruskal-Wallis ANOVA P = 0.407) motors, allowing the jumping frequency to be pooled for each motor. The median jumping frequencies for the 100 and 150 hp motors were 0.17 fish/second (0.07, 0.32) and 0.31 fish/min (0.13, 0.56), respectively, and were also not significantly different (Mann-Whitney P = 0.064).

The arc created by the centerline wake (viscous wake and propeller downwash) ranged from 26.4° ± 2.4° to 19.9° ± 3.1° (100 hp) and 31.1° ± 2.0° to 20.8° ± 3.0° (150 hp). However, there was no significant difference in the wake angle between the speeds for either the 100 hp (ANOVA P = 0.358) or 150 hp motors (ANOVA P = 0.257) allowing the data to be pooled and resulting in no significant difference in wake angle between the motor types (100 hp: 22.4° ± 3.8°; 150 hp: 25.2° ± 5.0°; P = 0.152). The Kelvin wake, which is often independent of boat speed and size, was verified to project astern in a 39° arc (160.5°–199.5°) and the Kelvin waves were observed radiating out approximately 15° on either side of the Kelvin wake (Reed and Milgram 2002).

Fish were observed jumping in the centerline wake for all motor and speed combinations except for the 100 hp motor trials at 32 km/hr. The percentages of fish jumping in the centerline wake ranged from 3.1% (100 hp at 24 km/hr) to 30.8% (150 hp at 24 km/hr) (Table 1). Of all the airborne fish, 2.4% (100 hp at 16 km/hr) to 22.3% (100 hp at 40 km/hr) jumped within the Kelvin wake area beyond the centerline wake (100 hp motor: ~160.5°–168.7° and 191.3°–199.5°; 150 hp motor: ~160.5°–167.2° and 192.8°– 199.5°) (Table 1). Finally, 9.5% (150 hp at 16 km/hr) to 30.0% (150 hp at 32 km/hr) jumped in the waves created by the Kelvin wake (~145.5°–160.5° and 199.5°–214.5°). The edge of these waves formed the outermost border of the wake (~145.5°–214.5°) and 21.9% (100 hp at 24 km/hr) to 57.7% (150 hp at 24 km/hr) jumped in the full boat wake (which includes the centerline wake, Kelvin wake, and Kelvin waves) (Table 1).

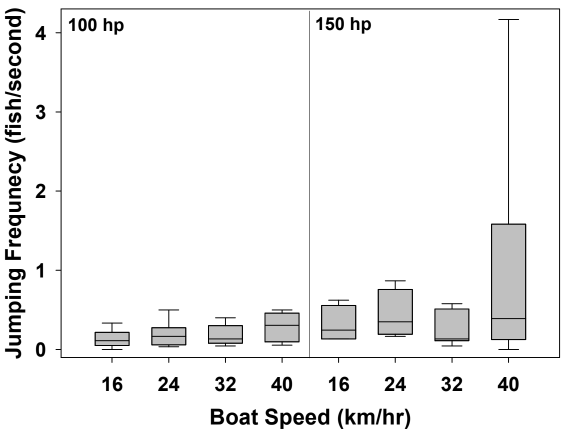


Figure 4. Median frequency of jumping carp responding to each of the eight motor speed combinations. The boxes represent the 25th (bottom) and 75th (top) quartiles and the line within the box indicates the median value, with the 10th and 90th percentiles shown as whiskers. There was no significant difference in jumping frequency among the motor type and speed variables (Kruskal-Wallis ANOVA P = 0.407).

For the 100 hp motor, 90.6% of the fish initiated their jumps in the region astern, after the boat had passed (> 90° and < 270°). The fish also primarily vectored their jumps away (84.8%) from the boat (Table 2, Figure 5). Jump origin was not randomly distributed (Rao's P < 0.01) and was initiated primarily in a 90° arc behind the boat, with the stern serving as the center, and median jump origin locations ranged from 135.9° (129.2°, 166.3°) at 24 km/hr to

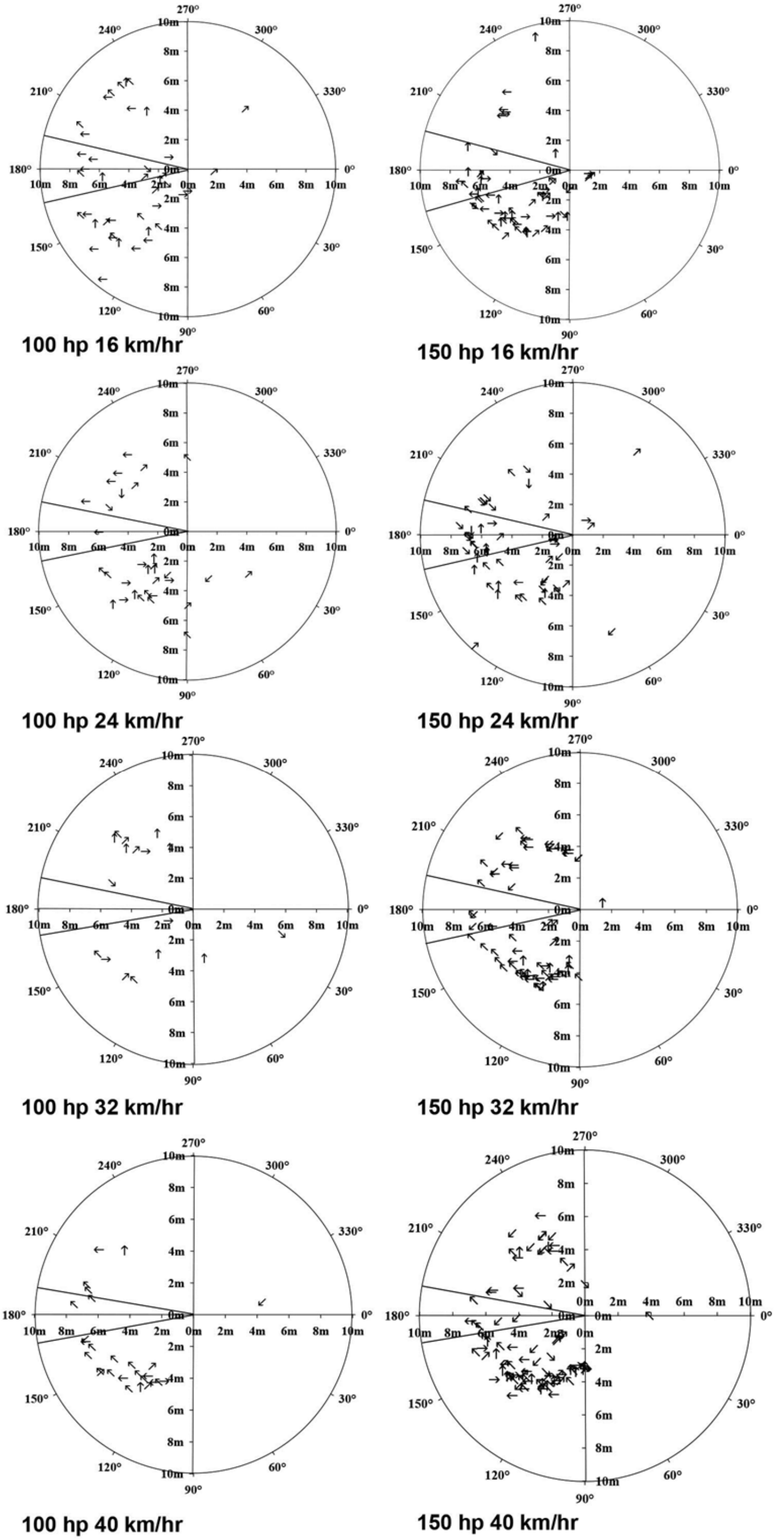


Figure 5. Polar plots for all eight treatments. Each arrow represents one fish and marks the estimated distance from and jump initiation angle in relation to the boat and its direction of movement (0º). To assess the jumping vector, each fish was categorized in one of eight arcs (0°–45°, 46°–90°, 91°–115°, 116°–135°, 136°–180°, 181°–270°, 271°–315°, and 316°–360°) and plotted with a corresponding arrow. The arrows point in the direction of each fish's trajectory (jumping vector). The solid lines represent the boat's wake.

Table 2. Summary of the angle data for all eight stimuli. N represents the number of fish analyzed for both the jump initiation angle and jumping vector (Median; 1st Q, 3rd Q) (Rao's P < 0.01). * indicates the only non-significant group.

Motor (hp) @ Speed (km/hr)	N	Jumping Initiation Angle Median° (1st Q°, 3rd Q°)	Jumping Vector Median° (1st Q°, 3rd Q°)
100 @16	41	156.7 (149.9, 180.0)	149.1 (119.3, 168.0)
100 @ 24	32	135.9 (129.2, 166.3)	121.8 (92.8, 167.4)*
100 @ 32	17	222.3 (158.1, 220.7)	102.2 (66.3, 118.3)
100 @ 40	27	145.0 (136.6, 165.1)	153.1 (142.2, 176.9)
150 @ 16	63	143.9 (138.7, 161.2)	129.6 (105.1, 141.6)
150 @ 24	52	168.6 (150.8, 176.1)	90.1 (45.2, 124.7)
150 @ 32	70	152.9 (150.0, 176.5)	186.0 (165.6, 193.1)
150 @ 40	96	140.9 (139.7, 159.5)	138.3 (116.3, 159.0)

222.3° (158.1°, 220.7°) at 32 km/hr. The jumping vectors were also not randomly distributed (Rao's P < 0.01) and median angles ranged from 102.2° (32 km/hr: 66.3°, 118.3°) to 153.1° (40 km/hr: 142.2°, 176.9°) (Table 2).

Similar to the 100 hp motor, fish responding to the 150 hp motor primarily jumped away (77.0%) from and behind (95.0%) the moving boat. The jump origins for the 150 hp motor favored the starboard/stern quadrant (Rao's P < 0.01), with median origination angles ranging from 140.9° (40 km/hr: 139.7°, 159.5°) to 168.6° (24 km/hr: 150.8°, 176.1°) (Table 2). Furthermore, the jumping vectors were non random (Rao's P < 0.01), varying from 90.1° (24 km/hr: 45.2°, 124.7°) to 186.0° (32 km/hr: 165.6°, 193.1°) (Table 2).

Jumping patterns for each motor type and speed are summarized in Figures 5 and 6. The pattern for both motor types shows a semicircle array astern with the motor as the center point and is more pronounced for the 150 hp motor (Figure 5). Fish avoided the area directly astern (< 4.0 m) within the motor wake (Figures 5, 6).

Figure 7 summarizes the jump origination by quantifying the number of jumps in 20° arcs centered around the boat, with each arc subdivided into 2.0 m lengths. There is a clear exclusion zone from 0.0–4.0 m between 140°–220° in the area directly astern (Figure 7). The majority of jumping occurred between 4.0–8.0 m in the 100°–180° arcs, within and just outside the Kelvin wake and in the distant portion of the centerline wake (> 4.0 m astern). The fish avoided the area directly astern (0–4.0 m) independent of motor and speed (Figures 5, 6, 7).

The median distance of the jump origin from the boat (100 hp) varied from 5.2 m (24 km/hr: 3.8 m, 6.1 m) to 6.4 m (16 km/hr: 3.1 m, 7.3 m), with no significant difference between the speeds (Kruskal-Wallis ANOVA P = 0.117). Similarly, there was no significant difference in distance for fish reacting to the 150 hp motor (Kruskal-Wallis ANOVA P = 0.274), with distances ranging from 4.9 m (40 km/hr: 3.9 m, 5.8 m) to 5.7 m (24 km/hr: 3.7 m, 6.5 m). Therefore, the distances were pooled by motor type. The fish jumped slightly further (5.6 m; 4.7 m, 6.8 m) from the boat during the trials with the 100 hp motor versus the 150 hp motor (5.1 m; 3.9 m, 5.9 m) (Mann-Whitney P < 0.001) (Figure 8A). The highest percentages of jumping fish occurred at distances 5, 6, and 7 m from the boat for both the 100 hp (19.2%, 22.1%, and 19.2%,) and 150 hp (20.4%, 31.1%, and 20.4%) motors (Figure 8B).

All jumping vectors were categorized as moving either towards or away from the boat. 79.8% of all observed jumps were angled away from the boat and significantly (P < 0.001) more fish (42.0 ± 6.3) fish jumped away from the boat (10.6 ± 3.4) when each motor type and speed was compared (Figure 9).

Discussion

This study is the first to quantitatively examine silver carp jumping behavior in response to motorized watercraft. The frequency of jumping was independent of boat speed and the two motor types examined. However, the results indicate that jumping is non-random as the fish primarily jumped behind and away from the boat but rarely in the area directly astern (< 4.0 m). Furthermore, there is a pattern in fish response to moving (> 15 km/hr) boats as both motor types elicited a semi circle arrangement of jumping behind the boat.

Although the exact jumping trigger remains unclear, the results indicate that the majority of fish moved away from the stimulus source. Unfortunately, only above water behavior was observed in the turbid water, with nearby turbidity readings between 22 and 97 FTU on the testing days (USGS 2016). Therefore, it is uncertain if jumping was initiated as a c-start, which is an evasive reflex in fish that occurs rapidly (< 100 ms) (Eaton et al. 1977; Fetcho et al. 1991), or slower neuromuscular pathways. The

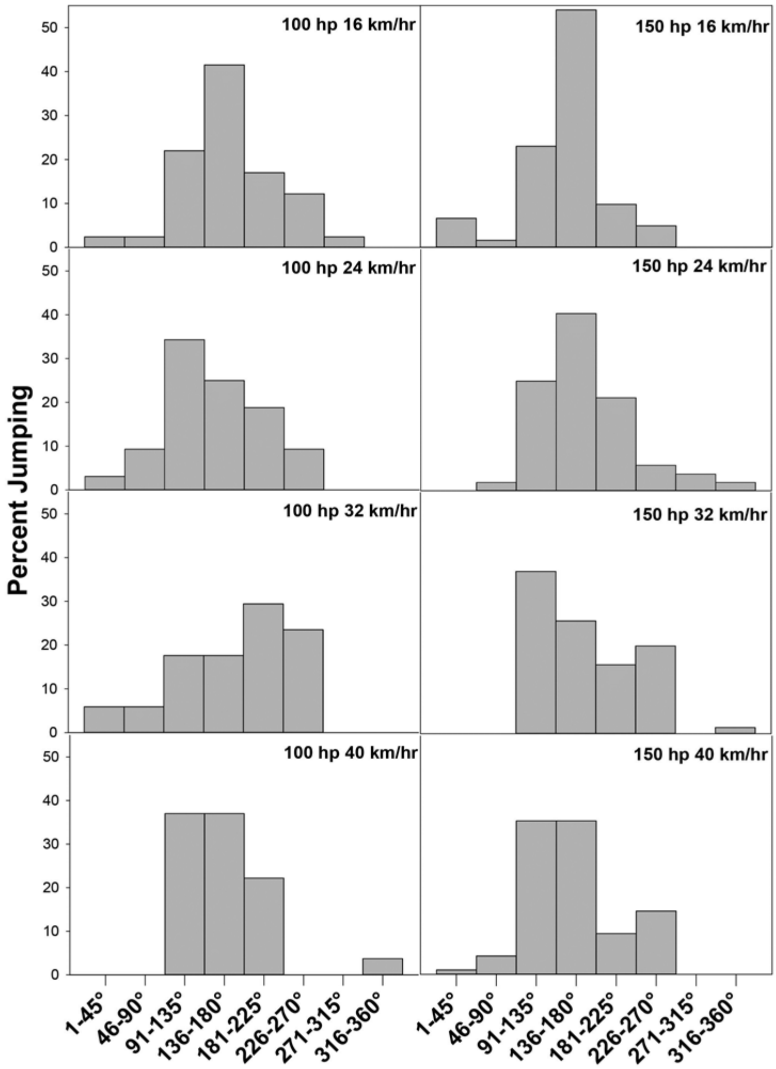


Figure 6. Histograms assessing the percentage of fish that initiated jumping in one of eight angular arcs (0°–45º, 46°–90°, 91°–115°, 116°–135°, 136°–180°, 181°–270°, 271°–315°, and 316°–360°) in relation to the boat.

results presented reflect the behavioral response of carp at the point when the fish broke the water surface, but the fish's location in relation to the boat when it first responded underwater is unknown. Both the fish's depth and the type of response (e.g. c-start) could have resulted in a time lag between when the fish reacted to the oncoming boat and when it broke the water surface. For instance, a fish that was near the surface of the water and reacting with a c-start response would break the water's surface quicker than a fish that was either deeper or had a slower reaction time. Observing jumping in clear water could provide insight into the biomechanics of jumping, however anecdotal evidence suggests that this behavior is reduced in these environments. Alternatively, sonar imaging could evaluate the depths at which fish respond and move to the surface to jump.

It is unlikely that vision was a factor in detecting the boat. In the turbid water, fish and submerged objects were only visible to human observers within a few centimeters of the surface. Although the silver carp's visual sensitivity remains to be determined, light adsorption and scattering in turbid environment degrade visual range relatively quickly for aquatic animals (Lythgoe 1979; Benfield and Minello 1996).

It is likely that the jumping fish were responding to mechanosensory cues from hydrodynamic water changes. The majority of fish initiated their jumps after the boat stern passed their position and therefore, the jumping location was compared with the hydrodynamic disturbance created by the boat to determine if any particular component influenced jumping. The boat wake consists of the Kelvin wake (originating at the bow), the waves created by the

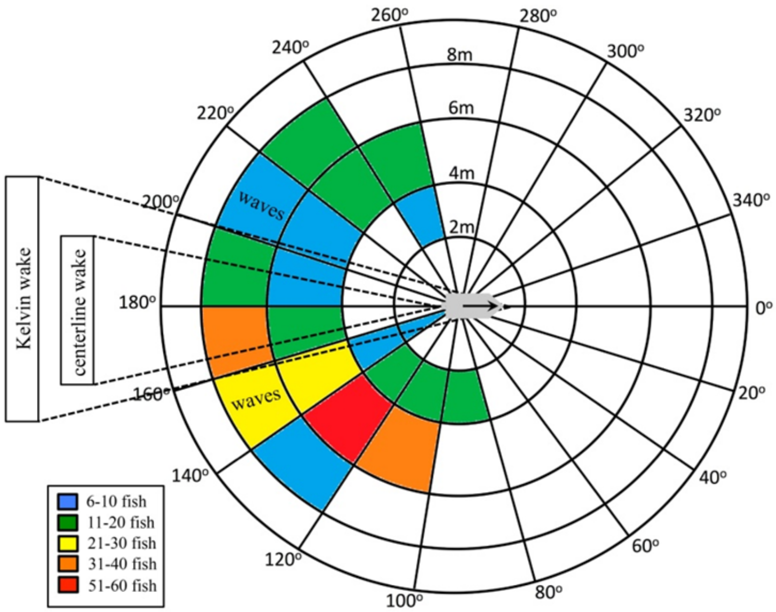


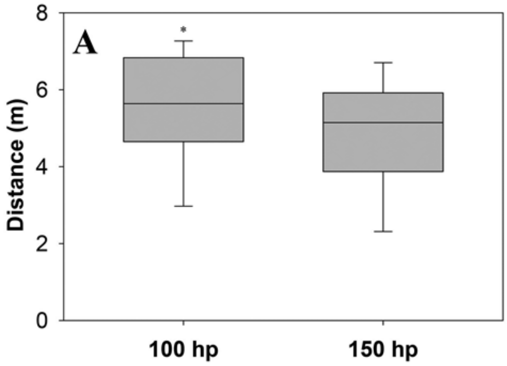
Figure 7. Summary of all jump initiation locations. The area around the boat was divided into 2 m segments in 20° arcs, giving 90 total sections. The colors represent the number of jumping fish in each section, see legend inset. White segments represent areas where 5 or less fish jumped. There were no sections in which the total number of jumping fish was between 41–50.

Kelvin wake, and the centerline wake (which includes the propeller downwash and the viscous wake) (Reed and Milgram 2002). The Kelvin wake begins at the bow and forms a 39° angle, extending past the stern of the boat. Additionally, a series of surface waves generated by the boat wake extends approximately 15° beyond and on either side of the Kelvin wake. Both motors generated a semi-circle jumping pattern around the full wake and the highest densities of jumping fish were observed within the centerline wake (100 hp: 168.7°–191.3°; 150 hp: 167.2°–192.8°), the Kelvin wake (which spanned approximately 160.5°–199.5°), and the waves that radiated out from the Kelvin wake (~145.5°–214.5°). Additionally, the propeller downwash may have deterred fish from jumping directly behind the boat. Jumping origin was independent of motor size and speed, suggesting that under certain conditions the jumping pattern can be predicted.

While the results from this study strongly suggest that jumping is largely associated with turbulence generated by the wake, the contribution of sound to behavior cannot be discounted. At least part of the sound (0.06–10 kHz) emitted by the outboard motors used in this experiment is within the hearing range of the silver carp, as Lovell et al. (2006) reported frequency sensitivity up to 3 kHz in this species. Furthermore, the lateral line, which is sensitive to low frequency water movement, has recently been determined to assist in sound detection. For instance, goldfish (*Carassius auratus* Linnaeus, 1758), another carp species, responded to sounds up to 200 Hz with their lateral line (Higgs and Radford 2013). Additionally, the particle motion component of the sound field may also influence behavior and its contribution still needs to be determined. Therefore, the fish in this study that jumped in the boat wake may have perceived and responded to sound and/or vibrations from the motor with both their lateral line and inner ear.

However, the jumping pattern observed strongly suggests that the sound of the approaching boat is insufficient to trigger jumping. The sound of the outboard motor was detected by the hydrophone well before the boat passed its location and an examination of sound pressure levels (130–137 dB re 1 µPa) indicates that, depending on boat speed and motor type, the silver carp should be able to detect the sound generated by the outboard motor well in advance of the boat approaching their position. This suggests that the pressure levels or particle motion may have to reach certain thresholds, which are only surpassed when the boat is near the fish, to trigger the jumping. Therefore, the sound thresholds that could modify silver carp behavior still need to be determined. Finally, jumping could be elicited by a combination of sound and water turbulence. Further examination of wild silver carp behavior in response to sound is imperative to better define the relationship between sound and jumping.

In Sites 1 and 2, which had more jumping fish than Site 3, there was a bias for jumping on the starboard (eastern) rather than the port (western) side



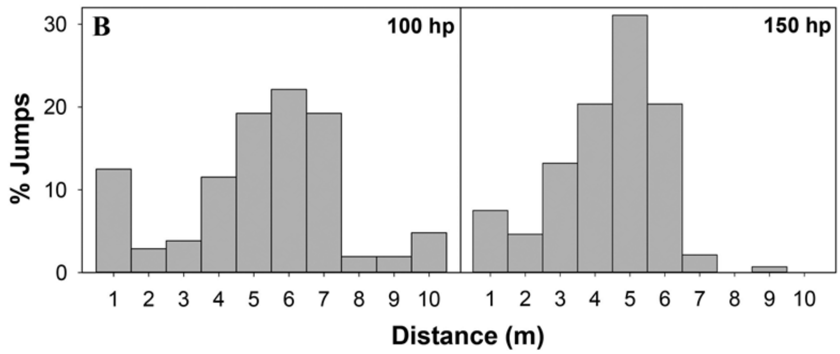


Figure 8. A) Median distance from the boat in which fish initiated jumping for the 100 and 150 hp motors. Each box represents the 24th and 75th quartiles with the median marked by the line within the box. The 10th and 90th percentiles are indicated by the whiskers (error bars). * indicates significantly different groups (Shapiro-Wilk P < 0.001) **B)** Histograms representing the percentage number of jumps that occurred at 1 m increments from the boat.

of the boat. An extensive shallow flat on the west side of the river, in which Sites 1 and 2 were located, forced the boat to favor the east side of the channel. This asymmetrical depth profile could have created a non-random distribution of fish underwater, as the fish may have preferred the deeper eastern side of the river, explaining the greater numbers that jumped between 100°–180°. Since Sites 1 and 2 were in close proximity, the results from these sites were pooled, as the boat could have influenced the fish in Site 2 as it passed through Site 1. However, it is unlikely that downstream boat movement impacted the fish in Site 3, as this location was 1 km upstream and separated from the first two sites by an island. Rather, the decreased jumping in Site 3 was probably related to the greater river width at this site. Therefore, there may have been less fish present in this region or the sound stimulus could have been attenuated. A sonar system to evaluate fish behavior underwater would aid in a better understanding of the differences between jumping at the sites.

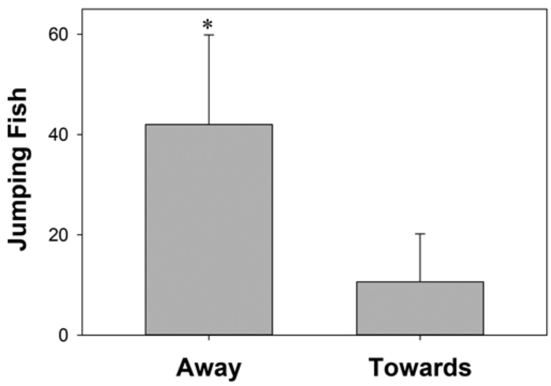


Figure 9. Average number of fish that jumped "away" from versus "towards" the boat. Averages were calculated based on pooling the total number of jumping fish in response to each outboard motor type at one of the four speeds (100 hp @ 16 km/hr, 100 hp @ 24 km/hr, 100 hp @ 32 km/hr, and 100 hp @ 40 km/hr, 150 hp @ 16 km/hr, 150 hp @ 24 km/hr, 150 hp @ 32 km/hr, and 150 hp @ 40 km/hr), * indicates significantly different groups (t-test P < 0.001). Error bars represent ± SE.

The two motor types and four speeds of travel were chosen based on anecdotal input from the researchers at the Illinois River Biological Station that different motors and speeds were most effective in stimulating jumping. Therefore, the study evaluated jumping in response to two motor types (100 and 150 hp) and four speeds (16–40 km/hr). However, the results indicated that there was no significant difference in jumping frequency for any of the motor or speed combinations. This could be related to redundancy in site testing or limited trials. Additional replicates and a greater number of sites could better evaluate jumping. Alternatively, as the hydrophone data suggests that the sound pressure levels and spectrum were similar across trials, wild silver carp may not differentially respond to the two boats used in the study.

Water clarity limited the current study to examining only carp jumping behavior, however the results presented can be applied to fisheries management strategies. Commercial fishermen currently drive fish towards nets by banging on their boat hulls and revving partially submerged outboard propellers, which suggests refinement of these techniques could allow a greater number of fish to be captured and removed. By modeling the jumping, managers will be able to optimize capture or killing methods using boom nets, solid screens, or towed collecting vessels. The results from this study, which indicate that carp responding to moving (16–40 km/hr) watercraft primarily jump behind the boat, suggest that two laterally extending nets mounted on the back of a boat may be successful in capturing or killing airborne fish. Alternatively, another method could involve using 2–3 boats spaced across a river channel to drive fish toward a block net for capture and removal. Further research is also needed to determine the jumping trigger, as this could be another useful tool in managing silver carp. Isolating the exact trigger for jumping, combined with the ability to consistently induce the behavior, could also be used to census areas for number of fish and refine herding technologies, as the airborne fish reveal their position and vector.

The results presented provide the first evidence that silver carp jumping can be modeled, as the fish demonstrated a distinct and consistent behavioral pattern. This study suggests that jumping is non-random and that the fish primarily moved away from the moving boats. A better understanding of silver carp jumping behavior can help officials determine the best methods for capturing fish.

Acknowledgements

We would like to thank the staff and interns at the Illinois River Biological Station (IRBS) in Havana, IL and their sponsors, Sport-fish Restoration Fund project F-101-R, Illinois DNR Great Lakes Restoration Initiative Asian Carp Removal Program, and Upper Mississippi River Restoration Long-term Resource Monitoring Element, for providing resources (including boat time and buoys) and input on the experimental design. We would also like to thank Graham Hanson for assisting with fieldwork. Funding was provided by University of Minnesota Duluth, the Minnesota Environment and Natural Resources Trust Fund, and the Illinois Natural History Survey. Finally, the authors wish to thank the reviewers for the time and effort put in to providing feedback on this manuscript.

References

Aronson LR (1971) Further studies on orientation and jumping behavior in the gobiid fish, *Bathygobius soporator*. *Annals of the New York Academy of Sciences* 188: 378–392, https://doi.org/10.1111/j.1749-6632.1971.tb13110.x

Bayliss JR (1982) Unusual escape response by two cyprinodontiform fishes, and a bluegill predator's counter-strategy. *Copeia* 1982: 455–457, https://doi.org/10.2307/1444631

Benfield MC, Minello TJ (1996) Relative effects of turbidity and light intensity on reactive distance and feeding of an estuarine fish. *Environmental Biology of Fishes* 46: 211–216, https://doi.org/10.1007/BF00005223

Bierman HS (2013) Aerial Jumping in the Trinidadian Guppy (*Poecilia reticulata*). *PLoS ONE* 8: e61617, https://doi.org/10.1371/journal.pone.0061617

Eaton RC, Bombardieri RA, Meyer DL (1977) The Mauthner-initiated startle response in teleost fish. *Journal of Experimental Biology* 66: 65–81

Fetcho JR (1991) Spinal network of the Mauthner cell. *Brain, Behavior and Evolution* 37: 298–316, https://doi.org/10.1159/000114367

Higgs DM, Radford CA (2013) The contribution of the lateral line to "hearing" in fish. *Journal of Experimental Biology* 216: 1484–1490, https://doi.org/10.1242/jeb.078816

Irons KS, Sass GG, McClelland MA, Stafford JD (2007) Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA—Is this evidence for competition and reduced fitness? *Journal of Fish Biology* 71: 258–273, https://doi.org/10.1111/j.1095-8649.2007.01670.x

Kolar KS, Chapman DC, Courtenay WR, Housel CM, Williams JD, Jennings DP (2005) Asian carps of the genus *Hypophthalmichthys* (Pisces, Cyprinidae)—a biological synopsis and environmental risk assessment. U.S. Fish and Wildlife Service, 183 pp

Kolar KS, Chapman DC, Courtenay WR, Housel CM, Williams JD, Jennings DP (2007) Bigheaded carps: a biological synopsis and environmental risk assessment. American Fisheries Society Special Publication 33. Bethesda, MD, 204 pp

Kondratieff M, Myrick C (2006) How High Can Brook Trout Jump? A Laboratory Evaluation of Brook Trout Jumping Performance. *Transactions of the American Fisheries Society* 135: 361–370, https://doi.org/10.1577/T04-210.1

Lauritzen DV, Hertel FS, Jordan LK, Gordon MS (2010) Salmon jumping: behavior, kinematics and optimal conditions, with possible implications for fish passageway design. *Bioinspiration and Biomimetics* 5, https://doi.org/10.1088/1748-3182/5/3/035006

Lythgoe JN (1979) The Ecology of Vision. Oxford University Press, New York, 244 pp

Lovell JM, Findlay MM, Nedwell JR, Pegg MA (2006) The hearing abilities of the silver carp (*Hypopthalmichthys molitrix*) and bighead carp (*Hypopthalmichthys nobilis*). *Comparative Biochemistry and Physiology A* 143: 286–291, https://doi.org/10.1016/j.cbpa.2005.11.015

Moy PB, Polls I, Dettmers JM (2011) The Chicago sanitary and ship canal aquatic nuisance species dispersal barrier. In: Chapman DC, Hoff MH (eds), Invasive Asian carps in North America, American Fisheries Society Special Publication 74. Bethesda, MD, pp 1–10

Murphy EA, Jackson PR (2013) Hydraulic and water-quality data collection for the investigation of Great Lakes tributaries for Asian carp spawning and egg-transport suitability: U.S. Geological Survey Scientific Investigations Report 2013–5106, http://pubs.usgs.gov/sir/2013/5106/

Reed AM, Milgram JH (2002) Ship wakes and their radar images. *Annual Review of Fluid Mechanics* 34: 469–502, https://doi.org/10.1146/annurev.fluid.34.090101.190252

Saidel WM, Strain GF, Fornari SK (2004) Characterization of the Aerial Escape Response of the African Butterfly Fish, *Pantodon buchholzi* Peters. *Environmental Biology of Fishes* 71: 63–72, https://doi.org/10.1023/B:EBFI.0000043153.38418.cd

Sampson SJ, Chick JH, Pegg MA (2009) Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi Rivers. *Biological Invasions* 11: 483–496, https://doi.org/10.1007/s10530-008-9265-7

Sass GG, Cook TR, Irons KS, McClelland MA, Michaels NN, O'Hara ETM, Stroub MR (2010) A mark-recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. *Biological Invasions* 12: 433–436, https://doi.org/10.1007/s10530-009-9462-z

Sass GG, Hinz C, Erikson AC, McCelland NN, McCelland MA, Epifanio JM (2014) Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. *Journal of Great Lakes Research* 40: 911–921, https://doi.org/10.1016/j.jglr.2014.08.010

Shih AM, Techet AH (2010) Characterization of the Jumping Behavior of Archer Fish, *Toxotes microlepis*. 6th World Congress of Biomechanics (WCB 2010). August 1–6, 2010 Singapore, pp 48–51, https://doi.org/10.1007/978-3-642-14515-5_13

USGS (2016) Historical Water Quality Data through the National Water Information System for Station 05586300 (Illinois River at Florence, IL) (accessed March 11, 2016)

Wiest FC (1995) The specialized locomotory apparatus of the freshwater family Gasteropelecidae. *Journal of Zoology* 236: 571–592, https://doi.org/10.1111/j.1469-7998.1995.tb02733.x

Journal of Great Lakes Research 43 (2017) 163–171

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Acoustic deterrence of bighead carp (*Hypophthalmichthys nobilis*) to a broadband sound stimulus

Brooke J. Vetter [a,*], Kelsie A. Murchy [a], Aaron R. Cupp [b], Jon J. Amberg [b], Mark P. Gaikowski [b], Allen F. Mensinger [a]

[a] Biology Department, University of Minnesota Duluth, 1035 Kirby Drive, Duluth, MN 55812, United States
[b] United States Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, WI 54603, United States

ARTICLE INFO

Article history:
Received 19 May 2016
Accepted 21 November 2016
Available online 30 November 2016

Associate Editor: John Janssen

Keywords:
Bighead carp
Broadband sound
Bioacoustics
Acoustic deterrent

ABSTRACT

Recent studies have shown the potential of acoustic deterrents against invasive silver carp (*Hypophthalmichthys molitrix*). This study examined the phonotaxic response of the bighead carp (*H. nobilis*) to pure tones (500–2000 Hz) and playbacks of broadband sound from an underwater recording of a 100 hp outboard motor (0.06–10 kHz) in an outdoor concrete pond (10 × 5 × 1.2 m) at the U.S. Geological Survey Upper Midwest Environmental Science Center in La Crosse, WI. The number of consecutive times the fish reacted to sound from alternating locations at each end of the pond was assessed. Bighead carp were relatively indifferent to the pure tones with median consecutive responses ranging from 0 to 2 reactions away from the sound source. However, fish consistently exhibited significantly ($P < 0.001$) greater negative phonotaxis to the broadband sound (outboard motor recording) with an overall median response of 20 consecutive reactions during the 10 min trials. In over 50% of broadband sound tests, carp were still reacting to the stimulus at the end of the trial, implying that fish were not habituating to the sound. This study suggests that broadband sound may be an effective deterrent to bighead carp and provides a basis for conducting studies with wild fish.

Introduction

The bighead carp (*Hypophthalmichthys nobilis*) is an invasive fish species in North America and has established breeding populations in the Mississippi River Watershed. Range expansion of these fish into the Great Lakes is a concern because they are present in the northern regions of the Illinois River (Kolar et al., 2007; Sass et al., 2010) and have been found in the Chicago Sanitary and Ship Canal (Moy et al., 2011) near Lake Michigan. These fish, along with the closely related silver carp (*H. molitrix*), evolved in Asia and were intentionally brought to the United States for use in wastewater treatment plants and aquaculture facilities (Kelly et al., 2011; Kolar et al., 2007). Both species are an ecological concern because they compete with native species, such as paddlefish (*Polyodon spathula*; Schrank et al., 2003), gizzard shad (*Dorosoma cepedianum*; Sampson et al., 2009), and bigmouth buffalo (*Ictiobus cyprinellus*; Irons et al., 2007), for food and space. While adults from both *Hypophthalmichthys* species can grow up to 40–50 kg, they are planktivores, which precludes them from being caught via angling or baited traps. Furthermore, these filter feeders will consume both zooplankton and phytoplankton and could alter the entire food web in rivers where they are abundant (Sass et al., 2014).

As part of an integrated pest management strategy, state and federal agencies throughout the Midwest are prioritizing the development of effective non-physical deterrents, including acoustic barriers, to prevent further bighead and silver carp range expansion. Acoustic deterrents, often in combination with other techniques such as bubbles or strobe lights, have been moderately successful at dam and power plant intakes (see Noatch and Suski, 2012 for a review). Barriers utilizing ultrasound (122–128 kHz; Ross et al., 1993) or varied low-frequency sound (20–600 Hz; Maes et al., 2004) successfully repelled 87% and 60% of clupeids, respectively. There is evidence that bighead carp are deterred by sound (20–2000 Hz) combined with bubbles in studies conducted on both captive (Pegg and Chick, 2004; Taylor et al., 2005) and wild fish (Ruebush et al., 2012). However, an investigation into the phonotaxic response of invasive carp to sound alone is important for the evaluation of acoustic deterrents.

Bighead carp are ostariophysans and possess Weberian ossicles, which connect the gas bladder to the inner ear (Fay and Popper, 1999), allowing for higher frequency hearing than many non-ostariophysan species. Lovell et al. (2006) indicated bighead carp frequency sensitivity up to 3 kHz. However, as the researchers did not test above 3 kHz, it is uncertain if bighead carp can hear beyond this frequency. Ladich (1999) studied species from four ostariophysan orders (Cypriniformes, Characiformes, Siluriformes, and Gymnotiformes) and elicited auditory brainstem responses up to at least 5 kHz in all species. Furthermore, brown bullhead (10–13 kHz; *Ameiurus nebulosus*;

* Corresponding author.
E-mail address: vett0114@d.umn.edu (B.J. Vetter).

http://dx.doi.org/10.1016/j.jglr.2016.11.009
0380-1330/

Poggendorf, 1952) and neotropical catfish (6 kHz; *Lophiobagrus cyclurus*; Lechner et al., 2011) have frequency sensitivity beyond 5 kHz. Therefore, it is possible that bighead carp can detect higher frequencies than those previously reported by Lovell et al. (2006).

The silver carp is notorious for its jumping behavior, which can be elicited when motorized watercraft move through carp-infested areas. Playbacks of the broadband (0.06–10 kHz) sound emitted by outboard motors caused wild silver carp to jump (Mensinger, unpublished) and elicited negative phonotaxis in captive fish (Vetter et al., 2015), however bighead carp do not jump (Kolar et al., 2007). Therefore, the effect of similar acoustic stimulation on bighead carp is unknown, as their underwater behavior is difficult to monitor in turbid water. Since silver and bighead carp coexist and will hybridize, if bighead carp are affected similarly by sound, the two species could be co-managed by acoustic deterrents.

The goal of this study was to examine the behavioral response of bighead carp to pure tones and broadband sound stimuli, which was successful in modulating silver carp swimming behavior. It was predicted that bighead carp would also demonstrate negative phonotaxis to broadband sound, providing further support for the development of acoustic barriers to manage these species.

Methods

Animal husbandry

All experiments were conducted at the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin. Bighead carp (n = 50; total length: 212 ± 7.7 mm; wet weight: 101.4 ± 12.3 g; mean ± standard deviation) were obtained in the summer of 2013 from Osage Catfisheries, a private aquaculture farm in Osage Beach, Missouri, USA. Fish were maintained in 1500 L flow-through indoor ponds and fed trout starter diet (Skretting, Tooele, UT) at a rate of 0.5% body weight per day (Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government). A Chapter NR 40 Permit for Possession, Transport, Transfer, or Introduction of Prohibited or Restricted Species was obtained from the Wisconsin Department of Natural Resources prior to acquisition of test animals and movement to outdoor ponds and experiments were conducted under UMESC Animal Care and Use Committee Protocol Number AEH-12-PPTAC-01.

Behavioral experiments

Behavioral experiments were conducted in an above ground 10 × 5 × 1.2 m (60 kL) outdoor concrete flow-through pond. Each group (N = 5) of ten naïve fish was allowed to acclimate in the outdoor pond for at least 48 h prior to the initiation of experiments. Five two-day trials were conducted from June through August 2014. At the conclusion of each trial, the pond was drained, refilled, and naïve fish (N = 10) added.

Sound stimuli

Sound was delivered via one of two pairs of underwater speakers (UW-30, Lubell Labs Inc., Whitehall, OH) that were placed 1.0 m from each end of the pond, 1.6 m from the nearest side-wall, 1.8 m apart, and positioned so that sound was projected along the longitudinal axis of the pond (Fig. 1). Acoustic stimuli consisted of pure tones (500, 1000, 1500, or 2000 Hz), generated by Audacity 2.0.5 software, and broadband sound, recorded underwater from an outboard motor (100 Hp 4-stroke, Yamaha, Kennesaw, GA). The outboard motor sound was recorded with a hydrophone (HTI-96-MIN, High Tech Inc., Long Beach, MS), in the Illinois River near Havana, Illinois, USA (40° 17′ 30″ N, 90° 04′ 20″ W). Sound was recorded in approximately 1 m of water while the boat transited past the hydrophone at 32 km/h at a nearest distance of 10 m.

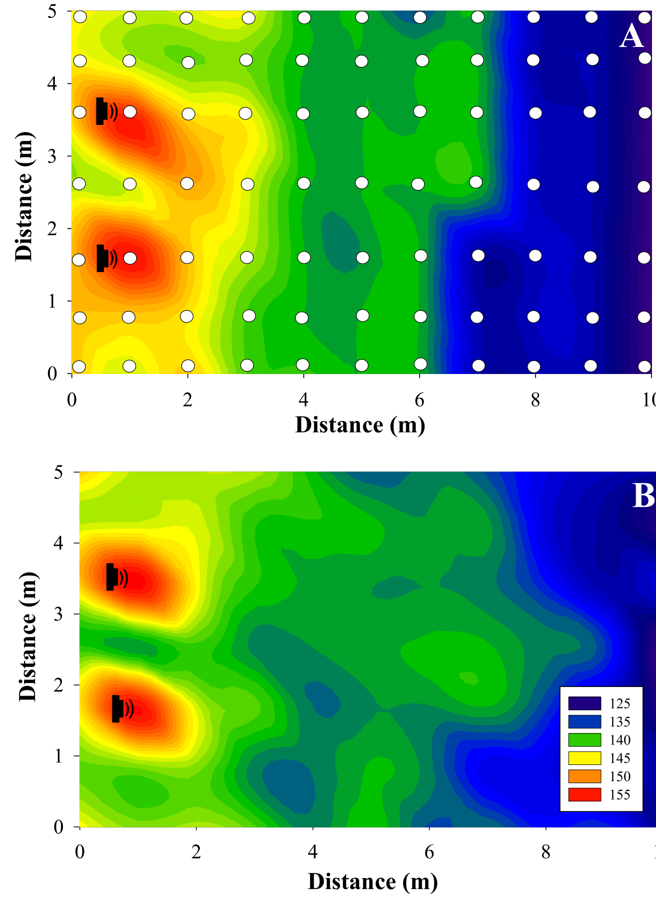


Fig. 1. Sound pressure level in the experimental pond. The sound intensity was measured using a hydrophone at a depth of 0.6 m at 77 intervals throughout the pond during broadband sound playback. The speakers and points of measurement (white circles in upper figure) are indicated. The colors represent the sound intensity level (dB re 1 μPa), indicated in the scale on the lower right. A) 1000 Hz pure tone; B) Broadband sound stimulus.

The sound was amplified with a UMA-752 amplifier (UMA-752, Peavey Electronics, Meridian, MS) and each speaker pair was controlled manually with a switchbox (MCM Electronics, Centerville, OH). Each pond contained a single hydrophone to monitor the sound stimuli, which were recorded using a PowerLab 4SP data acquisition system and LabChart 7 software (AD Instruments, Colorado Springs, CO). To map the acoustic field, recordings of the broadband sound and 1000 Hz pure tone were made at 77 positions throughout the tank at a depth of 0.6 m which was the depth at which fish were most often swimming. Sound pressure levels were approximately 155 dB re 1 μPa directly in front of the speakers for both pure tones and broadband sound and dropped below 120 dB re 1 μPa at the far end of the pond (Fig. 1). All pure tone stimuli showed a narrow energy peak at the dominant frequency (Fig. 2). The broadband sound produced a spectrum of sound from 0.06–10 kHz, with maximal energy contained in two peaks from 0.06–2 kHz and 6–10 kHz (Fig. 2).

Behavior was monitored with eight overhead SONY bullet 500 TVL video cameras connected to ProGold software (Security Camera World, Cooper City, FL). The cameras continuously monitored the fish during daylight hours on testing days and provided full coverage of the pond. The water remained clear throughout the entire study and fish were visible in all areas of the pond. All monitoring equipment (i.e. cameras, speaker switchbox, etc.) was contained within a shelter located approximately 50 m from the test pond, therefore eliminating any experimenter influence on fish behavior. Additionally, hydrophone

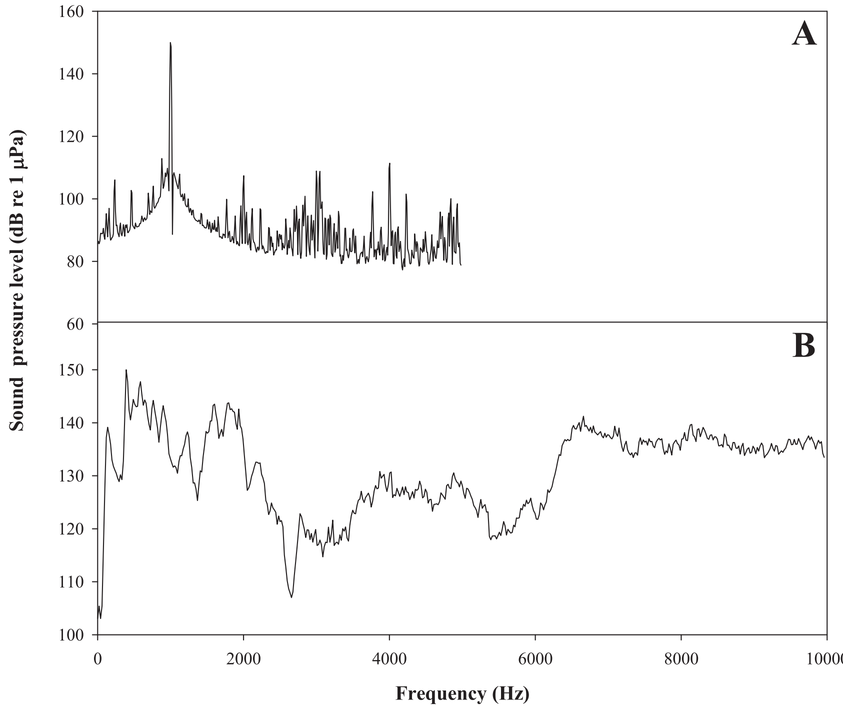


Fig. 2. The power spectrum of the A) pure tone (1000 Hz) and B) broadband sound is plotted versus frequency from a hydrophone a depth of 0.6 m directly in front of the speaker (<1 m) during sound playback in the pond (Modified from Vetter et al., 2015).

records were examined for sound artifacts during speaker onset and offset, and only the sound stimuli were detected.

The bighead carp demonstrated schooling behavior, consistently staying in the middle of the water column; therefore the fish in each trial were treated as a single unit, with position determined as the approximate center of the school. An “end zone” was established at each end of the pond and was defined as the area of the pond within 2.5 m of the end wall. The order of stimuli presentation (pure tones vs. broadband sound) was determined randomly before each trial. Four broadband and 12–16 pure tone sound trials (3–4 trials for each 500, 1000, 1500, and 2000 Hz pure tone) were conducted on each group. Experimental trials were initiated by playing a 30 s sound stimulus from the speaker pair in the end zone occupied by the fish. If the fish swam away from the sound and crossed the centerline of the pond within 30 s, then the sound source was switched to the opposite end of the pond when the fish reached the far end zone. This procedure was immediately repeated, and the sound stimulus replayed, until the fish stopped responding to the sound or 10 min elapsed. Consecutive responses were defined as fish reacting to two or more sound presentations from opposite ends of the pond. If the fish did not cross the centerline within 30 s, remained in the same location, or swam towards the sound, then this behavior was scored as no response and the stimulus was not played from the opposite speaker pair. PowerLab recordings were time synchronized with video recording, to compare the onset of sound with the carp's behavioral response. Furthermore, when alternating the sound source, the active speakers were turned off before the opposite speaker pair was powered on, leaving approximately a 1 s sound gap when speakers were switched during sound playback.

Pure tone trials

Fish position was monitored for 10 min prior to the acoustic stimulus. Each trial began with a 30 s pure tone (500, 1000, 1500, or 2000 Hz) initiated from the speaker pair in the end zone containing the fish. If the fish responded to the initial stimulus, the sound source was alternated to the other end of the pond as many times as was necessary, until the fish no longer responded. After the fish failed to respond to either the initial or subsequent stimuli, they were allowed a recovery time of 90 to 180 s before the next 30 s presentation of the same frequency. This was repeated two more times during each frequency trial. At the end of every pure tone trial, a 30 s clip of the broadband sound was played. If the fish responded to the sound during this 30 s broadband sound clip, the sound source was alternated in the same method as was employed when exposing fish to the pure tones. Fish were allowed at least 15 min of recovery after each pure tone trial.

Broadband trials

Broadband sound trials were conducted following a similar method to the pure tones. Because the fish were more responsive to the broadband sound, the protocol was modified slightly and the 30 s outboard motor recording was looped continuously (except for the approximate 1 s delay when one speaker pair was turned off and the opposite pair turned on), with only the speaker position changing. The sound stimulus was switched to the opposite speaker pair as soon as the school crossed into the opposite end zone. Each broadband sound trial was terminated after the fish no longer responded to the stimulus or 10 min elapsed. Fish were allowed at least 30 min to recover after each broadband sound trial.

Data analysis

Fish position was monitored during the 10 min before (control) and through the application of the sound stimulus for every trial by recording the position in meters (x, y) of the midpoint of the school every 5 s. Swim speed was quantified for experimental fish that reacted to the sound using frame by frame analysis of the video recording (30 frames per second). The elapsed time from when the fish turned and swam 2.0 m away from the sound stimulus was calculated and the swim speed determined. The swim speeds were only assessed for fish that reacted to the sound stimulus and swam 2.0 m in <30 s. Fish that took longer than 30 s or did not respond, were excluded from analysis, which included 68.3% of 500 Hz, 61.5% of 1000 Hz, 30.0% of the 1500 Hz, 48.4% of 2000 Hz, and 0% of broadband trials. For controls, fish were observed for a 10 min period of continuous swimming in the absence of sound, and the time it took the school to transverse

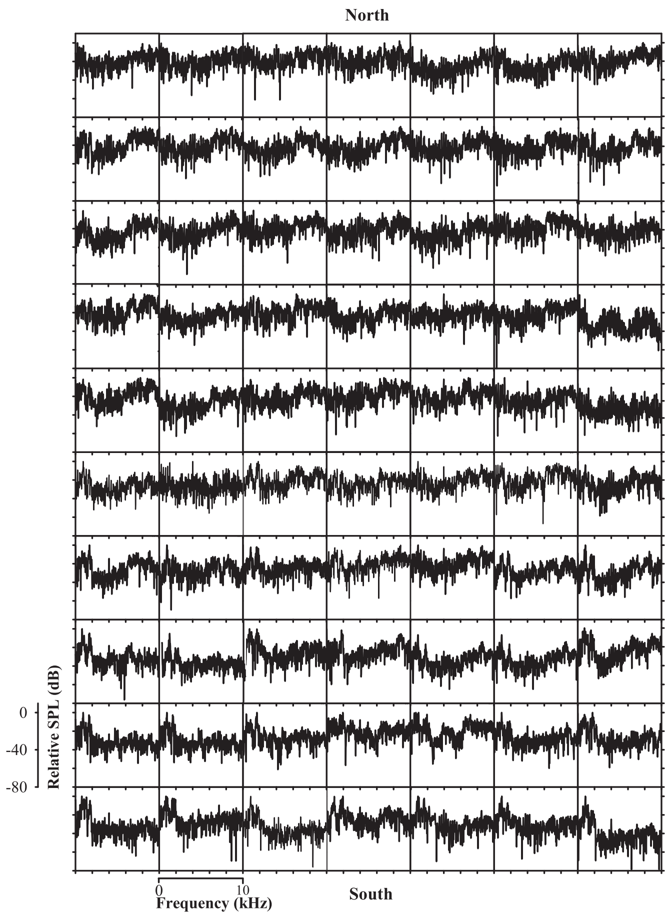


Fig. 3. The power spectrum from measurement locations in the pond is plotted versus frequency during playback of the broadband sound. The maximum sound pressure level at each location was assigned a decibel level of 0 and each spectrum is plotted relative to the maximum sound pressure level at each location.

2 m intervals was determined (15.6% of the time, fish exceeded 30 s to swim 2 m and these values were not included). Control speeds were determined prior to testing or at least an hour after the last exposure to sound stimuli.

All statistical tests were performed with SigmaPlot for Windows (version 12.5). Shapiro-Wilk tests indicated that the response number and swimming speeds data were not normally distributed ($P < 0.05$) and therefore a non-parametric Kruskal-Wallis ANOVA with a Dunn's post hoc test was used. The median along with the upper and lower quartiles for the response numbers and swimming speed are reported using the following format: (median; 1st Q, 3rd Q) or median (1st Q, 3rd Q).

Results

The sound recorded within 1 m of the front of the speaker showed that for the pure tones, most of the energy was centered at the dominant frequency, while the broadband sound ranged from 0.06–10 kHz, with the highest energy contained in frequencies <2 kHz (Fig. 2). Sound pressure levels peaked in front of and behind the speakers and the end zone nearest the active speakers contained the areas of highest sound pressure levels, with sound attenuating as it traveled towards the far end of the pond (Fig. 1). Fig. 3 illustrates the power spectrum for the broadband sound throughout the pond.

Swimming behavior

Figs. 4 and 5 show the swimming behavior from one representative school of bighead carp from control and experimental trials. During the control trials, the fish primarily moved around the perimeter at a relatively consistent speed, with the school shown completing approximately 5.5 circuits of the pond over the 10 min observation period (Fig. 4A). In contrast, the fish responding to the broadband sound favored the longitudinal center of the tank when moving away from the sound source (Fig. 4B). The school showed no response to the 500, 1000, or 2000 Hz pure tones, as the fish either remained in the area or swam towards the stimulus (Fig. 5). For the 1500 Hz tone, fish reacted once to the first playback and twice to the third, but then stopped responding and remained in the same area even though the sound was present (Fig. 5). However, at the end of every pure tone trial, the

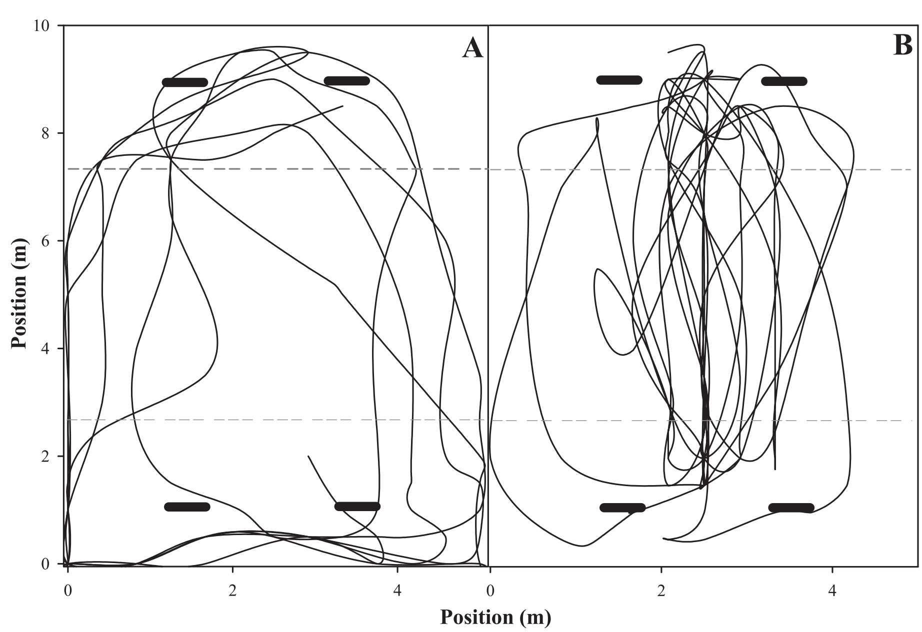


Fig. 4. Bighead carp swimming behavior. The solid black lines represent the speaker location and the dotted line represents the “end zones.” The traces mark the horizontal and longitudinal position (m) of the center of one representative school of bighead carp during a control and broadband sound trial, with the fish position mapped every 5 s. A) Control; B) Broadband sound.

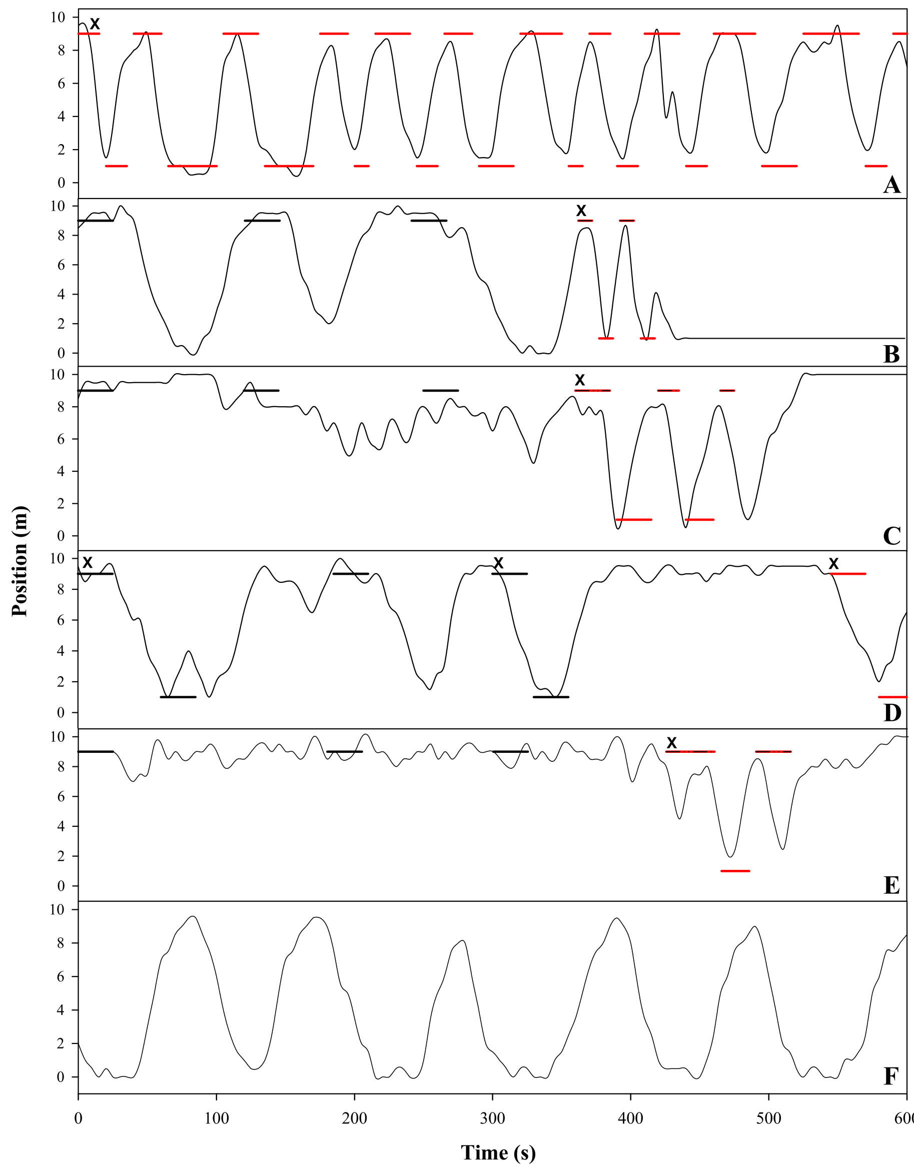


Fig. 5. Representative bighead carp behavioral response to acoustic stimulation for one school. The longitudinal position (m) of the center of the school is plotted versus time (s) with fish position mapped every 5 s. Solid lines above and below each fish position trace indicate the location and duration of the sound stimulus (black = pure tone; red = broadband sound). The X represents the first negative phonotaxis of a series or resumption after non-responsive trials. For clarity, successive responses in a series are not labeled. A) Broadband sound; B) 500 Hz; C) 1000 Hz; D) 1500 Hz; E) 2000 Hz; F) Control (no sound).

fish responded at least once to the broadband sound stimulus. The same school responded to the broadband sound stimulus 23 consecutive times over the 10 min trial (Fig. 5).

Responses and swim speed

The bighead carp were significantly (Kruskal-Wallis ANOVA $P < 0.001$; Dunn's $P < 0.05$; $H = 53.478$ with 4 degrees of freedom) more reactive to the broadband sound (20.0 consecutive responses; 12.0, 23.0) than to the pure tones [500 Hz: 0.0 (0.0, 2.0); 1000 Hz: 1.0 (0.0, 2.0); 1500 Hz: 2.0 (0.0, 4.0); 2000 Hz: 1.0 (0.0, 5.0)] (Fig. 6). Behavior during the pure tone trials was inconsistent and not sustained, as the median consecutive response did not exceed 2.0 for any frequency. While the fish always retreated from the broadband sound, they responded to only 53% of pure tone presentations with one third of these trials (~17% of total) eliciting more than one reaction.

The number of reactions throughout the two-day testing period remained consistent (Fig. 7) with no significant decrease in responses between consecutive trials to the broadband sound (500 Hz: Kruskal-Wallis ANOVA $P = 0.178$; $H = 4.917$ with 3 degrees of freedom; 1000 Hz: Kruskal-Wallis ANOVA $P = 0.782$; $H = 1.079$ with 3 degrees of freedom; 1500 Hz: Kruskal-Wallis ANOVA $P = 0.887$; $H = 0.642$ with 3 degrees of freedom; 2000 Hz: Kruskal-Wallis ANOVA $P = 0.359$; $H = 3.218$ with 3 degrees of freedom; Broadband sound: Kruskal-Wallis ANOVA $P = 0.212$; $H = 4.505$ with 3 degrees of freedom). Furthermore, in 58% of tests, the carp were still responding to the broadband sound when the 10 min trials were terminated. The bighead carp demonstrated significantly faster swimming (median swim speed: 0.47 m/s; 0.36 m/s, 0.60 m/s) when moving away from the broadband sound than the pure tones or control swimming (Kruskal-Wallis ANOVA $P < 0.001$; Dunn's $P < 0.05$; $H = 80.234$ with 5 degrees of freedom) (Fig. 8).

Discussion

Throughout the experiment, the bighead carp schooled and in the absence of sound, primarily swam circular routes along the pond walls. However, their behavior changed quickly when presented with

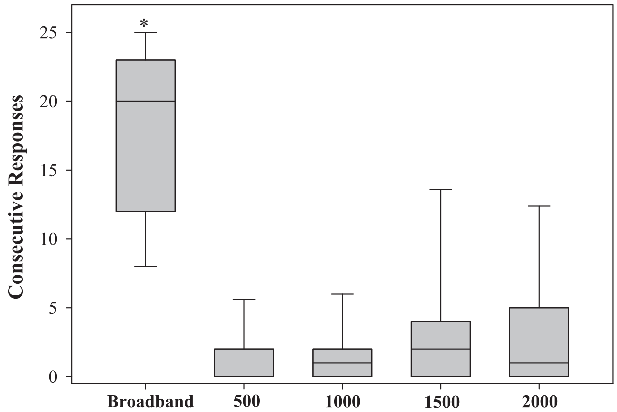


Fig. 6. Consecutive responses per trial to sound playback versus sound stimulus type (500, 1000, 1500, and 2000 Hz and broadband sound). For each box, the boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. * indicates significantly different group (ANOVA $P < 0.001$; Dunn's $P < 0.05$; $H = 53.478$ with 4 degrees of freedom).

broadband sound, and they moved directly away from the sound source by swimming through the middle, longitudinal axis of the pond. Furthermore, the highest number of consecutive responses and the fastest swim speeds were observed when bighead carp were reacting to the broadband sound.

Pure tones, which have been historically used in non-physical fish deterrent systems either alone or in combination with bubbles and/or electric barriers (Noatch and Suski, 2012), were ineffective in producing a consistent response in bighead carp. Responses were only observed in 53% of pure tone trials, with few schools responding >2- 3 times. Vetter et al. (2015) determined that silver carp responded during 100% of the broadband sound trials (mean: 11.8 responses), but to only 12% of the pure tone presentations (<1% of these trials elicited a subsequent response). However, when presented with broadband playbacks of boat motor recordings, bighead carp showed rapid and sustained responses, with a median of 20 consecutive responses.

While the complete hearing range of bighead carp remains unknown, these fish possess Weberian ossicles, allowing relatively higher frequency hearing than many non-ostariophysan fish. Using auditory evoked potentials (AEP), Lovell et al. (2006) reported frequency sensitivity up to 3 kHz, however the tuning curve was unusually flat compared with the audiograms of other teleosts, and higher frequencies were not tested. Additionally, the study was limited due to acoustic complications with the small tank and the use of auditory evoked potentials, in which thresholds vary between studies and with behaviorally derived thresholds (Ladich and Fay, 2013; Sisneros et al., 2016). In both behaviorally based (Popper, 1972) and AEP studies (Amoser and Ladich, 2005), common carp (*Cyprinus carpio*) were found to have similar hearing sensitivities, with a maximum ranging between 0.3 and 1 kHz and a decrease in sensitivity beyond 3 kHz (see Ladich and Fay, 2013, for a review). Based on the behavior evidence reported in the current study and the sensitivities of related carp species, it appears that the pure tones and at least a portion of the broadband stimulus were within the frequency sensitivity of bighead carp as identified in Lovell et al. (2006). However, it is crucial that the upper limit of bighead carp hearing sensitivity be determined, especially since related carp species, including common carp, were much less responsive than the bighead carp to the broadband sound stimulus used in this study (Murchy et al., unpublished).

An acoustic deterrent must balance high sound pressure, which provides greater range and/or increases its efficacy, with the risk of hearing damage in fish species. Smith et al. (2004) found that goldfish exposed to 130–170 dB white noise became acclimated after 10 min of exposure and experienced hearing loss at the higher sound pressure levels; however, these experiments were conducted in much smaller tanks (19–600 L) than the present study. The maximum SPL in the experimental ponds was 156 dB re 1 μPa in a small area near the speakers where the fish spent minimal time during playback. Although it is possible that some hearing loss occurred, the bighead carp's continued phonotaxic behavior suggests fish experienced minimal impact on hearing sensitivity. Furthermore, their repeated responses indicate that the fish could locate the approximate source of the sound and/or detect the sound gradient. It also did not appear that the bighead carp were habituating to the broadband sound as the carp were still reacting to the stimulus from the active speakers in 58% of trials when the test was terminated. Additionally, there was not a significant decrease in responsiveness to the broadband sound over the two-day testing period. As both swimming duration and speed were elevated during playbacks, non-responding fish may have been fatigued rather than habituated to the sound. However, the fish did appear to habituate to the pure tones. Therefore, it is imperative that long term studies exposing both bighead and silver carp to broadband sound are conducted to determine if the fish will habituate to the broadband sound and what conditions would minimize habituation (i.e. optimal stimulus duration and interval between playbacks).

Several studies have examined non-physical barriers including acoustic barriers, either alone or in combination with bubbles, which also generate low frequency sound (Zielinski et al., 2014). Sound (20–2000 Hz) combined with a bubble curtain prevented a majority of captive bighead and silver carp crossing attempts in outdoor raceways (Pegg and Chick, 2004; Taylor et al., 2005). The same broadband sound used in the current study effectively prevented both bighead and silver carp from passing through a small opening (1 m) in a concrete barrier (Murchy et al., unpublished). These experiments demonstrate the success of sound at deterring fish in a controlled setting; however, there is little research examining the efficacy of acoustic barriers in the field. A preliminary study by Ruebush et al. (2012) used a bubble-strobe-sound (500–2000 Hz) barrier on a tributary of the Illinois River, but the researchers were unable to quantify how many fish challenged the barrier or remained in the area. The effectiveness of acoustic deterrents in winter months has been questioned due to changes in fish behavior in cold water (Hawkins and Popper, 2014). However, these behavioral changes often mean reduced activity and could result from observed decreases in metabolic processes in colder water (David, 2006; Jones et al., 2008). Silver and bighead carp are less active in colder water (Murchy, unpublished) and therefore may be less likely to challenge an acoustic barrier during the late fall through early spring.

There are limitations with this study to wild fish because of the inherent challenges in small tank acoustics and the differences between captive and wild fish behavior. Echoes are produced from interactions of the sound with the water surface and with the pond's bottom and walls, creating a complex acoustic environment for the fish to localize the sound source, even in larger concrete ponds like the one used in this study (Gray et al., 2016). Compared to field conditions, the pond is suboptimal with a complex echoic environment complicating sound localization (Gray et al., 2016) and providing limited space for the fish to escape. However, the pond's concrete composition closely replicates a lock chamber (on a smaller scale), where the technology may be eventually placed. Although there are differences in the sound field of a concrete tank when compared with a natural environment, controlled experiments can be useful to compare fish behavior when other conditions (i.e. methods, speakers, tank, fish size, etc.) remain consistent (Rogers et al., 2016), which was the case with this experiment. Therefore, despite the limitations of the small pond, the results are encouraging for the use of a broadband acoustic deterrent as part of an integrated pest management system.

Two recent reviews have cautioned against applying behavioral results from captive fish to those in the wild (Popper et al., 2014; Hawkins et al., 2015). However, preliminary results from a field study

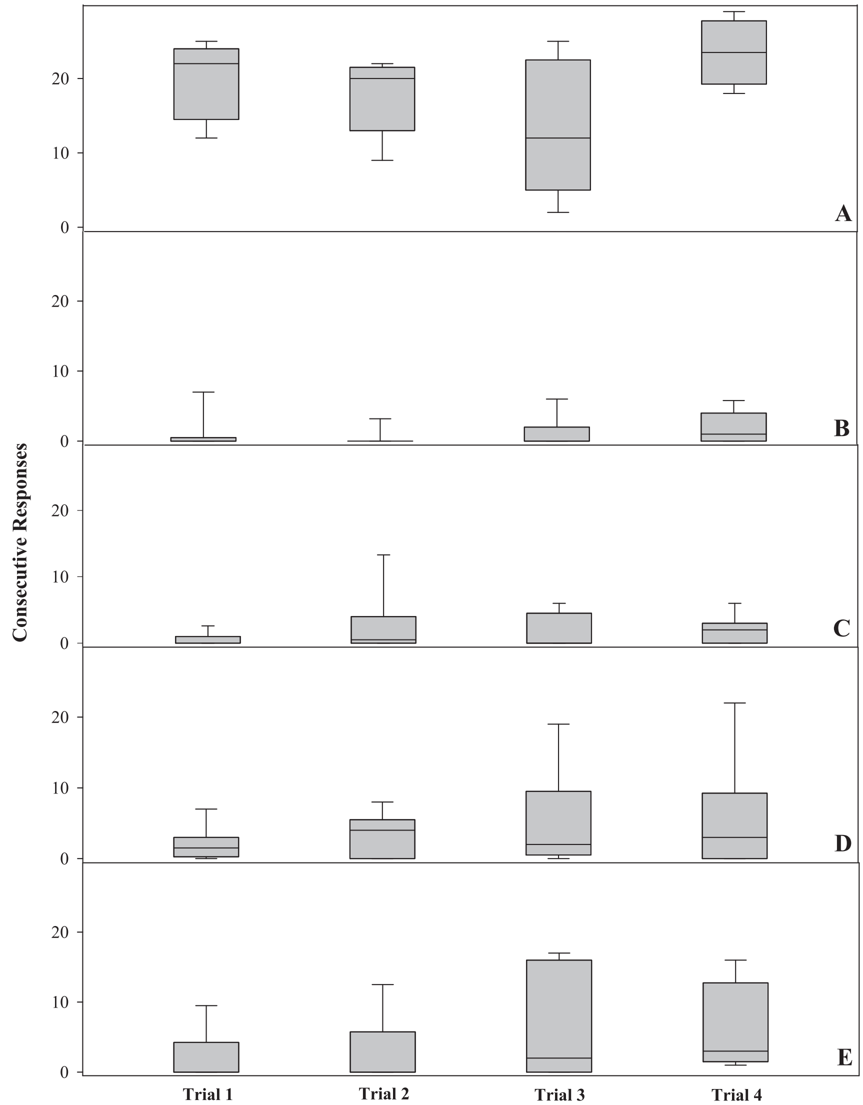


Fig. 7. Number of consecutive responses over time for each sound stimulus type: A) 500 Hz, B) 1000 Hz, C) 1500 Hz, D) 2000 Hz, E) Broadband sound. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Boxes compare the number of consecutive responses by bighead carp to the first, second, third, and fourth presentation of each stimulus type. There is no significant difference between the trials (500 Hz: ANOVA $P = 0.178$; $H = 4.917$ with 3 degrees of freedom; 1000 Hz: ANOVA $P = 0.782$; $H = 1.079$ with 3 degrees of freedom; 1500 Hz: ANOVA $P = 0.887$; $H = 0.642$ with 3 degrees of freedom; 2000 Hz: ANOVA $P = 0.359$; $H = 3.218$ with 3 degrees of freedom; Broadband sound: ANOVA $P = 0.212$; $H = 4.505$ with 3 degrees of freedom).

that exposed resident silver and bighead carp in the Spoon River near Havana, IL to broadband sound, demonstrated that silver carp jump in response to the acoustic stimulus alone (Vetter, unpublished). Furthermore, concurrent sonar indicated that all putative carp (species identification was not possible) exited the area and that the sound could displace fish at least 200 m from the source (Mensinger, unpublished). This suggests that sound could be effective in modulating wild fish behavior and provides a strong argument for further research exploring the efficacy of acoustic deterrents in carp infested waters. The pond was modest in size and prevented fish from swimming >9.0 m from the source and the continual alternation of the sound source probably generated fatigue in a portion of the schools, neither of which would be a factor with longer distance repulsion and less frequent sound exposure in a natural setting.

Playback of the outboard motor recording through the UW-30 did modify the sound due to the speaker characteristics, however the goal of the study was to identify sound that caused consistent negative phonotaxis and not rebroadcast the exact sound spectrum of the outboard motor in high fidelity. The playbacks were effective in accomplishing the goals of the study. Additionally, while particle motion, which was not measured in this study, may have given greater insights the acoustic environment in the pond, it was not necessary to accomplish the experimental objectives. Furthermore, future deterrents will be tested in much larger ponds or in the field and the same particle motion environment of a small pond would be difficult to recapitulate. However, it is important that the ambient sound field of the river be determined in field sites where broadband sound is tested or implemented as part of a deterrent barrier.

Bighead and silver carp are closely related, co-exist in the wild, and hybridize. However, silver carp can be readily stimulated to jump by boat traffic, electric shock, or loud sound, making it relatively easy to locate their presence. Even small silver carp (<10 cm sl) in relatively low densities (single fish jumping) have been observed to jump (Mensinger, unpublished). In turbid waters, it is difficult to assess the number of bighead carp, as they do not jump. To effectively manage both species, the response behavior of bighead carp must also be determined. This study suggests that, similar to silver carp, bighead carp swimming is also modulated by broadband sound.

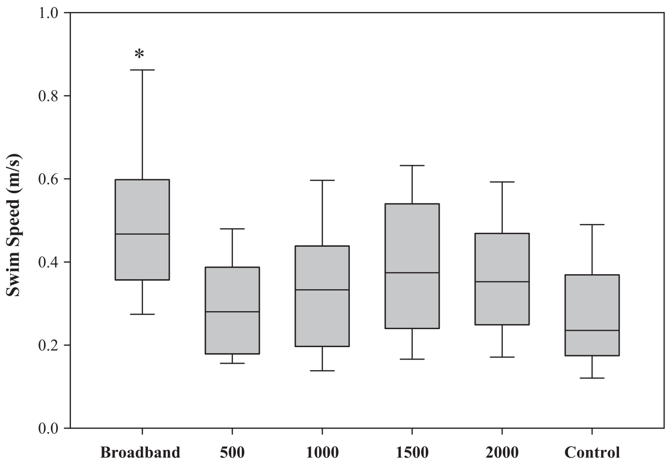


Fig. 8. Fish swim speeds. Box and whisker plots display the median swim speed while the bighead carp were retreating from the sound stimuli. Swim speed was calculated by determining the time the fish swam the first 2.0 m away from the sound source. The control represents the average time for fish to swim 2.0 m in the absence of sound. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. * indicates significantly different group (ANOVA $P < 0.001$; Dunn's $P < 0.05$; $H = 80.234$ with 5 degrees of freedom).

Finally, it is important to determine the impact of broadband sound on native species prior to field implementation. Preliminary studies suggest that many native species, including game fish (walleye, *Sander vitreus*; bluegill, *Lepomis macrochirus*; and rainbow trout, *Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), fathead minnow (*Pimephales promelas*), lake sturgeon (*Acipenser fulvescens*), paddlefish, gizzard shad, and bigmouth buffalo do not demonstrate a behavioral response to the same sound used in this study (Murchy, unpublished). This is especially interesting since bigmouth buffalo are ostariophysans and gizzard shad are clupeids, both of which can detect high frequency. However, many native species do not have specialized hearing structures, like the Weberian ossicles found in bighead carp, and therefore cannot detect higher frequency sounds. Lovell et al. (2005) examined paddlefish and lake sturgeon hearing and reported low thresholds between 200 and 300 Hz. Furthermore, other non-native carp, such as grass (*Ctenopharyngodon idella*) and common carp (Murchy, unpublished), also do not demonstrate the degree of negative phonotaxis seen in silver carp (Vetter et al., 2015) or the bighead carp in this study. Therefore, a refined broadband sound that targets the peak frequency sensitivity of bighead and silver carp could be effective in targeting these species with minimal impact on native species, as suggested in the Lovell et al. (2005) study.

Acoustic deterrents could be an effective means to herd or prevent upstream migration of both bighead and silver carp. While physical and electric barriers are expensive and not always practical, an acoustic deterrent has many applications. For instance, speakers playing a broadband sound stimulus could be used to move bighead and silver carp towards a net or shore, clear fish out of a lock before allowing a ship to pass through, or as reinforcement to an electric barrier during routine maintenance when the field is not active. The range expansion of invasive bighead and silver carp is a concern to many state and federal agencies as the fish threaten their environments. This study indicates that because bighead and silver carp (Vetter et al., 2015) are similarly responsive to broadband sound, the species can be co-managed and that broadband sound may be an important management tool which could be effective either on its own or integrated with other deterrent technology. These closely related species are already treated as one because of their population overlap and genetic relationship. The similar responses of bighead and silver carp to broadband sound stimuli suggest that incorporation of these sounds into the integrated pest management programs of natural resource agencies may be successful in altering fish behavior.

Acknowledgements

We would like to thank the UMESC staff and interns for their assistance with this project. Funding was provided by the Minnesota Environment and Natural Resources Trust Fund (ID # 101-D: Bioacoustics to Detect, Deter and Eliminate Flying Carp) and resources for the USGS component of the research were provided through the USGS Ecosystem Mission Area Invasive Species Program and from the US Environmental Protection Agency's Great Lakes Restoration Initiative.

References

Amoser, S., Ladich, F., 2005. Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? J. Exp. Biol. 208, 3533–3542.

David, J.A., 2006. Water quality and accelerated winter growth of European catfish using an enclosed recirculating system. Water Environ. J. 20, 233–239.

Fay, R.R., Popper, A.N., 1999. The auditory periphery in fishes. In: Fay, R.R., Popper, A.N. (Eds.), Comparative Hearing: Fish and Amphibians. Springer, New York, pp. 43–100.

Gray, M.D., Rogers, P.H., Popper, A.N., Hawkins, A.D., Fay, R.R., 2016. In: Fay, R.R., Popper, A.N. (Eds.), The Effects of Noise on Aquatic Life II. Springer, New York, pp. 363–369.

Hawkins, A.D., Popper, A.N., 2014. Assessing the impact of underwater sounds on fishes and other forms of marine life. Acoust. Today 30–41 (Spring).

Hawkins, A.D., Pembroke, A.E., Popper, A.N., 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish Biol. Fish. 25, 139–164.

Irons, K.S., Sass, G.G., McClelland, M.A., Stafford, J.D., 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA - is this evidence for competition and reduced fitness? J. Fish Biol. 71, 258–273.

Jones, E.A., Jong, A.S., Ellerby, D.J., 2008. The effects of acute temperature change on swimming performance in bluegill sunfish *Lepomis macrochirus*. J. Exp. Biol. 211, 1386–1393.

Kelly, A.M., Engle, C.R., Armstrong, M.L., Freeze, M., Mitchell, A.J., 2011. History of introductions and governmental involvement in promoting the use of grass, silver, and bighead carps. In: Chapman, D.C., Hoff, M.H. (Eds.), Am Fish Soc Spec Symp. 74, pp. 1–12 (Bethesda, MD).

Kolar, K.S., Chapman, D.C., Courtenay Jr., W.R., Housel, C.M., Williams, J.D., Jennings, D.P., 2007. Bigheaded carps: a biological synopsis and environmental risk assessment. American Fisheries Society Special Publication. 33 (Bethesda).

Ladich, F., 1999. Did auditory sensitivity and vocalization evolve independently in otophysan fishes? Brain, Behav. Evol. 53, 288–304.

Ladich, F., Fay, R.F., 2013. Auditory evoked potential audiometry in fish. Rev. Fish Biol. Fish. 23, 317–364.

Lechner, W., Heiss, E., Schwaha, T., Glösmann, M., Ladich, F., 2011. Ontogenetic development of Weberian ossicles and hearing abilities in the African bullhead catfish. PLoS One 6 (4), e18511.

Lovell, J.M., Findlay, M.M., Moate, R.M., Nedwell, J.R., Pegg, M.A., 2005. The inner ear morphology and hearing abilities of the Paddlefish (*Polyodon spathula*) and the Lake Sturgeon (*Acipenser fulvescens*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 142, 286–296.

Lovell, J.M., Findlay, M.M., Nedwell, J.R., Pegg, M.A., 2006. The hearing abilities of the silver carp (*Hypopthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 143, 286–291.

Maes, J., Turnpenny, A.W.H., Lambert, D.R., Nedwell, J.R., Parmentier, A., Ollevier, F., 2004. Field evaluation of a sound system to reduce estuarine fish intake rates at a power plant cooling water inlet. J. Fish Biol. 64, 938–946.

Moy, P.B., Polls, I., Dettmers, J.M., 2011. The Chicago sanitary and ship canal aquatic nuisance species dispersal barrier. In: Chapman, D.C., Hoff, M.H. (Eds.), Invasive Asian Carps in North America. American Fisheries Society Special Publication 74, pp. 1–10 (Bethesda, MD).

Noatch, M.R., Suski, C.D., 2012. Non-physical barriers to deter fish movements. Environ. Rev. 20, 71–82.

Pegg, M.A., Chick, J.H., 2004. Aquatic nuisance species: an evaluation of barriers for preventing the spread of bighead and silver carp to the Great Lakes. Final Report for the Illinois-Indiana Sea Grant A/SE (ANS) 01–01. Illinois-Indiana Sea Grant, Urbana, IL.

Poggendorf, D., 1952. Die absolute Hörschwelle des Zwergwelses (*Amiurus nebulosus*) und Beiträge zur Physik des Weberschen Apparates der Ostariophysen. Z. Vgl. Physiol. 34, 222–257.

Popper, A.N., 1972. Pure-tone auditory thresholds for the carp, *Cyprinus carpio*. J. Acoust. Soc. Am. 52, 1714–1717.

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D.A., Bartol, S., Carlson, T.J., et al., 2014. Sound Exposure Guidelines for Fishes and Sea Turtles: a Technical Report Prepared by ANSI-Accredited Standards Committee S3/SC1 and Registered with ANSI.

Rogers, P.H., Hawkins, A.D., Popper, A.N., Fay, R.R., Gray, M.D., 2016. Parvulescu revisited: small tank acoustics for bioacousticians. In: Fay, R.R., Popper, A.N. (Eds.), The Effects of Noise on Aquatic Life II. Springer, New York, pp. 933–941.

Ross, Q.E., Dunning, D.J., Thorne, R., Menezes, J.K., Tiller, G.W., Watson, J.K., 1993. Response of alewives to high-frequency sound at a power plant intake on Lake Ontario. N. Am. J. Fish. Manag. 13, 291–303.

Ruebush, B.C., Sass, G.G., Chick, J.H., Stafford, J.D., 2012. In-situ tests of sound-bubble-strobe light barrier technologies to prevent range expansions of Asian carp. Aquat. Invasions 7, 37–48.

Sampson, S.J., Chick, J.H., Pegg, M.A., 2009. Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. Biol. Invasions 11, 483–496.

Sass, G.G., Cook, T.R., Irons, K.S., McClelland, M.A., Michaels, N.N., O'Hara, E.T.M., Stroub, M.R., 2010. A mark-recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. Biol. Invasions 12, 433–436.

Sass, G.G., Hinz, C., Erikson, A.C., McCelland, N.N., McCelland, M.A., Epifanio, J.M., 2014. Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. J. Great Lakes Res. 40, 911–921.

Schrank, S.J., Guy, C.S., Fairchild, J.F., 2003. Competitive interactions between age-0 bighead carp and paddlefish. Trans. Am. Fish. Soc. 132, 1222–1228.

Sisneros, J.A., Popper, A.N., Hawkins, A.D., Fay, R.R., 2016. Auditory evoked potential audiograms compared with behavioral audiograms in aquatic animals. In: Fay, R.R., Popper, A.N. (Eds.), The Effects of Noise on Aquatic Life II. Springer, New York, pp. 1049–1056.

Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). J. Exp. Biol. 207, 427–435.

Taylor, R.M., Pegg, M.A., Chick, J.H., 2005. Response of bighead carp to a bioacoustic behavioural fish guidance system. Fish. Manag. Ecol. 12, 283–286.

Vetter, B.J., Cupp, A.R., Fredriks, K.T., Gaikowski, M.P., Mensinger, A.F., 2015. Acoustical deterrence of silver carp (*Hypophthalmichthys molitrix*). Biol. Invasions 17, 3383–3392.

Zielinski, D.P., Voller, V.R., Svendsen, J.C., Hondzo, M., Mensinger, A.F., Sorensen, P., 2014. Laboratory experiments demonstrate that bubble curtains can effectively inhibit movement of common carp. Ecol. Eng. 67, 95–103.

RESEARCH ARTICLE

Reexamining the frequency range of hearing in silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp

Brooke J. Vetter[1,2]*, **Marybeth K. Brey**[3], **Allen F. Mensinger**[1]

1 Biology Department, University of Minnesota Duluth, Duluth, MN, United States of America, **2** Department of Psychology, University of Washington, Seattle, WA, United States of America, **3** U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, United States of America

* bjvetter@uw.edu

Citation: Vetter BJ, Brey MK, Mensinger AF (2018) Reexamining the frequency range of hearing in silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp. PLoS ONE 13(3): e0192561. https://doi.org/10.1371/journal.pone.0192561

Editor: Harold A. Burgess, National Institutes of Health, UNITED STATES

Received: November 13, 2017

Accepted: January 25, 2018

Published: March 9, 2018

Data Availability Statement: All relevant data are available from ScienceBase at https://doi.org/10.5066/F7M61JH0.

Funding: Funding was provided by the USGS Ecosystem Mission Area Invasive Species Program to AFM and from the US Environmental Protection Agency's Great Lakes Restoration Initiative. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp (collectively bigheaded carp) are invasive fish that threaten aquatic ecosystems in the upper Midwest United States and the Laurentian Great Lakes. Controlling bigheaded carp is a priority of fisheries managers and one area of focus involves developing acoustic deterrents to prevent upstream migration. For an acoustic deterrent to be effective however, the hearing ability of bigheaded carp must be characterized. A previous study showed that bigheaded carp detected sound up to 3 kHz but this range is narrower than what has been reported for other ostariophysans. Therefore, silver and bighead carp frequency detection was evaluated in response to 100 Hz to 9 kHz using auditory evoked potentials (AEPs). AEPs were recorded from 100 Hz to 5 kHz. The lowest thresholds were at 500 Hz for both species (silver carp threshold: 80.6 ± 3.29 dB re 1 μPa SPL_{rms}, bighead carp threshold: 90.5 ± 5.75 dB re 1 μPa SPL_{rms}; mean ± SD). These results provide fisheries managers with better insight on effective acoustic stimuli for deterrent systems, however, to fully determine bigheaded carp hearing abilities, these results need to be compared with behavioral assessments.

Introduction

Silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp (collectively bigheaded carp) are invasive to the Mississippi River Drainage and these prodigious filter feeders threaten native species [1–4] by altering trophic structures in areas where their populations are high [5]. Although their initial introduction in North America was in the southern reaches of the Mississippi River Drainage, bigheaded carp have since migrated north and now threaten the Laurentian Great Lakes via the Chicago Sanitary and Ship Canal [6–8]. There is currently an electric dispersal barrier in the Chicago Sanitary and Ship Canal that separates Lake Michigan from the Illinois and Des Plaines Rivers, however, this barrier is costly and must be operated continuously to prevent further northward migration [9]. Therefore, alternate non-physical deterrents have been proposed both as a backup during maintenance of the electric barrier and to be implemented in additional areas of concern, such as lock chambers.

One promising non-physical barrier is an acoustic deterrent, either used alone [10] or in combination with bubbles and/or strobe lights [11, 12]. Bigheaded carp are ostariophysans and possess Weberian ossicles, which are bony structures that transmit vibrations from the swim bladder to the inner ear and allow sensitivity to higher frequencies [13]. Bigheaded carp displayed negative phonotaxic behavior in response to an outboard motor recording (0.06–10 kHz), suggesting both species can be deterred by broadband sound[14, 15]; however which portion of the frequency spectrum the fish were reacting to is unclear; thus further assessment of bigheaded carp hearing was warranted. Lovell et al. [16] examined auditory evoked potentials (AEPs) for silver and bighead carp and reported AEPs could be stimulated by 3 kHz tones but did not examine higher frequencies. As AEPs have been recorded above 3 kHz in other ostariophysans (see [17] for a review on AEP studies), the purpose of this study was to determine if silver and bighead carp have greater frequency range than previously reported to aid in the optimization of acoustic deterrents.

The AEP technique was first developed for mammals [18, 19] and adapted for fish by Kenyon et al. [20]. This method uses minimally invasive subcutaneous or cutaneous electrodes to record evoked potentials in response to acoustic stimuli. As electrodes are often placed above the brainstem, there has been some confusion as to the origin of the recorded AEPs, however, it is now believed that in most fish AEP studies, AEPs result from microphonic potentials from hair cells and/or their afferent nerves rather than brainstem activity [21]. As the hair cells have opposite orientation, they produce a characteristic double-frequency response [22] and this is evident in AEPs recorded from fish [23, 24]. It is important to note that while AEP studies provide valuable information about the frequencies that stimulate auditory end organs, they are not a comprehensive assessment of the fish's hearing ability and can only provide relative thresholds. To determine true frequency sensitivity, behavioral experiments, which assess higher order acoustical processing, must also be conducted [21].

In this study, the range of frequencies that silver and bighead carp can detect was evaluated using the AEP technique. Common carp (*Cyprinus carpio*) were also tested to serve as a reference, since multiple AEP studies have been published on this species [25, 23]. Additionally, although bigheaded carp are ostariophysans and are capable of detecting sound pressure, this study determined threshold curves for both sound pressure and acoustic particle motion, as recommended by Popper and Fay [26]. This information provides a basis on which behavioral assessments can be designed to better understand bigheaded carp hearing and evaluate effective acoustic deterrents.

Methods

Animal husbandry

All experiments were conducted at the University of Minnesota Duluth in Duluth, MN. Silver (n = 5; standard length (SL): 13.4 ± 1.2 cm, mean ± 1 SD), bighead (n = 5; 12.3 ± 1.2 cm SL), and common (n = 3; 6.7 ± 0.7 cm SL) carp were obtained in the spring of 2017 from the U.S. Geological Survey (USGS) in Columbia, MO. Silver and bighead carp were maintained in a circular 1230 L (2 m diameter) indoor tank equipped with a biological, chemical, and mechanical filtration system (Fluval FX6 High Performance Canister Filter, Fluval, Baie d'Urfé, Quebéc, Canada) and fed a diet of liquid algae mixture (~300 mL; 1:1 *Chorella* and *Spirulina*; Bulk Foods, Toledo, OH) daily. A Prohibited Invasive Species Permit (#391) from the Minnesota Department of Natural Resources and an Injurious Wildlife Permit (MA-98346B-0) from the U.S. Fish and Wildlife Service were obtained prior to acquisition of the animals and the fish were maintained in a locked room with restricted access. Common carp were housed in an 80 L rectangular tank (1.5 m x 0.25 m x 0.5 m) equipped with the same filtration system and fed goldfish flakes (Tetra Werke; Melle, Germany) daily. Water quality was monitored daily and the temperature ranged between 19 and 22˚C for both tanks. All experiments were conducted in accordance with protocol #1604-33658A approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Auditory evoked potentials

Prior to electrode implantation, fish were anesthetized using phosphate buffered tricaine methanesulfonate (0.005%; Western Chemical Inc., Ferndale, WA) and a tail pinch was used to ensure that the dosage was effective at anesthetizing the animal. Fish were given an intramuscular injection of the paralytic pancuronium bromide (0.001%; Sigma Aldrich, St Louis, MO) dissolved in 0.9% NaCl (Thermo Fisher Scientific; Waltham, MA) to reduce muscle activity, although opercular movements persisted allowing self-ventilation. Each fish was placed in a mesh sling and suspended in the middle of a 350 L circular tank (88 cm inside diameter, 57 cm water depth) such that the top of the cranium was 4 cm below the water surface and 35 cm above an underwater speaker (UW-30; Lubell Labs Inc.; Whitehall, OH). Water temperature was maintained between 19 and 22˚C. Two stainless steel electrodes (Rochester Electro-Medical Inc.; Tampa, FL) were insulated with finger nail polish, except for 1 mm at the tip, and implanted just beneath the surface of the skin between the nostrils (reference electrode) and above the brainstem (recording electrode). Prior to collecting data, electrode placement, which was guided by anatomical markers, such as the location of the eyes and opercular openings, was verified by testing individuals from each species to ensure the magnitude of the AEP at each frequency was consistent. The tank was elevated from the cement floor with cinderblocks (41 x 20 x 10 cm) and a 1 cm thick rubber mat was placed between the tank and cinderblock to dampen vibrations. A four sided frame (110 x 125 x 182 cm) constructed from galvanized angle iron surrounded the tank, with three of the sides and top covered with FOAMULAR Insulation Sheathing (2.54 cm thick; Owens Corning; Toledo, OH) to further reduce noise and to block the fish from seeing the observer.

The AEP signal was amplified with a headstage (gain = 10x; Dagan Corporation; Minneapolis, MN) connected to an extracellular differential amplifier (gain = 100x; Dagan Corporation; Minneapolis, MN) using 20 Hz high pass and 10 kHz low pass filters. The signal was then collected and digitized by a Cambridge Electronic Design data acquisition system (Micro3 1401; CED; Cambridge, UK), which was also used to control the sound presentation. The sound pressure level was controlled with a programmable attenuator (CED 3505; CED; Cambridge, UK). The sound pressure level output from the attenuator was measured and calibrated using a Brüel and Kjaer hydrophone (8103; Brüel and Kjaer; Naerum, Denmark), placed in the same position as the experimental fish. The hydrophone was connected to a Nexus Conditioning Amplifier (2692-01s; Brüel and Kjaer; Naerum, Denmark). Custom Spike2 (version 8; CED; Cambridge, UK) scripts were used to calibrate the attenuator, administer sound stimuli, and collect data during the AEP procedure. The acoustic particle motion at the fish position was measured using a three dimensional accelerometer (sensitivity: 100 mV g^{-1} (10.2mV ms^{-2}); model: W356A12/NC, PCB Piezotronics Inc., Depew, NY) modified to be neutrally buoyant and connected to a signal conditioner (482C15, PCB Piezotronics Inc.). The accelerometer was placed such that the x-dimension corresponded to the fish's anterior/posterior position, the y-dimension was left/right and the z-dimension was dorsal/ventral. Particle motion measurements were obtained in the x, y, and z-axes at all frequencies and sound pressure levels evaluated. These measurements were then individually converted to magnitude vectors. All reported particle motion thresholds were calculated using the following equation: $20\log(\sqrt{(x^2 + y^2 + z^2)})$, where x, y, and z were the magnitude vectors [27–29]. Finally, as suggested by Popper and Fay [26], the acoustic impedance of the tank, or the ratio of sound pressure level to particle motion level, was determined for three sound pressure levels: 119, 130, and 145 dB re 1 μPa SPL_{rms}.

Pure tone bursts (50 ms; 500 repetitions; 3 ms delay) were broadcast to silver and bighead carp between 100 Hz and 9 kHz. The first three silver and bighead carp tested showed inconsistent response to frequencies > 5 kHz and ≤ 7 kHz and no responses to frequencies > 7 kHz to 9 kHz; therefore, subsequent fish were only tested using frequencies from 100 Hz to 5 kHz. The common carp were only tested at 100, 200, 400, 600, 800, 1000, 2000, 4000, and 5000 Hz and served as a reference as there are multiple published tuning curves for this species [25, 23]. Responses were collected and averaged using the Spike2 ABR script (all scripts available at www.ced.co.uk).

The presence of an AEP was verified by two means: (1) through observation of the characteristic wave visible above the background noise, as is commonly used in AEP studies (e.g. [30–32]) and (2) through fast Fourier Transform (FFT) analysis (Hanning window = 1024) to calculate the power spectra of the average waveforms at two times the stimulus frequency, because of the opposed orientation of hair cells [22]. The auditory threshold at each frequency was defined as the lowest sound pressure level that elicited both a repeatable AEP, visible above background noise, and a FFT peak at twice the stimulus frequency. In determining the threshold, AEPs were first elicited using a sound pressure level above threshold (100 Hz to 2 kHz: 130 dB re 1 μPa SPL_{rms}; 3 to 5 kHz: 131–147 dB re 1 μPa SPL_{rms}). After this initial test, the sound pressure levels were decreased in 3 dB steps for each frequency until an auditory evoked potential could not be determined both visually and via FFT analysis. All fish were sacrificed using an overdose of MS-222 at the end of the study and no AEPs were elicited from a sacrificed fish that served as a "dead control".

To determine the relative amplitude for the FFT analyses, the raw voltages (μV2) were normalized based on the highest FFT peak. A repeated measures ANOVA with a Holm-Sidak test was used to compare the sound pressure and acceleration thresholds for all three species at each frequency examined using SigmaPlot (version 12.5). All threshold data were normally distributed (Shapiro-Wilk $P > 0.05$) and are reported as mean ± 1 SD.

Results

The ambient sound pressure level was 70 dB re 1 μPa SPL_{rms} for all experiments and the baseline particle acceleration level was -96.0 dB re 1 ms^{-2}. The acoustic impedance at all three sound pressure levels examined indicates that there were no major resonances in the tank at the test frequencies (Fig 1A). At 130 dB re 1 μPa SPL_{rms}, the dorsoventral (z: -51.6 ± 7.0 dB re 1 ms^{-2}) axis had the highest mean particle acceleration across all test frequencies compared with the x (-54.0 ± 9.0 dB re 1 ms^{-2}) and y (-58.7 ± 6.2 dB re 1 ms^{-2}) axes. Fig 1B shows the individual the acceleration levels in the x, y, and z-axes for all of the test frequencies at 130 dB re 1 μPa SPL_{rms}. Auditory evoked potentials were recorded for all carp species from 100 Hz to 5 kHz and the waveforms were similar across species type at each frequency. Figs 2 and 3 show representative AEP traces from a silver and bighead carp, respectfully.

For silver carp, AEPs were recorded up to 5 kHz (threshold: 142 ± 3.7 dB re 1 μPa SPL_{rms}; Table 1, Figs 4A and 5). The lowest mean threshold for silver carp was at 500 Hz (threshold: 80.6 ± 3.3 dB re 1 μPa SPL_{rms}; Table 1, Figs 2 and 5). Similarly, AEPs were recorded for bighead carp up to 5 kHz (threshold: 140.6 ± 1.3 dB re 1 μPa SPL_{rms}; Table 1, Figs 4B and 5) with the lowest mean threshold at 500 Hz (threshold: 90.5 ± 5.8 dB re 1 μPa SPL_{rms}; Table 1, Figs 3 and 5). For common carp, the lowest mean threshold was at 400 Hz (threshold: 96.0 ± 9.2 dB re 1 μPa SPL_{rms}; Table 1, Fig 5). Silver carp had significantly lower ($F_{4,38} = 70.46$; $P < 0.05$)

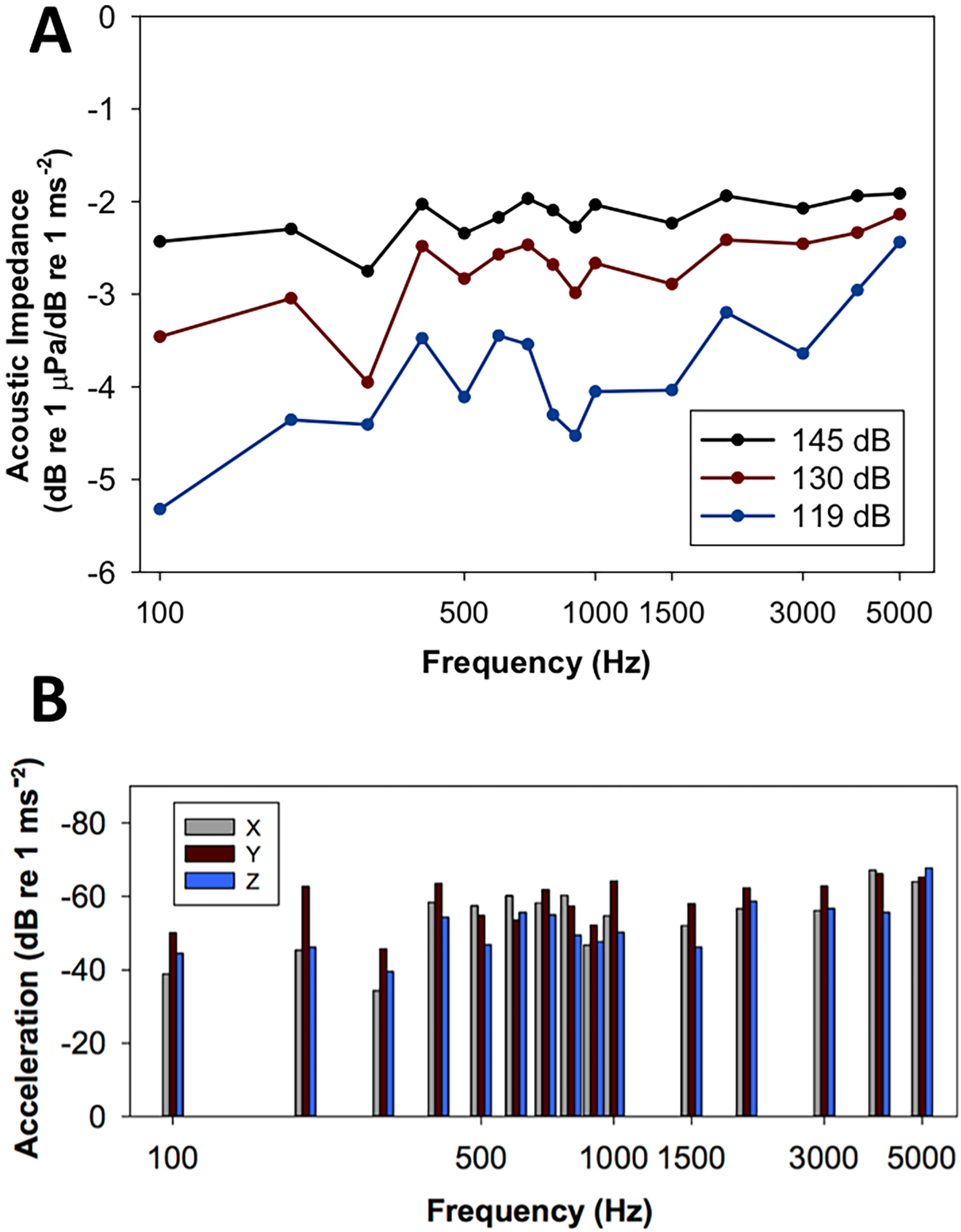

A
0
-1
-2
-3
-4
-5
-6
Acoustic Impedance
(dB re 1 μPa/dB re 1 ms-2)
145 dB
130 dB
119 dB
100
500
1000 1500
3000
5000
Frequency (Hz)
B
-80
-60
-40
-20
0
Acceleration (dB re 1 ms-2)
X
Y
Z
100
500
1000 1500
3000
5000
Frequency (Hz)

Fig 1. Acoustic characterization of the experimental tank. A) Acoustic impedance (ratio of sound pressure level to particle motion level) at three sound pressure levels (119, 130, and 145 dB 1 μPa SPL_{rms}) for all frequencies examined. There are no apparent resonances at any of the frequencies. **B)** Particle acceleration levels for each of the x, y, and z magnitude vectors at 130 dB re 1 μPa for all frequencies examined.

https://doi.org/10.1371/journal.pone.0192561.g001

thresholds than bighead and common carp at 500, 600, 700, 900, 1000, 1500, and 3000 Hz (Table 1). At 2000 Hz, the mean threshold for silver carp was significantly lower ($P < 0.05$) than that of bighead carp but not common carp (Table 1). Common carp and bighead carp were also not significantly different at 2000 Hz.

Acceleration thresholds were lowest between 400–1000 Hz for all three species (Fig 6). Silver carp had significantly lower ($F_{4,38} = 32.26$, $P < 0.05$) mean acceleration thresholds at 500, 600, 700, 900, 1000, 1500, and 3000 Hz (Table 1, Fig 6) than bighead or common carp. Similar to the sound pressure thresholds, common carp thresholds were not significantly different from either silver or bighead carp thresholds at 2000 Hz, but silver carp had significantly lower

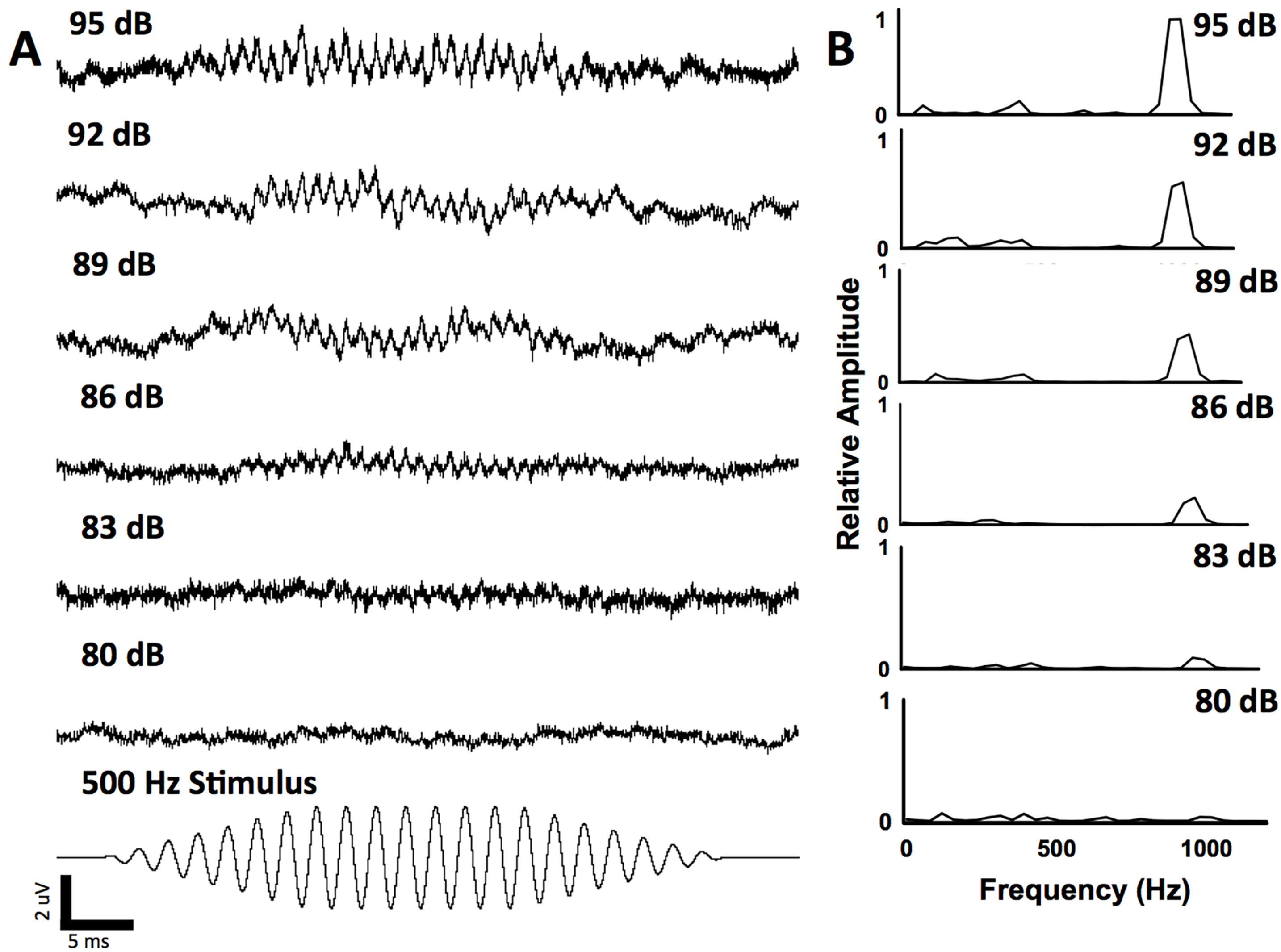


Fig 2. Example auditory evoked potentials (AEPs) recorded from a silver carp at 500 Hz. Averaged AEP traces **(A)** and FFT analysis **(B)** at six different sound pressure levels, including below the hearing threshold (80 dB re 1 μPa SPL_{rms}). FFT peaks are two times the stimulus frequency (1000 Hz). Hearing threshold was 83 dB re 1 μPa SPL_{rms} for this silver carp.

https://doi.org/10.1371/journal.pone.0192561.g002

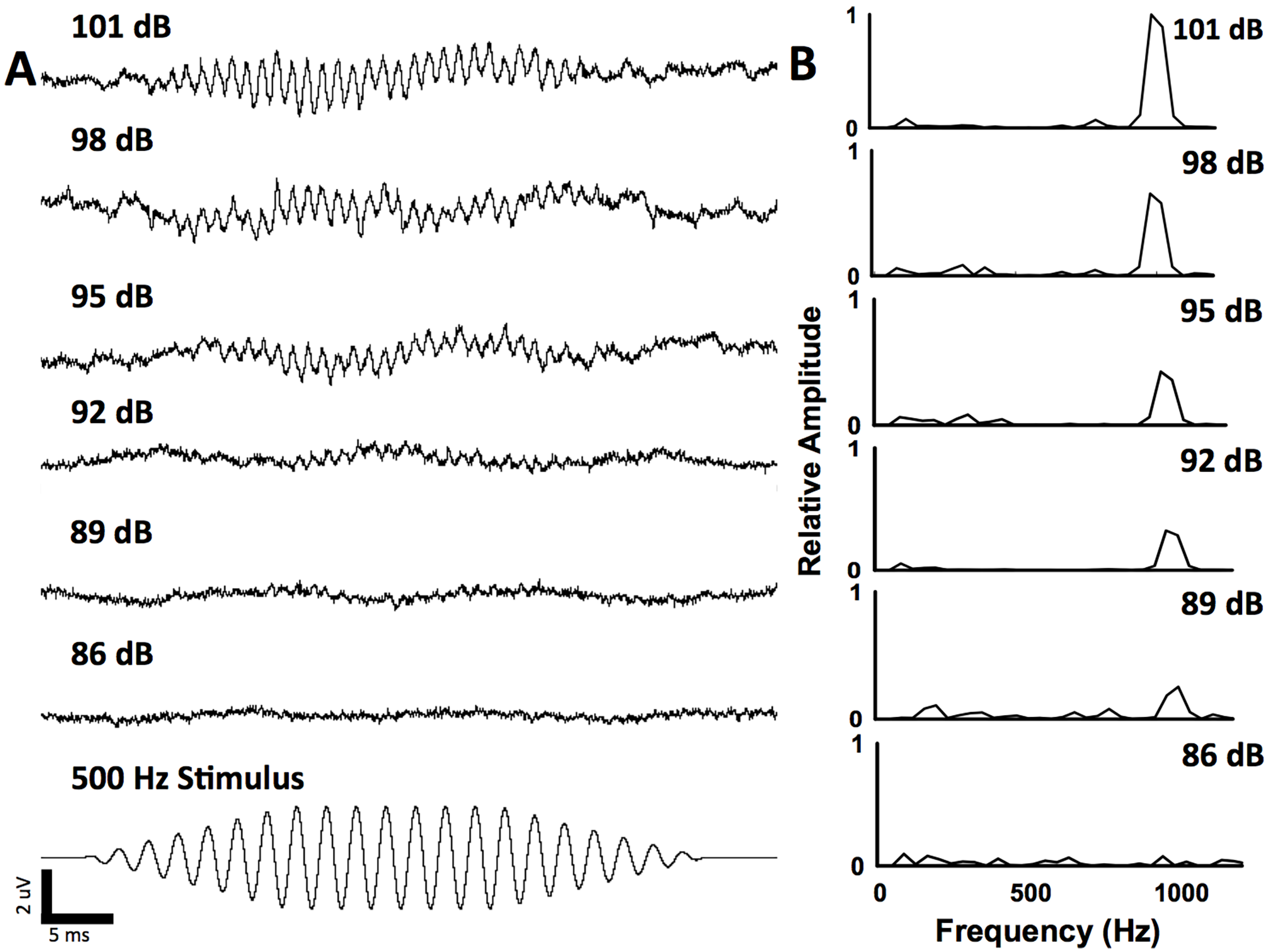


Fig 3. Example auditory evoked potentials (AEP) recorded from a bighead carp at 500 Hz. Averaged AEP traces **(A)** and FFT analysis **(B)** at six different sound pressure levels, including below the hearing threshold (86 dB re 1 μPa SPL_{rms}). FFT peaks are two times the stimulus frequency (1000 Hz). Hearing threshold was 89 dB re 1 μPa SPL_{rms} for this bighead carp.

https://doi.org/10.1371/journal.pone.0192561.g003

thresholds than bighead carp at this frequency (Table 1, Fig 6). The lowest mean acceleration threshold for all three species was at 400 Hz (silver carp: -77.0 ± 3.6 dB re 1 ms^{-2}; bighead carp: -73.2 ± 3.6 dB re 1 ms^{-2}; common carp: -71.3 ± 5.3 dB re 1 ms^{-2}; Table 1, Fig 6).

Discussion

Auditory evoked potentials were elicited from silver and bighead carp between 100 Hz– 5 kHz. The lowest mean sound pressure threshold was 500 Hz for both species. This demonstrates that bigheaded carp can detect higher frequencies than originally reported, which will be important in the future design of acoustic deterrents. Finally, the acoustic impedance of the tank was also determined and is reported so that it can be used in comparison with future fish hearing studies.

The need for investigating the upper range of bigheaded carp hearing originates from behavioral studies examining silver and bighead carp responses to pure tones (500–2000 Hz)

Table 1. Mean sound pressure and particle motion thresholds for each species at all frequencies examined. Letters indicate significant groups and * indicates significantly lower mean thresholds (ANOVA $P < 0.05$). BHC = bighead carp; SVC = silver carp; CC = common carp.

Species/Frequency (Hz)	Mean Threshold SPL (dB re 1 μPa) ± 1 SD	Mean Threshold Particle Motion (dB re 1 ms^{-2}) ± 1 SD
BHC 100	97.6 ± 4.5	-64.2 ± 3.5
SVC 100	96.4 ± 3.9	-65.2 ± 3.1
CC 100	102.0 ± 6.3	-60.7 ± 5.0
BHC 200	95.2 ± 4.6	-66.6 ± 3.1
SVC 200	91.6 ± 3.3	-69.1 ± 2.2
CC 200	100.0 ± 8.0	-63.4 ± 5.4
BHC 300	92.2 ± 7.5	-63.6 ± 6.0
SVC 300	85.6 ± 6.2	-68.8 ± 4.9
BHC 400	92.8 ±6.2	-73.2 ± 3.6
SVC 400	86.2 ± 6.2	-77.0 ± 3.6
CC 400	96.0 ± 9.2	-71.3 ± 5.3
BHC 500	90.5 ± 5.8	-67.2 ± 3.1
SVC 500	80.6 ± 3.3*	-72.5 ± 1.8*
BHC 600	102.5 ± 5.9	-61.8 ± 2.6
SVC 600	88.2 ± 4.6*	-68.0 ± 1.9*
CC 600	101.7 ± 0.58	-62.2 ± 0.25
BHC 700	107.6 ± 3.3	-67.3 ± 2.2
SVC 700	96.2 ± 5.0*	-75.0 ± 3.4*
BHC 800	105.2 ± 4.6	-65.7 ± 3.2
SVC 800	96.8 ± 5.0	-71.6 ± 3.5
CC 800	105.0 ± 9.6	-65.8 ± 6.8
BHC 900	105.8 ± 1.6	-59.7 ± 1.1
SVC 900	97.4 ± 2.5*	-65.3 ± 1.7*
BHC 1000	108.0 ± 2.1	-66.6 ± 1.8
SVC 1000	97.8 ± 2.7*	-75.1 ± 2.2*
CC 1000	106.0 ± 1.6	-68.2 ± 6.3
BHC 1500	113.0 ± 5.2	-54.6 ± 2.5
SVC 1500	104.6 ± 4.9*	-58.7 ± 2.4*
BHC 2000	125.6 ± 3.9[a]	-57.0 ± 2.3[a]
SVC 2000	116.6 ± 5.8[b]	-62.3 ± 3.4[b]
CC 2000	119.0 ± 3.0[ab]	-60.87 ± 1.8[ab]
BHC 3000	143.6 ± 2.5	-42.6 ± 1.7
SVC 3000	132.8 ± 2.7*	-50.0 ± 1.8*
BHC 4000	142.8 ± 5.1	-49.7 ± 2.4
SVC 4000	138.2 ± 3.4	-51.9 ± 1.6
CC 4000	140.7 ± 0.58	-50.7 ± 0.27
BHC 5000	140.6 ± 1.3	-50.8 ± 0.45
SVC 5000	142.0 ± 3.7	-48.6 ± 4.9
CC 5000	142.0 ± 1.7	-50.3 ± 0.58

https://doi.org/10.1371/journal.pone.0192561.t001

and broadband sound (0.06–10 kHz) recorded from an outboard motor. Both species showed negative phonotaxis to pure tone stimuli (150 dB re 1 μPa SPL_{rms}) in outdoor concrete ponds but habituated to these pure tone sounds after a few presentations [14, 15]. However, they demonstrated consistent negative phonotaxis to the broadband sound stimulus. Furthermore, the broadband sound also deterred both species from crossing a narrow opening in a concrete

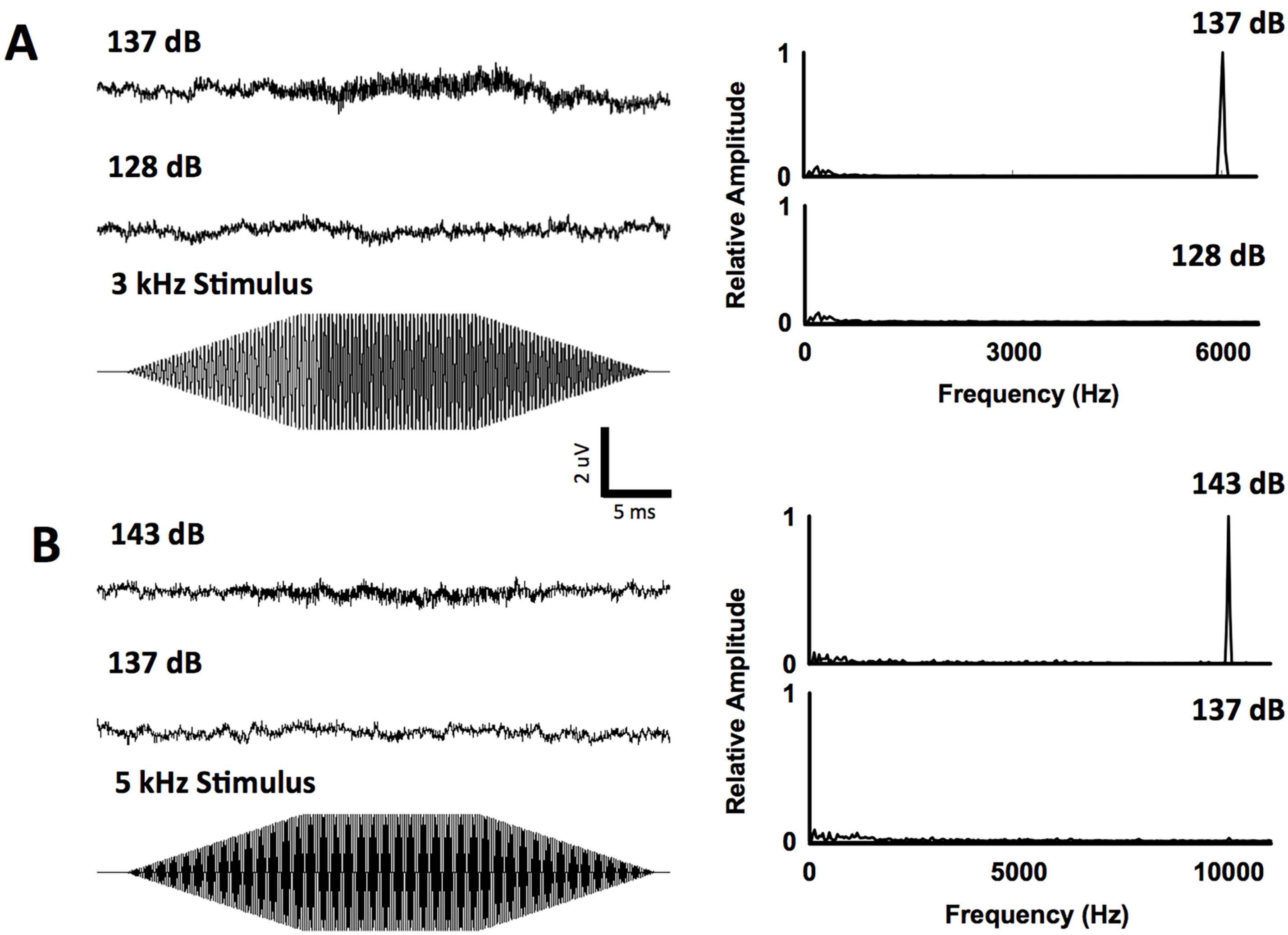


Fig 4. Example AEP traces recorded in response to high frequencies. Examples of AEPs (with FFT analysis) elicited at 3 kHz from a silver carp **(A)** and 5 kHz from a bighead carp **(B)**; upper traces were taken at sound pressure levels above the hearing threshold while the lower traces represent a baseline recorded at sound pressure levels below the hearing threshold.

https://doi.org/10.1371/journal.pone.0192561.g004

barrier [10]. While these studies demonstrate that the outboard motor recording is effective in altering bigheaded carp swimming, it is unclear what frequency components contained in the broadband sound impact fish behavior. The study by Lovell et al. [16] first characterized bigheaded carp hearing ability, but the researchers did not report thresholds greater than 3 kHz. Therefore the upper range of bigheaded carp hearing needed to be determined and was hypothesized to include higher frequencies than previously reported because (1) AEPs have been elicited at 5 kHz in other cyprinids [17] and (2) the behavior experiments demonstrated negative phonotaxis to broadband outboard motor recordings which had energy in frequencies up to 10 kHz. The present results indicate that both silver and bighead carp can detect a broad range of frequencies from 100 Hz up to 5 kHz with lowest thresholds at 500 Hz.

The tuning curves from the present study and those reported by Lovell et al. [16] demonstrate a broad sensitivity with the lowest thresholds between 300 Hz and 1.5 kHz for both bigheaded carp species. However, Lovell et al. [16] found higher hearing thresholds and different peak sensitivities (bighead carp: 1500 Hz; silver carp: 750 Hz). Comparisons between AEP

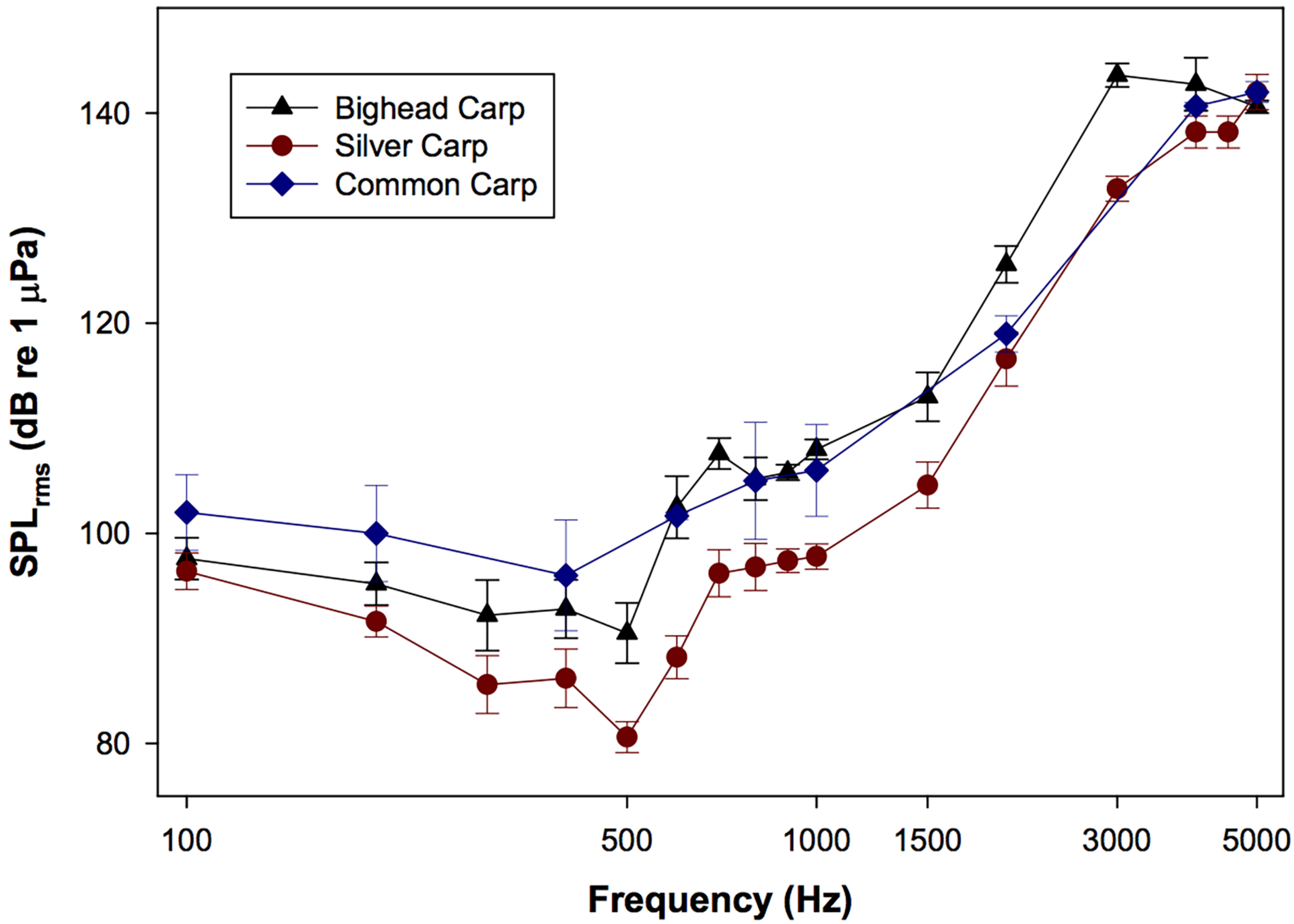


Fig 5. Audiogram for bighead, silver, and common carp. Each data point represents the minimum sound pressure level (SPL; dB re 1 μPa SPL_{rms}) necessary to invoke an AEP response at each frequency examined (100 Hz– 5 kHz). Data are plotted as mean ± SD. Silver carp had the lowest thresholds of the species examined.

https://doi.org/10.1371/journal.pone.0192561.g005

results are challenging because there are many variables between experimental design and methodology [21]. The present study used a single speaker that could produce greater sound pressure levels (153 dB re 1 μPa at 150 Hz) compared to the twin speakers used by Lovell et al. [16], which did not have an output greater than 134 dB re 1 μPa. Additionally, in the present study, both the visual and FFT analysis were used to determine the threshold sound pressure level for each frequency while Lovell et al. [16] only employed visually determined thresholds, which can be more subjective [21].

While comparisons between AEP thresholds generated using different setups can be difficult [21], several common carp were tested to qualitatively determine similarities in tuning curve shape and peak frequency detection between the current study and the published literature. The present findings show similar peak thresholds (400 Hz; [23]) and tuning curve shape as other AEP studies conducted on common carp [25, 23]. This suggests reliability of the AEP method utilized in the present study.

In addition to reporting AEP-derived threshold curves for the three carp species in regards to sound pressure, the tuning curves for acoustic particle motion were also determined. For all

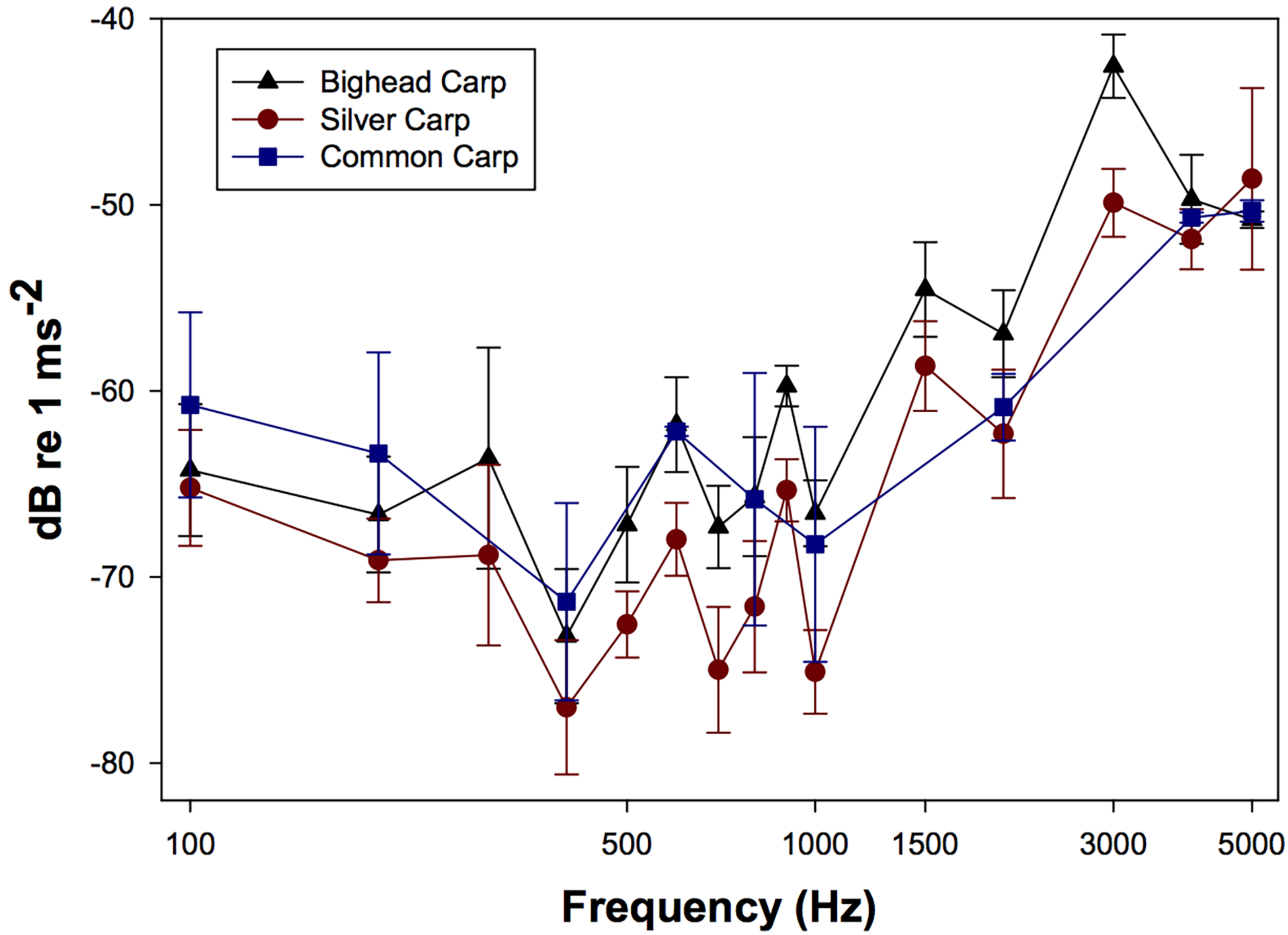


Fig 6. Particle acceleration thresholds (dB re 1 ms^{-2}) for the bighead, silver, and common carp. Each threshold was derived using a tri-axial accelerometer and are reported as the combined magnitude vector of the x, y, and z-axes Data are reported as mean (± SD).

https://doi.org/10.1371/journal.pone.0192561.g006

three species, the lowest mean particle acceleration thresholds were at 400 Hz. It is believed that all fish are capable of detecting acoustic particle motion through direct stimulation of the otoliths [33, 34]. However, many AEP studies only report sound pressure thresholds. As carp are likely capable of detecting both particle motion and sound pressure, the threshold curves for both were characterized and reported.

Recent reviews comparing the AEP and behavioral paradigms have concluded that while the two methods yield similar frequency ranges, the thresholds vary greatly, even among AEP studies [17, 21]. The AEP technique is therefore most useful as a means to determine range of frequencies that can be detected by a fish species' auditory end organs. In the Vetter et al. [14, 15] studies, although the fish demonstrated a robust avoidance response, the stimulus was a broadband sound and therefore the results could not identify a specific frequency or range of frequencies that most affected fish behavior. Ideally, to evaluate effective deterrent sound stimuli, these behavioral experiments would be repeated with many more frequencies examined. However, this would be logistically difficult as these studies were conducted in large outdoor ponds that take multiple days to fill and drain and require a lengthy acclimation period for the

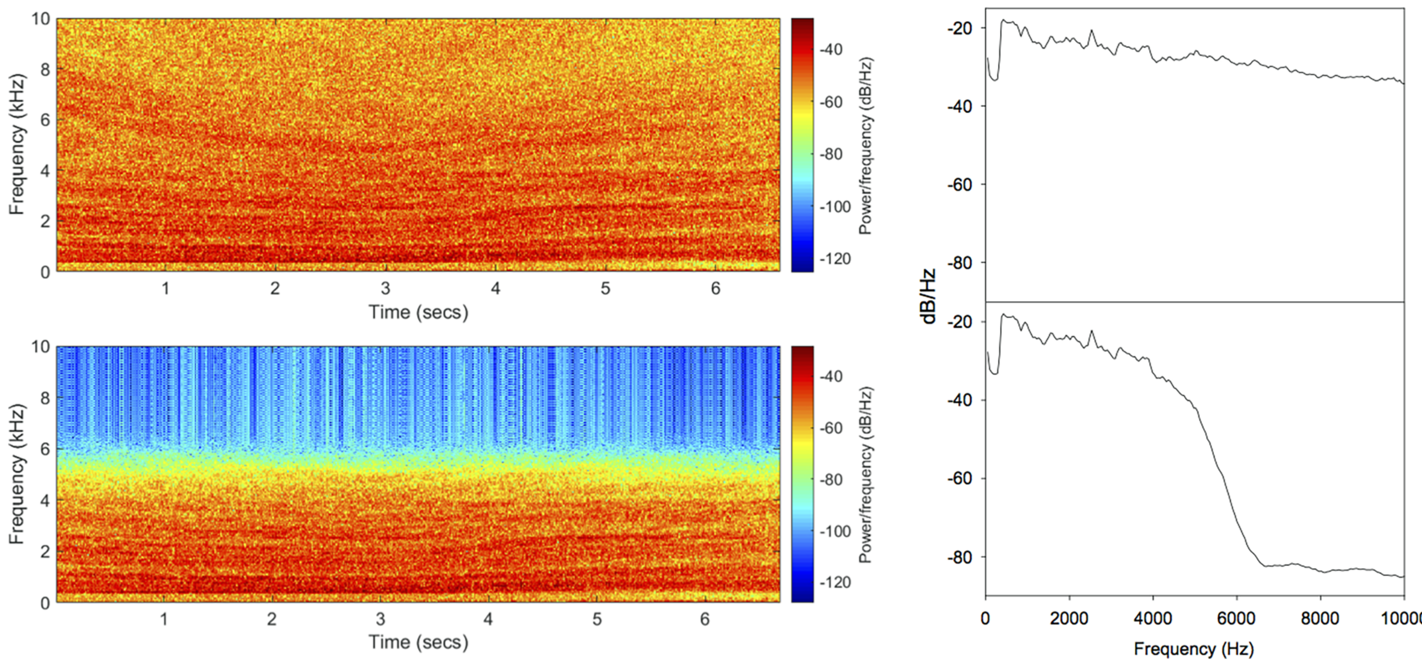


Fig 7. Low-pass filtered broadband sound. Spectrogram (left) and power spectrum (right) of the unfiltered (top) broadband sound used by Vetter et al. [14, 15] and Murchy et al. [10] to deter bigheaded carp. Bottom spectrogram and power spectrum represent the same broadband sound with a 5 kHz low-pass filter applied using Audacity (version 2). Spectrograms were generated using MatLab (version 9.3) and power spectra were analyzed in Audacity.

https://doi.org/10.1371/journal.pone.0192561.g007

fish. Furthermore, there are an infinite number of sound combinations that could be examined. Based on the results of the present study, these assessments can focus on frequencies between 100 Hz to 5 kHz and it is now imperative that behavioral studies examining bigheaded carp responses to sound be evaluated to better understand bigheaded carp hearing and to develop the most effective acoustic deterrent. In addition to examining the response behavior in fish exposed to a range of high frequency pure tones, applying a 5 kHz low-pass filter to the broadband sound will allow more energy to be broadcast in the hearing range of the bigheaded carp and may provide greater deterrence (Fig 7).

Finally, it is possible that bigheaded carp can hear frequencies above 5 kHz at sound pressure levels > 150 dB re 1 μPa SPL_{rms}, as other AEP studies have shown that ostariophysans can detect up to ~8 kHz [17]. When exposed to high sound pressure levels (i.e. 140–149 dB re 1 μPa SPL_{rms}) at frequencies between 6–9 kHz, small peaks on the FFT at two times the stimulus frequency were observed for some fish at 6, 7, 8, and 9 kHz, but these peaks appeared inconsistently and did not meet the established AEP criterion of the present study. However, given the constraints of tank size and speaker output, generating sound pressure levels above 150 dB re 1 μPa SPL_{rms} were not possible. Additional research could examine sensitivity to frequencies > 5 kHz at sound pressure levels above 150 dB re 1 μPa SPL_{rms} in both carp species.

The results of the present study provide important insight on the upper range of silver and bighead carp hearing, as they indicate higher frequency hearing than has been previously reported. Together with findings that bigheaded carp behavior can be modified using broadband sound, this research will aid in developing an effective acoustic deterrent. Particularly, the conclusion that bigheaded carp hearing extends up to 5 kHz could allow for refinement of the broadband stimulus to target bigheaded carp. Further research may allow for development of an effective acoustic deterrent primarily comprised of frequencies above the hearing range of non-ostariophysans, however care must to be taken to avoid disturbing native cyprinids.

Acknowledgments

The authors wish to thank Alayna Mackiewicz from the University of Minnesota Duluth and Mark Gaikowski, Jon Amberg, and Kelsie Murchy from the USGS Upper Midwest Environmental Science Center in La Crosse, WI. Fish were provided by Robin Calfee from the U.S. Geological Survey Columbia Environmental Research Center in Columbia, MO. Geoff Horseman and Simon Gray, from Cambridge Electronic Designs, provided technical support. Funding was provided by the USGS Ecosystem Mission Area Invasive Species Program and from the Great Lakes Restoration Initiative. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. All supporting data used in this publication are available from ScienceBase at https://doi.org/10.5066/F7M61JH0

Author Contributions

Conceptualization: Brooke J. Vetter, Marybeth K. Brey, Allen F. Mensinger.

Data curation: Brooke J. Vetter, Allen F. Mensinger.

Formal analysis: Brooke J. Vetter.

Funding acquisition: Marybeth K. Brey, Allen F. Mensinger.

Investigation: Brooke J. Vetter.

Methodology: Brooke J. Vetter, Allen F. Mensinger.

Project administration: Marybeth K. Brey, Allen F. Mensinger.

Resources: Allen F. Mensinger.

Software: Allen F. Mensinger.

Supervision: Allen F. Mensinger.

Validation: Brooke J. Vetter, Allen F. Mensinger.

Visualization: Brooke J. Vetter, Allen F. Mensinger.

Writing – original draft: Brooke J. Vetter.

Writing – review & editing: Marybeth K. Brey, Allen F. Mensinger.

References

1. Schrank SJ, Guy CS, Fairchild JF. Competitive interactions between age-0 bighead carp and paddlefish. Trans Am Fish Soc. 2003; 132: 1222–1228.
2. Irons KS, Sass GG, McClelland MA, Stafford JD. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA—Is this evidence for competition and reduced fitness?"J Fish Biol. 2007; 71: 258–273.
3. Sampson SJ, Chick JH, Pegg MA. Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. Biol Invasions. 2009; 11: 483–496.
4. Solomon LE, Pendleton RM, Chick JH, Casper AF. Long-term changes in fish community structure in relation to the establishment of Asian carps in a large floodplain river. Biol Invasions. 2016; 18: 2883–2895.
5. Sass GG, Hinz C, Erikson AC, McCelland NN, McCelland MA, Epifanio JM. Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. J Great Lakes Res. 2014; 40: 911–921.

6. Kolar KS, Chapman DC, Courtenay WR, Housel CM, Williams JD, Jennings DP. Bigheaded carps: a biological synopsis and environmental risk assessment. Bethesda: American Fisheries Society Special Publication 33; 2007. pp 204.
7. Sass GG, Cook TR, Irons KS, McClelland MA, Michaels NN, O'Hara ETM, et al. A mark-recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. Biol Invasions. 2010; 12: 433–436.
8. Moy PB, Polls I, Dettmers JM. The Chicago sanitary and ship canal aquatic nuisance species dispersal barrier. In: Chapman, DC., Hoff, MH, editors. Invasive Asian Carps in North America. Bethesda: American Fisheries Society Special Publication 74;2011. pp. 1–10.
9. Clarkson RW. Effectiveness of electrical fish barriers associated with the Central Arizona Project. N Am J Fish Manag. 2004; 24: 94–105.
10. Murchy KA, Cupp AR, Amberg JJ, Vetter BJ, Gaikowski MP, Mensinger AF. Evaluation of acoustical barriers for deterring silver (*Hypophthalmichthys molitrix*) and bighead carp (*H. noblis*) movement. Fish Mgmt Ecol. 2017; 24: 208–216.
11. Pegg MA, Chick JH. Aquatic nuisance species: an evaluation of barriers for preventing the spread of bighead and silver carp to the Great Lakes." Final Report for the Illinois- Indiana Sea Grant A/SE (ANS) 01–01. Illinois-Indiana Sea Grant, Urbana, IL. 2004
12. Taylor RM, Pegg MA, Chick JH. Response of bighead carp to a bioacoustic behavioural fish guidance system. Fish Manag Ecol. 2005; 12: 283–286.
13. Fay RR, Popper AN. The auditory periphery in fishes. In: Fay RR, Popper AN, editors. Comparative Hearing: Fish and Amphibians. New York: Springer Verlag; 1999. pp 43–100.
14. Vetter BJ, Cupp AR, Fredriks KT, Gaikowski MP, Mensinger AF. Acoustical deterrence of silver carp (*Hypophthalmichthys molitrix*). Biol Invasions. 2015; 17: 3383–3392.
15. Vetter BJ, Murchy KA, Cupp AR, Amberg JJ, Gaikowski MP, Mensinger AF. Acoustic deterrence of bighead carp (*Hypophthalmichthys nobilis*) to a broadband sound stimulus. J Great Lake Res. 2017; 43: 163–171.
16. Lovell JM, Findlay MM, Nedwell JR, Pegg MA. The hearing abilities of the silver carp (*Hypopthalmichthys molitrix*) and bighead carp (*Hypopthalmichthys nobilis*). Comp Biochem Physiol A Mol Integr Physiol. 2006; 143: 286–291. https://doi.org/10.1016/j.cbpa.2005.11.015 PMID: 16458557
17. Ladich F, Fay RF. Auditory evoked potential audiometry in fish. Rev Fish Biol Fish. 2013; 23: 317–364. https://doi.org/10.1007/s11160-012-9297-z PMID: 26366046
18. Jewett DL. Volume conducted potentials in response to auditory stimuli as detected by averaging in the cat. Electroencephalogr Clin Neurophysiol. 1970; 28: 609–618. PMID: 4192837
19. Jewett DL, Williston JS. Auditory evoked far fields averaged from the scalp of humans. Brain. 1971; 94: 681–696. PMID: 5132966
20. Kenyon TN, Ladich F, Yan HY. A comparative study of hearing ability in fishes: the auditory brainstem response approach. J Comp Physiol A. 1998; 182: 307–318. PMID: 9528109
21. Sisneros JA, Popper AN, Hawkins AD, Fay RR. Auditory evoked potential audiograms compared with behavioral audiograms in aquatic animals. In: Fay RR, Popper AN, editors. The Effects of Noise on Aquatic Life II. New York: Springer; 2016. pp. 1049–1056.
22. Fay RR. Sound reception and processing in the carp: saccular potentials. Comp Biochem Physiol A. 1974; 49: 29–42. PMID: 4153677
23. Kojima T, Ito H, Komada T, Taniuchi T, Akamatsu T. Measurements of auditory sensitivity in common carp *Cyprinus carpio* by the auditory brainstem response technique and cardiac conditioning method. Fish Sci. 2005; 71: 95–100.
24. Maruska KP, Boyle KS, Dewan LR, Tricas TC. Sound production and spectral hearing sensitivity in the Hawaiian seargent damselfish, *Abudefduf abdominalis*. J Exp Biol. 2007; 210: 3990–4004. https://doi.org/10.1242/jeb.004390 PMID: 17981867
25. Amoser S, Ladich F. Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? J Exp Biol. 2005; 208: 3533–3542. https://doi.org/10.1242/jeb.01809 PMID: 16155225
26. Popper AN, Fay RR. Rethinking sound detection by fishes. Hear Res. 2011; 273: 25–36. https://doi.org/10.1016/j.heares.2009.12.023 PMID: 20034550
27. Wysocki LE, Codarin A, Ladich F, Picciulin M. Sound pressure and particle acceleration audiograms in three marine fish species from the Adriatic Sea. J Acoust Soc Am. 2009; 126: 2100–2107. https://doi.org/10.1121/1.3203562 PMID: 19813819
28. Vasconcelos RO, Sisneros JA, Amorim MCP, Fonseca PJ. Auditory saccular sensitivity of the vocal Lusitanian toad fish: low frequency tuning allows acoustic communication throughout the year. J Comp Physiol A. 2011; 197: 903–913

29. Bhandiwad AA, Whitechurch EA, Colleye O, Zeddies DG, Sisneros JA. Seasonal plasticity of auditory saccular sensitivity in "sneaker" type II male plainfin midshipman fish, *Porichthys notatus*. J Comp Physiol A. 2017; 203: 211–222.
30. Mann DA, Higgs DA, Tavolga WN, Souza MJ, Popper AN. Ultra- sound detection by clupeiform fish. J Acoust Soc Am. 2001; 109: 3048–3054. PMID: 11425147
31. Higgs DM, Souza MJ, Wilkins HR, Presson JC, Popper AN. Age- and Size-Related Changes in the Inner Ear and Hearing Ability of the Adult Zebrafish (*Danio rerio*). JARO. 2001; 03: 174–184.
32. Egner SA, Mann DA. Auditory sensitivity of sergeant major damselfish *Abudefduf saxatilis* from post-settlement juvenile to adult. Mar Ecol Prog Ser. 2005; 285: 213–222.
33. De Vries HL. The mechanics of the labyrinth otoliths. Acta Otolaryngol. 1950 38(3):262–273
34. Hawkins AD. Underwater sound and fish behavior. In: Pitcher TJ, editor. The behaviour of teleost fishes. London: Chapman and Hall; 1993. pp 114–151.

Biol Invasions (2015) 17:3383–3392
DOI 10.1007/s10530-015-0964-6

ORIGINAL PAPER

Acoustical deterrence of Silver Carp (*Hypophthalmichthys molitrix*)

Brooke J. Vetter · Aaron R. Cupp · Kim T. Fredricks · Mark P. Gaikowski · Allen F. Mensinger

Received: 25 November 2014 / Accepted: 18 August 2015 / Published online: 25 August 2015

Abstract The invasive Silver Carp (*Hypophthalmichthys molitrix*) dominate large regions of the Mississippi River drainage and continue to expand their range northward threatening the Laurentian Great Lakes. This study found that complex broadband sound (0–10 kHz) is effective in altering the behavior of Silver Carp with implications for deterrent barriers or potential control measures (e.g., herding fish into nets). The phonotaxic response of Silver Carp was investigated using controlled experiments in outdoor concrete ponds (10 × 4.9 × 1.2 m). Pure tones (500–2000 Hz) and complex sound (underwater field recordings of outboard motors) were broadcast using underwater speakers. Silver Carp always reacted to the complex sounds by exhibiting negative phonotaxis to the sound source and by alternating speaker location, Silver Carp could be directed consistently, up to 37 consecutive times, to opposite ends of the large outdoor pond. However, fish habituated quickly to pure tones, reacting to only approximately 5 % of these presentations and never showed more than two consecutive responses. Previous studies have demonstrated the success of sound barriers in preventing Silver Carp movement using pure tones and this research suggests that a complex sound stimulus would be an even more effective deterrent.

B. J. Vetter (✉) · A. F. Mensinger
Biology Department, University of Minnesota Duluth, 1035 Kirby Drive, Duluth, MN 55812, USA
e-mail: vett0114@d.umn.edu

A. R. Cupp · K. T. Fredricks · M. P. Gaikowski
Upper Midwest Environmental Sciences Center, United States Geological Survey, 2630 Fanta Reed Road, La Crosse, WI 54603, USA

Keywords Silver Carp · Acoustics · Phonotaxis · Deterrent barriers · Management · Behavior

Introduction

Silver Carp (*Hypothalmichthys molitrix*) were introduced to aquaculture facilities in the southern region of the United States from eastern Asia in the 1970's (Kolar et al. 2005). The carp initially were used as a biological method of controlling algal growth in sewage treatment and fish farming facilities. Through a series of flooding events, the fishes subsequently escaped and established populations throughout the Mississippi River Basin and are currently threatening the Laurentian Great Lakes (Sass et al. 2010; Murphy and Jackson 2013). Carp have negatively impacted native fish such as Paddlefish (*Polyodon spathula*) (Schrank et al. 2003), Gizzard Shad (*Dorosoma cepedianum*) (Sampson et al. 2009), and Bigmouth Buffalo (*Ictiobus cyprinellus*) (Irons et al. 2007) due to their fast growth, prolific spawning, and ability to outcompete native fish for food and space. Additionally, Silver Carp demonstrate an unusual jumping behavior, which presents a hazard to boaters.

Invasive Silver Carp continue to migrate northwards through the Mississippi River Basin and expand their range via interconnected waterways, impeded only by large physical barriers. The Laurentian Great Lakes are currently threatened as these carp have been found in shipping canals that connect the Illinois River and Lake Michigan (Moy et al. 2011). Additionally, prevention efforts are ongoing to prevent Silver Carp expansion into Mississippi River tributaries and lakes (Kelly et al. 2011). Considerable effort has gone into erecting electric barriers on the Chicago Ship and Sanitary Canal to prevent spread into Lake Michigan, however, electrical barriers have inherent risks and must be continuously operated to prevent upstream migration (Clarkson 2004). Non-physical barriers, such as noxious sound stimuli, are promising methods that can be deployed in addition to electric barriers or when such systems are not feasible (Noatch and Suski 2012).

Since the early 1950's, researchers have examined sound to control fish movement (Burner and Moore 1953). Historically, research efforts have focused on using acoustic deterrents to prevent fish from entering hydropower dams or power plants (Schilt 2007). Ultrasound (122–128 kHz) was 87 % effective in preventing Alewives (*Alosa pseudoharengus*) from approaching a dam intake in Lake Ontario (Ross et al. 1993). Maes et al. (2004) used a variety of frequencies (20–600 Hz) to repel Atlantic Herring (*Clupea harengus*, 94.7 %) and European Sprat (*Sprattus sprattus*, 87.9 %) from a power plant intake. In the past 20 years, acoustic deterrents, often coupled with bubbles or lights, have been used to modulate invasive fish behavior with the intent on preventing their range expansion (Noatch and Suski 2012). Pegg and Chick (2004) found 20–2000 Hz sound was more effective (95 %) in preventing Silver and Bighead (*Hypothalmichthys noblis*) Carp from crossing a bubble-sound barrier than frequencies in the 20–500 Hz range (57 % effective). Similarly, sound (20–2000 Hz) combined with a bubble curtain, successfully repelled Bighead Carp (95 %) in an enclosed raceway (Taylor et al. 2005). Sound (500–2000 Hz), bubbles, and light impeded the upstream migration of Silver and Bighead Carp in a small tributary (Ruebush et al. 2012). Laboratory experiments demonstrate that bubble curtains, which generate 200 Hz frequency sound, can inhibit movement of Common Carp (*Cyprinus carpio*) (75–85 %) (Zielinski et al. 2014). While strobe lights have some success in affecting fish movement, they also appear more effective when paired with sound or bubbles (Noatch and Suski 2012). These studies demonstrate the potential of acoustic deterrents for modulating fish behavior.

For sound barriers to be effective, fish must be able to detect the frequency, localize the sound source, and stop or move away from the source. Silver Carp are cyprinids in the superorder ostariophysi, which possess Weberian ossicles that form a connection between the swim bladder and inner ear (Popper and Carlson1998; Fay and Popper 1999). These ossicles provide Silver Carp with relatively broad hearing (up to at least 3 kHz) and greater sensitivity than many other Midwestern and Great Lakes fishes that lack the connection (Lovell et al. 2006). For example, Lake Sturgeon (*Acipenser fulvescens*) and Paddlefish only detect sounds up to approximately 400 Hz, with peak sensitivity between 200 and 300 Hz (Lovell et al. 2006), and the frequency sensitivity of Bluegill Sunfish (*Lepomis machrochirus*) is 200–300 Hz (Scholik and Yan 2002a). Other carp species have demonstrated the ability to detect and/or localize sound stimuli associated with food reward. Grass Carp (*Ctenopharyngodon idella*) (Willis et al. 2002) were trained to localize pure tones (600–1000 Hz) and carp feeding sounds, and Common Carp (Sloan and Mensinger 2013) were classically conditioned to associate feeding with a 400 Hz pure tone. Therefore, the use of higher frequency sounds for Silver Carp management has the potential to modulate carp behavior while minimizing the effect on native game fish.

Previous studies on effective sound barriers utilized pure tone stimuli. The present study investigated both pure tones (0.5–2 kHz) and higher frequency (0–10 kHz) complex sound on Silver Carp behavior during a set of controlled experiments in outdoor concrete ponds. The goal was to determine the optimal frequency or frequencies for deterring Silver Carp movement and it was predicted that the complex sound stimulus would be more successful in affecting fish swimming behavior.

Material and methods

Animal husbandry

All experiments were conducted at the Upper Midwest Environmental Sciences Center (UMESC) of the

United States Geological Survey (USGS) in La Crosse, WI. Silver Carp (18–24 cm) were maintained in 1500 L flow through indoor ponds and fed trout starter diet (Skretting, Tooele, UT) at a rate of 0.5 % body weight per day (Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government). All experimental fish were tagged with passive integrated transponder (PIT) tags (Biomark Inc, Boise, ID) at least 1 week prior to experimentation. During the tagging process, fish were sedated with 100 mg/L AQUI-S ® 20E (10 mg/L eugenol) (AQUI-S New Zealand Ltd., Lower Hutt, New Zealand) in the culture pond to minimize jumping when removed for tagging. Fish were hand netted from the culture pond and then placed in 300 mg/L AQUI-S ® 20E (30 mg/L eugenol) until loss of equilibrium and failure to respond to caudal peduncle pinch. Each fish was wiped with 1 % topical iodine and injected with PIT tags into the abdomen about 2 cm anterior to the vent and placed in fresh flowing water to recover. To facilitate transport to the pond, fish ($N = 10$) were sedated with 50 mg/L AQUI-S ® 20E (5 mg/L eugenol) to minimize jumping and potential injury. Food was withheld for 24 h prior to transport and fish were not fed while in the outdoor ponds (<7 days). Each group ($N = 5$) was allowed to acclimate in the outdoor pond for at least 48 h prior to the initiation of experiments. Two-day trials were conducted from July through September 2013.

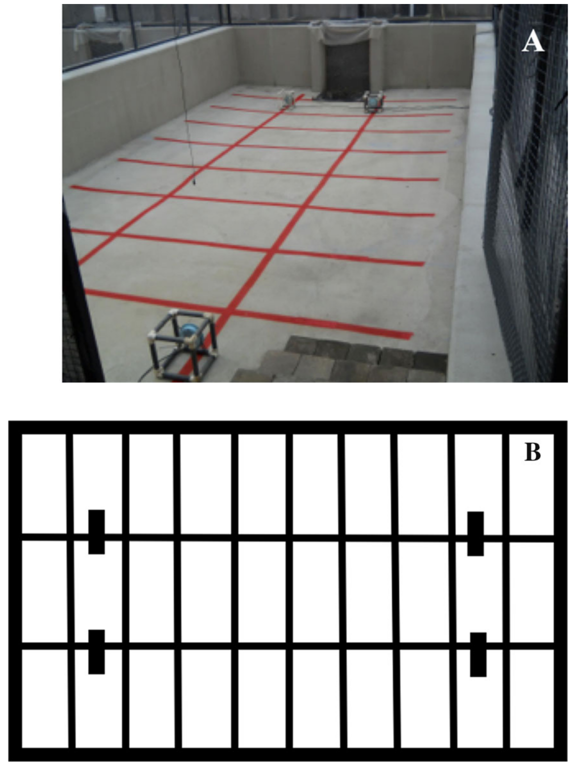


Fig. 1 **a** View from the entry door of a drained experimental pond. Speakers are at the near (only one visible) and the far (pair) end of the pond. Water level was maintained within 5 cm of the top of the concrete walls. The fence enclosing the pond is visible at the top of the walls. Gridlines painted on pond bottom assisted in assessing fish position. **b** Overhead schematic of the experimental pond showing approximate location of gridlines and speakers (*solid rectangle*)

Behavioral experiments

Behavioral experiments were conducted in 10 m × 5 m × 1.2 m (60 k L) outdoor concrete flow through ponds (Fig. 1). Flow rate into the ponds was adjusted to maintain a water temperature range of 17–21 °C. Water was pumped into the ponds directly from UMESC wells. Although water quality was not measured, fish showed no signs of being stressed due to poor water quality. Each pond was fully enclosed vertically by a 2 m wire fence on the top of the pond walls with anti-bird netting draped across the top of the fence. Pond access was restricted to a 2 m × 1 m wire door that remained locked throughout the experiment.

Sound stimuli

Sound was delivered via one of two pairs of underwater speakers (UW-30, Lubell Labs Inc., Whitehall, OH) that were placed 1.5 m from each end of the pond and 1.6 m from the nearest side-wall (Fig. 1). Acoustic stimuli consisted of pure tones (500, 1000, 1500, or 2000 Hz) generated by Audacity 2.0.5 software and complex tones recorded underwater from an outboard motor (100 Hp Honda 4-stroke). The outboard motor sound was recorded using a hydrophone (HTI-96-MIN, High Tech Inc., Long Beach, MS), in a section of the Illinois River near Havana, IL, which contained Silver Carp populations. The sound was recorded in approximately 1 m of water while the boat transited past the hydrophone at 32 km/h, which also stimulated carp to jump in the area.

Sound was amplified (UMA-752 amplifier, Peavey Electronics, Meridian, MS) and each speaker pair was controlled manually with a switchbox (MCM Electronics, Centerville, OH). Each pond contained a single hydrophone (HTI-96-MIN, High Tech Inc., Long Beach, MS) to verify the sound stimuli, which was recorded using a PowerLab 4SP data acquisition system and LabChart 7 software (AD Instruments, Colorado Springs, CO).

Sound pressure levels were maintained constant for the pure tones and complex sound and were approximately 150 dB re 1 μPa @ 1 m directly in front of the speakers, which was approximately 30 dB re 1 μPa @ 1 m above the minimum ambient noise (Fig. 2). All pure tone responses showed a narrow energy peak at the dominant frequency (Fig. 3). The complex sound produced a broad spectrum of sound from 0 to 10 kHz with maximal energy contained in two relatively broad peaks from 0 to 2 kHz and 6 to 10 kHz (Fig. 3).

Behavior was monitored remotely with eight overhead SONY bullet 500 TVL video cameras connected to ProGold software (Security Camera World, Cooper City, FL). An observer was situated in a shelter approximately 50 m from the test pond. The cameras continuously monitored the fish and provided full coverage of the pond. Gridlines (1.6 m × 1.0 m) on the pond bottom (Fig. 1) assisted in determining fish position.

Silver Carp demonstrated schooling behavior and therefore the group of fish in each trial was treated as a single unit with position determined as the approximate center of the school. Trials (i.e., sound stimuli) were not initiated until the school was positioned within an end zone, which was defined as the area of the pond within 2.5 m of the end wall.

The experimental trials consisted of playing sound from one speaker pair, observing the behavioral

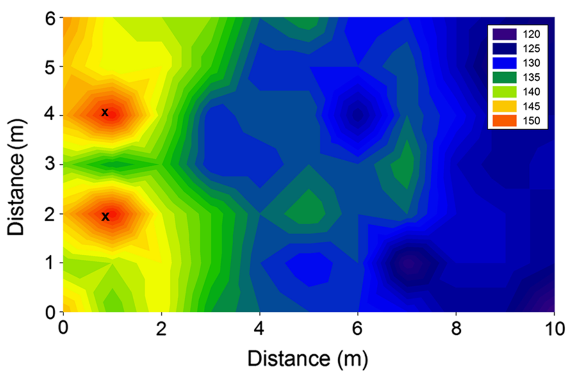


Fig. 2 The sound intensity level (dB re 1 μPa @ 1 m) is plotted during active broadcast of the two underwater speakers (indicated by *X*). Recordings were made at 1 m intervals and a depth of 0.6 m. Intensity level is indicated by color in *upper right inset*

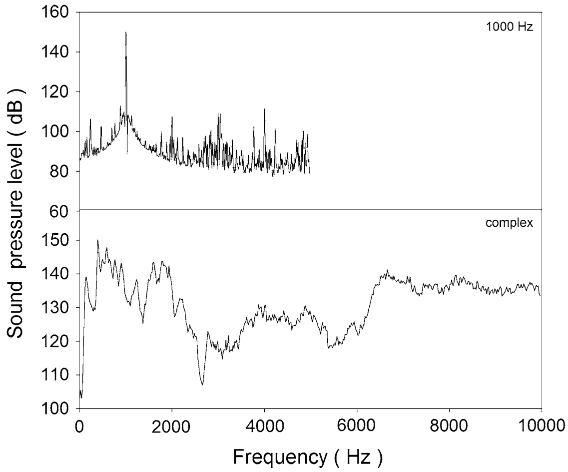


Fig. 3 The power spectrum in dB of the 1000 Hz and complex sound stimulus is plotted versus frequency (Hz)

response, and alternating the sound location if the fish swam away from the sound. Negative phonotaxis was defined as the group of fish orienting and swimming away from the end zone closest to the sound source within the first 15 s of sound onset and crossing the centerline (5 m) within 30 s. During these responses, the observer would continue to administer sound until fish reached the far end zone. Once the fish entered the opposite end zone from the midline, the sound source was changed to the speakers in that end zone. All behaviors not conforming to the criteria established for a negative phonotaxis, such as no reaction, swimming towards the speaker, or failure to cross the midline in 30 s, were categorized as no response. Consecutive responses were defined as fish reacting to two or more consecutive sound presentations from opposite ends to the pond. Sound trials were conducted with pure tones and complex sounds with the order of presentation (pure tones vs. complex) randomly determined prior to each trial. Trials were completed over a 2-day period for each of the five groups of fish with 3–4 pure tone and 4–11 complex sound trials conducted on each group.

Pure tone trials

Fish position was monitored for 10 min prior to initiation of sound. Each trial began with a 30 s pure tone (500, 1000, 1500 or 2000 Hz) initiated from the nearest speaker pair to the fish. Once the fish failed to respond, the sound was terminated and the fish were allowed a recovery time of 90–180 s before the next sound presentation of the same frequency. Each trial consisted of three to five presentations of the same frequency and was concluded with 30 s of continual complex sound (outboard motor underwater recording). For both the pure tone and complex sound presentation, the sound source was alternated if the fish reacted to the sound and crossed into the opposite end zone. Fish were allowed to rest for 10–15 min after the presentation of the complex sound at the conclusion of the pure tone trial, before a different frequency was tested using the same procedure. The four frequencies were tested consecutively with presentation order of the frequencies randomized. Fish were allowed to rest for at least 30 min after each set of all four frequencies was tested before subsequent sound trials (pure tone or complex).

Complex sound trials

Complex sound trials were conducted following a similar protocol with the underwater recording of an outboard motor used as the stimulus. Preliminary trials showed that this stimulus produced consistent and repeated negative phonotaxis so the protocol was modified slightly, and the 30 s complex sound file was continuously looped throughout the trial. The sound stimulus was switched to the opposite speaker pair as soon as the school crossed into the opposite end zone. Based on fish response and position, the sound source was alternated for 10 min or until the fish failed to respond. Fish were allowed to rest for at least 1 h after each complex sound trial before any other sound trials were conducted.

Data analysis

Fish position was monitored from 10 min prior to and throughout the sound presentation for sound trials. The position of the midpoint of the school was recorded every 5 s.

Swim speed was quantified for experimental fish that reacted to the sound using frame-by-frame analysis of the video recording (30 frames/s). The elapsed time from when the fish turned away from the sound and swam 2 m away was calculated. The swim speeds were only assessed when the group of fish turned in response to sound playback and swam the 2 m in <30 s. In order to accurately compare response times, groups that took longer than 30 s, or did not respond, were excluded from analysis. Control swim speeds were determined prior to testing or at least an hour after fish had been exposed to sound by monitoring. For a control, fish were observed for a 10-min period of continuous swimming in the absence of sound and the duration that it took the school to transverse each 2 m interval was recorded and averaged.

Sound mapping

Acoustic properties of the speakers and pond were mapped using an HTI hydrophone connected to the PowerLab 4SP data acquisition system and LabChart 7 software. The pond was divided into a 1 m × 1 m grid and a total of 77 recordings were made at 1 m intervals. Relative sound pressure levels (SPL) were calculated for each frequency by measuring the root mean square (rms) voltage and converting to SPL in dB re 1 μPa @ 1 m using Avisoft-SASLab Pro ver 5.2.07. The frequency components and power spectrum of the sound were calculated with a 1024-point fast Fourier transform (Hamming window) and sampling rate of 40 kHz.

All statistical tests were performed with Sigmaplot for Windows, version 12.5. Shapiro–Wilk tests indicated that the response number and swimming speeds data were not normally distributed and therefore non-parametric Kruskal–Wallis ANOVAs with Dunn's post hoc tests were used to analyze the data. Although the response data were analyzed using non-parametric tests, the mean ± 1 SE is reported for illustrative purposes as the median and quartiles for the pure tone frequencies were all 0. The median and upper and lower quartile is reported for the swim speeds ($P < 0.05$).

Results

Behavioral responses

Fish behavior, in the absence of sound, alternated between slow swimming throughout the pond (one circuit approximately every 2 min) and remaining in one location, typically a shady area of the pond. For pure tones trials, fish demonstrated negative phonotaxis to approximately 12 % of the initial sound presentations, with <1 % of the pure tone stimuli trials eliciting a subsequent reaction from the fish and zero responses to three or more consecutive presentations (Fig. 4). However, the fish always displayed negative phonotaxis when subjected to the complex sound at the conclusion of each trial. Fish were slightly more responsive to higher frequencies, showing an average of 0.18 ± 0.06 responses to 2000 Hz compared 0.13 ± 0.06 reactions for the 500 Hz. In contrast, the Silver Carp responded during 100 % of the complex trials with an average of 11.8 ± 1.3 (range 3–37) consecutive responses per trial. Furthermore, the number of average consecutive responses to the complex sound was significantly greater ($H = 144.06$, $P < 0.001$) than in the pure tone trials.

Representative Silver Carp behavior to acoustic stimulation from two of the five groups is displayed in Fig. 5. Controls demonstrate the typical slow swimming over the course of 10 min in the absence of sound stimuli (Fig. 5 control). Group A did not respond to the 500 and 1000 Hz and at the two higher frequencies (1500 and 2000 Hz), the fish responded to only the second of three pure tone presentations. Similarly, for Group B, the fish did not respond to pure tones at the 500, 1000, and 2000 Hz frequencies. During the first, second, and third 1500 Hz pure tone presentations, Group B demonstrated one response. In contrast, both groups responded to the complex sound after all the pure tone presentations. Consistent back and forth swimming along the length of the pond away from the active speaker pair during complex sound trials was observed (Fig. 5 complex) with Group B demonstrating 37 consecutive negative phonotaxic responses to the complex sound. Following the fourth sound presentation, Group A swam to the opposite wall of the pond but remained behind the speakers. They remained at this end for about a minute but then continued to react to the complex sound stimuli, demonstrating 26 consecutive responses for a total of 31 responses during this trial.

Fish averaged approximately 13 s to swim 2 m (0.15 m/s) during the times they were actively swimming in the absence of sound (Fig. 6). Median times for fish to respond to pure tones ranged from 9.2 (1500 Hz) to 26.0 s (2000 Hz) (0.22 to 0.08 m/s), while fish reacting to the complex sound swam away significantly faster with a median time of 4.8 s ($H = 75.306$, $P < 0.001$) or 0.42 m/s.

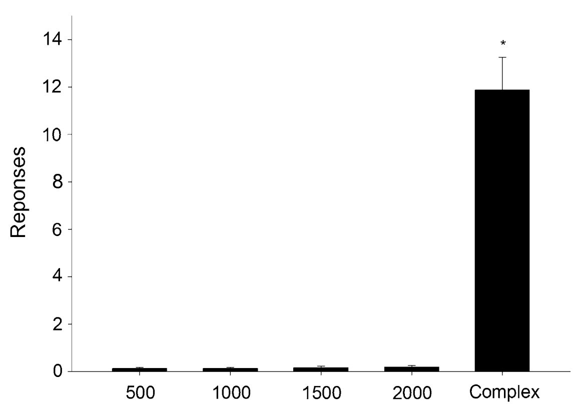


Fig. 4 Average number of responses per trial to sound playback versus sound stimulus type (500, 1000, 1500, and 2000 Hz and complex sound). All data show the mean ± 1 standard error. *Asterisk* indicates significantly different group ($P < 0.001$)

Discussion

Silver Carp demonstrated consistent movement away from complex sounds whereas pure tones were less successful in eliciting a reaction. At best, fish responded to two consecutive pure tones, but failed to react to over 95 % of the presentations. Conversely, the complex sound alone was sufficient to reliably drive carp away from the source eliciting an average of eleven consecutive responses. This suggests that complex broadband sound (0–10 kHz), such as the outboard motor recording used, is more effective in affecting Silver Carp swimming than pure tones.

The Silver Carp habituated quickly to the pure tones as they demonstrated the characteristic decrease in responsiveness upon repeated exposure to the stimuli (Rankin et al. 2008; Thompson and Spencer 1966). For the complex sound, the fish usually stopped responding by the end of the 10 min test period but it was unclear whether this was due to habituation or fatigue. In contrast to the pure tones, subsequent playbacks of the complex sound, after a recovery period, continued to elicit a response. This suggests that fatigue may have factored into reduced responses as the fish continually reacted to the alternating complex sound source at a significantly greater swim speed than during the pure tones or controls. Furthermore, despite repeated trials, the schools would still respond to at least three consecutive sound presentations. Finally, the decreased responsiveness to pure

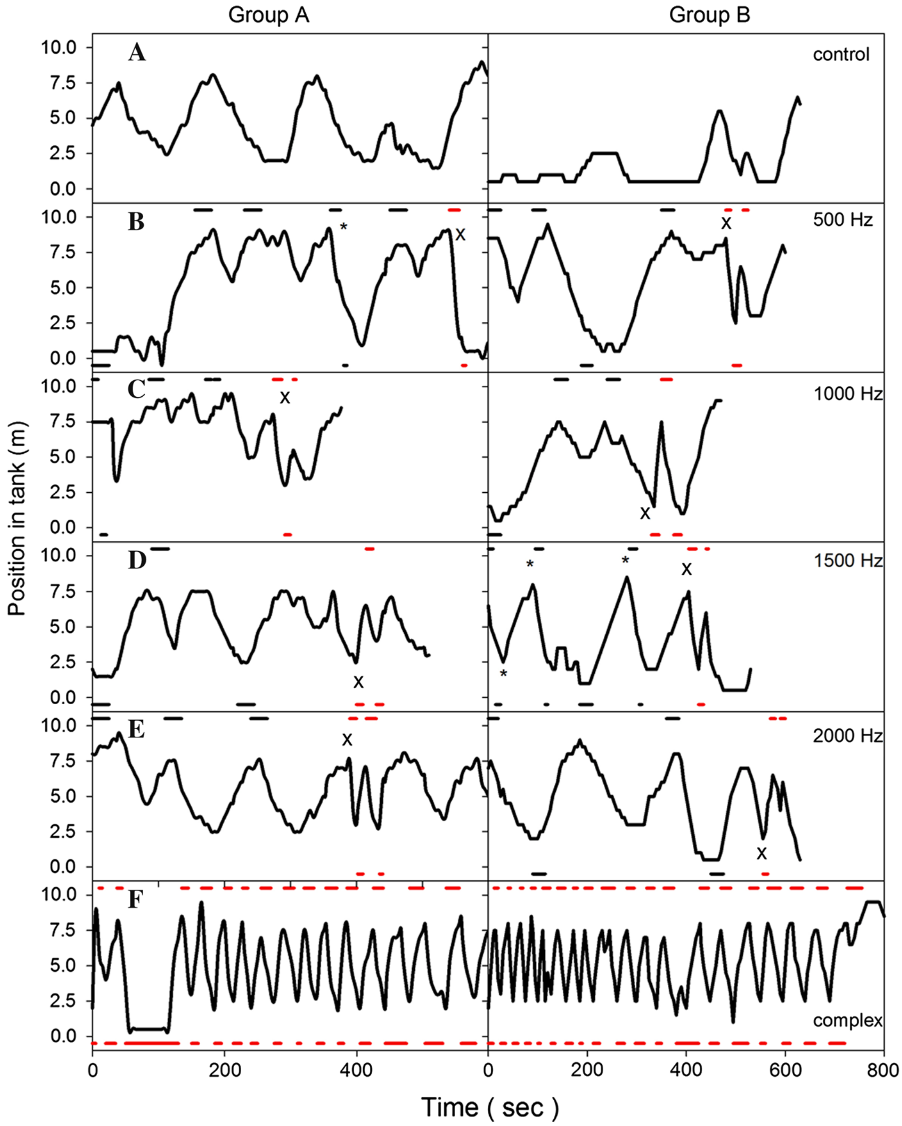


Fig. 5 Representative Silver Carp behavioral response to acoustic stimulation for two groups of fish (*Group A* and *Group B*). For each figure, the longitudinal position (m) of the center of the school is plotted versus time (s) with fish position mapped every 5 s. *Solid lines* above and below each fish position trace indicate the location and duration of the sound stimulus. *Black* indicates pure tones and *red* indicates complex motor sounds. *Asterisk* indicates no response and *X* represents negative phonotaxis; in situations where the fish demonstrated consecutive responses, the first response is indicated by an *X*. **a** control (no sound); **b** 500 Hz; **c** 1000 Hz; **d** 1500 Hz; **e** 2000 Hz; **f** complex sound

tones was behaviorally based as subsequent playbacks of the complex sound resulted in rapid movement away from the sound, indicating that the auditory system was functional and the fish were able to locate the sound source.

Both the pure tones and a portion of the complex stimulus used in this study were within the known frequency sensitivity of Silver Carp (up to 3 kHz) and the intensities presented were well above their auditory thresholds (Lovell et al. 2006). Although the typical c-start startle response characterized by rapid contraction of the axial muscles and movement away from the stimulus was sometimes observed at the first sound presentation (video quality and speed was insufficient to quantify c-start mechanics), subsequent responses did not elicit this behavior. Thus, the prolonged negative phonotaxis exhibited appeared to be directed swimming behavior away from the complex sound and not a sudden or rapid escape response.

The effects of high frequency anthropogenic sound on native ostariophysans, such as minnows, suckers, and catfish, remains to be determined. In a laboratory study involving Fathead Minnows (*Pimephales promelas*), exposure to white noise (0.3–4.0 kHz dB re µ1 Pa) significantly increased auditory thresholds, especially in the higher frequency range (0.8–2.0 kHz)

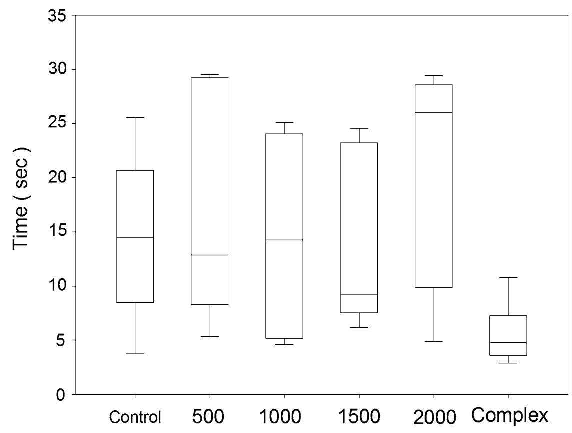


Fig. 6 Fish swim speeds. *Box* and *whisker* plots display the median and upper and lower quartile for the time for fish to swim 2 m after sound stimulation. The control represents the average time (<30 s) to swim 2 m in the absence of sound ($P < 0.001$)

and persisted for at least 14 days after exposure (Scholik and Yan 2001). Anthropogenic noise is also thought to affect fish behavior. For example, Blacktail Shiners (*Cyprinella venusta*) increased the amplitude and rate of mating calls in the presence of background noise (Holt and Johnston 2014). One of the most prominent sources of anthropogenic noise is recreational and commercial motorized watercraft and negative effects of these sounds on fish are well documented (Scholik and Yan 2002b; Liu et al. 2013; Voellmy et al. 2014; Popper and Hastings 2009; Whitfield and Becker 2014). More research on the effect of high frequency sound on native species, especially ostariophysans, is essential before acoustic deterrents can be implemented.

The impetus to determine if sound could be used to modulate behavior was based on the jumping behavior of Silver Carp in response to motorized watercraft and anecdotal reports of commercial fisherman using noise to concentrate fish for capture. Although their propensity for jumping has been well documented, especially in popular videos, few if any studies address the sensory input that elicits this behavior. Understanding the behavior and sensory physiology of an invasive fish species is imperative when developing methods to for management and control (Popper and Carlson 1998).

The Silver Carp in the current study did not jump in response to sound. Fish have been documented to jump using higher intensity sound in the Illinios River in the absence of motorized watercraft, howevever they tended to be larger than the fish used in this study (Mensinger, unpublished). Furthermore, the water clarity was also much higher in the outdoor ponds compared to the Illinois River (Arnold et al. 1999). Increased turbidity may enhance the tendency of Silver Carp to jump, as it reduces the fish's visual field. It is unclear whether boat movement and/or waves plus sound is the basis for this behavior. It should also be noted that each group of fish was naïve to the sound stimuli. Furthermore, the fish were collected as young of the year and reared in the lab so any exposure to outboard motors would have been limited to their early life history.

Previous studies have investigated sound to control both Bighead and Silver Carp using primarily pure tones. Taylor et al. (2005) tested a bubble-curtain barrier combined with a random sound generator (pure tones from 20 to 2000 Hz) in outdoor experimental raceways and reported that the bubble-sound barrier was effective at preventing 95 % of the Bighead Carp's attempts to cross. Ruebush et al. (2012) used a bubble-strobe-sound (500–2000 Hz) barrier on a tributary of the Illinois River and assessed the number of marked Silver and Bighead Carp that crossed the barrier while migrating upstream. Only two tagged Silver Carp ($N = 575$) and no Bighead Carp ($N = 101$) crossed the barrier; however it was unclear how many fish challenged the barrier or remained in the area.

Lovell et al. (2006) demonstrated that Silver Carp respond to frequencies up to 3 kHz, however as their hearing sensitivity decreased relatively slowly at the higher frequencies tested, the fish may retain higher frequency sensitivity past the end point (3 kHz) of their study. Therefore, the carp were able to detect the complex sound stimulus. The results suggest that complex sound, containing frequencies from 0 to 10 kHz, is capable of consistently modulating behavior and has potential to be developed as part of an acoustic or multi-modal deterrent system. An acoustic deterrent has advantages over electrical or physical barriers in that sound can travel a considerable distance underwater, poses minimal environmental risk, and is relatively inexpensive to deploy. Furthermore, a barrier that uses this complex sound, either alone or in combination with light and bubbles, is an ideal strategy to restrict Silver Carp range expansion because the higher frequency components target Silver Carp, and will have minimal, if any, impact on most native game fish, though further testing is needed to evaluate the effect on native ostariophysans. Research examining the efficacy of a sound deterrent in an open rather than closed system is also necessary, as the Silver Carp had limited (<10 m) distance to escape the sound in the experimental ponds. An open system, such as a river, might allow the Silver Carp to swim a greater distance from the sound and could lengthen the time that the fish would stay away. Ruebush et al. (2012) reported that many carp moved back down stream, away from their bubble-strobe-sound barrier and out of the study system. A barrier using the complex sound stimuli might have a similar effect as wild fish can leave the area.

The range expansion of invasive Silver Carp is a concern to many state and federal agencies as the fish threaten entire food webs and the jumping behavior of Silver Carp endangers recreational and commercial boaters. This study's objective was to determine the effects of sound on modulating Silver Carp behavior. The results suggest that the complex sound may be an important management tool and could be effective either on its own or integrated with other deterrent technology.

Acknowledgments We would like to thank the USGS Upper Midwest Environmental Sciences Center (UMESC) staff and interns, especially Justin Smerud, Riley Buley, and Allison Zwarycz for their assistance with this project. The Illinois River Biological Station staff, including Andrew Casper, Levi Solomon, and Thad Cook, provided additional support. 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). Resources for the USGS component of the research were provided through the USGS Ecosystem Mission Area Invasive Species Program and from the US Environmental Protection Agency's Great Lakes Restoration Initiative. The University of Minnesota Duluth Small Seed Research Grant provided additional funding.

References

Arnold TL, Sullivan DJ, Harris MA, Fitzpatrick FA, Scudder BC, Ruhl PM, Hanchar DW, Stewart JS (1999) Environmental setting of the upper Illinois River basin and implications for water quality. US Department of the Interior and US Geological Survey National Water-Quality Assessment Program Water-Resources Investigations Report 98-4268

Burner CJ, Moore HL (1953) Attempts to Guide Small Fish with Underwater Sound. US Department of the Interior Special Scientific Report: Fisheries No. 111. US Department of the Interior

Clarkson RW (2004) Effectiveness of electrical fish barriers associated with the Central Arizona Project. N Am J Fish Manag 24(1):94–105

Fay RR, Popper AN (1999) The auditory periphery in fishes. In: Fay RR, Popper AN (eds) Comparative hearing: fish and amphibians. Springer, New York, pp 43–100

Holt DE, Johnston CE (2014) Evidence of the Lombard effect in fishes. Behav Ecol 25(4):819–826

Irons KS, Sass GG, McClelland MA, Stafford JD (2007) Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, USA—is this evidence for competition and reduced fitness? J Fish Biol 71:258–273

Kelly AM, Engle CR, Armstrong ML, Freeze M, Mitchell AJ (2011) History of introductions and governmental involvement in promoting the use of grass, silver, and bighead carps. In: Chapman DC, Hoff MH (eds) Invasive Asian Carps in North America. American Fisheries Society, Symposium, 74. Bethesda, MD, pp 163–174

Kolar KS, Chapman DC, Courtenay WR Jr, Housel CM, Williams JD, Jennings DP (2005) Asian carps of the genus Hypophthalmichthys (Pisces, Cyprinidae)—a biological synopsis and environmental risk assessment. US Fish and Wildlife Service

Liu M, Wei QW, Du H, Fu ZY, Chen QC (2013) Ship noise-induced temporary hearing threshold shift in the Chinese sucker *Myxocyprinus asiaticus* (Bleeker, 1864). J Appl Ichthyol 29:1416–1422

Lovell JM, Findlay MM, Nedwell JR, Pegg MA (2006) The hearing abilities of the silver carp (*Hypopthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). Comp Biochem Physiol A: Mol Integr Physiol 143(3):286–291

Maes J, Turnpenny AWH, Lambert DR, Nedwell JR, Parmentier A, Ollevier F (2004) Field evaluation of a sound system to reduce estuarine fish intake rates at a power plant cooling water inlet. J Fish Biol 64(4):938–946

Moy PB, Polls I, Dettmers JM (2011) The Chicago sanitary and ship canal aquatic nuisance species dispersal barrier. In: Chapman DC, Hoff MH (eds) Invasive Asian carps in North America. American Fisheries Society Special Publication 74. Bethesda, MD, pp. 121–137

Murphy EA, Jackson PR (2013) Hydraulic and water-quality data collection for the investigation of Great Lakes tributaries for Asian carp spawning and egg-transport suitability: US Geological Survey Scientific Investigations Report 2013-5106. http://pubs.usgs.gov/sir/2013/5106/. Accessed 11 Nov 2014

Noatch MR, Suski CD (2012) Non-physical barriers to deter fish movements. Environ Rev 20:71–82

Pegg MA, Chick JH (2004) Aquatic nuisance species: an evaluation of barriers for preventing the spread of bighead and silver carp to the Great Lakes. Final report for the Illinois-Indiana Sea Grant A/SE (ANS)-01–01. Illinois-Indiana Sea Grant, Urbana, IL

Popper AN, Carlson TJ (1998) Application of sound and other stimuli to control fish behavior. Trans Am Fish Soc 127:673–707

Popper AN, Hastings MC (2009) The effects of human-generated sound on fish integrative. Zoology 4:43–52

Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, Coppola G, Geyer MA, Glanzman DL, Marsland S, McSweeney FK, Wilson DA, Wu C, Thompson RF (2008) Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem 92:135–138

Ross QE, Dunning DJ, Thorne R, Menezes JK, Tiller GW, Watson JK (1993) Response of alewives to high-frequency sound at a power plant intake on Lake Ontario. N Am J Fish Manag 13(2):291–303. doi:10.1577/1548-8675(1993)013<0291:ROATHF>2.3.CO;2

Ruebush BC, Sass GG, Chick JH, Stafford JD (2012) In-situ tests of sound-bubble-strobe light barrier technologies to prevent range expansions of Asian carp. Aquat Invasions 7(1):37–48

Sampson SJ, Chick JH, Pegg MA (2009) Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi rivers. Biol Invasions 11:483–496

Sass GG, Cook TR, Irons KS, McClelland MA, Michaels NN, O'Hara ETM, Stroub MR (2010) A mark-recapture population estimate for invasive silver carp (*Hypophthalmichthys molitrix*) in the La Grange Reach, Illinois River. Biol Invasions 12:433–436

Schilt CR (2007) Developing fish passage and protection at hydropower dams. Appl Anim Behav Sci 104(4):295–325

Scholik AR, Yan HY (2001) Effects of underwater noise on auditory sensitivity of a cyprinid fish. Hear Res 152:17–24

Scholik AR, Yan HY (2002a) The effects of noise on the auditory sensitivity of the bluegill sunfish, *Lepomis macrochirus*. Comp Biochem Physiol A 133:43–52

Scholik AR, Yan HY (2002b) Effects of boat engine noise on the auditory sensitivity of the fathead minnow, *Pimephales promelas*. Environ Biol Fishes 63:203–209

Schrank SJ, Guy CS, Fairchild JF (2003) Competitive interactions between age-0 bighead carp and paddlefish. Trans Am Fish Soc 132:1222–1228

Sloan JL, Mensinger AF (2013) Acoustical conditioning and retention in the common carp (*Cyprinus carpio*). J Great Lakes Res 39:507–512

Taylor RM, Pegg MA, Chick JH (2005) Response of bighead carp to a bioacoustic behavioural fish guidance system. Fish Manag Ecol 12:283–286

Thompson RF, Spencer WA (1966) Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol Rev 73:16–43

Voellmy IK, Purser J, Flynn D, Kennedy P, Simpson SD, Radford AN (2014) Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim Behav 89:191–198

Whitfield AK, Becker A (2014) Impacts of recreational motorboats on fishes: a review. Mar Pollut Bull 83:24–31

Willis DJ, Hoyer MV, Canfield DE, Lindberg WJ (2002) Training grass carp to respond to sound for potential lake management uses. N Am J Fish Manag 22:208–212

Zielinski DP, Voller VR, Svendsen JC, Hondzo M, Mensinger AF, Sorensen P (2014) Laboratory experiments demonstrate that bubble curtains can effectively inhibit movement of common carp. Ecol Eng 67:95–103