

**Bioacoustic Deterrence of Invasive Bigheaded Carp**

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## **Dedication**

This thesis is dedicated to my wonderful parents, Michael and Marsha Murchy, for without their support and love none of this would have been possible.

## Abstract

Since their accidental release into the Mississippi River 30 years ago, invasive bighead and silver carp (*Hypophthalmichthys noblis* and *H. molitrix*) comprise a large percent of the biomass in many watersheds throughout the Midwest and Southern regions of the United States. These filter feeding fish, with no natural predators as adults in North America, threaten native species. Local, state, and federal agencies are working to prevent further migration of these invasive species, especially into Lake Michigan, which could open the entire Laurentian Great Lakes system to invasion. One solution currently undergoing evaluation is an acoustic barrier. Controlled experiments in restricted access outdoor ponds outfitted with a cinder-block barrier evaluated the effectiveness of a broadband sound stimulus on fish movement. An acoustic barrier broadcasting a broadband sound was found to be >90% effective in restricting bighead and silver carp movements in a controlled setting. I also tested the impact of this sound on native species which is critical prior to field deployments. The swimming behaviors of native ostariophysians (fathead minnow *Pimephales promelas*, channel catfish *Ictalurus punctatus*, bigmouth buffalo *Ictiobus cyprinellus*), invasive ostariophysians (bighead carp, silver carp, common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella*), and native non-ostariophysians (paddlefish *Polyodon spathula*, lake sturgeon *Acipenser fulvescens*, gizzard shad *Dorosoma cepedianum*, walleye *Sander vitreus*, bluegill *Lepomis macrochirus*, rainbow trout *Oncorhynchus mykiss*) were monitored in response to broadband sound. Non-bigheaded carp react less to broadband sound and native species do not appear to be impacted by acoustic deterrents using broadband sound.

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## **Foreword**

This master's thesis is submitted as partial fulfillment of the degree of Master of Science in Integrated Biosciences at the University of Minnesota. Chapter one includes a review of literature on the evolution of hearing in fish. Chapters two and three are manuscripts prepared for publication in Fisheries Management and Ecology and Journal of Fish Biology. Coauthors for Chapter two include Aaron R. Cupp, Jon J. Amberg, Brooke J. Vetter, Kim T. Fredricks, Mark P. Gaikowski, and Allen F. Mensinger. Coauthors for Chapter three include Brooke J. Vetter, Marybeth Brey, Jon J. Amberg, Mark P. Gaikowski, Allen F. Mensinger.

## **Chapter One: Evolution of Fish Hearing**

Fish, like other vertebrates, need to differentiate relevant acoustical cues, like intraspecific communication, from insignificant sounds in their aquatic environments. Both abiotic (e.g. waves, current) and biotic (e.g. communication, prey, predators) factors can generate sound stimuli in an ecosystem and each factor has an important role in developing hearing abilities in fish (Schulz-Mirbach and Ladich 2016). Sound travels over 4 times faster in water compared to air. This is due to the greater density of water compared to air, making water a more efficient sound medium. To detect relevant acoustical signals in their environment, fish require specialized structures, like Weberian ossicles. Along with these specialized connections, some other hearing structures, like the inner ear, in fish have been evolutionarily conserved.

Similar to other vertebrates, fish have a bilateral pair of internal ears located on either side of the cranium. The inner ear consists of three semicircular canals with an associated chamber (sacculle, utricle and lagena) that contains an otolith and sensory hair cells (Popper and Lu 2000). Hair cells are elongated epithelial cells with an anteriorly located ciliary bundle that aids in hearing. Recent studies in goldfish (*Carassius auratus*) suggest, the length of the ciliary bundle allows different frequencies to be detected. Long ciliary bundles respond to low frequencies and short ciliary bundles respond to high frequencies (Smith *et al.* 2011). In fish, sound is detected when differential amplitude and phase motion cause the otolith to shift with respect to its canal, mechanically bending

some of the cilia and stimulating a neural transmission to the auditory portion of the brain (Moyle and Cech 2004).

Compared to low frequency sounds (100-500 Hz), high frequency stimuli (>1000 Hz) requires specialized structures to move the otolith and bend the cilia (Moyle and Cech 2004). The main structure responsible for heightened hearing abilities are Weberian ossicles. Weberian ossicles are a double chain of ossicles that join the swim bladder to the inner ear. The curved posterior region is in contact with the swim bladder and the anterior portion is connected to the concha scaphii by interossicular ligaments. This configuration allows changes in volume of the swim bladder to be transmitted to the inner ear (Diogo 2009), thus enhancing the detection of sound because the swim bladder acts as a large drum.

Once the Weberian ossicles connect the inner ear to the swim bladder, the swim bladder becomes important for enhancing the detection of high frequencies which might not otherwise be poorly detected by the ciliary bundles. Though the main function of the swim bladder is to control buoyancy, some fish are able to use their swim bladder to transfer acoustical energy from the swim bladder to the inner ear. This happens because gas inside the swim bladder is more compressible and the surface pulsates when it is exposed to sound (Moyle and Cech 2004).

The size and shape of the swim bladder is therefore important for high frequency hearing. Some fish species have a modified swim bladder that extends anteriorly into the inner ear and these species are sensitive to high frequency sounds. The Mojarra (*Eucinostomus argenteus*) is a fish that has a specialized swim bladder, which runs the

entire length of their body and divides into two horns that are connected to the auditory bulla. Mojarra have a frequency range of 100 Hz to 1800 Hz. They are less sensitive to sounds compared to fish species that have Weberian ossicles, but they validate that the swim bladder contributes to hearing in fish (Parmentier *et al.* 2011).

The inner ear anatomy, swim bladder and Weberian ossicles provide fish with the ability to detect high frequency sounds in their aquatic environments. The conditions under which these structures evolved are not entirely clear, due to the large variety of fish that can detect high frequency sounds. Fish represent the largest group of vertebrates with the most diversity of auditory structures, so understanding the evolution of fish can enhance the understanding of evolution of hearing in other vertebrates.

### **Evolutionary development of high frequency hearing**

All vertebrates that evolved hearing have two key tasks in common, the need to determine relevant sound sources in their environment and localize the sound source. These natural selection pressures have shaped and maintained the sense of hearing throughout vertebrate evolution (Fay and Popper 2000). From this, there are two logical reasons why hearing evolved in fish: a byproduct of acoustic communication or environmental conditions.

#### ***Acoustic Communication***

Many different species of fish use acoustic cues for intraspecific communication, like swim bladder drumming or knocking sounds. One study was conducted using red eye bass (*Micropterus coosae*) and Alabama bass (*Micropterus henshalli*) to test if their hearing range matched with the frequencies emitted by males during aggressive

interactions (Holt and Johnston 2011b). Holt and Johnston (2011b) discovered that *M. henshalli* and *M. coosae* have the highest sensitivity to low frequency sounds, specifically below 300 Hz. This was expected, since the fundamental frequency of their intraspecific calls is about 170 Hz. However, for *M. henshalli* and *M. coosae*, their audiograms had a stronger linear shape compared to normal audiograms that have a U shape (higher thresholds at upper and lower hearing limits). Holt and Johnston (2011b) believe this difference can be explained through the lateral line system that has sensitivity between 100 and 200 Hz for *M. henshalli* and *M. coosae*. Since the lateral line system was not excluded in this study, intraspecific communication of these fish is believed to be detected by the lateral line (Holt and Johnston 2011b). These findings suggest that the hearing specialization is not a byproduct of acoustic communication, because there are other structures, like the lateral line, that can also detect intraspecific communication.

There are also phylogenetic studies that support the idea that specialized structures, like Weberian ossicles, evolved before sound generating mechanisms. Studies conducted on ostariophysan species, fish with Weberian ossicles, showed very similar auditory sensitivity between vocal and non-vocal ostariophysans. These conclusions lead researchers to believe that high frequency hearing structures, like Weberian ossicles, evolved earlier than structures used to produce sound since all species have similar hearing ranges. This conclusion demonstrates further that high frequency hearing is not the result of acoustic communication. A phylogenetic analysis also demonstrated that Weberian ossicles is a feature found in all ostariophysans, while acoustic communication evolved independently in a few Cypriniformes and in most Characiphysi (Ladich 1999).

All of this evidence strongly suggests that acoustic communication was not the driving force behind the evolution of hearing in fish.

### ***Environmental Conditions***

The more likely mechanism for the evolution of hearing in fish is due to environmental conditions. When there is high ambient noise in an environment, signal detection is low, while in low ambient noise conditions, like lakes and shallow waters, signal detection would be high (Tavolga 1967). This idea was tested using common carp (*Cyprinus carpio*) and European perch (*Perca fluviatilis*). The hearing abilities of both fish were tested using auditory evoked potential (AEP) recordings during a base line test and four different ambient noise levels. The results disclosed that the carp had excellent hearing abilities in the quiet habitats (100-4000 Hz); yet, in conditions with large amounts of ambient noise, the carps high frequency hearing was masked (100-1000 Hz). This was compared to the perch, whose hearing ability remained between 100-1000 Hz in all environments. This shows that specialized hearing abilities would have evolved in quiet habitats rather than in loud habitats (Amoser and Ladich 2005).

Studies have also shown that high ambient noise can lead to high frequency hearing loss in fish. In the family Amblyopsidae, there are both surface dwelling species and cave dwelling species. By using these two different species from the same family, researchers were able to explore the effects of ambient noise on the hearing of fish. They tested the auditory thresholds and hair cell histology of one surface dwelling amblyopsids (*Forbesichthys agassizii*) and two cave dwelling ablyopsids (*Typhlichthys subterraneus* and *Amblyopsis spelaea*). The results demonstrated that the cave amblyopsids had similar

hearing abilities to their surface dwelling kin at 800 Hz and lower. However, neither cave dwelling species could hear above 800 Hz, compared to the surface dwelling species which could hear up to 2 kHz (Niemiller *et al.* 2013). Furthermore, the cave dwelling species had a decrease in their hair cell densities compared to the surface dwelling species. Niemiller *et al.* (2013) also examined the sound profiles in the cave and the surface habitats and discovered a high peak in background noise at 1000 Hz with higher noise levels between 1000-3000 Hz in the cave environment compared to the surface habitats. This significant decrease in hearing abilities of cave amblyopsids is an excellent example of regressive evolution. These findings illustrate that cave amblyopsids could have lost their high frequency hearing due to the extremely noisy environment they live in (Niemiller *et al.* 2013). These studies are strong indicators that hearing has evolved in fish due to environmental conditions.

### **Adaptive advantage to high frequency hearing**

High frequency hearing most likely evolved under quiet environmental conditions. However, the reason why high frequency hearing would be adaptive to specific species of fish is unknown. Increased hearing range and sensitivity to acoustic stimuli could increase the chance of finding food or decrease the risk of predation, or both. Either way, high frequency hearing could increase the survival of the individual or population compared to similar species that do not have this ability.

### ***Prey Detection***

In omnivorous fish, like piranhas, the ability to hear noises in their environment could aid in prey localization by being able to detect prey movements. Piranhas will

attack noisy prey more often than it will attack prey that is silent (Reviewed in: Holt and Johnston 2011a). This suggests that hearing could be adaptive in finding food when the prey is not stationary.

Holt and Johnston (2011a) reinforced this idea by playing white noise and a rock shuffling noise, to mimic an invertebrate moving in the water, to cyprinid fish. This was used to test whether the fish localized the prey by the noise. They found that fish were attracted to naturally occurring noises over the white noise. The results of this study conclude that increased hearing range and sensitivity could be adaptive to detect prey and increase fitness, because these fish species are better adapted at finding and catching prey.

### ***Predator detection***

Hearing could also be an adaptation in fish to detect predators. Clupeidae fishes, like herrings and shads, are one prey option for echolocating dolphins and porpoises. In a study conducted on American shad (*Alosa sapidissima*), pure tone ultrasound stimuli was used to determine if fish could detect and respond to echolocation clicks. American shad can detect ultrasound signals in the frequency range of echolocation beams and have a directional, fast, and evasive behavior. This behavior occurs when the fish detects continuous echolocation frequencies (below 60 kHz), then the fish turns slowly away from the signal, but if they detect dolphin clicks (70-110 kHz), they form a compact group to decrease the chance of the dolphin discriminating individual fish. These findings suggest that American shad have evolved a mechanism to detect predators and make them less preyed upon, increasing their survival in the wild (Plachta and Popper 2003).

The Gulf toadfish (*Opsanus beta*) can also detect predators acoustically. Gulf toadfish live in seagrass beds and males emit ‘boat whistles’ to attract mates and compete with rival males. The Gulf toadfish are also a desired prey of bottlenose dolphins (*Tursiops truncatus*). It would be a risk for the toadfish to produce calls while dolphins are around, especially because their calls average 126 dB re 1 mPa (Barimo and Fine 1998). To avoid detection, toadfish evolved a way to detect the low frequency pops emitted by foraging dolphins. Since these pops are at a frequency similar to other non-predatory animals, toadfish use a combination of the frequency, repetition rate, and pulse duration to distinguish dolphin pops from harmless background noise (Remage-Healey *et al.* 2006). Since toadfish use sound to find a mate but do not want to be vocalizing when predators are around, this ability to detect dolphins is an evolutionary tradeoff between increasing fitness and risk of predation. Predator and/or prey detection are two possible reasons why hearing specialization are adaptive for fish, and they both have supportive evidence.

### **Sensory physiology application**

Research into fish hearing evolution has come a long way and now the knowledge about differences in hearing sensitivity among species of fish provides a novel approach to application of sensory physiology. One of the primary high frequency hearing species used in scientific research is the invasive common carp (*Cyprinus carpio*). Recently, two close-relatives to the common carp are becoming a concern for the United States: silver and bighead carp (hereafter, bigheaded carp).

Bigheaded carp are invasive species accidentally released into ecosystems in the United States around the 1980's. Both of these species belong to a group of fish known as ostariophysans, which have heightened hearing abilities due to Weberian ossicles, which increase their hearing range and sensitivity. Bigheaded carp have a hearing range of 100 Hz - 3000 Hz (Lovell *et al.* 2006) unlike native non-ostariophysan species like paddlefish (*Polyodon spathula*) and lake sturgeon (*Acipenser fulvescens*) which can only hear between 100 Hz and 500 Hz (Lovell *et al.* 2005). Not only do bigheaded carp have a wider range, but they can hear the same frequencies as native species at lower intensities (110 dB compared to 130-140 dB). This increase in hearing range and sensitivity allows researchers to use high frequency sound to modify swimming behavior of bigheaded carp, which is within the hearing range of bigheaded carp, but is well above most native species (Lovell *et al.* 2006).

Bigheaded carp are native to larger rivers and lakes associated with flood plains along the east coast of China and Siberia, which are quiet environments where keen hearing is expected to evolve (Kolar *et al.* 2007). Both species also have an adaptive advantage to high frequency hearing. The Baiji, Yangtze River Dolphin (*Lipotes vexillifer*) is a natural predator of carp species native to the east coast of China (Klinowska 1991). Also the Orinoco Dolphins (*Inia geoffrensis humboldtiana*) consume live carp in captivity at the Duisburg Zoo (Sylvestre 1985). Since carp have natural predators that locate them through echolocation, high frequency hearing could be adaptive in knowing when dolphins are in the area.

The invasions of these species are major threats to aquatic ecosystems in the United States. Bigheaded carp are filter feeders that consume phytoplankton and are out competing native gizzard shad (*Dorosoma cepedianum*) (Sampson *et al.* 2009a), paddlefish (*Polyodon spathula*) (Schrank *et al.* 2003) and bigmouth buffalo (*Ictiobus cyprinellus*) (Irons *et al.* 2007) populations. The continued spread of bigheaded carp throughout the Mississippi River watershed led to the creation of barriers to prevent them from invading new habitats, such as the Laurentian Great Lakes system.

Silver carp exhibit a unique behavioral response to acoustic stimuli, propelling themselves out of the water when motorized watercrafts are in the area. Once a motorized boat is in close proximity to a silver carp, the carp produce a distinct response by swimming fast near the surface and jumping vertically out of the water and sometimes into the boat (Berg 1962; Kolar *et al.* 2007). This jumping behavior is very energetically expensive and can cause potential injury to themselves or humans (Kolar *et al.* 2007).

The sensory biology and unique jumping behavior of bigheaded carp, specifically silver carp, presents researchers with an interesting question: how does an acoustic stimulus in the environment modify the swimming behavior of fish? Previous studies have been conducted to assess the effectiveness of acoustical stimuli at altering behavior in silver and bighead carp (Vetter *et al.* 2015; Vetter *et al.* in preparation). However, further evaluation on these species and other fish species is necessary to fully understand application of acoustics to modify swimming behavior in fish.

## **Chapter Two: Potential implications of acoustic stimuli as a non-physical barrier to silver (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*)**

### **Introduction**

Silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844) and bighead carp (*H. nobilis* Richardson, 1845) (hereafter, bigheaded carps) were originally imported to the southern United States in the 1970's from eastern Asia to control algal growth in sewage treatment and fish farming facilities (Kolar *et al.* 2007). After heavy flooding, their subsequent escape into the wild resulted in detrimental environmental effects. The species' filter feeding ability, fast growth, and prolific spawning has allowed them to impact native fishes such as paddlefish (*Polyodon spathula* Walbaum, 1792) (Schrank *et al.* 2003), gizzard shad (*Dorosoma cepedianum* Lesueur, 1818) (Sampson *et al.* 2009a) and bigmouth buffalo (*Ictiobus cyprinellus* Valenciennes, 1844) (Irons *et al.* 2007). Furthermore, the resulting decline of lower trophic level organisms and/or community shifts in zooplankton populations have likely affected additional native aquatic species (Xie and Chen 2001; Cooke *et al.* 2009).

Other than an electric barrier in the Chicago Sanitary and Ship Canal (near Lake Michigan), solid structure gravity dams (or high head dams) are currently the only barriers slowing the upstream expansion and potential colonization of the Laurentian Great Lakes by bigheaded carp (Sass *et al.* 2010; Moy *et al.* 2011). To limit their range expansion, especially into the Great Lakes, numerous states are evaluating the efficacy of non-physical barriers to deter invasive carp (Kelly *et al.* 2011). Non-physical barriers to

deter or control fish movement were originally developed to reduce movement into hydroelectric dams and/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, and/or electric fields (Popper and Carlson 1998; Noatch and Suski 2012). 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 and Suski 2012).

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, IL. The barrier was originally installed in 2002 to slow the downstream movement of the round goby (*Neogobius melanostomus* Pallas, 1814) from the Great Lakes to the Illinois River (Sparks *et al.* 2010; Moy *et al.* 2011), but later improvements to the barrier were made with the goal of blocking the northern expansion of bigheaded carp into Lake Michigan (Sparks *et al.* 2010). The electric field targets the neuromuscular junctions, causing temporary paralysis or death and can block upstream fish movement. Although moderately effective, electrical barriers are costly, need continual power, present danger to non-target species including humans, may be ineffective against small fish, and can be disrupted by metal-hulled barges (Dettmers *et al.* 2005; Moy *et al.* 2011; Noatch and Suski 2012; Parker *et al.* 2015). Additionally, during times of power disruption or maintenance, alternative systems are necessary to block movement (Clarkson 2004). These shortcomings preclude electric barrier insertion into many waterways.

Other studies have evaluated non-physical barriers, such as light (Hamel *et al.* 2008), sound (Taylor *et al.* 2005; Vetter *et al.* 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 and Carlson 1998; Welton *et al.* 2002). For example, Atlantic menhaden (*Brevoortia tyrannus* Latrobe, 1802), spot (*Leiostomus xanthurus* Lacepède, 1802), and white perch (*Morone americana* Gmelin, 1789) demonstrated increased avoidance to strobe lights combined with bubbles compared to either stimulus alone (McIninch and Hocutt 1987). Patrick *et al.* (1985) found that strobe lighting was more effective in deterring alewife (*Alosa pseudoharengus* Wilson, 1811), smelt (*Osmerus mordax* Mitchill, 1814), and gizzard shad than constant illumination; however, a combined strobe light/bubble barrier maximized avoidance behavior. Finally, bubble curtain barriers that generate 200 Hz frequency sound can reduce common carp crossing attempts (*Cyprinus carpio* Linnaeus, 1758) (Zielinski *et al.* 2014).

In past studies, acoustic stimuli have been used to deter fish from approaching power plants or hydropower dams (Burner and Moore 1962; Schilt 2007). Frequencies ranging from 20 – 600 Hz were effective (60% repels) in preventing fish from approaching a power plant (Maes *et al.* 2004) and ultrasound deterred (87% repels) alewives from entering a dam intake (Ross *et al.* 1993). More recently, sound is being examined as a barrier to invasive fish movement (Popper and Carlson 1998; Lovell *et al.* 2005; Noatch and Suski 2012; Vetter *et al.* 2015).

To use sound as a non-physical barrier, silver and bighead carp need to be able to perceive the sound, localize where it is coming from, and alter their behavior to avoid the

sound. Grass carp (*Ctenopharyngodon idella* Valenciennes, 1844; 600-1000 Hz) (Willis *et al.* 2002) and common carp (400 Hz) (Sloan *et al.* 2013) were classically conditioned using sound for feeding, which suggests that close relatives of bigheaded carp can localize sound. Silver and bighead carp have demonstrated the ability to detect and alter their behavior due to sound. Pegg and Chick (2004) found that sound stimuli between 20 and 2000 Hz was more effective at preventing bigheaded carp from crossing an electric/sound barrier compared to frequencies between 20 and 500 Hz. Also, a combination of sound (20-2000 Hz) and bubbles successfully repelled 95% of bighead carp in a shallow, narrow raceway (Taylor *et al.* 2005). Vetter *et al.* (2015) showed that complex or broadband sounds (60 Hz to 10 kHz) were more effective than pure tones (500 to 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 but was unable to monitor the fish movements at the barrier (Ruebush *et al.* 2012).

The goal of the present study was to examine if a complex, broadband sound (60 Hz to 10 kHz) could block the movement of silver and bighead carp through a barrier, so that the potential for field application, specifically in locks, could be assessed.

## **Material and Methods**

### *Animal Husbandry*

All experiments were conducted at the United States Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI. Silver carp or bighead carp (18 - 24 cm TL) were maintained in 1500 L flow through indoor rearing tanks 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). Each experimental fish was tagged with a passive integrated transponder (PIT) tag (Biomark Inc, Boise, ID) at least one week prior to experimentation. Prior to tagging, fish were sedated with 100 mg/L AQUIS® 20E (10 mg/L eugenol AQUIS New Zealand Ltd., Lower Hutt, New Zealand) in the rearing tank. Fish were hand netted and placed in 300 mg/L AQUIS® 20E (30 mg/L eugenol) until the fish lost equilibrium and did not respond to a caudal peduncle pinch. A 1% iodine solution was applied to the injection sites, and a passive integrated transponder (PIT) tag 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 AQUIS® 20E (5 mg/L eugenol) to minimize jumping, stress, and potential injury. Food was withheld for 24 h prior to transport, and fish were not fed while in the outdoor ponds (< 7 days) to avoid food conditioning. Each group (N=10) was allowed to acclimate in the pond for at least 48 hours prior to the initiation of experiments. 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 x 5 m x 1.2 m (60 kL) outdoor concrete flow-through pond (Fig. 1). Water was pumped into the ponds directly from UMESC wells and flow rate was adjusted to maintain a temperature range of 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.

Two concrete-block barriers (0.4 x 2 x 1.2 m) 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 barriers 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 (Fig.1). The ponds were located outdoors, and to maintain water temperature within the previous noted experimental window, trials could only be conducted in July and August.

#### *Sound stimuli*

Sound was delivered via one of two pairs of underwater speakers (UW-30, Lubell Labs Inc., Whitehall, OH) that were placed 1 m from each end of the barrier opening, approximately 2 m from the nearest side-wall (Fig. 1). One HTI-96-MIN (High Tech Inc., Long Beach, MS) hydrophone was placed in the middle of each end of the pond, 2 m from the end wall, to monitor the sound stimulus, which was recorded using a PowerLab 4SP data acquisition system and LabChart 7 software (AD Instruments, Colorado Springs, CO). Acoustic stimuli consisted of a 30 second broadband sound recorded underwater using a stationary HTI hydrophone from a moving 6 m aluminum boat equipped with a 100 Hp Honda 4-stroke outboard motor 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 Hz to 10 kHz with maximal energy contained in two broad peaks with the first between 500 to 2000 Hz and the second peaking at 7.5 kHz (Fig. 2).

An UMA-752 amplifier (Peavey Electronics, Meridian, MS) regulated sound intensity and each speaker pair was controlled manually with a switchbox (MCM Electronics, Centerville, OH). The acoustic properties of the speakers and pond were mapped using the HTI hydrophone at 60 points evenly distributed throughout the experimental pond. Sound pressure levels for both ambient and broadband sound was collected at each site and the ambient noise was subtracted from the maximum broadband sound to determine the sound pressure levels. Sound pressure levels (SPL) were calculated by measuring the root mean square (rms) voltage and converting to SPL in dB re 1 $\mu$ Pa @ 1m using Avisoft-SASLab Pro version 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.

### *Behavioral Experiments*

Behavior 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). The cameras continuously monitored the fish and provided full coverage of the pond.

### *Experimental Design*

One trial consisted of five consecutive periods: 1) pre-sound (120 minutes); 2) sound playback 1 (30 minutes); 3) inter-sound (60-270 minutes); 4) sound playback 2 (30 minutes); and 5) post-sound (120 minutes). During the pre-, inter-, and post-sound periods, fish were free to swim throughout the pond and the speakers were inactive.

During the two experimental periods, the speaker pair on the side of the barrier opposite to the fish was activated whenever the fish entered the “reaction zone,” or the area within the rectangle formed by the four speakers which measured approximately 2 m<sup>2</sup> on each side of the barrier (Fig. 1). The sound was terminated 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. All fish demonstrated schooling behavior, 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 center of the school.

Swimming behavior was monitored during the pre-sound, inter-sound, and post-sound intervals and the 30 minute sound playback periods with fish position recorded every 5 seconds. The number of attempted and successful crossings, and residence time in each side were documented for each trial. The fish position in the reaction zone when sound was first initiated was monitored and swim speed in the meter preceding the reaction zone was quantified. Reaction time was defined as the time from sound onset to when the leading edge of the school exited the reaction zone during successful repels.

Silver 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 and bighead carp equally mixed schools (N=20) were tested. Each school was tested between four and six times (variation due to weather curtailing later trials) with the overall number of tests 16, 11, and 10 for silver, bighead carp and mixed schools, respectfully.

The initial location (i.e. north vs. south) of fish was randomly chosen at the beginning of each trial 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.

### *Data Analyses*

All video and data analysis was 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. 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 enter 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 two to three days.

Barrier crossings per minute, percent successful repels, residence time, and time to exit reaction zone were tested for normality using Shapiro-Wilk tests. The data was not 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 quartile is reported using the following format (median; 1<sup>st</sup> quartile, 3<sup>rd</sup> quartile).

## Results:

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. 1: A), crossing the barrier approximately every three to five minutes. However, when confronted with sound after entering the reaction zone, the majority of schools turned away and did not cross the barrier (Fig. 1: B). Fish maintained school formation through sound playbacks with only one instance of a 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 of the active speakers and reaching a maximum level of 155 SPL  $\text{dB}_{\text{rms}}$  re:  $1\mu\text{Pa}$  @ 1m. The sound stimulus projected through the barrier and reached 146 SPL  $\text{dB}_{\text{rms}}$  re:  $1\mu\text{Pa}$  @ 1m at the barrier midpoint then attenuated throughout the other half of the pond (Fig. 1: C). SPL levels were asymmetrical in each pond half and during sound trials fish had a tendency to remain in the area of lowest sound pressure (i.e. northeastern edge in figure 1: C).

Silver carp averaged significantly (Kruskal-Wallis,  $p = 0.002$ ) fewer attempted crossings per minute during each of the five periods (0.16 median; 0.10 1<sup>st</sup> quartile, 0.23 3<sup>rd</sup> quartile) compared to bighead carp (0.26; 0.11, 0.48) 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,  $p = 0.11$ ) (Fig. 3).

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,  $p < 0.001$ ) from 0.16 (0.10, 0.21) to 0.02 (0.00, 0.07) crossings per minute (Fig. 3). Bighead carp showed a significant decline (Mann-Whitney,  $p < 0.001$ ) from 0.22 (0.13, 0.42) to 0.00 (0.00, 0.02) crossings per minute. The mixed schools also were significantly inhibited from crossing (Mann-Whitney,  $p < 0.001$ ), showing a reduction from 0.32 (0.20, 0.44) to 0.03 (0.00, 0.07) crossings per minute (Fig. 4).

Sound playbacks were successful in stopping fish transiting through the barrier in all three groups with 82.4%, 93.7% and 92.2% repulsion rates for the combined trials of silver carp, bighead carp, and mixed species, respectively (Fig. 5). The initial sound playback for each group of silver carp was the most successful with sound stopping the fish during all 13 attempts (Table 1). Success rates dropped slightly during subsequent playbacks before rebounding to 91% during sound playback 5 (Fig. 6). Bighead carp were less likely to cross the sound barrier with four of the six sound playbacks achieving 100% repulsion and 89 out of 95 attempts repulsed. The mixed school also displayed sound avoidance behavior with high (> 90%) repulsion rates observed until the last playback.

The time spent in each half of the pond during the 120 minute pre-sound interval was not significantly different for either the silver or bighead carp (Fig. 7). Silver carp averaged slightly more time in the north end (4380 s; 3674 s, 4869 s) than south (2796 s; 2399 s, 3571 s); however, the results were not significantly different (Mann Whitney,

$p=0.12$ ). In contrast, bighead carp spent more time in the south (4483 s; 1503 s, 5353 s) than in the north end (2716 s; 2104 s, 5771 s); however, there was no significant difference (Mann Whitney,  $p = 0.94$ ). The mixed schools did show a decided preference (Mann Whitney,  $p < 0.001$ ) for the north end (5083 s; 3410, 6858) over the south end (2103 s; 1269 s, 2744 s). Following active sound periods, all fish favored the side furthest from the previously active speakers (i.e., they did not cross the barrier) during the inter- and post-sound interval. Silver carp resided significantly longer (64%, Mann Whitney,  $p = 0.014$ ) in the near side (5344 s; 3467 s, 9349 s) vs. the far side (2951 s; 2365 s, 4107 s). Bighead carp spent significantly more time (74%, Mann Whitney,  $p = 0.036$ ) away from the speakers (5462 s; 3367 s, 6514 s vs 1918 s; 344 s, 3826 s). The mixed school also spent the majority of the time (69%) in the near end (4671 s; 2085, 7467 s vs 2145 s; 498 s, 4831 s) although the difference was not significant (Mann Whitney,  $p = 0.085$ ).

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 s (3.0, 11.3) while bighead and mixed schools were significantly faster (Mann Whitney,  $p < 0.001$ ), with identical median times of 3.0 s (2.0, 4.0) (Fig. 8). As very few schools showed aversive behavior upon entering the reaction zone with the sound off, it was not possible to directly compare time to exit the zone with controls.

## **Discussion**

Playback of underwater noise recorded from motorboats was effective at deterring silver and bighead carp egress 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 carp 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 repulsion success rate remained high throughout multiple trials over several days.

Silver and bighead carp belong to the ostariophysians group of fish, who have relatively higher hearing sensitivity than non-ostariophysians fish, due to the presence of Weberian ossicles, and previous work has demonstrated that both carp species can detect frequencies up to at least 3 kHz (Lovell *et al.* 2006)(Lovell *et al.* 2006). Recent studies have established that silver (Vetter *et al.* 2015) and bighead carp (Vetter *et al.* submitted) showed significantly greater movement away from broadband (60 Hz to 10 kHz) sound stimuli compared to pure tones (500 to 2000 Hz). Therefore, the underwater recording of an outboard motor was used as the deterrent.

The sound pressure levels (145 to 155 dB re 1 $\mu$ Pa @ 1m) were well above the carp's reported hearing threshold, 104 dB re 1 $\mu$ Pa (Lovell *et al.* 2006). The carp 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 *et al.* 2004).

It was predicted that attempted crossings would decline over time because the fish would start to associate the barrier opening with the sound, however contrary to our original hypothesis; carp continuously challenged the sound barrier throughout the seven-

hour 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 favor 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 shadier side of the pond by the mixed schools during the pre-sound intervals. However, these tests were conducted during warmer days with minimal cloud cover, and the behavior was consistent with fish preference in shallow water for shaded areas (Gibson and Power 1975). As the initiation of sound playback was randomized, carp distribution during sound playback was found to be more dependent on sound origination than shade, indicating that even when carp favored a section of the pond, the sound barrier could override this preference.

Although the pond size provided sufficient opportunity for the carp 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. For example, 75% of the silvers, 85% of the bigheads and 75% of the mixed schools would continue through the barrier after entering the reaction zone during control trials in the absence of sound. However, the small reaction zone only provided a brief period to manually activate the sound before fish would transgress the barrier. Since fish swim speed fluctuated, the observer needed to visually confirm carp

location and manually activate the trigger, and 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. Additionally, the speakers were offset from the opening to reduce any impediment to swimming; therefore, the sound source was never greater than 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. We hypothesize that 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 seconds and bighead and mixed groups responded in 3 seconds. 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 additionally slow upstream swimming speeds (Jones 1963), resulting in greater exposure time to the sound barrier, which could result in higher repulsion rates.

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. Additionally, 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. However, the pond size did restrict the ability of the fish to travel more than 5 m from the sound source.

The results demonstrated consistent sound aversion, however longer observation periods could further refine the behavior, and address potential hearing damage and/or habituation to the acoustic stimuli. Variability was observed with the silver and mixed schools during later trials however weather curtailed several day 3 trials, resulting in lower sample numbers (see table). Future trials will examine fish reactions over a prolonged period to determine when and if habituation to sound will transpire.

The small sample size was necessitated by limiting the carp to a single concrete pond to avoid acoustic interference from concurrent trials. Temperature has been observed to effect carp swimming behavior (Murchy unpublished) and trials were limited to the period when ambient temperature was sufficient to maintain the outdoor ponds 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 showed lower responses to the sound than succeeding groups. Water temperature was warmer for the bighead 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 behavior.

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. Echoes were produced from interactions of the sound with the pond's bottom, side, end and barrier walls in addition to the water surface, creating a challenging acoustic

environment for the fish to localize the sound source. Also, wild fish would have the opportunity to leave the area in response to a sound stimulus; the experimental fish were constrained to a 25 m<sup>2</sup> area. Similar acoustic tests in the wild displaced silver carp greater than 200 meters from the sound (Mensing unpublished). The most effective deterrent locations may be in dam and lock structures with sound used to deter fish from entering the locks with vessel traffic. The ponds mimicked the lock chambers composition, although on a much smaller scale, and despite the study's limitation, demonstrated consistent and sustained repulsion of the bigheaded carp.

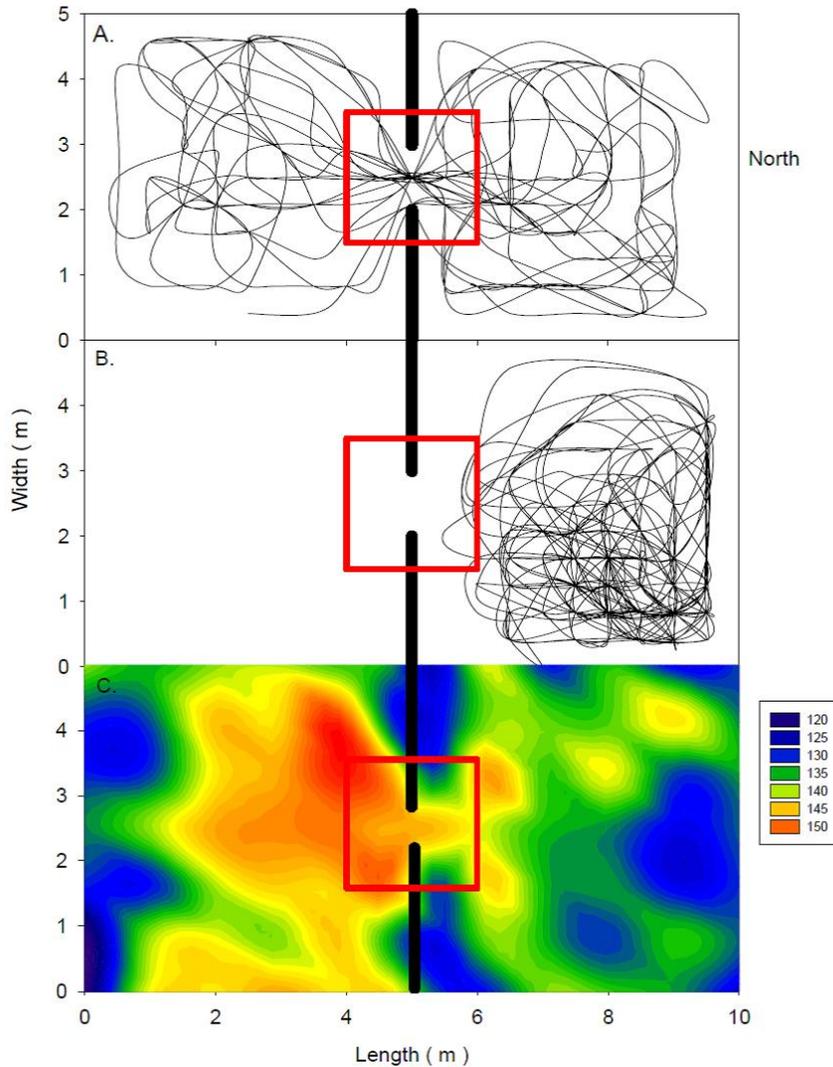
Sound barriers present advantages over other non-physical barriers. The speakers are relatively inexpensive and require a modest power supply compared to electrical barriers. Small backup generators or batteries could be used to power the speakers in the event of a power failure and the low cost could 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 by using acoustic stimuli above their hearing range (Lovell *et al.* 2006); however their effects on other ostariophysians remain to be determined.

The current experiments deployed only sound to mediate carp behavior and achieved relatively high success rates compared to multi-stimuli combination studies such as bubble and sound barrier (Zielinski *et al.* 2014), sound and electric barrier (Pegg and Chick 2004) and strobe light and bubble barrier (McIninch and Hocutt 1987). Repulsion rates approaching 100% are often the goal for invasive species prevention, however, the high repulsion rates noted in this study may be effective at creating choke

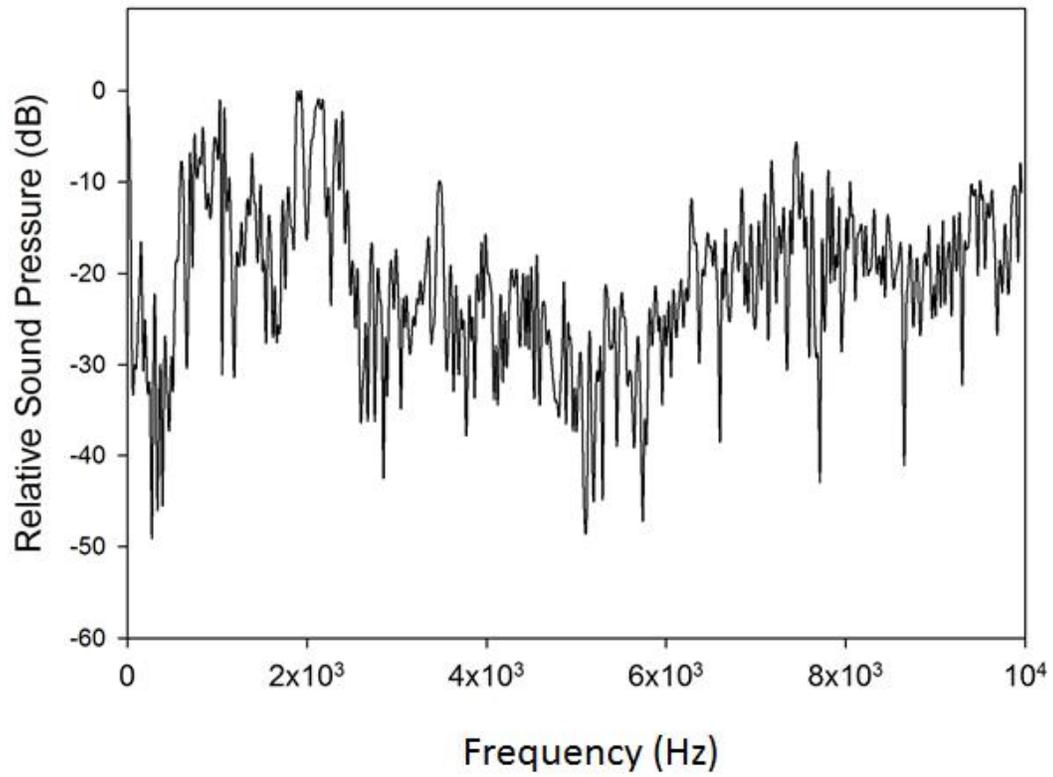
points at locks that would allow commercial fisherman to bring down the populations between points or broadband sound could be combined with other deterrent methods to increase effectiveness. It also remains unclear what specific sub set of this acoustical stimulus causes repulsion and further refinement of the broadband sound may lead to greater repulsion rates.

The results suggest that an acoustic deterrent could be an effective means to prevent upstream migration of both bighead 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 and silver carp toward a net or shore, clear fish out of a lock, as a part of a bubble and/or strobe light barrier in a river channel, or as backup system in an electric barrier, especially during routine maintenance. This study indicates that because bighead and silver carp are responsive to broadband sound they can be co-managed, and that acoustic stimuli may be an important management tool that could be effective either on its own or integrated with other deterrent technology.

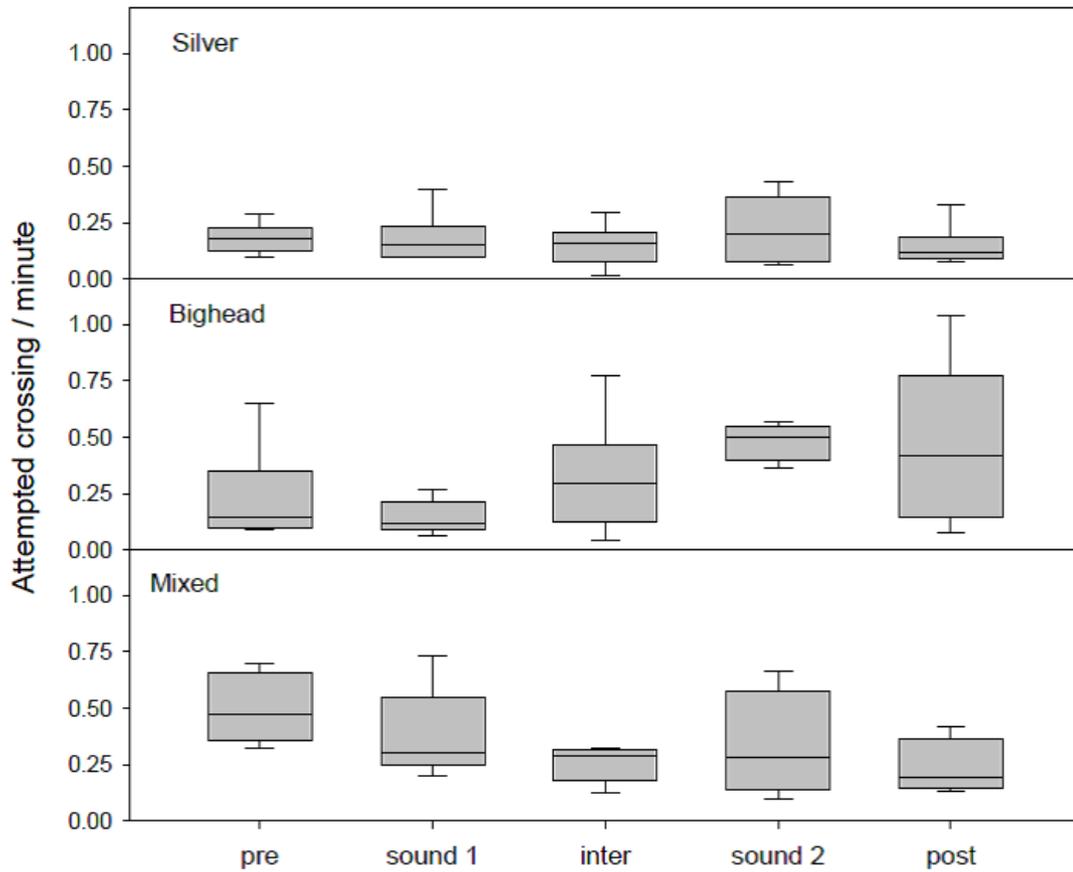
**Figures:**



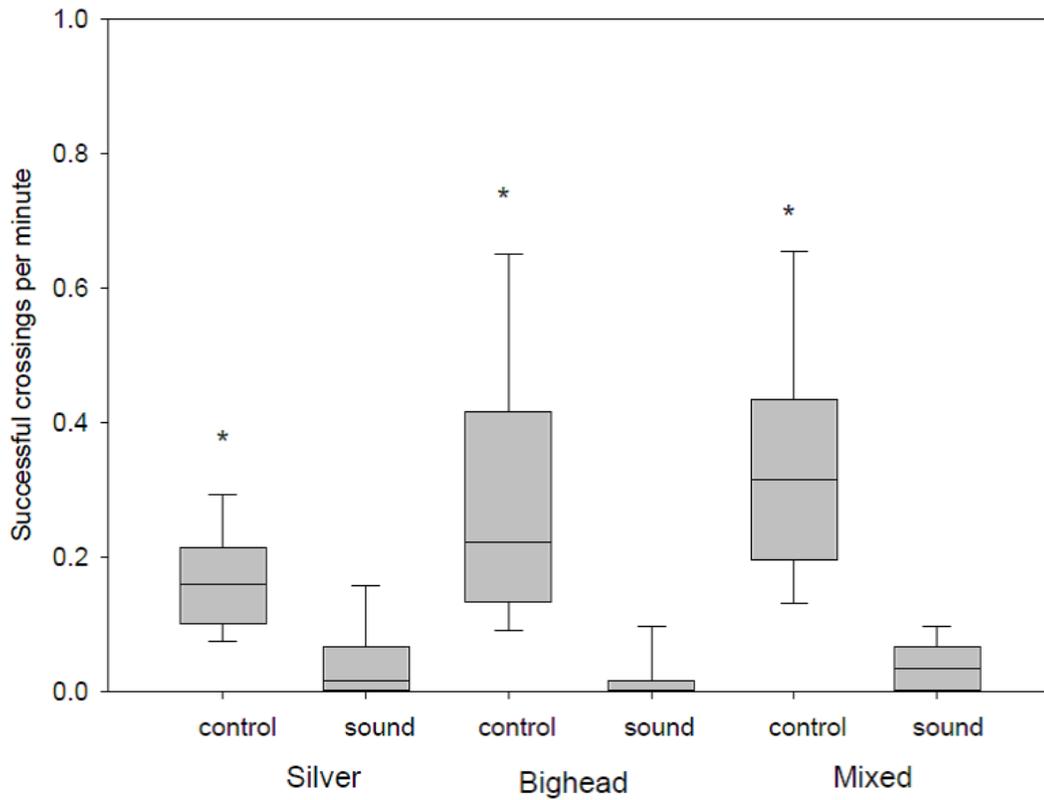
**Figure 1.** Overhead schematic of the experimental pond. The thick black lines indicate the barrier that divided the pond. The length and width of the pond is indicated in meters. The red box indicates the reaction zone with the corners of the box representing speaker locations. The location of the fish school was determined every five seconds and the x, y coordinates plotted and connected with spline lines. Each trace represents 30 minutes of swimming for one hetero-specific group; A) speakers inactive B) speakers activated when fish entered reaction zone C) Sound map of pond with pseudo color indicating sound intensity level dB re 1 $\mu$ Pa @ 1m during active broadcast of the two underwater speakers on the south side of the barrier. Intensity level is indicated by color panel.



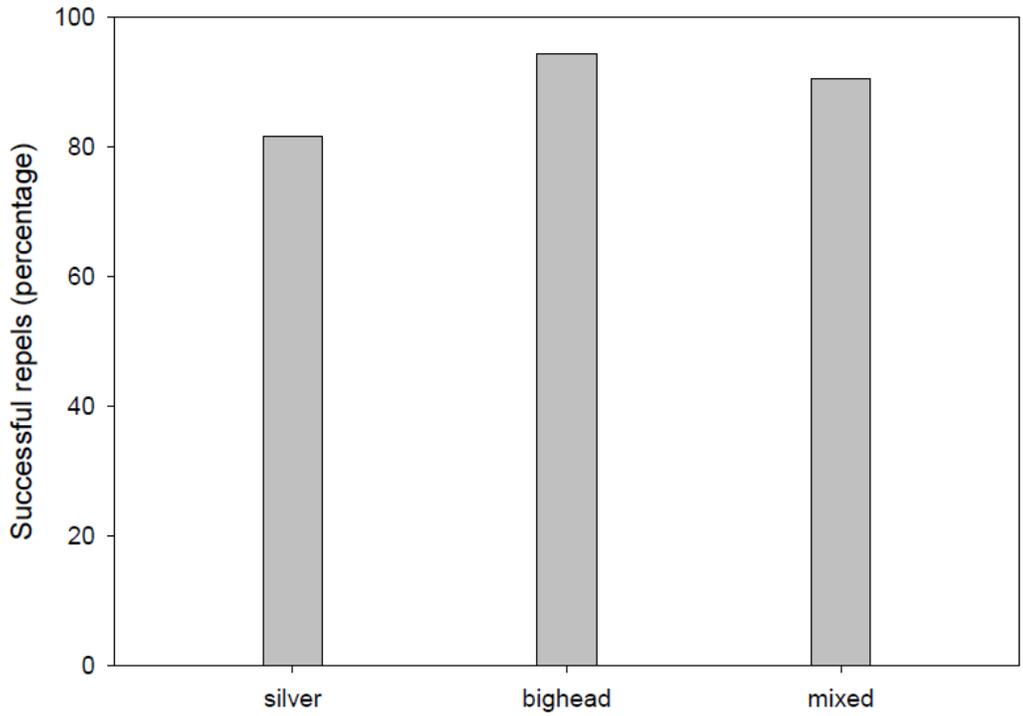
**Figure 2.** The power spectrum in relative dB of the broadband sound stimulus is plotted versus frequency (Hz).



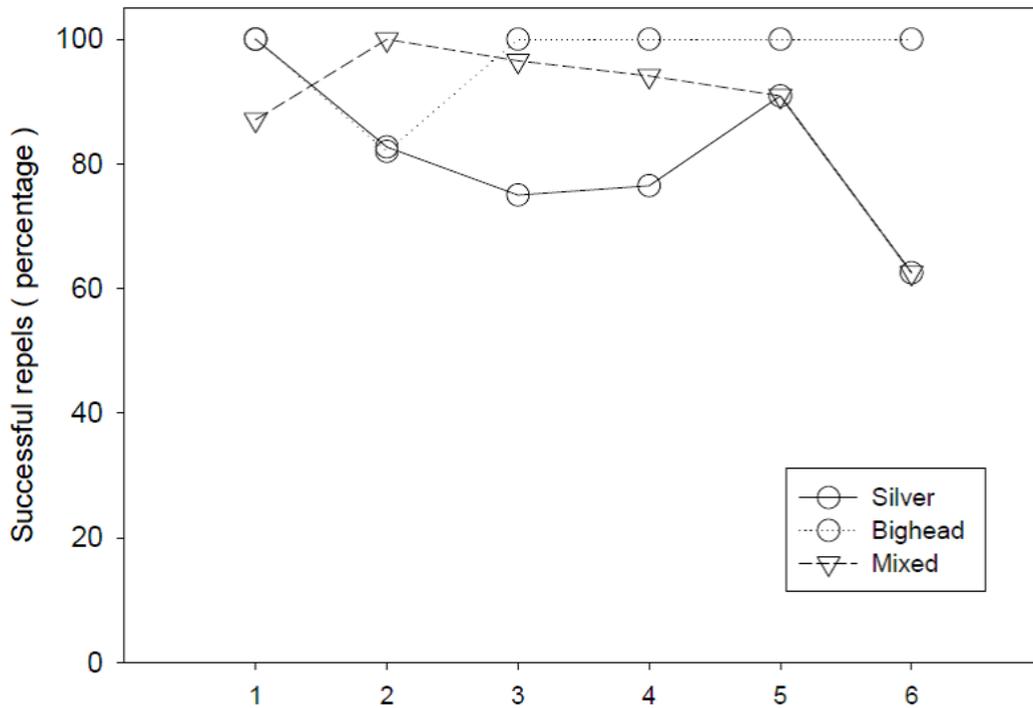
**Figure 3.** Box whisker plots show the attempted crossings (the number of times carp entered the reaction zone) per minute for silver, bighead, and mixed schools of carp for the five different intervals (pre – pre sound; sound 1 – sound playback 1; inter – inter sound interval between the two sound playbacks; sound 2 – sound playback 2; post – post sound interval after playback 2). The horizontal line in each box shows the median value, the bottom and top of the box indicate 1<sup>st</sup> and 3<sup>rd</sup> quartiles respectively, and the error bars represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles.



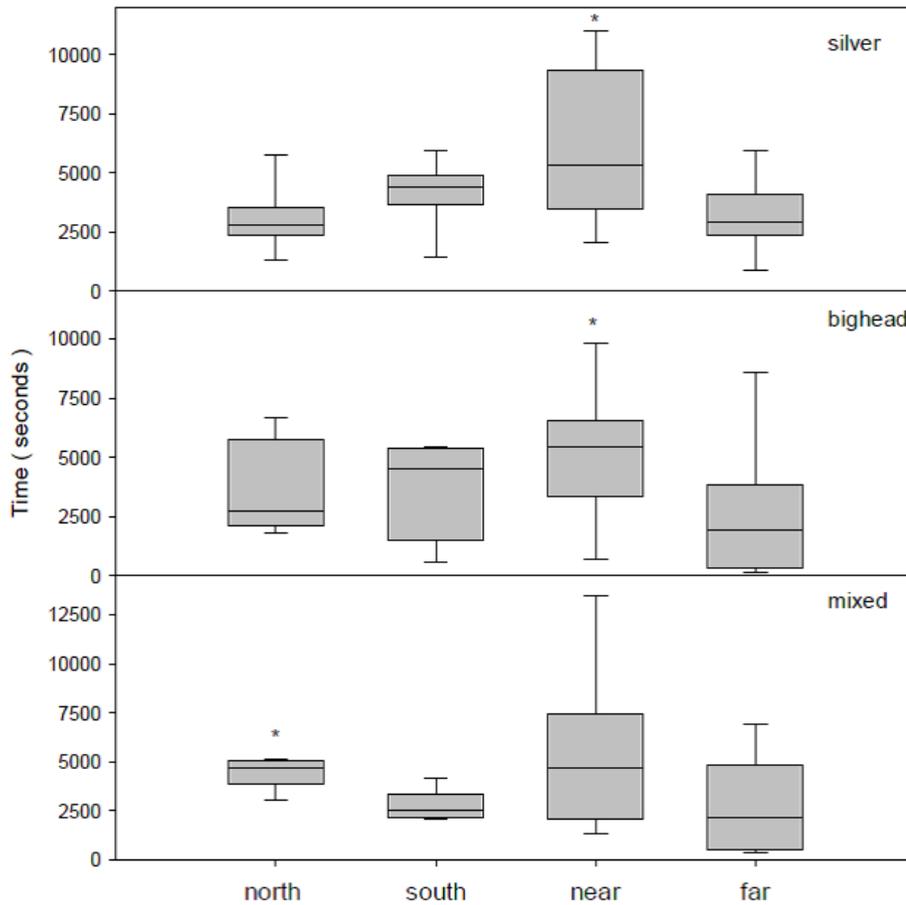
**Figure 4.** Box whisker plots show the successful barrier crossings per minute for silver, bighead, and mixed schools of carp during the control (sound off) and sound activation intervals. The horizontal line in each box shows the median value, the bottom and top of the box indicate 1<sup>st</sup> and 3<sup>rd</sup> quartiles respectively, and the error bars represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Asterisks denote significantly different crossing for each group (Mann-Whitney,  $P < 0.001$ ).



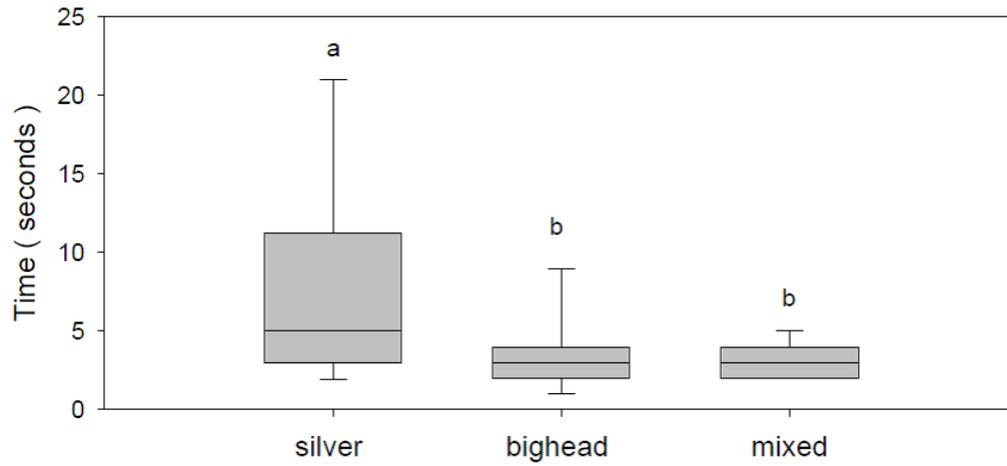
**Figure 5.** The overall percentage of successful repels (unsuccessful crossing / attempted crossing) is shown for the three experimental groups during the intervals when the speakers were active. The percentages were obtained by dividing the number of repulsions by the number of times the groups entered the reaction zone for all trials combined.



**Figure 6.** The percentage of successful repels (unsuccessful crossing / attempted crossing) for each sound playback. Each data point represents the percentage of all the attempts for each experimental group (silver carp, bighead carp, and mixed schools) during successive sound playbacks.



**Figure 7.** Box whisker plots show the median resident time for each side of the pond for the pre-sound interval (north/south) and the inter- and post-sound interval combined for silver, bighead, and mixed schools of carp. 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 1<sup>st</sup> and 3<sup>rd</sup> quartiles, respectively, and the error bars indicate the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Asterisks denote significantly different intervals (Mann-Whitney,  $P < 0.001$ ).



**Figure 8.** Box whisker plots show the median reaction following sound onset for fish to exit the reaction zone following successful repels. Each box shows the median value, the bottom and top of the box indicate 1<sup>st</sup> and 3<sup>rd</sup> quartiles, respectively, and the error bars indicate the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Letter indicates significantly different means.

Table 1: Acoustic deterrent results

Sound playback	SVC 1	SVC 2	SVC 3	BHC 1	BHC 2	Mixed 1	Mixed 2
1	3/3	3/3	6/6	3/3	4/4	7/9	20/22
2	11/11	9/11	4/6	8/11	4/5	6/6	3/3
3	5/5	5/8	3/3	2/2	15/17	19/20	9/9
4	2/2	7/10	0/1	13/13	6/6	8/8	8/9
5		5/6	4/4	3/3	16/16		10/11
6		4/5	0/2	15/15			5/8
Total	21/21	32/42	17/22	44/47	45/48	40/43	55/60
%	100%	76.2%	77.3%	93.6%	93.8%	93.2%	91.7%
Combined		70/85		89/95		95/103	
%		82.4%		93.7%		92.2%	

SVC=Silver carp; BHC=Bighead carp. Data indicate success repels/total crossing attempts

### **Chapter Three: The effects of anthropogenic sound on native and invasive fish behavior in the Upper Mississippi River**

#### **Introduction:**

Bighead and silver carp (*Hypophthalmichthys nobilis* and *H. molitrix*; hereafter, bigheaded carp) are native to the rivers of eastern China and Siberia (Kolar *et al.* 2007). In 1973, bigheaded carp were imported to the United States for phytoplankton control in eutrophic bodies of water (Buck *et al.* 2010). However, in the 1980's, both silver and bighead carp escaped from captivity and have continued to spread throughout the Mississippi River watershed and are causing major ecological and economical damage (Buck *et al.* 2010).

Bighead and silver carp eat between 5-20% of their body weight each day through filter-feeding for phytoplankton and zooplankton (Kolar *et al.* 2007), leading to depletion of nutrients essential for native food webs (Kolar *et al.* 2007), specifically blue-green algae (*Microcystis spp.*) which comprise 80% of the carp's foregut contents (Cooke *et al.* 2009). As they exhibit relatively fast growth, these fish have high energy demands and out-compete many native species, including gizzard shad (*Dorosoma cepedianum*) (Sampson *et al.* 2009b), bigmouth buffalo (*Ictiobus cyprinellus*) (Irons *et al.* 2007), and paddlefish (*Polyodon spathula*) (Schrank *et al.* 2003) for resources.

Bigheaded carp range expansion, especially into the Laurentian Great Lakes, is a concern. The main limiting factor for these species' establishment in new habitats is food availability, since bigheaded carp require high plankton densities to flourish (Cooke *et al.* 2009). While there is a low risk of bigheaded carp colonization in open water regions

with low plankton biomass, there are areas in Great Lakes that have the plankton productivity needed to support the energetic requirements of silver and bighead carp. These regions, which are primarily embayments and tributaries, are most susceptible to invasion. However, the offshore pelagic and nearshore habitats may be indirectly affected by establishment of silver and bighead carp in adjoining embayments and wetlands (Cooke and Hill 2010).

Silver and bighead carp are ostariophysans and are sensitive to a wider hearing range than non-ostariophysan fish due to specialized structures called Weberian ossicles. Weberian ossicles connect and facilitate the transfer of acoustical energy between the swim bladder and the inner ear (Fay and Popper 1999). This bony connection allows silver and bighead carp to have a broader and higher frequency hearing range than non-ostariophysans. Lovell *et al.* (2006) reported frequency sensitivity up to 3 kHz for both species but the researchers did not test beyond 3 kHz and there were limitations to the methods used in this study, specifically the small tanks that were used (Ladich and Fay 2013; Sisneros *et al.* 2016) and therefore bigheaded carp could hear up to 5 kHz, like other ostariophysans (Popper and Fay 2011). Further research is needed to confirm hearing thresholds reported by Lovell *et al.* (2006).

Native non-ostariophysan species, such as paddlefish, rainbow trout (*Oncorhynchus mykiss*), and lake sturgeon (*Acipenser fulvescens*) cannot hear beyond 500 Hz (Lovell *et al.* 2005; Wysocki *et al.* 2007). However, some other native non-ostariophysan species have a wider hearing range. Members of the families Percidae (walleye *Sander vitreus* : Speares *et al.* 2011), and Centrarchidae (bluegill *Lepomis*

*macrochirus*: Scholik and Yan 2002) have hearing limits between 1000-2000 Hz and the family Clupeidae (gizzard shad) can hear ultrasound stimuli up to 100 kHz (Mann *et al.* 1998). There are also native ostariophysans, channel catfish (*Ictalurus punctatus*: Wysocki *et al.* 2009), fathead minnows (*Pimephales promelas*: Scholik and Yan 2001) and bigmouth buffalo (Mann *et al.* 2007), which have upper limit hearing thresholds between 2000-4000 Hz, similar to that of bigheaded carp. Bigheaded carp have a wider hearing range than many native non-ostariophysians; therefore, it is possible that broadband sound (up to 5 kHz) sound could elicit avoidance behavior from bighead and silver carp with minimal impacts on native non-ostariophysian species. However, impacts an acoustical barrier would have on native ostariophysans are unknown.

Currently, researchers are evaluating acoustic deterrents as part of an integrated pest management approach to preventing bigheaded carp range expansion. Vetter *et al.* (2015, 2016) determined that a broadband sound stimulus effectively modulated captive bighead and silver carp behavior and Murchy *et al.* (2016) successfully used sound as an acoustic deterrent. However, before sound stimuli can be implemented to deter bigheaded carp movement, the impact to native species must be evaluated. Additionally, assessing broadband sound impacts on other invasive species, such as common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*), is important in the development of acoustic deterrents. The goal of this study was to compare the behavioral responses between native fish species (non-ostariophysans and ostariophysans) and invasive carp species (silver, bighead, grass and common carp) in response to a broadband acoustic stimulus.

## **Material and Methods:**

### *Animal Husbandry:*

All experiments were conducted at the U.S. Geological Survey (USGS) Upper Midwest Environmental Science Center (UMESC) in La Crosse, Wisconsin. Native non-ostariophysans: lake sturgeon (13-20 cm TL), paddlefish (16-30 cm TL), gizzard shad (5-10 cm TL), walleye (10-15 cm TL), bluegill (15-20 cm TL), and rainbow trout (15-20 cm TL); native ostariophysans: channel catfish (9-13 cm TL), fathead minnow (6-9 cm TL), and bigmouth buffalo (19-25 cm TL); and invasive ostariophysans: common carp (20-25 cm TL) and grass carp (10-25 cm TL) were maintained in rearing tanks using flow through well water and fed a diet of classic fry (Skretting, Tooele, UT) everyday via a belt feeder (an use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government). Food was withheld for 24 hours prior to transport to the experimental tanks and fish were not fed during the testing periods. Each group was allowed to acclimate in the experimental tank for at least 24 hours prior to the initiation of experiments. Trials were conducted July – October 2014 and June 2015 – March 2016.

### *Indoor set up:*

Experiments were conducted in four 69 cm x 292 cm x 15 cm (735 L) indoor flow-through tanks (Figure 1) that were placed 1 m apart to avoid disturbance in concurrent trials. The tank water was sourced directly from onsite UMESC wells and was heated to maintain a temperature of  $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Mesh netting completely covered each tank to prevent fish from escaping during acclimation or the sound trials. Black plastic

sheeting surrounded all four tanks to separate them from the observer. Room access was restricted during the trials and only a single observer was present 5 m away from the nearest tank.

*Indoor sound stimulus:*

One underwater speaker (UW-30, Lubell Labs Inc., Whitehall, OH) was placed approximately 31 cm from each end of the experimental tank. Plastic netting restricted fish from accessing the area behind the speaker and sound was only played from one speaker at a time. In each tank, a hydrophone (HTI-96-MIN; High Tech Inc., Long Beach, MS) was placed approximately 80 cm from the right speaker to record sound. Hydrophone data was collected using a PowerLab 4SP data acquisition system controlled by LabChart 7 software (AD Instruments, Colorado Springs, CO). The sound stimulus broadcast to the fish was recorded from a 100 Hp Honda 4 stroke outboard motor moving at 32 km/hr in the Illinois River. The stimulus contained a broad spectrum of sound (0.06 – 10 kHz) and had maximal energy in two broad peaks from 0.06 – 2 kHz and 6 – 10 kHz.

Sound was regulated using an amplifier (UMA-752; Peavey Electronics, Meridian, MS) and speakers were controlled separately with a switchbox (MCM Electronics, Centerville, OH). Individual tanks were sound mapped using the hydrophone to evaluate the acoustic properties of the speakers. Relative sound pressure levels (SPL) were calculated by measuring the root mean square (rms) voltage in LabChart 7 and converting to SPL in dB re 1 $\mu$ Pa @ 1m using Avisoft-SASLab Pro ver. 5.2.07.

*Indoor experimental design:*

To monitor fish behavior, each tank was equipped with three overhead SONY bullet 500 TVL video cameras that were connected to a computer with ProGold software (security Camera World, Cooper City, FL). Approximately 10 m away from the experimental set up, an observer manually controlled the speakers and recorded observations on swimming behavior. Each camera covered about 1/3 of the experimental tank, providing full coverage of each of the tanks for the duration of the trials. To aid in tracking fish position in the tank, black grid lines (23 cm x 20.8 cm) were placed on the bottom. These grid lines allowed the observer to denote fish position along the tank's length and width.

*Indoor behavior experiments:*

Species were tested in mono-specific groups ( $n=10$ ) and were not run simultaneously in the tanks. Sound trials were not initiated until the majority of fish ( $>5$ ) were within 50 cm of one speaker (end zone). Trials were initiated by broadcasting the sound from the nearest speaker and the species' behavioral response was then observed. A negative phonotaxis response was defined as at least 6 fish swimming away from the active speaker and reaching the midpoint (146 cm) of the tank in 30 seconds or less. If the fish moved within 50 cm of the inactive speaker at the opposite end of the tank in less than 60 seconds, the original speaker was inactivated and input was switched to the opposite speaker end. This process was repeated until the fish failed to respond or until 10 minutes elapsed. A non-response was defined as fish not moving, swimming towards active speaker, or failing to react within the time limits. Each group of fish was tested

three times with at least an hour interval between trials. For each species, four groups were tested creating a total of 12 replicates.

*Outdoor set up:*

Experiments were conducted in a 5 m x 10 m x 1.2 m (60 kL) outdoor concrete pond (Figure 2). Water depth in the pond was maintained at 1.1 m. Well water was pumped into the pond directly from UMESC and allowed to warm to the experimental temperature of  $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$  over two days. Metal fencing (2 m high) topped by anti-bird netting surrounded the pond to prevent fish escape. An observer was positioned approximately 50 m away from the experimental pond in a trailer and access to the pond was restricted during the sound trials.

*Outdoor sound stimulus:*

Two pairs of underwater speakers (UW-30, Lubell Labs Inc., Whitehall, OH) were placed at each end of the outdoor experimental pond. Hydrophones (HTI-96-MIN; High Tech Inc., Long Beach, MS) were placed on each side of the pond to monitor sound output from the speaker pairs. Each hydrophone was connected to a PowerLab 4SP data acquisition system running LabChart 7 software (AD Instruments, Colorado Springs, CO) and the same sound stimulus from the indoor trials was used in these experiments.

Sound was regulated using an amplifier (UMA-752; Peavey Electronics, Meridian, MS) and individual speakers were controlled separately using a switchbox (MCM Electronics, Centerville, OH). The outdoor pond was sound mapped using the hydrophone to evaluate the acoustic properties of the concrete pond and speakers. Sound pressure levels were measured at 70 pointed spaced approximately 1 m apart throughout

the pond. Relative sound pressure levels (SPL) were calculated by measuring the root mean square (rms) voltage and converting to SPL in dB re 1 $\mu$ Pa @ 1m using Avisoft-SASLab Pro ver. 5.2.07.

*Outdoor behavior experiments:*

Eight overhead SONY bullet 500 TVL video cameras, connected to a computer equipped with ProGold (Security Camera World, Cooper City, FL), continuously recorded the swimming behavior throughout the trials. A trailer, which housed the computer, was located approximately 50 m from the experimental pond.

*Outdoor experimental design:*

During each sound trial, speaker activation was alternated so that only one speaker pair broadcast the sound stimulus at a time. The decision on which speaker was activated was selected based on observations of swimming behavior and fish location within the tank. Each species was presented with broadband sound in mono-specific grouping, with the exception of one instance when three species (walleye, bluegill and grass carp) were tested at once because of time constraints. When the majority (>5) of the fish were within 2 m of one speaker pair, sound was initiated. A negative phonotaxis response was defined when at least 5 fish oriented and swam away from the active speakers, reaching at least the midpoint (5 m) of the tank within 30 seconds and the opposite end of the tank within 60 seconds. Sound broadcast continued until the fish reached < 2 m of the inactive speakers. At that point, the original speaker pair was inactivated and the speakers at the opposite were turned on. This process continued until the fish failed to respond within the time designated for a negative phonotaxis or until 10

minutes transpired. Non reactions were defined as fish not orienting away from sound, not crossing the midline by 30 seconds, not reaching the opposite end of the tank by 60 seconds, or swimming back towards active speaker. Each group of fish was exposed to the broadband sound two times per day with at least an hour break between trials for two consecutive days.

*Data Analysis:*

Fish position was monitored from the video recorded during the experiments. If fish consistently schooled, the school was treated as a single unit and the midpoint of the school (x, y) was tracked in meters every 5 seconds. Alternatively, if a species did not school, fish were individually tracked every five seconds. Either individual fish or congregates of fish were tracked during broadband sound playback and during no sound controls (10 minutes). Additionally, the total number of consecutive negative phonotaxis reactions to the broadband sound was counted for each sound trial and averaged across species. Density plots were created for indoor trials from school or individual fish locations. Number of fish in each of the 42 squares created by the 23 x 20.8 cm gridlines was tabulated and density plots were created in Sigmaplot version 12.5.

Non-parametric Kruskal-Wallis ANOVA's with Dunn's post hoc tests were performed on all data, since data was not normally distributed. All statistical tests were performed with SigmaPlot version 12.5. The median, lower (1<sup>st</sup>) and upper (3<sup>rd</sup>) quartile is reported.

**Results:**

*Indoor Experiments:*

The native ostariophysans (channel catfish and fathead minnow) and native non-ostariophysans (lake sturgeon and paddlefish) did not show any reaction to the sound (all species: 0.0; 0.0, 0.0). Gizzard shad (0.167; 0.0, 1.33), had significantly more (Kruskal-Wallis,  $p < 0.05$ ) reactions to the broadband sound compared to other non-ostariophysan species. However, reactions were inconsistent with most groups showing no reactions or few (1-3 consecutive responses) with a maximum 8 consecutive responses by one school. A previous study, using the same methods and sound stimulus as this experiment, reported a median of 7.5 (5.6, 24.5) consecutive responses from the bighead carp and 6.0 (1.0, 10.5) responses from the silver carp (Murchy et al. 2016). Compared with these results, the native species from this study had significantly fewer reactions (Kruskal-Wallis,  $p < 0.001$ ) to the broadband sound than was observed in bighead and silver carp.

Sound pressure levels for each speaker reached a maximum of 145 dB re 1 $\mu$ Pa @ 1m immediately in front of the speaker and quickly attenuated to 130 dB re 1 $\mu$ Pa @ 1m approximately 20 cm away from the speaker (Figure 1B). At the tank midpoint, the sound pressure levels were 120 dB re 1 $\mu$ Pa @ 1m.

Density plots for paddlefish (Figure 4: A), lake sturgeon (Figure 4: B), channel catfish (Figure 4: C) and gizzard shad (Figure 4: D) showed no visual difference in tank distribution between control times and sound trials. However, the densities of bighead and silver carp during a sound trial were high at on the opposite end of the active each speaker, which was not seen in the no sound controls (Figure 5). Lake sturgeon and channel catfish remained in consistent locations throughout both control times and sound trials. Paddlefish continually moved throughout tank, but their overall distribution did not

change. The only fish that schooled were fathead minnows, silver carp, and bighead carp, however, the fathead minnows did not alter their swimming behavior when presented with broadband sound.

*Outdoor Experiments:*

The broadband sound used in the outdoor portion of this study, as well as the experimental methods, were the same as that used in a previous experiment by Vetter et al. (2015). Each speaker pair emitted high sound pressure (150 dB re 1 $\mu$ Pa @ 1m) in the immediate area around (< 2 m) each speaker (Figure 2: B). Sound pressure levels dropped to 120 dB re 1 $\mu$ Pa @ 1m on the opposite end of the pond.

Native species (ostariophysan and non-ostariophysan) showed a significantly lower (Kruskal-Wallis,  $p < 0.001$ ) in responses to the broadband sound stimulus compared with invasive bighead (20.0; 12.0, 23.0; Vetter et al. unpublished) and silver carp (11.8  $\pm$  1.3; Vetter et al. 2015). Bigmouth buffalo, were the only native species to visibly react to the broadband sound, responding a median of 1.67 times (0.33, 7.875). The rainbow trout, walleye, and bluegill, all native non-ostariophysans, showed no reaction (all species =0.0; 0.0, 0.0) to the broadband sound.

The other invasive ostariophysan species, common and grass carp, were significantly less responsive (Kruskal-Wallis,  $p < 0.001$ ) to the broadband sound compared with bighead and silver carp (Figure 6). Both common and grass carp demonstrated a median of 3.0 (common carp: 1.0, 6.0; grass carp: 0.75, 7.0) consecutive negative phonotaxis reactions. Overall, the native and invasive ostariophysan species

showed significantly more (Kruskal-Wallis,  $p < 0.001$ ) reactions to the broadband sound compared with non-ostariophysan species (Figure 6).

### **Discussion:**

All species tested in this study had reduced reactions to the broadband sound compared with the bigheaded carp tested in previous experiments, with only bigmouth buffalo, common carp, grass carp, and gizzard shad visibly reacting to the sound. In contrast, silver and bighead carp showed consistent negative phonotaxis to broadband sound in both indoor and outdoor experiments (Vetter et al. 2015, Vetter et al. unpublished, Murchy et al. 2016).

The indoor and outdoor tanks provided unique and challenging acoustical environments for the fish to localize sound. Sound in a small tank or a concrete pond can echo off the walls, bottom, and surface presenting a challenge for fish to determine the sound source (Gray *et al.* 2016; Rogers *et al.* 2016). Furthermore, these echoes can create elevated sound pressure levels and distort the sound stimulus. However, there was a gradient of sound present in both tank types with the highest sound pressure levels (145 – 150 dB re 1 $\mu$ Pa @ 1m) at the speakers and a decrease of 30 dB re 1 $\mu$ Pa @ 1m at the far end. Therefore, since the highest sound pressure levels remained above the hearing threshold of all species tested (Mann *et al.* 1998, 2007; Scholik and Yan 2001, 2002; Lovell *et al.* 2005, 2006; Wysocki *et al.* 2007, 2009; Speares *et al.* 2011; Maiditsch and Ladich 2014), the fish should have been able to detect the sound gradient. Smith et al. (2004) found that hearing loss occurred in goldfish at >170 dB re 1 $\mu$ Pa @ 1m and since the sound pressure levels were much lower than this in both the indoor and outdoor

ponds, it is unlikely that any fish suffered from hearing damage that would have affected their behavior.

Both indoor and outdoor tanks were used in this experiment based on the size of fish available to test. Larger fish were tested in the outdoor ponds because they could be easily seen with the overhead cameras. However, many of the native species could not be visualized using the cameras mounted above the outdoor ponds, so they were tested in smaller indoor tanks. This allowed for comparison between a broad size range of bighead and silver carp, tested in both tank types, all of which had consistently high responses. The fish from the indoor trials were less reactive overall and this is probably because the experiments were conducted in a small tank indoors, which is further removed from natural conditions and has more complicated acoustics (Gray *et al.* 2016; Rogers *et al.* 2016).

Despite all of these challenges, the experimental methodology remained consistent among all species examined and the bigheaded carp were more responsive to the broadband sound in both environments. Rogers *et al.* (2016) reported differences in the acoustical environment of large concrete tanks (similar to the ones used in this study) compared with how sound is propagated in the field, however, concluded that experiments conducted in concrete ponds can be useful when comparing the behavioral responses among species. It is possible that the broadband sound stimulus used is distorted from the sound emitted by an outboard motor in the Illinois River. The hydrophone used was only capable of recording the sound pressure and not the particle component of the motor sound and the speakers probably altered the recording.

Regardless, the objective of this study was to compare behavioral response of native species and invasive carp to broadband sound and the results from both tank types strongly suggest that the bigheaded carp are more responsive to broadband sound than the other fish tested, including the native species. Finally, one potential application for broadband sound is use in a lock chamber, which would have similar acoustic properties to the concrete tanks used, therefore this study provides strong support for the efficacy of this stimulus as part of an acoustic deterrent at a lock and dam.

These results indicate that native species' behavior is minimally affected by the broadband sound. Except for the gizzard shad, all native non-ostariophysans did not visibly react to the sound stimulus and their behavior did not alter from non-sound patterns. The native ostariophysans had varying responses. Channel catfish and fathead minnow did not react to the sound stimuli, but bigmouth buffalo exhibited lower responses compared to bigheaded carp. However, the reactions of the bigmouth buffalo were inconsistent and they habituated to the sound quickly. It is possible that native species cannot hear the higher frequency components of the sound in which case, a high frequency sound may not impact native species, or, they can detect the sound but do not react to it. Therefore care must be taken not use high sound pressure levels (>170 dB) that might deafen native species and also further analysis is needed to make sound the sound levels are not harmful.

Broadband sound elicits a consistent negative phonotaxis response from silver and bighead carp. However, common and grass carp, invasive species and close relatives of bigheaded carp, had reduced responses to the sound compared to their relatives. Common

carp are more sensitive to sound stimuli between 800 Hz and 1000 Hz (Kojima *et al.* 2005) compared to bigheaded carp which appear to be equally sensitive to stimuli up to at least 3000 Hz (Lovell *et al.* 2006). There has been some recent critique of the auditory brainstem response Lovell *et al.* (2006) used to test bigheaded carp hearing (Ladich and Fay, 2013; Sisneros *et al.*, 2016), therefore the carp could be capable of hearing beyond 3000 Hz. Nevertheless, these results suggest that not all closely related species have the same reactions to anthropogenic noises in their environment.

Morphological differences in the swim bladder and ossicle configuration could be responsible for the varied hearing sensitivities between ostariophysan species (Bird and Hernandez 2007; Lechner and Ladich 2008). The most common difference between ostariophysan species is the shape and length of the tripus, intercalarium, and scaphium. These minor morphological differences cause variations within the high frequency hearing ability (Bird and Hernandez 2007). Benthic catfishes, who do not use their swim bladders to control buoyancy, have reduced swim bladder size as well as number and size of ossicles, which lessens their hearing sensitivity compared to other ostariophysans (Lechner and Ladich 2008).

One unexpected result from this study was the elevated responses ( $> 3$ ) to the broadband sound by the non-ostariophysan, gizzard shad, compared to other non-ostariophysans. Gizzard shad are members of the order clupeiformes and other members of this group have documented hearing sensitivity to ultrasound stimuli (Mann *et al.* 2001). Current literature suggests that ultrasound detection is the result of specialization of the inner ear, specifically the utricle. The inner ear of the clupeiform ear has a unique

arrangement of the sensory epithelium and air-filled bulla that are closely associated with the utricle (Nestler *et al.* 1992; Mann *et al.* 2001). A closely related species, American shad (*Alosa sapidissima*), has a hearing range of 0.1-100 kHz, which suggests that gizzard shad and other native species from the family Clupeidae might be able to hear high frequency sounds and could be impacted by an acoustic barrier broadcasting broadband sound.

Bigheaded carp pose a danger to many ecosystems, including the Laurentian Great Lakes. Areas of the Great Lakes with adequate food resources, like the eutrophic western and central basins of Lake Erie, are possible places for invasion of silver and bighead carp. However, since bigheaded carp are generalist feeders, they can switch to feeding on detritus and sediment when plankton is low, making a wider range of habitats susceptible to invasion (Cooke *et al.* 2009).

Bigheaded carp cause major ecological impacts in regions that they have invaded. They consume substantial amounts of larger zooplankton and phytoplankton species, creating an ecosystem primary composed of smaller plankton species. This shift in plankton could cause changes in the food web, by diminishing native species that depend on larger zooplankton and phytoplankton (Kolar *et al.* 2007). Native planktivorous fish are also negatively impacted through competition. After bigheaded carp were introduced in the Aral Sea basin, native Aral barbell (*Barbus brachycephalus*) populations declined drastically (Pavlovskaya 1995). Even when bigheaded carp have been translocated within China, native planktivorous species declined (Xie and Chen 2001; Li and Xie 2002).

Native endemic barbless carp (*Cyprinus Pellegrini*) dropped to below 1% of total catch only 30 years after a bigheaded carp introduction (Xie and Chen 2001).

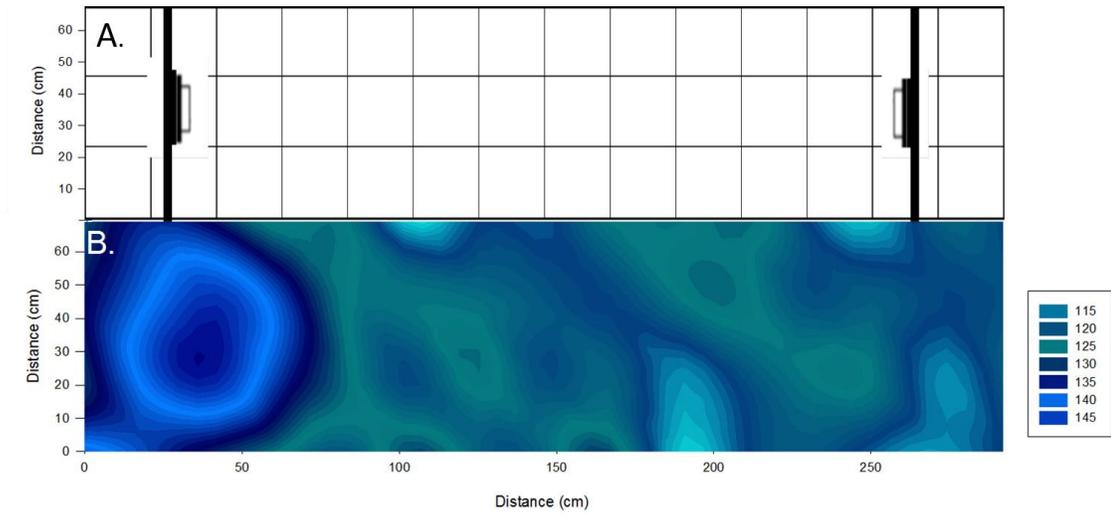
Bigheaded carp not only impact native fish species, but can also affect humans (Kolar *et al.* 2007). The high filter feeding ability of bigheaded carp has the potential to amplify noxious algal blooms, specifically species that cannot be digested by the carp, like *Microcystis aeruginosa* (Xie 2003; Xie *et al.* 2005; Kolmakov *et al.* 2006). Blue-green algae (*Microcystis aeruginosa*) produces a cyanotoxin that can cause hepatotoxicosis and other health issues in humans (Carmichael 2001). Amplification of these cyanotoxins poses a significant threat to human or animal populations that consume fish.

As bigheaded carp populations continue to grow, range restriction and removal of these species are major areas of research. However, before any control measures can be implemented, potential impacts on native species must be evaluated. Bigheaded carp are already causing population declines in many native species (Schrank *et al.* 2003; Irons *et al.* 2007; Kolar *et al.* 2007; Sampson *et al.* 2009b), so further negative impacts to these native species should be prevented.

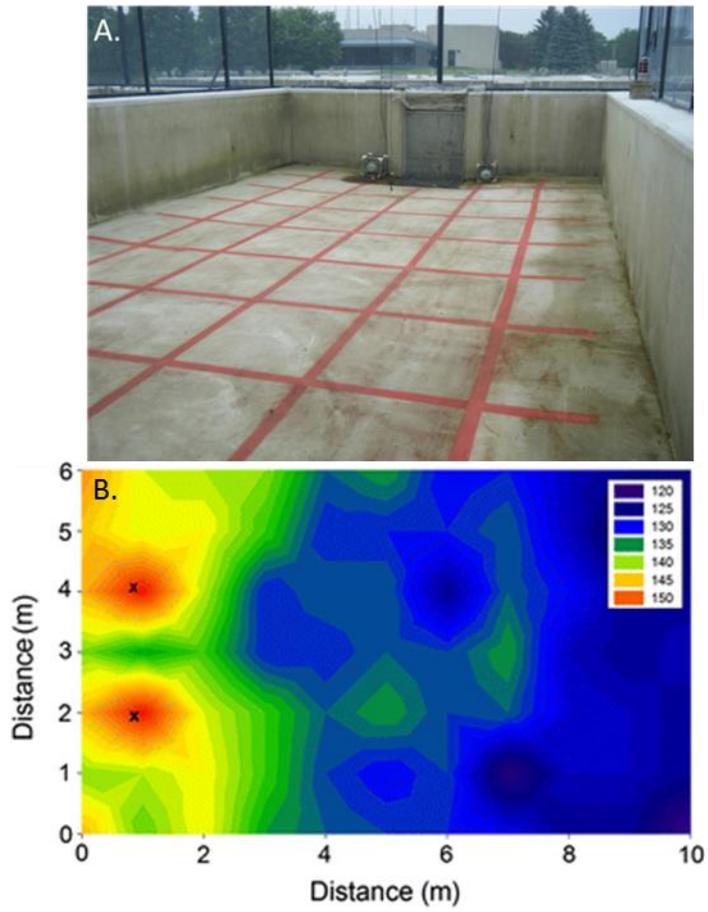
With increases in temperature and nutrient input in the Great Lakes, zooplankton and phytoplankton community structures will change, creating the offshore pelagic habitats of the Great Lakes more suitable for an invasion of silver and bighead carp (Cooke and Hill 2010). Non-physical barriers are needed to prevent their expansion into the Laurentian Great Lakes and other naïve habitats. Using a high frequency broadband sound stimulus (outboard motor) provides an optimal non-physical barrier to reduce

range expansion of silver and bighead carp. Bigheaded carp are ostariophysans and are more sensitive to high frequency sounds compared to non-ostariophysans. The current study demonstrates the potential for a broadband sound stimulus to be used as a non-physical barrier to restrict the movements of silver and bighead carp while having minimal impacts on many native species.

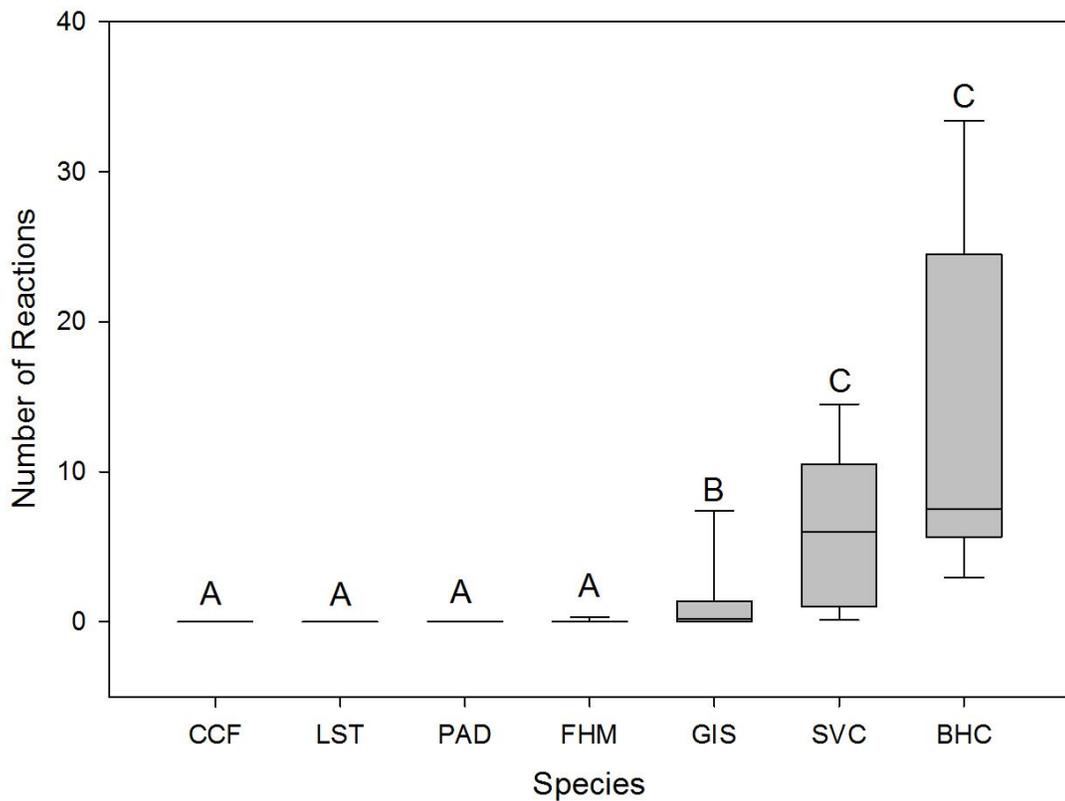
**Figures:**



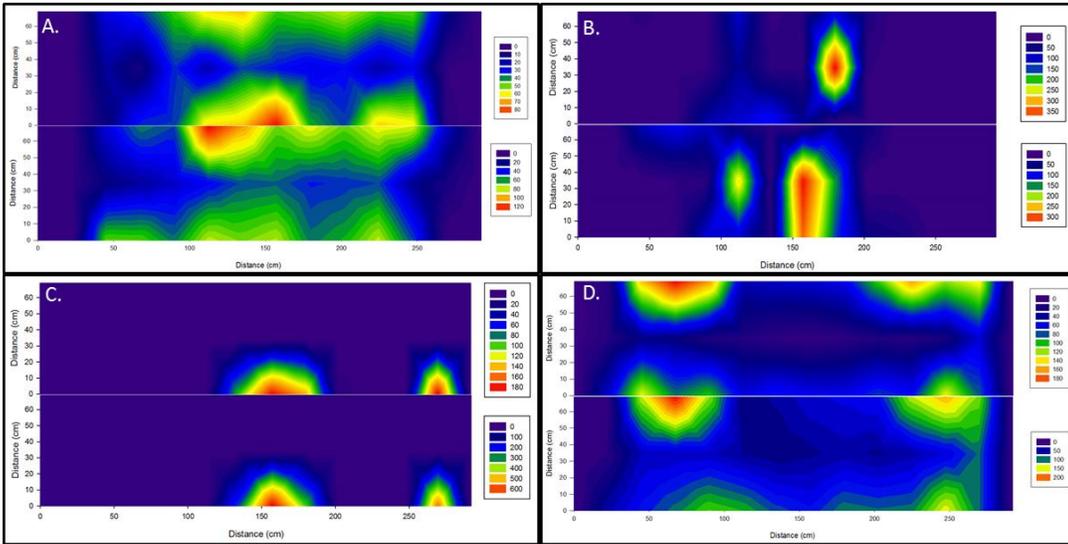
**Figure 1:** Indoor experimental tank setup. A) Schematic of one indoor experimental tank with speaker locations denoted. B) Sound map of indoor tank with colors indicating sound intensity level dB re 1µPa @ 1m during active broadcast of the underwater speaker on the left end of the tank. Intensity level (dB re 1µPa @ 1m) is indicated by color panel.



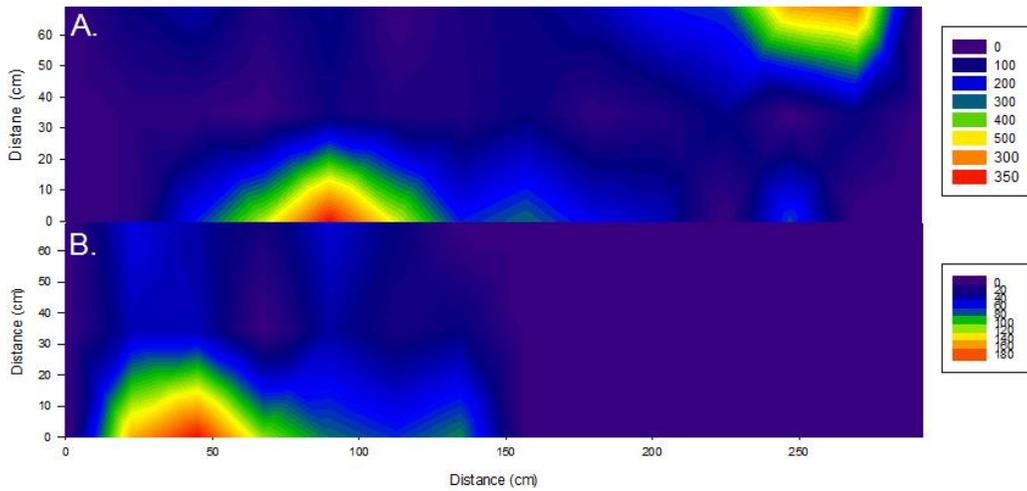
**Figure 2:** Outdoor experimental pond setup A) Picture of the outdoor experimental pond  
B) Sound map from Vetter et al. (2015).



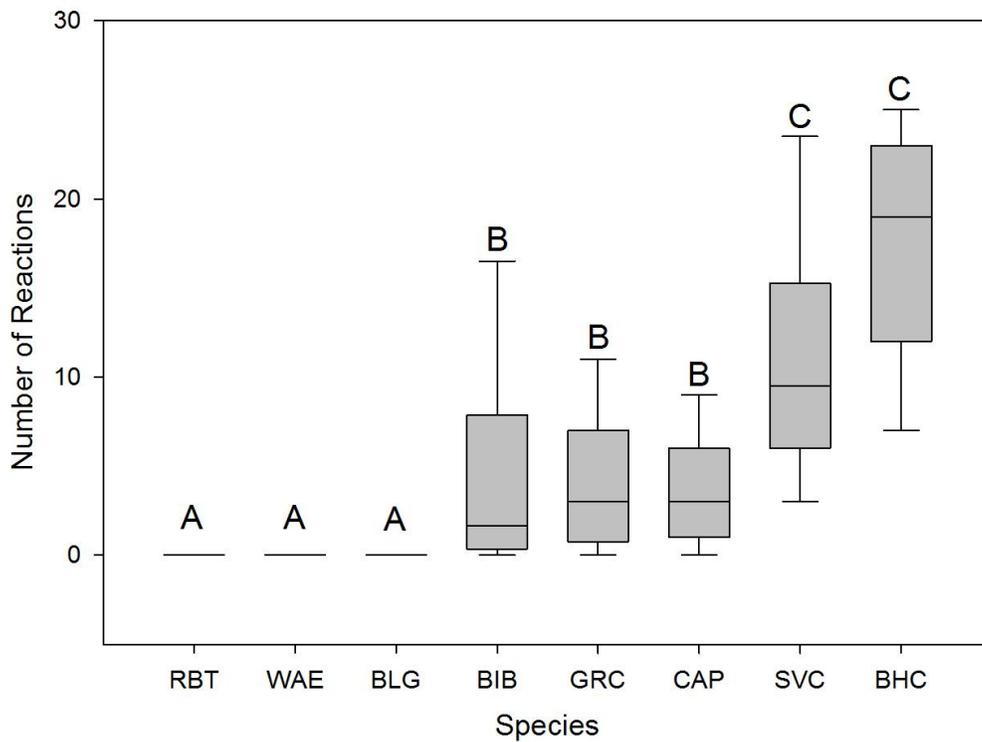
**Figure 3:** Box whisker plots show the number of reactions to the broadband sound stimulus by each species in indoor trials. CCF: channel catfish, LST: lake sturgeon, PAD: paddlefish, FHM: fathead minnow, GIS: gizzard shad, SVC: silver carp, and BHC: bighead carp. The horizontal line in each box shows the median value, the bottom and top of the box indicate 1<sup>st</sup> and 3<sup>rd</sup> quartiles respectively, and the error bars represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Letters denote significantly different means (Kruskal-Wallis  $P < 0.05$ ).



**Figure 4:** Density plots representing native fish distribution in experimental tank. A) paddlefish B) lake sturgeon C) channel catfish D) gizzard shad. Control time when speakers were inactive is the top density plot for each species and sound trials with active speakers is located in the bottom density plot.



**Figure 5:** Density plots representing bighead carp distribution in experimental tank. A) Control time when speakers were inactive B) Sound trials with the right speaker active.



**Figure 6:** Box whisker plots show the number of reactions to the broadband sound stimulus by each species in outdoor trials. RBT: rainbow trout, WAE: walleye, BLG: bluegill, BIB: bigmouth buffalo, GRC: grass carp, CAP: common carp, SVC: silver carp, and BHC: bighead carp. The horizontal line in each box shows the median value, the bottom and top of the box indicate 1<sup>st</sup> and 3<sup>rd</sup> quartiles respectively, and the error bars represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Letter indicates significantly different means. (Kruskal-Wallis  $p < 0.001$ )

Table 1: Indoor Sound Trial Results

Species	Known Hearing Range	Reaction Total: Median (1 <sup>st</sup> Quartile, 3 <sup>rd</sup> Quartile)
Silver carp (SVC)	100-3000 Hz (Lovell <i>et al.</i> 2006)	6.0 (1.0, 10.5) (Murphy unpublished)
Bighead carp (BHC)	100-3000 Hz (Lovell <i>et al.</i> 2006)	7.5 (5.6, 24.5) (Murphy unpublished)
Channel catfish (CCF)	100-4000 Hz (Wysocki <i>et al.</i> 2009)	0.0 (0.0, 0.0)
Fathead minnow (FHM)	300-4000 Hz (Scholik and Yan 2001)	0.0 (0.0, 0.0)
Lake sturgeon (LST)	100-500 Hz (Lovell <i>et al.</i> 2005)	0.0 (0.0, 0.0)
Paddlefish (PAH)	100-500 Hz (Lovell <i>et al.</i> 2005)	0.0 (0.0, 0.0)
Gizzard shad (GIS)	0.1-100 kHz (Mann <i>et al.</i> 1998)	0.137 (0.0, 1.33)

Table 2: Outdoor Sound Trial Results

Species	Known Hearing Range	Reaction Total: Median (1 <sup>st</sup> Quartile, 3 <sup>rd</sup> Quartile)
Silver carp (SVC)	100-3000 Hz (Lovell <i>et al.</i> 2006)	11.8 ± 1.3 (Vetter <i>et al.</i> 2015)
Bighead carp (BHC)	100-3000 Hz (Lovell <i>et al.</i> 2006)	20.0 (12.0, 23.0) (Vetter <i>et al.</i> submitted)
Common carp (CAP)	100-4000 Hz (Maiditsch and Ladich 2014)	3.0 (1.0, 6.0)
Grass carp (GRC)	Estimated 100-4000 based on other carp species	3.0 (0.75, 7.0)
Bigmouth buffalo (BIB)	100-2000 Hz (Mann <i>et al.</i> 2007)	1.67 (0.33, 7.875)
Walleye (WAE)	100-1000 Hz (Speares <i>et al.</i> 2011)	0.0 (0.0, 0.0)
Bluegill (BLG)	300-2000 Hz (Scholik and Yan 2002)	0.0 (0.0, 0.0)
Rainbow trout (RBT)	100-500 Hz (Wysocki <i>et al.</i> 2007)	0.0 (0.0, 0.0)

## **Chapter Four: Management of Invasive Bigheaded Carp**

The high frequency hearing abilities of invasive bigheaded carp provides a novel management approach for invasive species. Using an acoustical stimulus is a practical, non-physical barrier because it targets specific species hearing ranges. Previous studies assessed the effectiveness of acoustical stimuli at altering behavior in silver and bighead carp (Vetter *et al.* 2015; Vetter *et al.* in preparation). The results of both of these studies showed that the broadband sound (motor boat) was effective at deterring silver and bighead carp.

Broadband sound could be an effective integrated management tool for controlling invasive bigheaded carp. Speaker arrays are relatively inexpensive and have a wide range of applications. Locks provide a great opportunity to implement a broadband sound barrier; the concrete walls would mimic the set-up of the previous study (Ch. 2) and could create high sound pressure levels in the lock that could deter passage while barges are moving through. Speakers broadcasting acoustic stimuli could also be used to herd carp into nets, removing them from wild populations. Wild bigheaded carp have been driven over 200 m downstream by sound alone (Mensing unpublished). So, the fish could be driven into a cove and then fishermen would be able to remove them. Overall, acoustic systems are inexpensive and multiple systems could be deployed in a single area. This deployment would allow for amplified sound gradients and would allow for backup systems.

For invasive species, management and prevention are the main goals of research. Silver and bighead carp are a major ecological and economical pest in areas where they have already invaded, so prevention through barriers is very important. The evolution of high frequency hearing, due to environmental conditions in silver and bighead carp, allows researchers an opportunity to develop alternative non-physical barriers to dampen the effects on native species while still being effective at controlling the spread of silver and bighead carp.

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