Behavior and Bioacoustics of Invasive Silver (*Hypophthalmichthys molitrix*) and Bighead (*H. nobilis*) Carp

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Brooke Janney Vetter

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Allen F. Mensinger, Adviser

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Abstract

Invasive silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp (collectively bigheaded carp) dominate the Mississippi River Drainage, outcompete native species, and continue to expand northward. Silver carp demonstrate an unusual jumping behavior to boat traffic. Understanding this response is important from a behavioral standpoint and critical to developing effective control methods. Experiments in outdoor concrete ponds (10 x 5 x 2 m), investigated the impact of pure tones (500 – 2000 Hz) and a recording from an outboard motor (0.06 – 10 kHz) on bigheaded carp swimming. Speakers broadcasting the sound stimuli were placed at either end of the concrete pond and bigheaded carp behavior was assessed using overhead cameras. Silver carp were consistently directed away (mean: 11.8 ± 1.3 consecutive responses) from the broadband sound (outboard motor recording) and the bighead carp were more responsive, exhibiting a median of 20.0 consecutive responses (1st Q: 12.0, 3rd Q: 23.0). However, both species were less reactive and habituated quickly to pure tones (after 1-2 trials). Field observations of silver carp jumping frequency, orientation, and direction in response to fast moving (16 – 40 km/hr) boats suggest that jumping is non-random, as the fish primarily move away from (79.8%) and behind the boat (> 90%), avoiding the area directly astern (< 4 m) in the most turbulent part of the wake. Finally, broadband sound (> 150 dB) stimulated wild silver carp to jump in a small tributary of the Illinois River. The fish demonstrated a different pattern than those reacting to the fast moving boats, with carp jumping all around the slow moving boat. This research suggests that sound can be used to alter the behavior of bigheaded carp with implications for deterrent barriers or other uses (e.g., herding fish to increase harvest).

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This doctoral dissertation is submitted as partial fulfillment for the degree of Doctor of Philosophy in the Integrated Biosciences Program at the University of Minnesota. Chapter 2 is a 2015 publication from *Biological Invasions* (17(2):3383-3392) and the co-authors are: Aaron R. Cupp, Kim T. Fredricks, Mark P. Gaikowski, and Allen F. Mensinger. Chapter 3 was submitted for publication and is undergoing the review process. Kelsie A. Murchy, Aaron R. Cupp, Jon J. Amberg, Mark P. Gaikowski, and Allen F. Mensinger were collaborators on this study. Chapters 4 and 5 are manuscripts in preparation for publication. Co-authors for Chapter 4 are: Andrew F. Casper and Allen F. Mensinger and the co-author for Chapter 5 is Allen F. Mensinger.

Chapter 1: Introduction

The effort to eradicate and prevent the range expansion of aquatic invasive species is a priority for natural resource managers, especially in the Laurentian Great Lakes and Mississippi River Drainage regions. There are over 180 invasive species with established breeding populations in the Great Lakes Basin, 35 of which are fish (Riccardi 2006, Melles et al. 2015). Invasion biologists have characterized two modes of establishment by a non-native species: reaction-diffusion and stratified diffusion. Reaction-diffusion describes invasion as the species expanding their range in a wave-like pattern from a central population. It first involves a colonization phase and subsequent expansion that is directly proportional to the time since first establishment and is inversely related to the distance from this initial establishment site (MacIsaac 2001). The other method, stratified diffusion, involves a population "jump" from one site of colonization to another mediated by vectors such as wind or humans.

Humans have facilitated such jumps by nonindigenous species from native populations across the Atlantic Ocean into the Great Lakes Region through shipping commerce and many researchers believe that the Great Lakes are the most susceptible freshwater system to invasion because of this heavy shipping activity (Pagnucco et al. 2015). Humans also unintentionally introduced silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carp (collectively bigheaded carp) to the Mississippi River Drainage from Eastern China. Similar to the many species that colonized the Great Lakes, these fish have undergone reaction-diffusion after the initial introduction, and now inhabit a much larger region in North America, including many rivers of the southern and mid-western states. Researchers and legislators alike are prioritizing the prevention of silver and bighead carp range expansion from the Illinois River into Lake Michigan, via the Chicago Sanitary and Ship Canal.

As silver and bighead carp are filter feeders, they were employed in fish farming, wastewater treatment, and aquaculture facilities in the southern United States in the 1970's as a

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biological control method to consume algae and phytoplankton (Kolar et al. 2007). They escaped

during periodic flooding events and were first discovered in tributaries of the Mississippi in the

early 1980's. Bighead carp are present in the waterways of 23 states while silver carp have

colonized 15 states. Although there are distinct physical characteristics that distinguish the two

species, they are similar and can hybridize. Successful invading species often have genetic

variability, phenotypic plasticity, high dispersal rates, and quick growth (Elton 1958). Bigheaded

carp are of ecological concern because they have high fecundity, few natural predators, and

opportunistically feed on abundant sources of phytoplankton and zooplankton, effectively altering

the lowest trophic level (Kolar et al. 2007, Sass et al. 2014). Furthermore, silver and bighead carp

experience rapid growth during the first year with both species exceeding 2 kg, quickly moving

beyond the size most susceptible to predation (Kolar et al. 2007). Bighead carp adults typically

reach about 40 kg while adult silver carp usually weigh no more than 30 kg.

Although silver and bighead carp negatively impact native species populations, these fish

are perhaps best known for silver carp's unusual jumping behavior in response to watercraft. In

carp infested water, entire river segments have been known to erupt with jumping fish when a

motorized boat moves through the area. While the bighead carp do not jump, they are often

associated with silver carp because the two species are so related and many of the hybrid

offspring demonstrate this behavior. The numbers of jumping carp seen in North America are not

present anywhere else in the world, including in their native range (Kolar et al. 2007, Duane

Chapman, personal communication). However, the behavior has been documented in other

regions invaded by silver carp. V.K. Soldatov first reported jumping silver carp in Russia in 1928,

speculating that the fish were afraid of the boat noise (Berg 1964). The behavior is not only

energetically costly, it is also harmful to the fish as they often collide with partially submerged

woody debris and boat hulls and can become stranded on shore. Silver carp jumping has been the

focus of news and social media outlets; however, little research has examined its physiological

and behavioral basis. Particularly, the stimulus for jumping and its relationship to both outboard motor sound and silver carp's hearing abilities remains unknown.

Understanding the hearing sensitivity of silver and bighead carp is important because researchers are investigating acoustic deterrents to prevent these fish from migrating further north. Sound has distinct characteristics in the underwater environment. Similar to sound in the air, underwater sound is propagated as a longitudinal wave, which consists of alternating periods of particle compression and rarefaction. However, as the molecules are closer together in a liquid than in a gas, sound moves about four times faster underwater than in air, at a rate of 1440 m/s. From the initial sound source, energy moves through water and air as both sound pressure and particle motion. For terrestrial animals, since particle movement attenuates so quickly in air, it is the sound pressure that the ear primarily senses. Similarly, sound pressure in water is also perceptible in the long range, however the properties of water allow for particle motion to be propagated further away from the source compared with sound in air. For instance, the wavelength of a 100 Hz sound is 15 m, meaning that particle motion can be detected up to at least that distance from the source (Sisneros and Rogers 2016). Fish perceive particle motion through direct stimulation on the bones of the inner ear, while sound pressure detection is less straightforward. It was originally thought that only fish with structures connecting the ear and the swim bladder or a gas bubble could hear sound pressure, however, it is now believed that fish without these hearing specializations can detect sound pressure in the inner ear, but are probably not as sensitive (Popper and Fay 2011).

In most teleosts, the inner ear is comprised of three semicircular canals and three otolith organs: the saccule, utricle, and lagena. The semicircular canals, like in many other vertebrate species, function in detecting angular accelerations of the head (Popper et al. 2000, Popper et al. 2005). The otolith organs house highly dense calcareous structures known as otoliths (sagitta in the saccule, lapillus in the utricle, and asteriscus in the lagena). Since fish tissue is only slightly

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more dense than water, their bodies move with equal phase and amplitude to the surrounding water when a sound is propagated through the environment. However, otoliths are three times denser than the fish's body and move with a different amplitude and phase than the water particles (Popper et al. 2000, Popper and Fay 2011). When the otoliths move, they bend long ciliary bundles that are embedded in the sensory epithelium and are connected to nerve endings, which send mechanical signals to the eighth cranial nerve. It is these ciliary bundles that allow fish to sense movement and hear, through a direct influence from the sound source via particle acceleration (Popper et al. 2000). It is also thought that the ciliary bundles of some otoliths can respond to pressure waves, though these mechanisms are not as well understood (Popper and Fay 2011).

Ostariophysans, including cypriniformes (minnows and carp), characiformes, siluriformes (catfish), and gymnotiformes (eels and knifefish), are characterized as having 1 – 4 bony structures that transmit vibrations from the swim bladder to the bones of the inner ear. The German physiologist Ernst Weber first described these structures, known as Weberian ossicles, in the 1800's (Weber 1819, 1820). Since Weber's initial conjecture that the ossicles function in hearing, researchers have investigated the relationship between these structures' connection to the swim bladder and fish sensitivity to high frequencies. For instance, deflating the swim bladder or removing the Weberian ossicles caused a reduction in goldfish (cyprinid) hearing sensitivity at lower frequencies (0.1 – 2 kHz) and loss of high frequency hearing (> 2 kHz) (Fay and Popper 1974, Ladich and Wysocki 2003). Poggendorf (1952) observed a less dramatic shift in hearing thresholds (for a frequency range of 0.06 – 6 kHz) for catfish with deflated swim bladders.

Silver and bighead carp are ostariophysans and have higher frequency hearing. Lovell et al. (2006) examined the auditory brainstem response (ABR) of silver and bighead carp to assess their hearing abilities and reported frequency sensitivity up to 3 kHz for both species. However, as there has been some criticism of the unusually flat tuning curve reported in this study (Ladich

and Fay 2013, Sisneros et al. 2016) and since the researchers did not test past 3 kHz, both species could be capable of hearing up to 5 kHz, which is the upper limit for other ostariophysans (Popper and Fay 2011). Although there are ostariophysans native to North America in the Great Lakes and Mississippi River Drainage regions, many of the indigenous species in these areas, such as lake sturgeon, paddlefish, and a variety of game fish (percids, esocids, and salmonids), do not have Weberian ossicles and cannot hear high frequency sound. Therefore, high frequency deterrents (up to 5 kHz) could be an effective management tool to target bigheaded carp.

The objective of this dissertation was to better understand the impetus for jumping in silver carp (i.e. the jumping trigger) and to identify sound stimuli capable of modulating bigheaded carp behavior. Captive silver and bighead carp were more reactive to broadband sound (0.06 – 10 kHz) recorded from an outboard motor than pure tone stimuli (500 – 2000 Hz), as both species demonstrated consistent negative phonotaxis to the broadband sound (Chapters 2 and 3). Chapters 4 and 5 examined silver carp jumping in the field. Wild carp primarily jumped behind and away from fast moving (16 – 32 km/hr) boats equipped with 100 and 150 hp motors in the Illinois River (Chapter 4). Finally, broadband sound alone stimulated jumping from wild silver carp in the Spoon River, a small tributary of the Illinois River (Chapter 5). Characterizing bigheaded carp responsiveness to sound stimuli and defining silver carp's jumping trigger is not only important from a behavioral standpoint but it is also critical to developing effective control methods as this behavior could prove to be silver carp's "Achille's fin."

Chapter 2: Acoustical Deterrence of Silver Carp (*Hypophthalmichthys molitrix*)

Abstract

The invasive Silver Carp (*Hypophthalmichthys molitrix*) dominate large regions of the

Mississippi River drainage and continue to expand their range northward threatening the

Laurentian Great Lakes. This study found that complex broadband sound (0 – 10 kHz) is effective

in altering the behavior of Silver Carp with implications for deterrent barriers or potential control

measures (e.g., herding fish into nets). The phonotaxic response of Silver Carp was investigated

using controlled experiments in outdoor concrete ponds (10 x 4.9 x 1.2 m). Pure tones (500 -

2000 Hz) and complex sound (underwater field recordings of outboard motors) were broadcast

using underwater speakers. Silver Carp always reacted to the complex sounds by exhibiting

negative phonotaxis to the sound source and by alternating speaker location, Silver Carp could be

directed consistently, up to 37 consecutive times, to opposite ends of the large outdoor pond.

However, fish habituated quickly to pure tones, reacting to only approximately 5% of these

presentations and never showed more than two consecutive responses. Previous studies have

demonstrated the success of sound barriers in preventing Silver Carp movement using pure tones

and this research suggests that a complex sound stimulus would be an even more effective

deterrent.

Introduction

Silver Carp (*Hypothalmichthys molitrix*) were introduced to aquaculture facilities in the

southern region of the United States from eastern Asia in the 1970's (Kolar 2005). The carp

initially were used as a biological method of controlling algal growth in sewage treatment and

fish farming facilities. Through a series of flooding events, the fishes subsequently escaped and

established populations throughout the Mississippi River Basin and are currently threatening the

Laurentian Great Lakes (Sass et al. 2010; Murphy and Jackson 2013). Carp have negatively

impacted native fish such as Paddlefish (*Polyodon spathula*) (Schrank et al. 2003), Gizzard Shad

(*Dorosoma cepedianum)* (Sampson et al. 2009), and Bigmouth Buffalo (*Ictiobus cyprinellus*) (Irons et al. 2007) due to their fast growth, prolific spawning, and ability to outcompete native fish for food and space. Additionally, Silver Carp demonstrate an unusual jumping behavior, which presents a hazard to boaters.

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

Since the early 1950's, researchers have examined sound to control fish movement (Burner and Moore 1953). Historically, research efforts have focused on using acoustic deterrents to prevent fish from entering hydropower dams or power plants (Schilt 2007). Ultrasound (122-128 kHz) was 87% effective in preventing Alewives (*Alosa pseudoharengus*) from approaching a dam intake in Lake Ontario (Ross and Dunning 1993). Maes et al. (2004) used a variety of frequencies (20-600 Hz) to repel Atlantic Herring (*Clupea harengus,* 94.7%) and European Sprat (*Sprattus sprattus,* 87.9%) from a power plant intake. In the past 20 years, acoustic deterrents, often coupled with bubbles or lights, have been used to modulate invasive fish behavior with the intent on preventing their range expansion (Noatch and Suski 2012). Pegg and Chick (2004) found 20 - 2000 Hz sound was more effective (95%) in preventing Silver and Bighead

(*Hypothalmichthys noblis*) Carp from crossing a bubble-sound barrier than frequencies in the 20-500 Hz range (57% effective). Similarly, sound (20 - 2000 Hz) combined with a bubble curtain successfully repelled Bighead Carp (95%) in an enclosed raceway (Taylor et al. 2005). Sound (500-2000 Hz), bubbles, and light impeded the upstream migration of Silver and Bighead Carp in a small tributary (Ruebush et al. 2012). Laboratory experiments demonstrate that bubble curtains, which generate 200 Hz frequency sound, can inhibit movement of Common Carp (*Cyprinus carpio*) (75-85%) (Zielinski et al. 2014). While strobe lights have some success in affecting fish movement, they also appear more effective when paired with sound or bubbles (Noatch and Suski 2012). These studies demonstrate the potential of acoustic deterrents for modulating fish behavior.

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

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

Material and Methods

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

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Each group (N=5) was allowed to acclimate in the outdoor pond for at least 48 hours prior to the

initiation of experiments. Two-day trials were conducted from July through September 2013.

Behavioral Experiments: Behavioral experiments were conducted in 10 m x 5 m x 1.2 m

(60k L) outdoor concrete flow through ponds (Fig. 1). Flow rate into the ponds was adjusted to

maintain a water temperature range of 17°C - 21°C. Water was pumped into the ponds directly

from UMESC wells. Although water quality was not measured, fish showed no signs of being

stressed due to poor water quality. Each pond was fully enclosed vertically by a 2 m wire fence

on the top of the pond walls with anti-bird netting draped across the top of the fence. Pond access

was restricted to a 2 m x 1 m wire door that remained locked throughout the experiment.

Sound stimuli: Sound was delivered via one of two pairs of underwater speakers (UW-30,

Lubell Labs Inc., Whitehall, OH) that were placed 1.5 m from each end of the pond and 1.6 m

from the nearest side-wall (Fig. 1). Acoustic stimuli consisted of pure tones (500, 1000, 1500, or

2000 Hz) generated by Audacity 2.0.5 software and complex tones recorded underwater from an

outboard motor (100 Hp Honda 4-stroke). The outboard motor sound was recorded using a

hydrophone (HTI-96-MIN, High Tech Inc., Long Beach, MS), in a section of the Illinois River

near Havana, IL, which contained Silver Carp populations. The sound was recorded in

approximately 1 m of water while the boat transited past the hydrophone at 32 km/hr, which also

stimulated carp to jump in the area.

Sound was amplified (UMA-752 amplifier, Peavey Electronics, Meridian, MS) and each

speaker pair was controlled manually with a switchbox (MCM Electronics, Centerville, OH).

Each pond contained a single hydrophone (HTI-96-MIN, High Tech Inc., Long Beach, MS) to

verify the sound stimuli, which was recorded using a PowerLab 4SP data acquisition system and

LabChart 7 software (AD Instruments, Colorado Springs, CO).

Sound pressure levels were maintained constant for the pure tones and complex sound

and were approximately 150 dB re 1 µPa @ 1m directly in front of the speakers, which was

approximately 30 dB re 1 µPa @ 1m above the minimum ambient noise (Fig. 2). All pure tone responses showed at narrow energy peak at the dominant frequency (Fig 3). The complex sound produced a broad spectrum of sound from 0.06 - 10 kHz with maximal energy contained in two relatively broad peaks from 0.06 - 2 kHz and 6 - 10 kHz (Fig. 3).

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

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

The experimental trials consisted of playing sound from one speaker pair, observing the behavioral response, and alternating the sound location if the fish swam away from the sound. Negative phonotaxis was defined as the group of fish orienting and swimming away from the end zone closest to the sound source within the first 15 seconds of sound onset and crossing the centerline (5 m) within 30 seconds. During these responses, the observer would continue to administer sound until fish reached the far end zone. Once the fish entered the opposite end zone from the midline, the sound source was changed to the speakers in that end zone. All behaviors not conforming to the criteria established for a negative phonotaxis, such as no reaction, swimming towards the speaker or failure to cross the midline in 30 seconds were categorized as no response. Consecutive responses were defined as fish reacting to two or more consecutive sound presentations from opposite ends to the pond. Sound trials were conducted with pure tones and complex sounds with the order of presentation (pure tones vs. complex) randomly determined

prior to each trial. Sound trials were completed over a two-day period for each of the five groups of fish with 3 to 4 pure tone and 4 to 11 complex sound trials conducted on each group.

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

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

Data Analysis: Fish position was monitored from 10 minutes prior to and throughout the sound presentation for sound trials. The position of the midpoint of the school was recorded every 5 seconds.

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

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

All statistical tests were performed with Sigmaplot for Windows, version 12.5. Shapiro-Wilk tests indicated that the response number and swimming speeds data were not normally distributed and therefore non-parametric Kruskal-Wallis ANOVAs with Dunn's post hoc tests were used to analyze the data. Although the response data were analyzed using non-parametric

tests, the mean ± 1 SE is reported for illustrative purposes as the median and quartiles for the pure tone frequencies were all 0. The median and upper and lower quartile is reported for the swim speeds ($P < 0.05$).

Results

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

Representative Silver Carp behavior to acoustic stimulation from two of the five groups is displayed in Figure 5. Controls demonstrate the typical slow swimming over the course of 10 minutes in the absence of sound stimuli (Fig. 5 control). Group A did not respond to the 500 and 1000 Hz and at the two higher frequencies (1500 and 2000 Hz), the fish responded to only the second of three pure tone presentations. Similarly, for Group B, the fish did not respond to pure tones at the 500, 1000, and 2000 Hz frequencies. During the first, second, and third 1500 Hz pure tone presentations, Group B demonstrated one response. In contrast, both groups responded to the complex sound after all the pure tone presentations. Consistent back and forth swimming along the length of the pond away from the active speaker pair during complex sound trials was

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observed (Fig. 5 complex) with Group B demonstrating 37 consecutive negative phonotaxic responses to the complex sound. Following the fourth sound presentation, Group A swam to the opposite wall of the pond but remained behind the speakers. They remained at this end for about a minute but then continued to react to the complex sound stimuli, demonstrating 26 consecutive responses for a total of 31 responses during this trial.

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

Discussion

Silver Carp demonstrated consistent movement away from complex sounds whereas pure tones were less successful in eliciting a reaction. At best, fish responded to two consecutive pure tones, but failed to react to over 95% of the presentations. Conversely, the complex sound alone was sufficient to reliably drive carp away from the source eliciting an average of eleven consecutive responses. This suggests that complex broadband sound (0.06 to 10 kHz), such as the outboard motor recording used, is more effective in affecting Silver Carp swimming than pure tones.

The Silver Carp habituated quickly to the pure tones as they demonstrated the characteristic decrease in responsiveness upon repeated exposure to the stimuli (Rankin et al. 2008, Thompson and Spencer 1966). For the complex sound, the fish usually stopped responding by the end of the 10 minute test period but it was unclear whether this was due to habituation or fatigue. In contrast to the pure tones, subsequent playbacks of the complex sound, after a recovery period, continued to elicit a response. This suggests that fatigue may have factored into reduced responses as the fish continually reacted to the alternating complex sound source at a significantly

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greater swim speed than during the pure tones or controls. Furthermore, despite repeated trials, the schools would still respond to at least three consecutive sound presentations. Finally, the decreased responsiveness to pure tones was behaviorally based as subsequent playbacks of the complex sound resulted in rapid movement away from the sound, indicating that the auditory system was functional and the fish were able to locate the sound source.

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

The effects of high frequency anthropogenic sound on native ostariophysans, such as minnows, suckers, and catfish, remains to be determined. In a laboratory study involving Fathead Minnows (*Pimephales promelas*), exposure to white noise (0.3 to 4.0 kHz dB re µ1 Pa) significantly increased auditory thresholds, especially in the higher frequency range (0.8 to 2.0 kHz) and persisted for at least 14 days after exposure (Scholik and Yan 2001). Anthropogenic noise is also thought to affect fish behavior. For example, Blacktail Shiners (*Cyprinella venusta*) increased the amplitude and rate of mating calls in the presence of background noise (Holt and Johnston 2014). One of the most prominent sources of anthropogenic noise is recreational and commercial motorized watercraft and negative effects of these sounds on fish are well documented (Scholik and Yan 2002b, Liu et al. 2013, Voellmy et al. 2014, Popper and Hastings 2009, Whitfield and Becker 2014). More research on the effect of high frequency sound on native species, especially ostariophysans, is essential before acoustic deterrents can be implemented.

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

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

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

(N=101) crossed the barrier; however it was unclear how many fish challenged the barrier or remained in the area.

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

The range expansion of invasive Silver Carp is a concern to many state and federal agencies as the fish threaten entire food webs and the jumping behavior of Silver Carp endangers recreational and commercial boaters. This study's objective was to determine the effects of sound on modulating Silver Carp behavior. The results suggest that the complex sound may be an

important management tool and could be effective either on its own or integrated with other

deterrent technology.

Acknowledgements

Figure 1: A) View from the entry door of a drained experimental pond. Speakers are at the near (only one visible) and the far (pair) end of the pond. Water level was maintained within 5 cm of the top of the concrete walls. The fence enclosing the pond is visible at the top of the walls. Gridlines painted on pond bottom assisted in assessing fish position. **B).** Overhead schematic of the experimental pond showing approximate location of gridlines and speakers (solid rectangle).
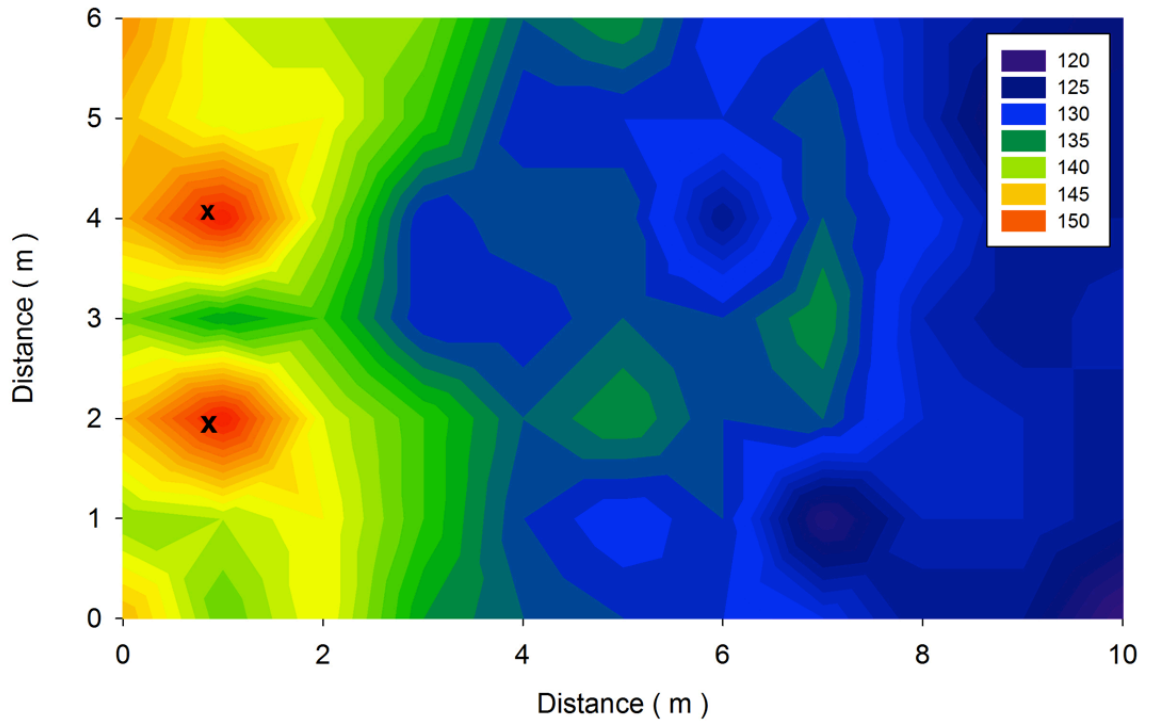
Figure 2: The sound intensity level (dB) is plotted during active broadcast of the two underwater speakers (indicated by X). Recordings were made at 1 m intervals and a depth of 0.6 m. Intensity level is indicated by color in upper right insert.
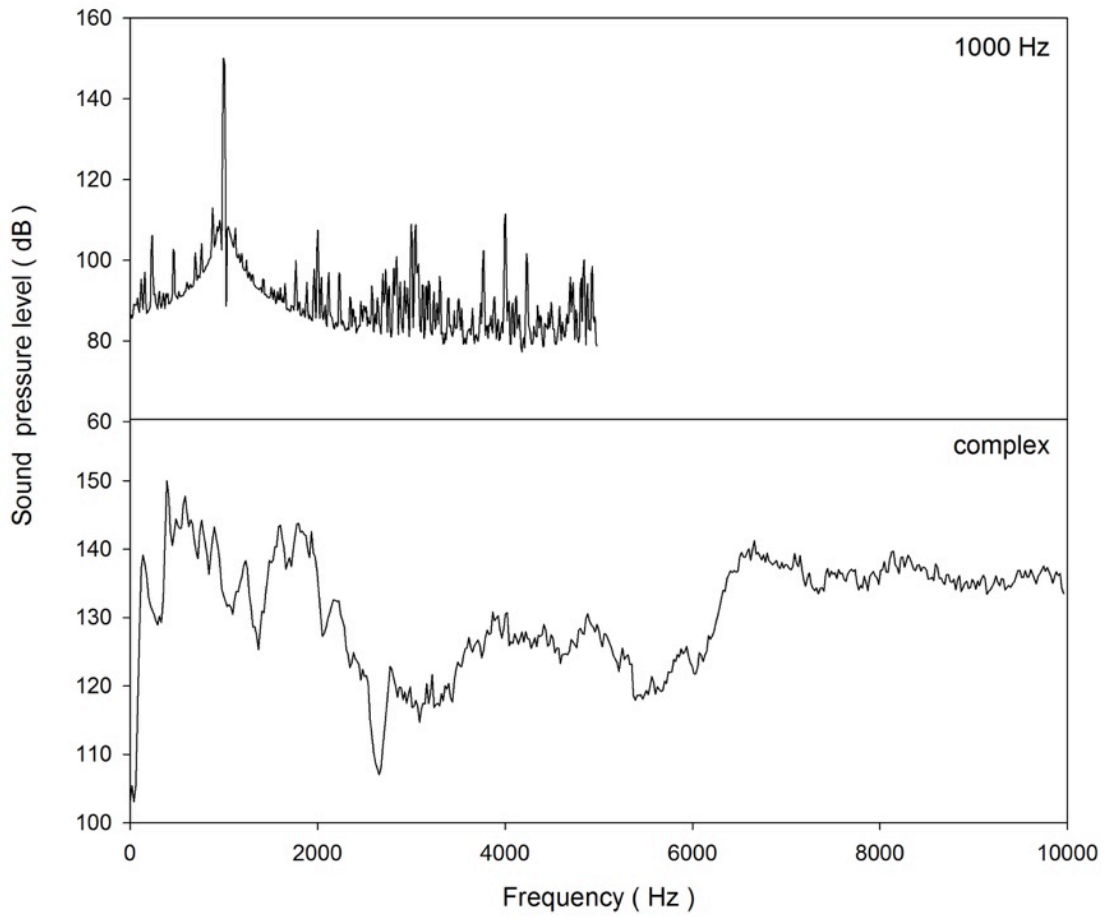
Figure 3: The power spectrum in dB of the 1000 Hz and complex sound stimulus is plotted versus frequency (Hz).
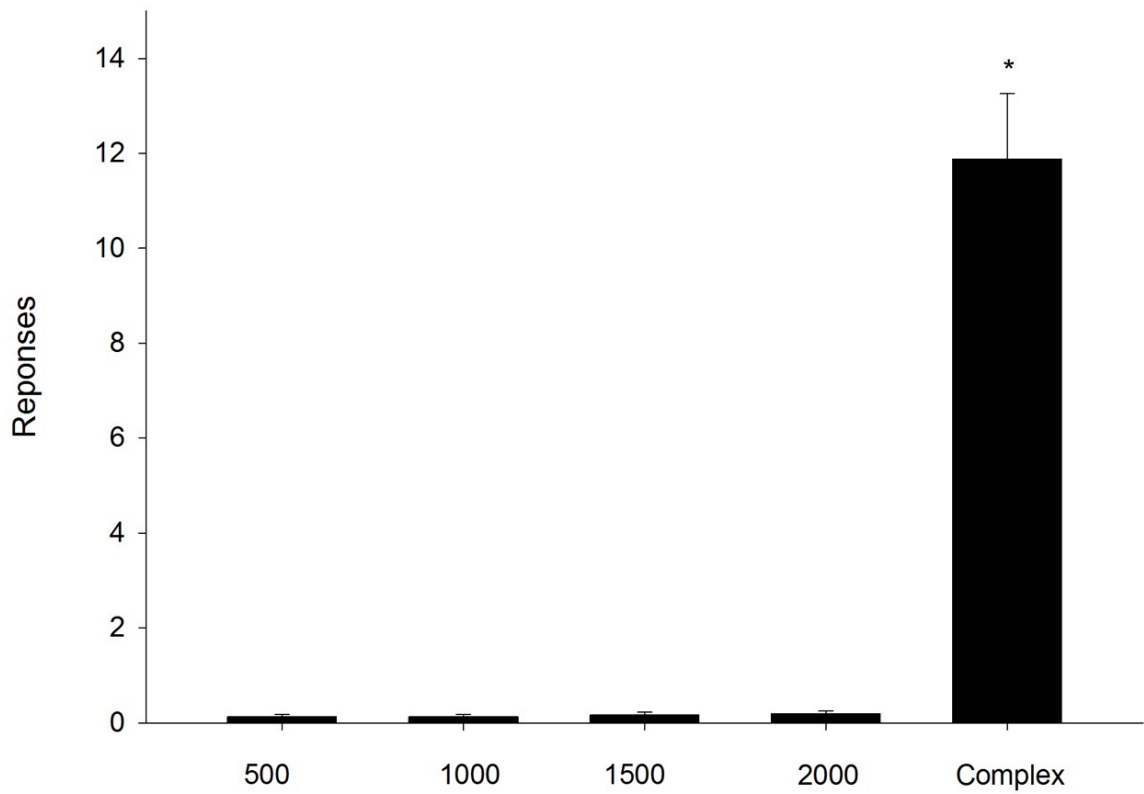
Figure 4: Average number of responses per trial to sound playback versus sound stimulus type (500, 1000, 1500, and 2000 Hz and complex sound). All data show the mean ± 1 standard error. * indicates significantly different group (P < 0.001).
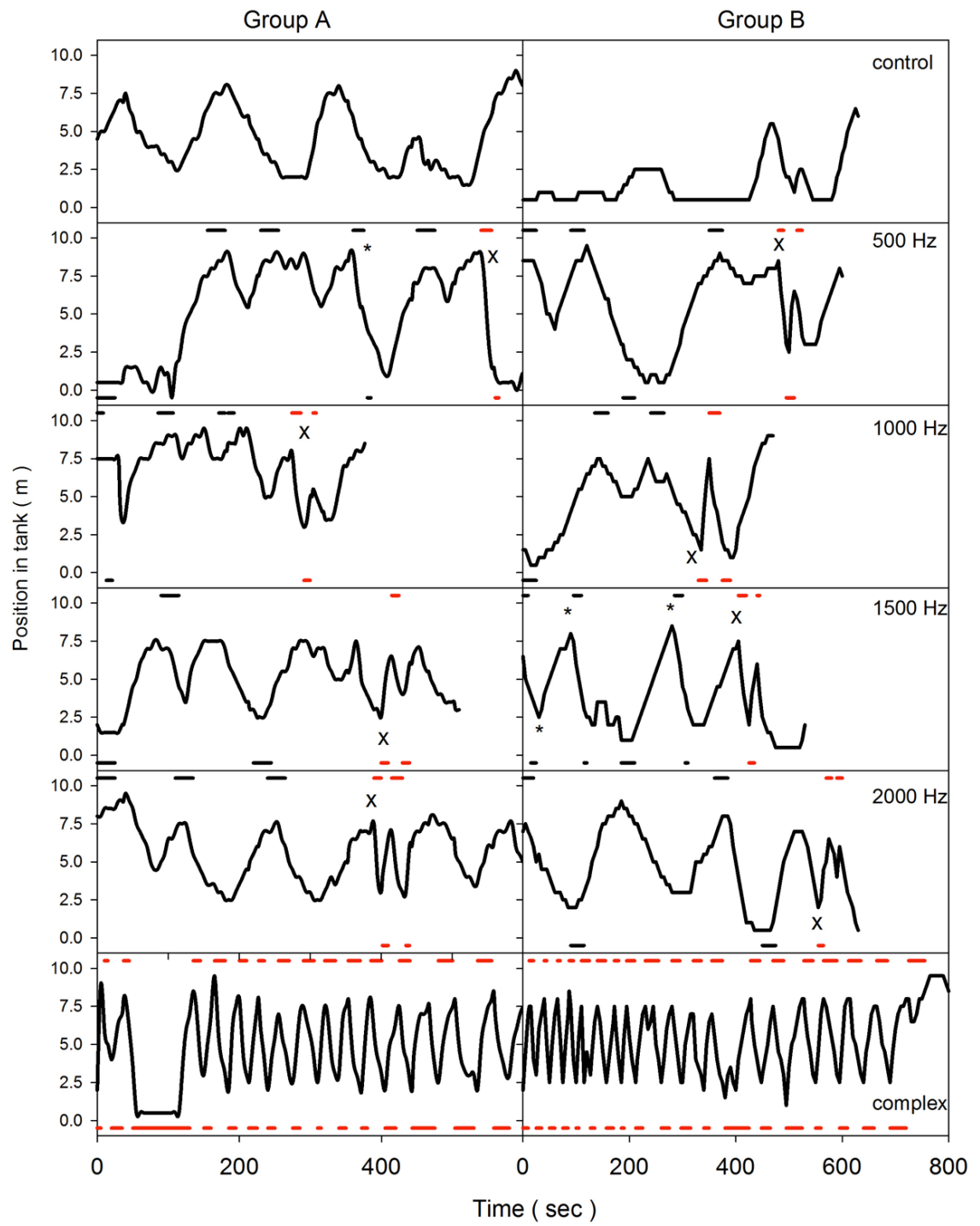
Figure 5: Representative Silver Carp behavioral response to acoustic stimulation for two groups of fish (Group A and Group B). For each figure, the longitudinal position (m) of the center of the school is plotted versus time (s) with fish position mapped every 5 seconds. Solid lines above and below each fish position trace indicate the location and duration of the sound stimulus. Black indicates pure tones and red indicates complex motor sounds. * indicates no response and X represents negative phonotaxis; in situations where the fish demonstrates consecutive responses, the first response is indicated by an X **A)** Control (no sound); **B)** 500 Hz; **C)** 1000 Hz; **D)** 1500 Hz; **E)** 2000 Hz; **F)** Complex sound.
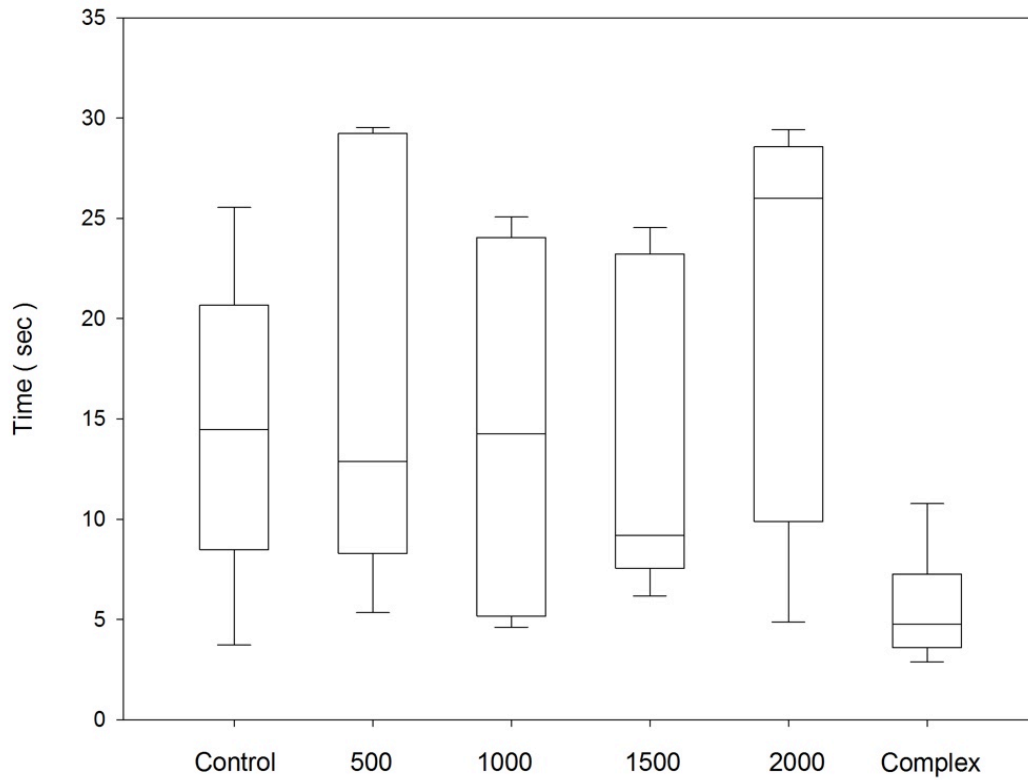
Figure 6: Fish swim speeds. Box and whisker plots display the median and upper and lower quartile for the time for fish to swim 2 meters after sound stimulation. The control represents the average time (< 30 seconds) to swim 2 m in the absence of sound. (P < 0.001).

Chapter 3: The effect of broadband sound on the movement of bighead carp

(*Hypophthalmichthys nobilis*)

Abstract

Recent studies have shown the potential of acoustic deterrents against invasive silver carp

(*Hypophthalmichthy molitrix*). This study examined the phonotaxic response of the bighead carp

(*H. noblis*) to pure tones (500 – 2000 Hz) and playbacks of broadband sound from an underwater

recording of a 100 hp outboard motor (0.06 – 10 kHz) in an outdoor concrete pond (10 x 5 x 1.2

m) at the U.S. Geological Survey Upper Midwest Environmental Science Center in La Crosse,

WI. The number of consecutive times the fish reacted to sound from alternating locations at each

end of the pond was assessed. Bighead carp were relatively indifferent to the pure tones with

median consecutive responses ranging from 0 to 2 reactions away from the sound source.

However, fish consistently exhibited significantly ($P < 0.001$) greater negative phonotaxis to the

broadband sound (outboard motor recording) with an overall median response of 20 consecutive

reactions during the 10 minute trials. In over 50% of broadband sound tests, carp were still

reacting to the stimulus at the end of the trial, implying that fish were not habituating to the

sound. This study suggests that broadband sound may be an effective deterrent to bighead carp

and provides a basis for conducting studies with wild fish.

Introduction

The bighead carp (*Hypophthalmichthys nobilis*) is an invasive fish species in North

America and has established breeding populations in the Mississippi River Watershed. Range

expansion of these fish into the Great Lakes is a concern because they are present in the northern

regions of the Illinois River (Kolar et al., 2005; Sass et al., 2010) and have been found in the

Chicago Sanitary and Ship Canal (Moy et al., 2011) near Lake Michigan. These fish, along with

the closely related silver carp (*H. molitrix*), evolved in Asia and were intentionally brought to the

United States for use in wastewater treatment plants and aquaculture facilities (Kolar et al., 2005;

Kelly et al., 2011). Both species are an ecological concern because they compete with native species, such as paddlefish (*Polyodon spathula*; Schrank et al., 2003), gizzard shad (*Dorosoma cepedianum*; Sampson et al., 2009), and bigmouth buffalo (*Ictiobus cyprinellus*; Irons et al., 2007), for food and space. While adults from both species can grow up to 40 – 50 kg, they are planktivores, which precludes them from being caught via angling or baited traps. Furthermore, these filter feeders will consume both zooplankton and phytoplankton and could alter the entire food web in rivers where they are abundant (Sass et al., 2014).

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

Bighead and silver carp are ostariophysans and possess Weberian ossicles, which connect the gas bladder to the inner ear (Fay and Popper, 1999), allowing for higher frequency hearing than non-ostariophysan species. Lovell et al. (2006) indicated silver and bighead carp sensitivity up to 3 kHz. However, this study has been criticized because higher frequency sound (> 3 kHz) was not examined and the tuning curve was unusually broad (Ladich and Fay, 2013; Sisneros et al., 2016). Other studies have indicated that frequency sensitivity in ostariophysans can reach 5 kHz and this may be the upper level for bighead carp (see Popper and Fay, 2011 for a review).

The silver carp is notorious for its jumping behavior, which can be elicited when motorized watercraft move through carp infested areas. Playbacks of the broadband (0.06 – 10

kHz) sound emitted by outboard motors caused wild silver carp to jump (Mensinger, unpublished) and elicited negative phonotaxis in captive fish (Vetter et al., 2015), however bighead carp do not jump (Kolar et al., 2005). Therefore, the effect of similar acoustic stimulation on bighead carp is unknown, as their underwater behavior is difficult to monitor in turbid water. Since silver and bighead carp coexist and will hybridize, if bighead carp are affected similarly by sound, the two species could be co-managed.

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

Methods

Animal Husbandry

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

Behavioral Experiments

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

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

The sound was amplified with a UMA-752 amplifier (Peavey Electronics, Meridian, MS) and each speaker pair was controlled manually with a switchbox (MCM Electronics, Centerville, OH). Each pond contained a single hydrophone to verify the sound stimuli, which were recorded using a PowerLab 4SP data acquisition system and LabChart 7 software (AD Instruments, Colorado Springs, CO). To map the acoustic field, recordings of the broadband sound and 1000 Hz pure tone were made at 75 positions throughout the tank at a depth of 0.6 m. Sound pressure levels were 156 dB re 1 µPa directly in front of the speakers for both pure tones and broadband sound and dropped to 122 dB re 1 µPa at the far end of the pond (Fig. 1). All pure tone stimuli showed a narrow energy peak at the dominant frequency (Fig. 2). The broadband sound produced

a spectrum of sound from 0.06 – 10 kHz, with maximal energy contained in two peaks from 0.06 – 2 kHz and 6 – 10 kHz (Fig. 2).

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

The bighead carp demonstrated schooling behavior and therefore the fish in each trial were treated as a single unit, with position determined as the approximate center of the school. An "end zone" was established at each end of the pond and was defined as the area of the pond within 2.5 meters of the end wall. The order of stimuli presentation (pure tones vs. broadband sound) was randomly determined before each trial. Four broadband and 12 – 16 pure tone sound trials (3 – 4 trials for each 500, 1000, 1500, and 2000 Hz pure tones) were conducted on each group. Experimental trials were initiated by playing a 30 second sound stimulus from the speaker pair in the end zone occupied by the fish. If the fish swam away from the sound and crossed the centerline of the pond within 30 seconds, then the sound source was alternated to the opposite end of the pond when the fish reached the far end zone. This procedure was immediately repeated, and the sound stimulus replayed, until the fish stopped responding to the sound or 10 minutes elapsed. Consecutive responses were defined as fish reacting to two or more sound presentations from opposite ends of the pond. If the fish did not cross the centerline within 30 seconds, remained in the same location, or swam towards the sound, then this behavior was scored as no response and the stimulus was not played from the opposite speaker pair. PowerLab recordings

were time synchronized with video recording, to compare the onset of sound with the carp's behavioral response. Furthermore, when alternating the sound source, the active speakers were turned off before the opposite speaker pair was powered on, leaving approximately a one second sound gap when speakers were switched during sound playback.

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

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

Data Analysis

Fish position was monitored during the 10 minutes before (control) and through the application of the sound stimulus for every trial by recording the position in meters (x, y) of the midpoint of the school every 5 seconds. Swim speed was quantified for experimental fish that reacted to the sound using frame by frame analysis of the video recording (30 frames per second). The elapsed time from when the fish turned and swam 2.0 m away from the sound stimulus was calculated and the swim speed determined. The swim speeds were only assessed for groups of fish that reacted to the sound stimulus and swam 2.0 m in less than 30 seconds. To accurately compare response times, groups that took longer than 30 seconds or did not respond, were excluded from analysis which included 68.3% of 500 Hz, 61.5% of 1000 Hz, 30.0% of the 1500 Hz, 48.4% of 2000 Hz, and 0% of broadband trials. For controls, fish were observed for a 10 minute period of continuous swimming in the absence of sound and the time it took the school to transverse 2 m intervals was determined (15.6% of the time, fish took longer than 30 seconds to swim 2 m and these values were not included). Controls were determined prior to testing or at least an hour after the last exposure to sound stimuli.

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

Results

The sound recorded within 1 m of the front of the speaker showed that for the pure tones, most of the energy was centered around the dominant frequency, while the broadband sound ranged from 0.06 – 10 kHz, with the highest energy contained in frequencies less than 2 kHz (Fig.

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2). Sound pressure levels peaked in front of and behind the speakers and the end zone nearest the active speakers was saturated with the sound stimulus, with sound attenuating before reaching the far end of the pond (Fig. 1). Figure 3 indicates the attenuation and change in the power spectrum at all locations in the pond.

Swimming Behavior: Figures 4 and 5 show the swimming behavior from one representative school of bighead carp from control and experimental trials. During the control trials, the fish primarily moved around the perimeter at a relatively consistent speed, with the school shown completing approximately 5.5 circuits of the pond over the 10 minute observation period (Fig. 4A, Fig. 5 Control). In contrast, the fish responding to the broadband sound favored the longitudinal center of the tank when moving away from the sound source (Fig. 4B). The school in Figure 5 showed no response to the 500, 1000, or 2000 Hz pure tones, as the fish either remained in the area or swam towards the stimulus (Fig. 5). For the 1500 Hz tone, fish reacted once to the first playback and twice to the third but then stopped responding and remained in the same area even though the sound was present. However, at the end of every pure tone trial, the fish responded at least once to the broadband sound stimulus. The same school responded to the broadband sound stimulus 23 consecutive times over the 10 minute trial (Fig. 5 Broadband).

Responses and Swim Speed: When comparing the results from all five groups, the carp were significantly (ANOVA $P < 0.001$) more reactive to the broadband sound (20.0 consecutive responses; 12.0, 23.0) than to the pure tones [500 Hz: 0.0 (0.0, 2.0); 1000 Hz: 1.0 (0.0, 2.0); 1500 Hz: 2.0 (0.0, 4.0); 2000 Hz: 1.0 (0.0, 5.0)] (Fig. 6). Behavior during the pure tone trials was inconsistent and not sustained, as the median consecutive response did not exceed 2.0 for any frequency. While the fish always retreated from the broadband sound, they responded to only 53% of pure tone presentations with one third of these trials (~17% of total) eliciting more than one reaction.

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The number of reactions throughout the two-day testing period remained consistent with no significant decrease (ANOVA $P > 0.10$) in responses between consecutive trials to the broadband sound (Fig. 7). Furthermore, in 58% of tests, the carp were still responding to the broadband sound when the 10 minute trials were terminated. The bighead carp demonstrated significantly faster swimming (median swim speed: 0.47 m/s) when moving away from the broadband sound than the pure tones or control swimming ($P < 0.001$) (Fig. 8).

Discussion

Throughout the experiment, the bighead carp schooled and in the absence of sound, primarily swam circular routes along the pond walls. However, their behavior changed quickly when presented with broadband sound and they moved directly away from the sound source by swimming through the middle, longitudinal axis of the pond. Furthermore, the highest number of consecutive responses and the fastest swim speeds were observed when bighead carp were reacting to the broadband sound.

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

While the exact hearing range and sensitivity of bighead carp remains unknown, these fish possess Weberian ossicles, allowing relatively higher frequency hearing than non-ostariophysan fish. Using auditory evoked potentials (AEP), Lovell et al. (2006) reported

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frequency sensitivity up to 3 kHz, however the tuning curve was unusually flat and higher frequencies were not tested. Additionally, the study was limited due to acoustic challenges with the small tank and the use of auditory evoked potentials, a technique that is not always an accurate measure of fish hearing sensitivity (Ladich and Fay, 2013; Sisneros et al., 2016). Based on the behavior evidence reported in the current study, it appears that the pure tones and at least a portion of the broadband stimulus were within the frequency sensitivity of bighead carp.

The ability of bighead and silver carp to localize sound is also necessary for acoustic deterrents to have practical application. Experiments on related cyprinids suggest that silver and bighead carp can also localize sound. Common carp (*Cyprinus carpio*; Sloan et al., 2013) and grass carp (*Ctenopharyngodon idella*; Willis et al., 2002) were trained to associate pure tones (400 – 1000 Hz) with a food stimulus and retained the behavior for months after initial conditioning. The negative phonotaxis exhibited by the bighead carp demonstrated that the fish could approximate the sound source location, at least by detecting the sound gradient.

An acoustic deterrent must also balance high sound pressure, which provides greater range and/or increases its efficacy, with the risk of causing hearing damage in fish species. Smith et al. (2004) found that goldfish exposed to 130 – 170 dB white noise became acclimated after 10 minutes of exposure and experienced hearing loss at the higher sound pressure levels, however these experiments were conducted in much smaller tanks (19 – 600 L) than the present study. The maximum SPL in the experimental ponds was 156 dB re 1 µPa in a small area near the speakers where the fish spent minimal time during playback. Although it is possible that some hearing loss occurred, the bighead carp's continued phonotaxic behavior suggests fish experienced minimal impact on hearing sensitivity. Furthermore, their repeated responses indicate that the fish could approximate the sound location and/or detect the sound gradient. It also did not appear that the bighead carp were habituating to the broadband sound, as the carp were still reacting to stimulus from the active speakers in 58% of trials when the test was terminated. Additionally, there was

not a significant decrease in responsiveness to the broadband sound over the two-day testing period. As both swimming duration and speed were elevated during playbacks, non-responding fish may have been fatigued rather than habituated to the sound.

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

There are limitations with applying this study to wild fish because of the inherent challenges in small tank acoustics, the differences between captive and wild fish behavior, and the modified broadband sound broadcast to the bighead carp. Echoes are produced from interactions of the sound with the water surface and with the pond's bottom and walls, creating a difficult acoustic environment for the fish to localize the sound source, even in larger concrete ponds like the one used in this study (Gray et al., 2016). Compared to field conditions, the pond is

suboptimal with a complex echoic environment complicating sound localization (Gray et al., 2016) and providing limited space for the fish to escape. However, the pond's concrete composition closely replicates a lock chamber (on a smaller scale), where the technology may be eventually placed. Although there are differences in the sound field of a concrete tank when compared with a natural environment, controlled experiments can be useful to compare fish behavior when other the conditions (i.e. methods, speakers, tank, fish size, etc.) remain consistent (Rogers et al., 2016), which was the case with this experiment. Therefore, despite the limitations of the small pond, the results are encouraging for the use of acoustic deterrents as part of an integrated pest management system.

Two recent reviews have cautioned against applying behavioral results from captive fish to those in the wild (Popper et al., 2014; Hawkins et al., 2015). However, preliminary results from a field study that exposed resident silver and bighead carp in the Spoon River near Havana, IL to broadband sound, demonstrated that silver carp jump in response to the acoustic stimulus alone. Furthermore, concurrent sonar indicated that all putative carp (species identification was not possible) exited the area and that the sound could displace fish at least 200 meters from the source (Mensinger, unpublished). This suggests that sound could be effective in modulating wild fish behavior and provides a strong argument for further research exploring the efficacy of acoustic deterrents in carp infested waters. The pond was modest in size and prevented fish from swimming more than 9.0 m from the source and the continual alternation of the sound source probably generated fatigue in a portion of the schools, neither of which would be a factor with longer distance repulsion and less frequent sound exposure in a natural setting.

Finally, playback of the outboard motor recording through the UW-30 did modify the sound due to the speaker characteristics, however the goal of the study was to identify sound that caused consistent negative phonotaxis and not rebroadcast the exact sound spectrum of the outboard motor in high fidelity. The playbacks were effective in accomplishing the goals of the

study. Additionally, while particle motion, which was not measured in this study, may have given greater insights the acoustic environment in the pond, it was not necessary to accomplish the experimental objectives. Furthermore, future deterrents will be tested in much larger ponds or in the field and the same particle motion environment of a small pond would be difficult to recapitulate.

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

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

relationship. The similar responses of bighead and silver carp to broadband sound stimuli suggest that incorporation of these sounds into the integrated pest management programs of natural resource agencies may be successful in altering fish behavior.

Acknowledgements

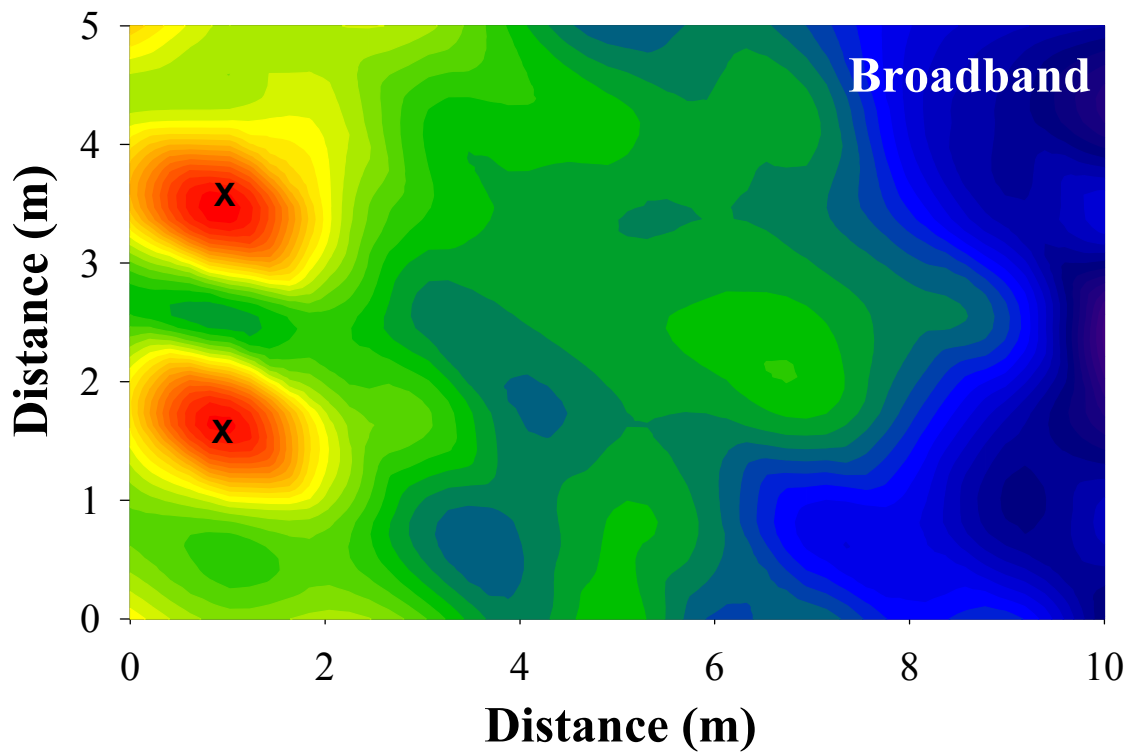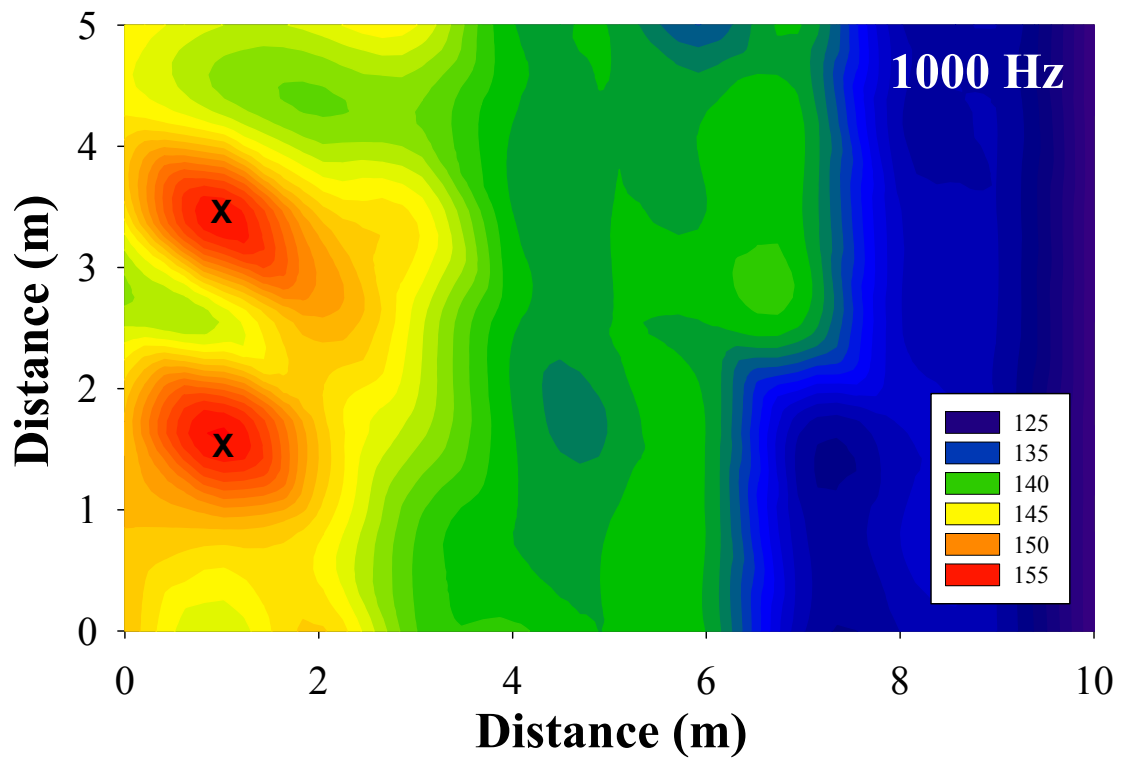**Figure 1:** Sound intensity in the experimental pond. Speaker location is indicated by an X. The sound intensity was measured using a hydrophone at a depth of 0.6 m at 1 m x 1 m intervals throughout the pond during broadband sound playback. The colors represent the sound intensity level (dB re μPa @ 1 m), indicated in the scale on the right, for the **A)** Broadband sound stimulus and the **B)** 1000 Hz pure tone.
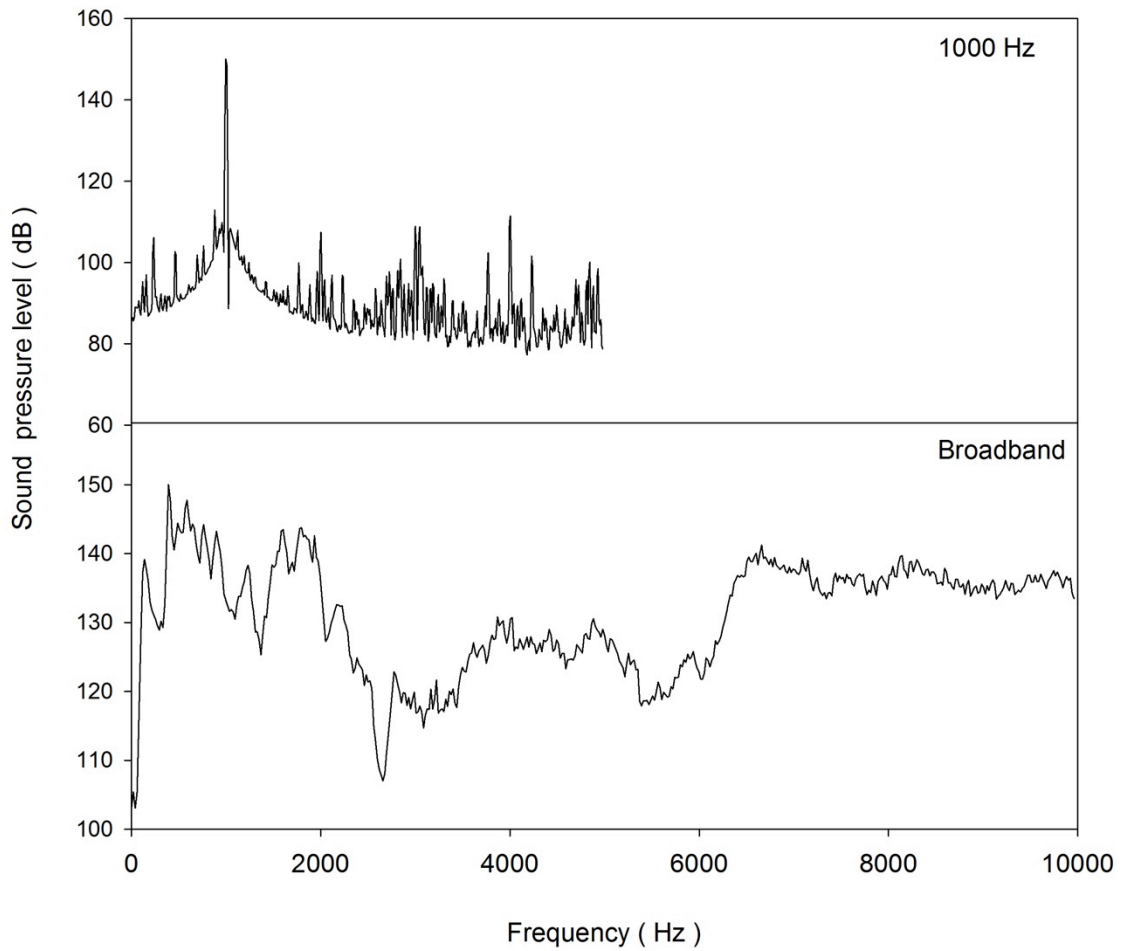
Figure 2: The power spectrum of the pure tone (1000 Hz) and broadband sound is plotted versus frequency from a hydrophone a depth of 0.6 m directly in front of the speaker (< 1 m) during sound playback in the pond. (Modified from Vetter et al. 2015)

North

Relative SPL (dB)

0
-40
-80

0   10
Frequency (kHz)   **South**

44
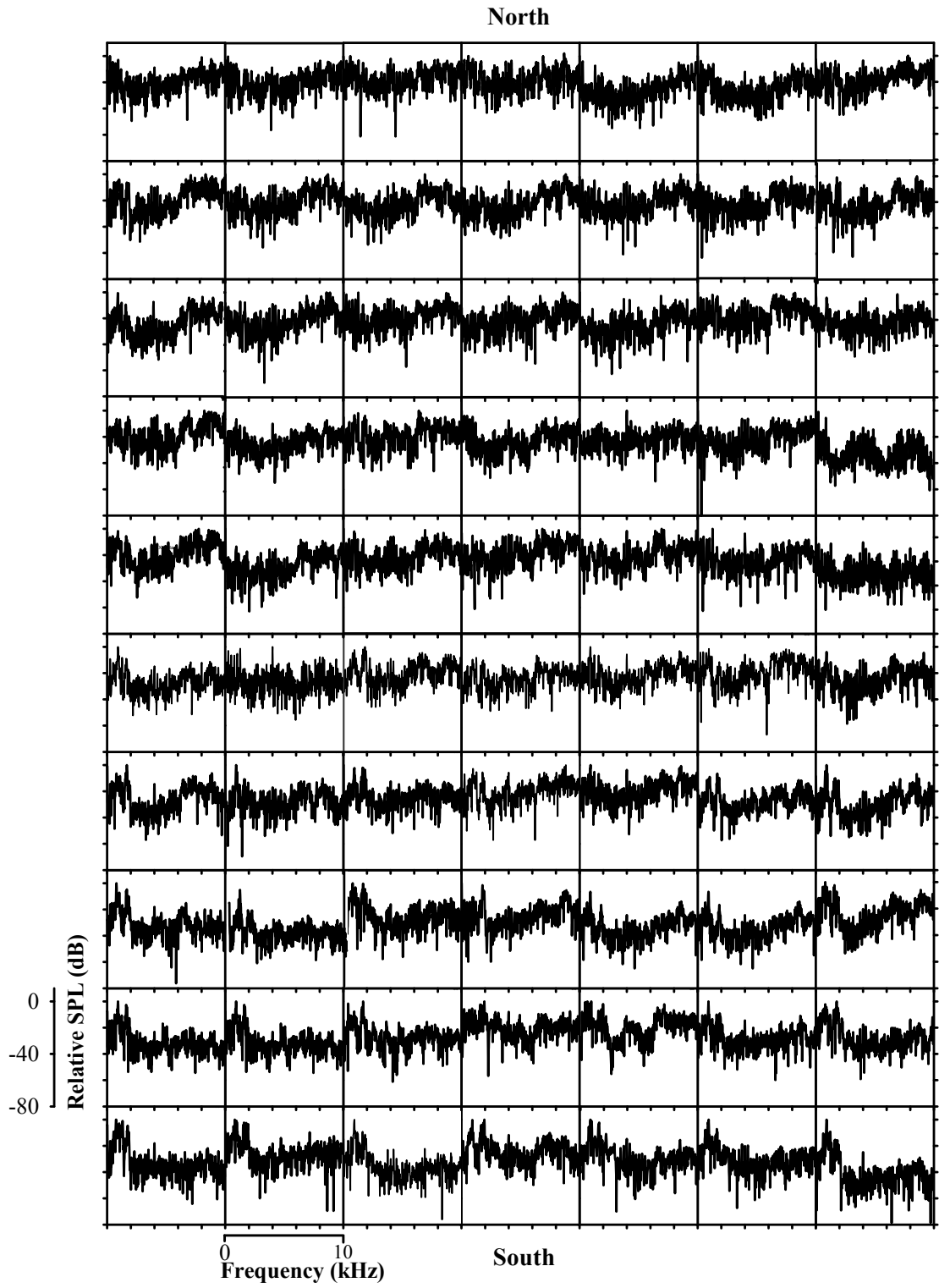
Figure 3: The power spectrum from 70 locations in the pond is plotted versus frequency during playback of the broadband sound. The maximum sound pressure level for each location was assigned a decibel level of 0 and the rest of the frequencies in each spectrum are relative to maximum sound pressure.
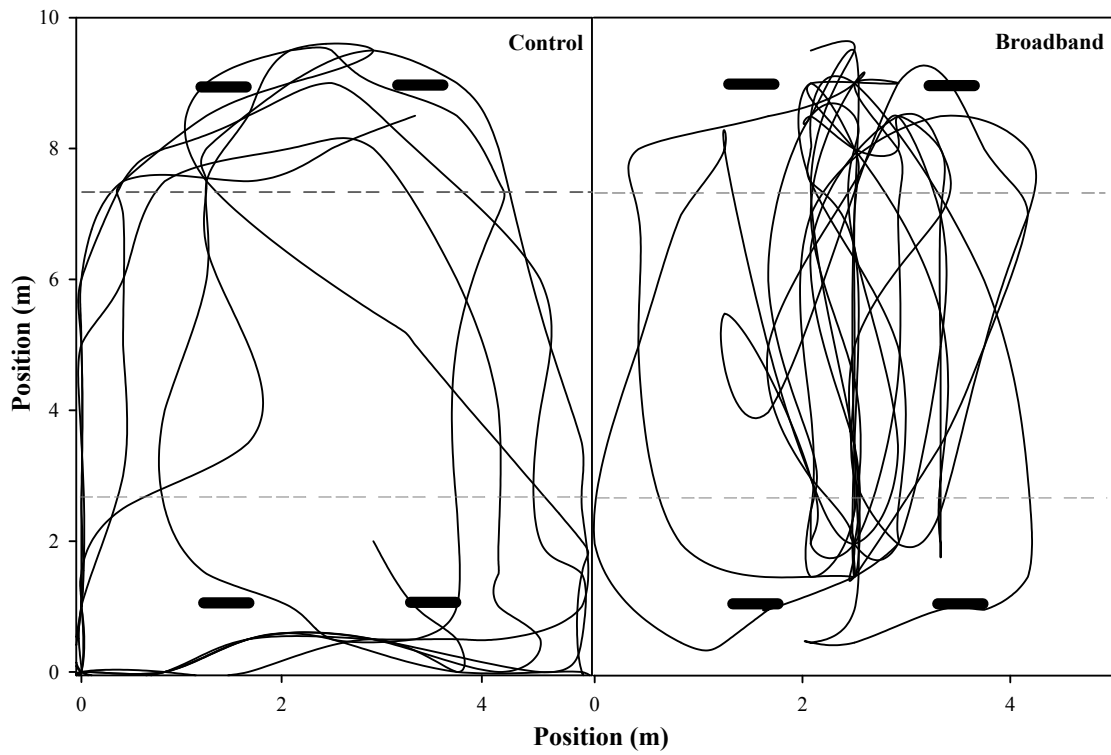
Figure 4: Bighead carp swimming behavior. The solid black lines represent the speaker location and the dotted line represents the "end zones." The traces mark the horizontal and longitudinal position (m) of the center of one representative school of bighead carp during a 10 minute broadband sound and control trial, with the fish position mapped every 5 seconds. **A)** Broadband sound trial **B)** Control swimming behavior

Broadband

500 Hz

1000 Hz

1500 Hz

2000 Hz

Control

Position (m)

Time (s)

47
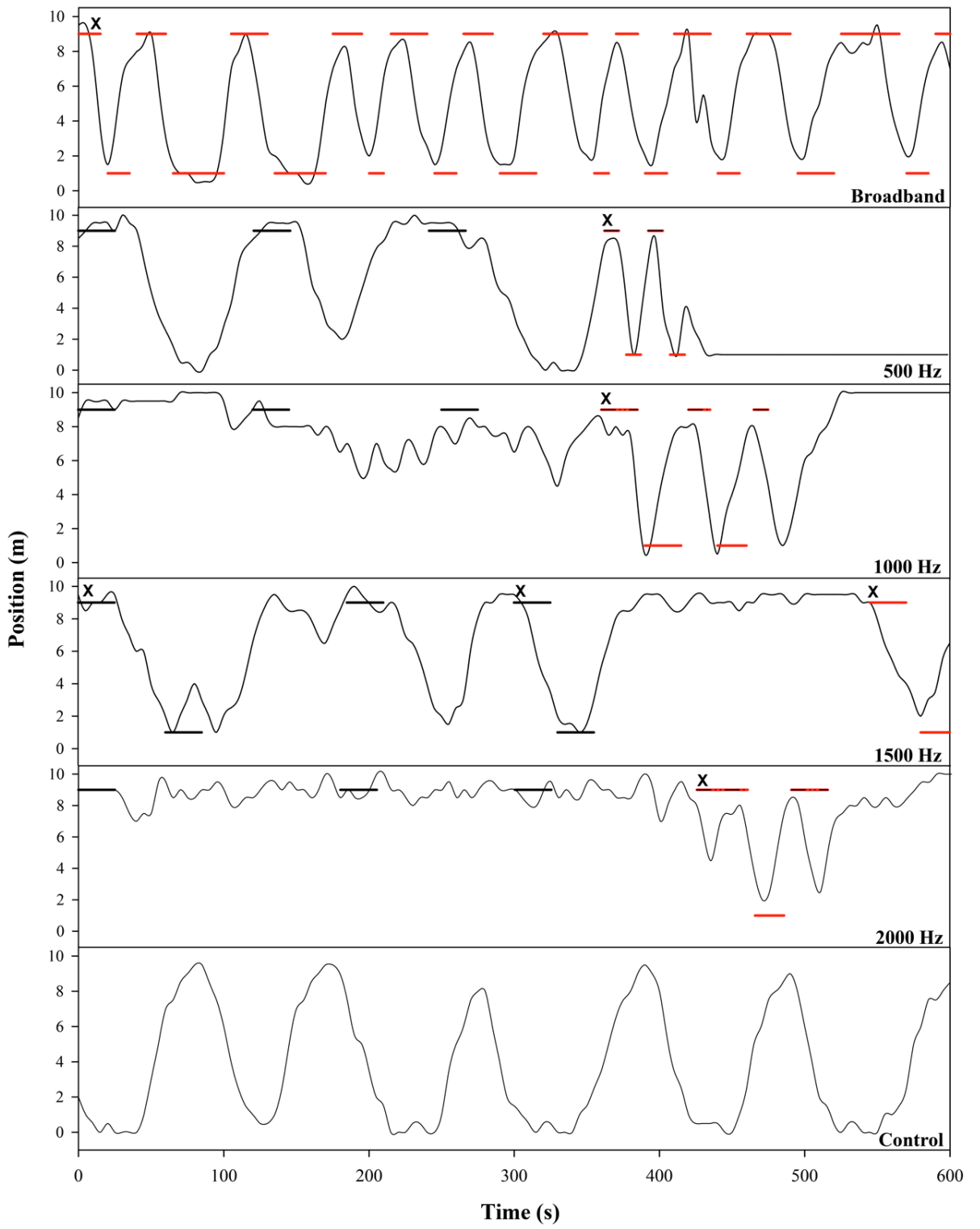
Figure 5: Representative bighead carp behavioral response to acoustic stimulation for one group of fish. The longitudinal position (m) of the center of the school is plotted versus time (s) with fish position mapped every 5 seconds. Solid lines above and below each fish position trace indicate the location and duration of the sound stimulus (black = pure tone; red = broadband sound). The X represents the first negative phonotaxis of a series or resumption after non-responsive trials. For clarity, successive responses in a series are not labeled. **A)** Broadband sound **B)** 500 Hz **C)** 1000 Hz **D)** 1500 Hz **E)** 2000 Hz **F)** Control (no sound)
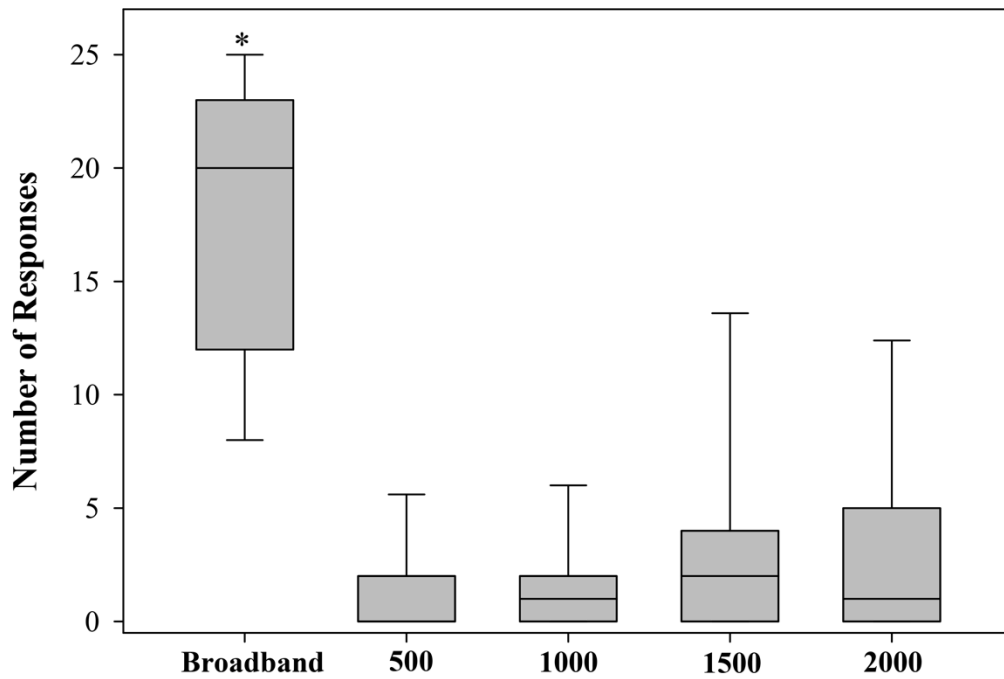
Figure 6: Consecutive responses per trial to sound playback versus sound stimulus type (500, 1000, 1500, and 2000 Hz and broadband sound). For each box, the boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. * indicates significantly different group (P < 0.001).
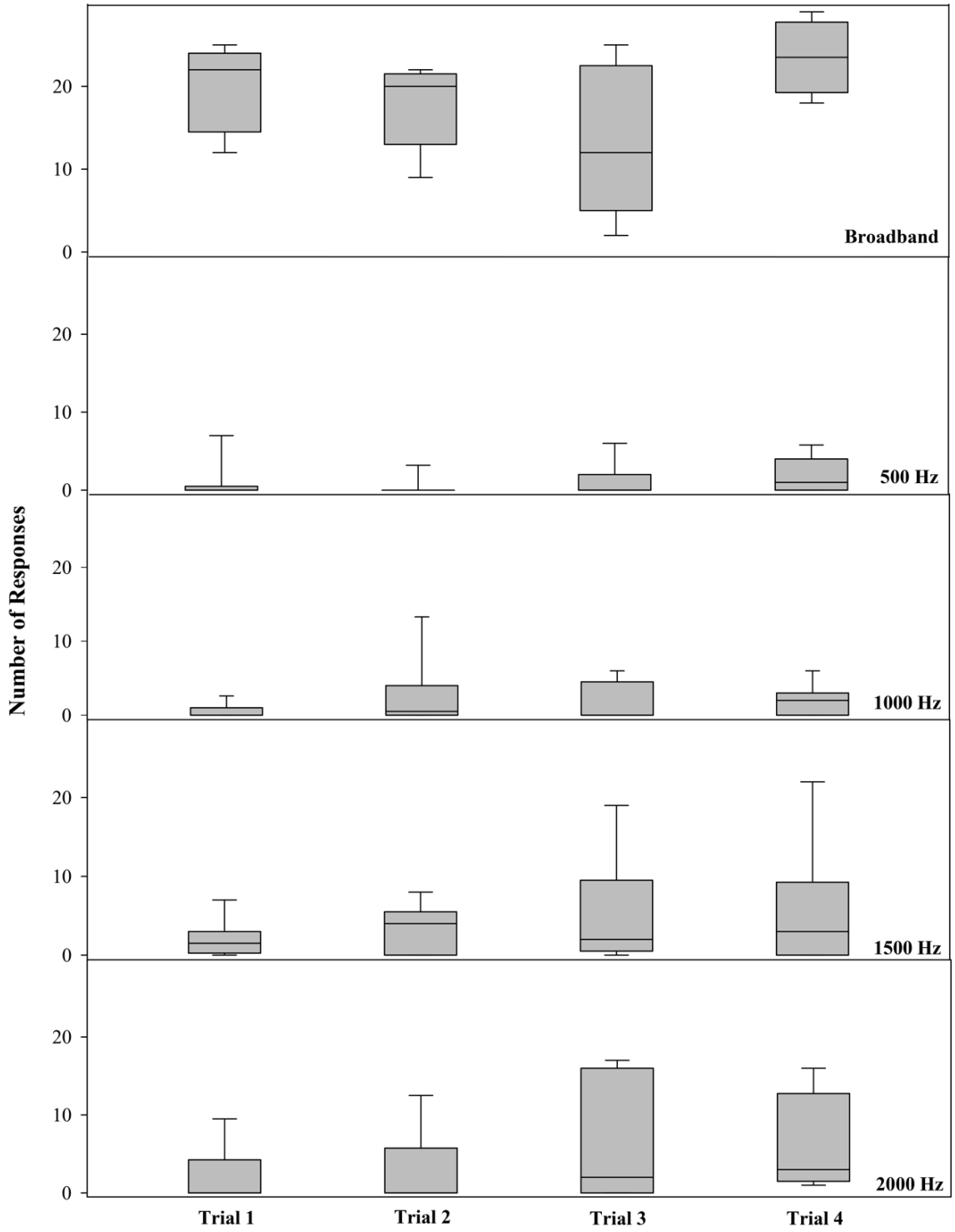
Figure 7: Number of consecutive responses over time for each sound stimulus type: **A)** 500 Hz, **B)** 1000 Hz, **C)** 1500 Hz, **D)** 2000 Hz, **E)** Broadband sound. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Boxes compare the number of consecutive responses by bighead carp to the first, second, third, and fourth presentation of each stimulus type. There is no significant difference between the medians ($P > 0.10$).
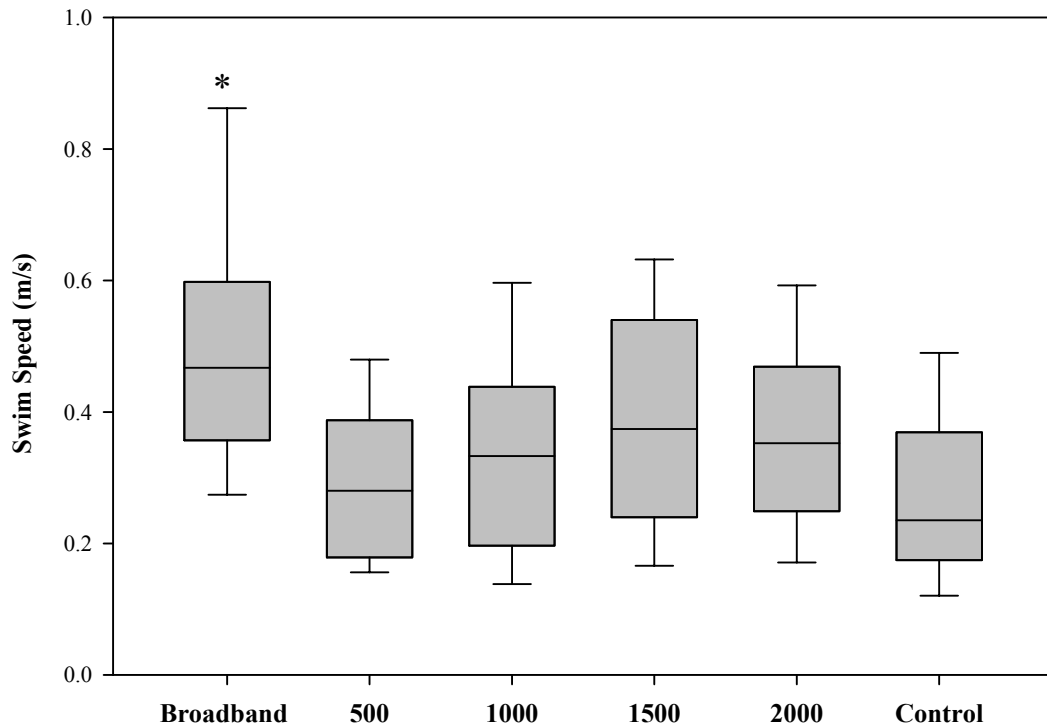
Figure 8: Fish swim speeds. Box and whisker plots display the median swim speed while the bighead carp were retreating from the sound stimuli. Swim speed was calculated by determining the time elapsed while the fish swam the first 2.0 m away from the sound source. The control represents the average time for fish to swim 2.0 m in the absence of sound. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. * indicates significantly different group ($P < 0.001$).

Chapter 4: Characterization of *in situ* silver carp (*Hypophthalmichthys molitrix*) jumping behavior in response to outboard motors

Abstract

Silver carp, an invasive planktivorous fish species in North America, pose a threat to aquatic ecosystems throughout the Mississippi River Drainage. These fish are well known for their airborne leaps in response to passing watercraft, but the trigger for and functional significance of jumping remains unknown. The behavior of wild silver carp responding to fast moving (16, 24, 32, and 40 km/hr) 6 m aluminum boats equipped with 4-stroke outboard motors (100 or 150 hp) was quantified. Experiments were conducted at three sites on the Illinois River near Havana, IL. All boat transits elicited jumping, with most (57.9%) stimulating five or more fish to jump. The frequency of jumping (fish/min) was independent of the speeds and motor type examined and more fish jumped in the narrower reach (180 m width) of the river than the wider channel (370 m). The vast majority of fish (> 90.0%) jumped behind the boat although they avoided the area directly astern (< 4.0 m). Furthermore, 79.8% of fish vectored away the moving watercraft. The results suggest that jumping direction is not random and fish can localize the stimulus source. The "delayed" jumping until after the boat had transited the area indicates that the trigger may be turbulence and/or higher sound pressure levels.

Introduction

Silver carp (*Hypophthalmichthys molitrix*) are an invasive fish species that escaped from captivity in the southern part of the United States in the 1980's and have since moved northward, colonizing much of the Mississippi River Drainage (Kolar et al. 2005, Kolar et al. 2007). In areas where carp are abundant, these planktivorous fish have drastically altered the composition of the lowest trophic levels (Kolar et al. 2005, Sass et al. 2014). Furthermore, they are competitively superior to other native filter feeders such as such as bigmouth buffalo (*Ictiobus cyprinellus*; Irons et al. 2007), paddlefish (*Polyodon spathula*; Schrank et al. 2003), and gizzard shad (*Dorosoma*

cepedianum; Sampson et al. 2009). Silver carp are abundant in the northern reaches of the Illinois River where they threaten to expand into the Laurentian Great Lakes, which would expose the entire system to ecological disruption (Sass et al. 2010, Moy et al. 2011, Murphy and Jackson 2013).

An additional reason these fish have gained notoriety is their jumping behavior. Both juvenile and adult silver carp jump in response to moving watercraft, and there are reports of these fish injuring boaters (Kolar et al. 2007). Jumping in freshwater fish has been associated with upstream migration, circumventing barriers, or escaping predators (Aronson 1971, Bayliss 1982, Bierman 2013). Prior to the introduction of silver carp, however, this behavior has not been observed in response to watercraft. Although jumping by carp has been well documented in the popular literature and numerous social media outlets, the trigger and functional significance of this behavior is unclear.

Silver carp, like all members of the superorder ostariophysi, possess Weberian ossicles, which form a connection between the swim bladder and bones of the inner ear. This adaptation allows the fish to detect higher frequency sound than non-ostariophysans (Popper and Carlson 1998; Fay and Popper 1999). Lovell et al. (2006) determined that silver carp are sensitive to sound frequencies up to 3 kHz, however recent studies have re-evaluated these results based on the unusually flat tuning curve reported (Ladich and Fay 2013, Sisneros et al 2016). Vetter et al. (2015) determined that broadband sound (0.06 – 10 kHz) from an underwater recording of a 100 hp outboard motor elicits consistent negative phonotaxis in captive silver carp. The findings from these two studies indicate that silver carp are sensitive to at least 3 kHz, but it is possible they can hear higher frequencies.

Sound has been proposed as a method for early detection, capture, and deterrence of silver carp. The efficacy of acoustic barriers has been evaluated (Pegg and Chick 2004, Taylor et al. 2005, Noatch and Suski 2012, Ruebush et al. 2012), with broadband sound successfully

repelling captive silver carp in > 90% of attempts to cross a sound barrier (Murchy et al. *in press*). It may also be possible to use sound to herd carp into nets or shallow areas for capture and subsequent removal. Additional information is needed about the jumping behavior of silver carp to evaluate sound as an acoustic barrier or deterrent, however detailed studies examining jumping behavior have not been conducted. This study evaluated wild silver carp jumping in response to different outboard motors and speeds to characterize silver carp's jumping behavior in response to moving watercraft.

Methods

Behavioral Observations

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

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

A trial consisted of the boat (powered by the 100 or 150 hp outboard motor) transiting the three sites at one of the four speeds (16, 24, 32, or 40 km/hr). For every trial, the boat started downstream of Site 1, attained the randomly selected speed before entering the first site, and maintained the speed through all sites (Fig 1B). Immediately after exiting Site 3, the boat turned

180° to port, moved downstream paralleling the western side of Site 3 (Fig. 1A), and continued

west of the island that separates the main channel from Sites 1 and 2, to a waiting point

downstream of Site 1. After at least a one hour recovery period, the sequence was repeated at a

different speed. One session consisted of either four morning or afternoon trials, with the order of

speeds randomized for each session and the motor type alternated between each session. All tests

were conducted on August 8th and 9th, 2013 and October 7th, 2013.

Sound pressure levels and boat motor frequencies were recorded using two hydrophones

(HTI-96-MIN, High Tech Inc., Long Beach, MS) connected to a Zoom H4n Handy Recorder

(Ronkonkoma, NY). The hydrophones were placed on the western edge of the sites, either

between Sites 1 and 2 or at the halfway point (100 m) of Site 3 (Fig. 1A), and were situated in the

middle of the water column (3 – 4 m deep) about 10 m from the transit area. Sound pressure

levels were a maximum of 130 dB and the 100 and 150 hp motors emitted broadband sound up to

10 kHz (Vetter et al. 2015).

Data Analysis

Number of Jumping Fish: The GoPro video files (mp4 format) from all four cameras

were analyzed using frame-by-frame analysis (30 frames per second) in Adobe Photoshop CS6

(version 13; San Jose, CA). Each fish that jumped above the water surface was counted. A fish

was only counted if it initiated jumping in the camera's field of view, since it is possible that two

cameras could capture the jumping progression of one fish. Boat transit time through each site

varied with speed so the jumping frequency (number of observed jumping fish/min) was used for

analysis.

Jumping Angles: Each jump was analyzed for: distance from boat at jump origin and the

position and vector of the jump in relation to the boat. The jump origin was determined as the

angle between the origin of jump (when the fish first broke the surface) and the boat (bow = 0°;

starboard = 90°; stern = 180°; port = 270°) (Fig. 1B, Angle 1). The jumping vector was defined as

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the angle of the fish's jumping trajectory (from jump initiation to reentry) (Fig. 1B, Angle 2) in relation to the boat. Jumps also were categorized as either "towards" or "away" from the boat. For example, bow camera jumping vectors between $91^{\circ} - 269^{\circ}$ indicated that the fish jumped towards the boat.

Jumping distance from the boat was determined by calculating the number of pixels for each 0.25 m segment of the 2 m PVC pipe, plotting a linear regression, and extrapolating these measurements for jumps originating beyond this marker. The extent of the centerline wake created by the outboard motor, which includes the propeller downwash and viscous wake as outlined by the white water directly astern (Reed and Milgram 2002), was determined from the stern camera. The Kelvin wake, which is often independent of boat speed and size, was verified to be between $160^{\circ} - 200^{\circ}$ (Reed and Milgram 2002).

Statistical Analyses:

The data evaluating the number of observed jumping fish in each site, the jumping frequency, and the distance of the jump origin from the boat failed the Shapiro-Wilk test of normality ($P < 0.05$), therefore non-parametric statistics were used in these analyses. A Kruskal-Wallis ANOVA with a post-hoc Tukey Test was used in analyzing the median number of jumping fish in Sites 1 – 3. For each motor, jumping frequency and distances were compared across the 16, 24, 32, and 40 km/hr speeds (Kruskal-Wallis ANOVAs). The speed data was then pooled and the jumping frequency and distances were evaluated based only on motor type (Mann-Whitney Rank Sum Test). The median and upper and lower quartile is reported using the following format (median; 1st Q, 3rd Q) or median (1st Q, 3rd Q). All analyses were conducted using SigmaPlot for Windows (version 12.5; SYSTAT Software; San Jose, CA).

Jumping Angles: The jump origin and vector were analyzed using Oriana (version 4; Kovach Computing Systems; Wales, UK) for each combination of outboard motor and speed. Rao's Spacing Test was implemented to assess the distribution of the jump initiation angles and

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jumping vectors. This test was utilized because it compares angular data to a uniform distribution around a circle to determine if the data is random or clustered around the median.

The jumping direction data (towards vs. away from the boat) was normally distributed among the eight motor/speed combinations, therefore a t-test was performed in SigmaPlot and the mean towards and away jumps are represented as mean ± SE.

Results

The boats stimulated jumping from silver carp during all the transits. The number of observed fish jumping at each site ranged from 1 to 75 and during 57.9% of the transits, at least five fish jumped (Sites 1 – 3). Overall, significantly more fish jumped in Sites 1 (median: 6.5; 1st Q: 3.0, 3rd Q: 11.5) and 2 (4.5; 1.8, 8.3) than in Site 3 (2.0; 0.0, 5.0) (Kruskal-Wallis ANOVA P = 0.001) (Fig. 2).

Boat transit time varied with speed (0.75 min for 16 km/hr to 0.3 min for 40 km/hr), therefore the jumping frequency (number of observed jumping fish/min) was calculated. There was no significant difference in jumping frequency between the four speeds for either the 100 hp (Kruskal-Wallis ANOVA P = 0.371) or the 150 hp (Kruskal-Wallis ANOVA P = 0.513) motors, so the jumping frequency data was pooled for each motor. The median jumping frequencies for the 100 and 150 hp motors were 10.0 fish/min (1st Q: 4.0, 3rd Q: 19.2) and 18.7 fish/min (8.0, 33.3), respectively, and were not significantly different (Mann-Whitney P = 0.064).

For the 100 hp motor, 90.6% of the fish initiated their jumps behind (> 90° and < 270°) the boat. The fish also primarily moved away (84.8%) from the boat (Table 1, Fig. 3A). Jump origin was not randomly distributed (Rao's P < 0.01). Fish jumping was initiated primarily in a 90° arc behind the boat, with the stern serving as the center and median angles ranging from 135.9^{o} (129.2^{o}, 166.3^{o}) at 24 km/hr to 222.3^{o} (158.1^{o}, 220.7^{o}) at 32 km/hr. The jumping vectors were also not randomly distributed (Rao's P < 0.01) and median angles ranged from 102.2^{o} (32 km/hr: 66.3^{o}, 118.3^{o}) to 153.1^{o} (40 km/hr: 142.2^{o}, 176.9^{o}) (Table 1).

Similar to the 100 hp motor, fish responding to the 150 hp motor primarily jumped behind (95.0%) and away (77.0%) from the moving boat. The jumping origination for the 150 hp motor favored the starboard/stern quadrant (Rao's $P < 0.01$), with median origination angles ranging from 140.9^o (40 km/hr: 139.7^o, 159.5^o) to 168.6^o (24 km/hr: 150.8^o, 176.1^o) (Table 1, Fig. 3A). Furthermore, the jumping vectors were non random (Rao's $P < 0.01$), varying from 90.1^o (24 km/hr: 45.2^o, 124.7^o) to 186.0^o (32 km/hr: 165.6^o, 193.1^o) (Table 1).

Jumping patterns for each motor type and speed are summarized in Figures 3B and 3C. Jumps initiated in response to the 150 hp motor were concentrated in a semi circle behind the boat with avoidance of the area directly astern (< 4.0 m) within the motor wake. Although this pattern is not as distinct in fish responding to the 100 hp motor, most fish also avoided the same region astern in these trials.

Figure 3D summarizes the jump origination data by quantifying the number of jumps in 20° arcs around the boat, which are further divided into 2.0 m lengths. There is a clear exclusion zone from $0.0 - 4.0$ m between $140^o - 220^o$, behind the boat. (Fig. 3D). This figure also indicates the average centerline wake angle and the 39° Kelvin wake. The majority of jumping occurred between $4.0 - 8.0$ m in the $100^o - 180^o$ arcs within and just outside the Kelvin wake and in the distant portion of the centerline wake (> 4.0 m astern).

The median distance of the jump origin from the boat (100 hp) varied from 5.2 m (24 km/hr: 3.8 m, 6.1 m) to 6.4 m (16 km/hr: 3.1 m, 7.3 m), with no significant difference between the speeds (Kruskal-Wallis ANOVA $P = 0.117$). Similarly, there was no significant difference in distance for fish reacting to the 150 hp motor (Kruskal-Wallis ANOVA $P = 0.274$), with distances ranging from 4.9 m (40 km/hr: 3.9 m, 5.8 m) to 5.7 m (24 km/hr: 3.7 m, 6.5 m). Therefore, the distances were pooled by motor type. The fish jumped significantly further (5.6 m; 4.7 m, 6.8 m) from the boat during the trials with the 100 hp motor versus the 150 hp motor (5.1 m; 3.9 m, 5.9 m) (Shapiro-Wilk $P < 0.001$) (Fig. 4A).

For most of the boat and motor combinations, the number of jumping fish was highest between 4 – 6 m with percentages varying from 65.4% (150 hp motor at 24 km/hr) to 85.2% (100 hp motor at 40 km/hr) (Fig. 4B). However, fish responding to the100 hp motor at 16 km/hr were more varied in distances jumped, with 14.6% jumping at 1 m, 19.5% between 4 – 5 m, and 48.7% between 6 – 7 m. Finally, the carp reacting to the 100 hp motor at 32 km/hr jumped slightly further away from the boat, as 68.7% of jumps occurred between 7 – 10 m.

The boundary of the centerline wake, which included the viscous wake and the propeller downwash, decreased slightly with increasing speed from $26.4^o \pm 2.4^o$ to $19.9^o \pm 3.1^o$ (100 hp) and $31.1^o \pm 2.0^o$ to $20.8^o \pm 3.0^o$ (150 hp). However, there was no significant difference in this wake angle among the speeds for either the 100 hp (ANOVA P = 0.358) or 150 hp motors (ANOVA P = 0.257). Furthermore, when the speed data was pooled, there was also no significant difference in wake angle between the motor types (100 hp: $22.4^o \pm 3.8^o$; 150 hp: $25.2^o \pm 5.0^o$; Shapiro-Wilk P = 0.152). The fish avoided the area directly astern (0 – 4.0 m) independent of motor and speed (Fig. 3B,D).

In addition to fish jumping in the centerline wake, the percentage of fish that jumped within the Kelvin wake ($160^o – 200^o$) was also assessed for each boat motor type and speed combination. For the 100 hp motor, Kelvin-wake jumping varied from 5.9% of fish (32 km/hr) to 26.0% (40 kph). The percent of fish jumping in the Kelvin wake was higher overall for the 150 hp and ranged from 14.3% (32 km/hr) to 48.1% (24 km/hr).

All jumping vectors were categorized as moving either towards or away from the boat and summed based on the eight boat type and speed combinations. 79.8% of all observed fish jumped away from the boats. In comparing the eight boat motor type and speed combinations, an average of 42.0 ± 6.3 fish jumped away per motor/speed stimulus type, which was significantly (t-test P < 0.001) more than the fish that jumped towards the boat (10.6 ± 3.4) (Fig. 5).

Discussion

      This study is the first to quantitatively examine silver carp jumping behavior in response to motorized watercraft. The frequency of jumping was independent of boat speed and the two motor types examined. Fish primarily jumped behind and away from the boat but rarely jumped in the area directly astern (< 0.4 m). Both motor types elicited a distinct semi circle pattern of jumping behind the boat, although this was most pronounced for the 150 hp motor.

      The focus of this research was to evaluate sensory stimuli that promote jumping behavior in silver carp. Sound travels approximately 1440 m/sec in freshwater and, as maximum boat speed was 11 m/sec, the sound generated by the watercraft was probably detected by fish well before the boat arrived in the area, with hydrophones detecting boat sound $10 - 20$ seconds prior to area transit. Sound playbacks of a 100 hp motor at 32 km/hr evoked consistent negative phonotaxis from captive silver carp (Vetter et al. 2015) and stimulated wild fish to jump in a small tributary of the Illinois River (Mensinger, unpublished). In the present study, however, as the vast majority of the jumps occurred after the boat passed the fish, sound detection alone was insufficient to trigger jumping. One possibility is that a minimum sound pressure level is necessary to stimulate the jumping and these levels were not achieved until the boat was within meters of the fish. The jumping behavior may also be triggered by near field sound, which would be limited in range compared to the far field sound. In either case, the presence of fish jumping behind the boat would simply reflect the time required for the fish to respond to the stimulus. Alternatively, the pattern of jumping behind the boat may indicate that fish are responding to some aspect of the wake produced and that jumping is a multifactor response.

      The boat wake is made up of the whitewater (originating at the bow), the centerline wake (which includes the propeller downwash and the viscous wake), and the Kelvin wake (Reed and Milgram 2002). The Kelvin wake begins at the bow and forms a 39° angle, extending past the stern of the boat. Beyond the Kelvin wake, there is a series of waves. For both motor types, but

especially the 150 hp, there was a distinct semi-circle pattern of jumping within the wake area. The propeller downwash deterred fish from jumping directly behind the boat (< 4.0 m) while the majority of fish jumped within the distant (> 4.0 m) part of the centerline wake, the Kelvin wake (which occurred approximately between $160^{\circ} – 200^{\circ}$), and the waves that radiated out from the Kelvin wake. In situations when boat transit is less than 6 km/hr, very few jumps are observed (Mensinger, unpublished), implying that there is a minimum speed necessary to consistently stimulate jumping. Therefore, it appears that for fast (> 15 km/hr) moving boats, there is a correlation between silver carp responses and boat location.

The preference for jumping on the starboard rather than the port side of the boat was probably influenced by the river section in Sites 1 and 2 (high density of jumps), where there was an extensive shallow flat on the west side, forcing the boat to favor the east side of the channel. This asymmetrical depth profile may also have created a non-random distribution of fish underwater explaining the greater numbers that jumped between $100^{\circ} – 180^{\circ}$.

Although the exact jumping trigger remains unclear, the results indicate that the majority of the fish moved away from the stimulus source. It is unlikely that vision was a factor in locating the boat, as the water was very turbid, with nearby turbidity readings between 22 and 97 FTU on the days of the testing (USGS 2016) and fish were only visible within centimeters of the surface. An evaluation of silver carp's visual sensitivity would aid in determining whether or not vision is involved in localization of moving watercraft.

Since only above water behavior was visible, it is uncertain if Mauthner cells have a role in jumping, as presence of the c-start response could not be observed. Mauthner cells are reflex neurons that govern the c-start startle response in fish. It is possible that the carp were detecting and responding to the near field sound and/or vibrations by the outboard motor with their lateral line. However, as fish jumped up to 10 m away from the boat, it is more likely that the fish detected the moving boats by hearing the sound emitted from the motor.

Other carp species are capable of localizing pure tones between 0.4 – 1 kHz (Willis et al. 2002, Sloan et al. 2013) and the sound emitted by these motors ranged between 0.06 – 10 kHz (Vetter et al. 2015) which is at least partially within silver carp's hearing range (Lovell et al. 2006). Studies on both captive (Vetter et al. 2015, Murchy et al. unpublished) and wild silver carp (Mensinger, unpublished) have determined that in general, outboard motor recordings can effectively move fish out of an area. However, the stimulus used in these earlier experiments was broadcast to fish at a higher sound pressure level (> 150 dB) than the outboard motor sounds (130 dB) in this study. Examination of wild silver carp behavior in response to high intensity sound (>150 dB) is imperative to further define the jumping stimulus.

The silver carp's jumping behavior is unusual for cyprinids. Although jumping may help larval and juvenile carp evade predation, mature animals have few, if any, natural predators. Therefore, the functional significance of jumping in adult silver carp is unknown. Furthermore, jumping in response to boat traffic can have deleterious consequences for carp such as self-stranding (into boats or on shore) or hard impacts with boat hulls or woody debris that often cause hemorrhaging. However, from a management view, it could prove to be the carp's "Achilles' fin" if the behavior can be controlled or directed. Understanding silver carp jumping behavior can help officials determine the best methods for herding or capturing fish. For instance, results from this study, which indicate that fish primarily jump behind and away from the boat, could be useful in developing effective nets for catching jumping fish.

Acknowledgements:

Motor (hp) @ Speed (km/hr)	N	Jumping Initiation Angle Median° (1st Q°, 3rd Q°)	Jumping Vector Median° (1st Q°, 3rd Q°)
100 @ 16	41	156.7 (149.9, 180.0)	149.1 (119.3, 168.0)
100 @ 24	32	135.9 (129.2, 166.3)	121.8 (92.8, 167.4)*
100 @ 32	17	222.3 (158.1, 220.7)	102.2 (66.3, 118.3)
100 @ 40	27	145.0 (136.6, 165.1)	153.1 (142.2, 176.9)
150 @ 16	62	143.9 (138.7, 161.2)	129.6 (105.1, 141.6)
150 @ 24	52	168.6 (150.8, 176.1)	90.1 (45.2, 124.7)
150 @ 32	70	152.9 (150.0, 176.5)	186.0 (165.6, 193.1)
150 @ 40	96	140.9 (139.7, 159.5)	138.3 (116.3, 159.0)

Table 1: Summary of the angle data for all eight stimuli. N represents the number of fish analyzed for both the jump initiation angle and jumping vector (Median; 1st Q, 3rd Q) (Rao's P < 0.01). * indicates the only non-significant group
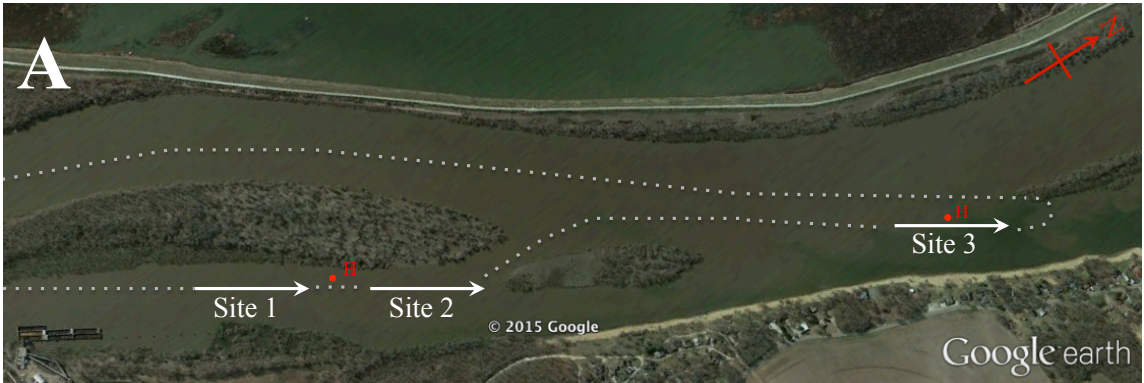
Figure 1: A) Aerial view of the three testing sites near Havana, IL on the Illinois River. Each site

is marked with an arrow indicating the direction of boat travel. The dashed line represents the

boat path through all three testing sites. Red dots marked with an "H" indicate the location of

hydrophones. **B)** Screen shot from the stern camera taken while using Adobe Photoshop CS6,

version 13. The 2 m PVC rod used to estimate distance jumped from boat is visible. The red circle indicates a jumping carp from which the jump initiation angle (**1**), distance from the boat, and jumping vector (**2**) were measured. The angle of the wake (**3**) is also specified.
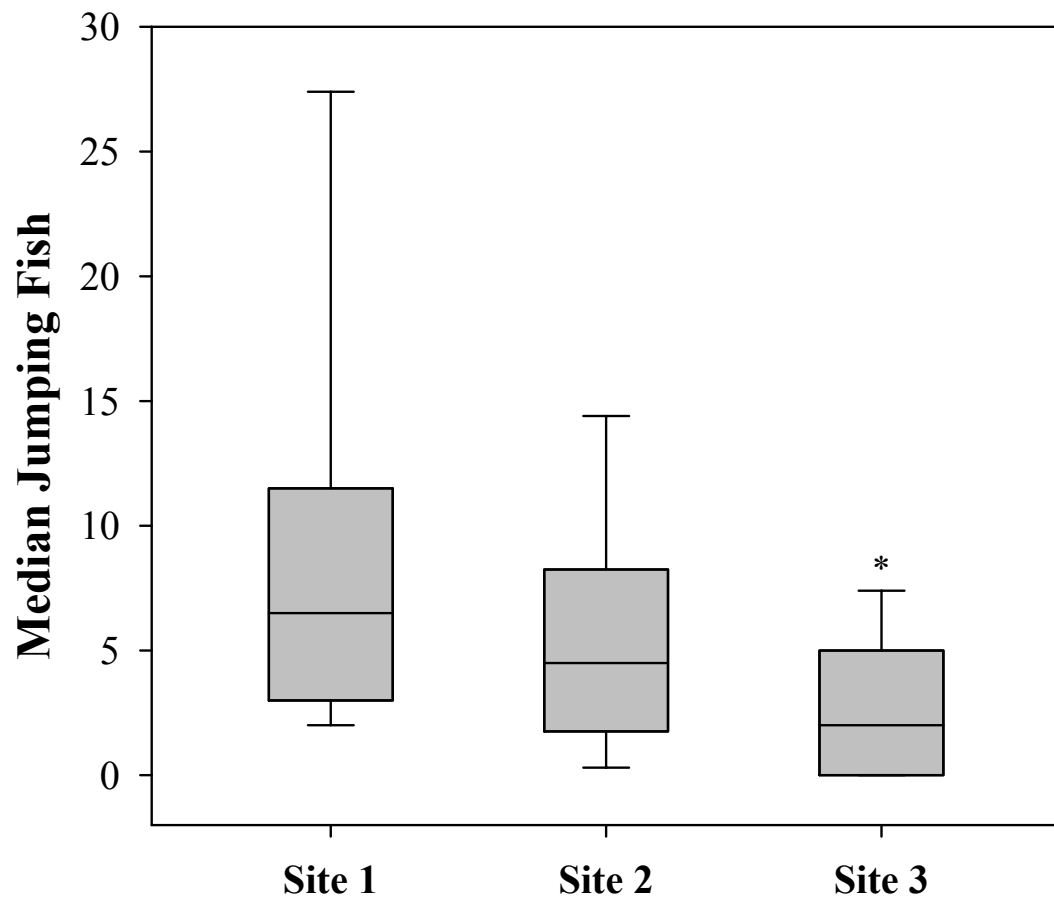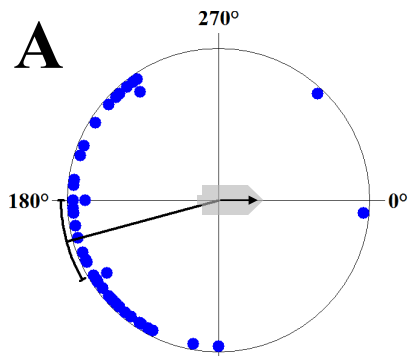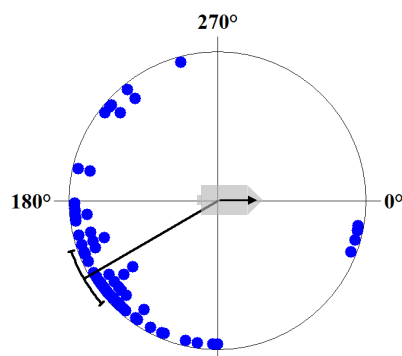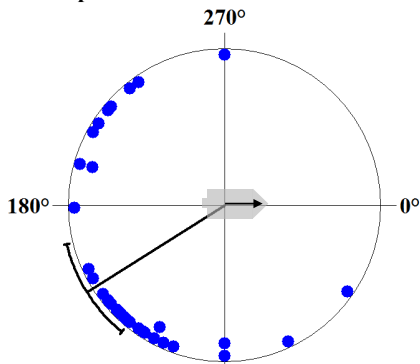
Figure 2: Median jumping silver carp in Sites 1 – 3. Each box represents the 25th (bottom of box) and 75th (top of box) quartiles with the median marked by the line within the box. Whiskers (error bars) above and below the box indicate the 10th and 90th percentiles. * indicates significantly different group (Tukey P = 0.001).
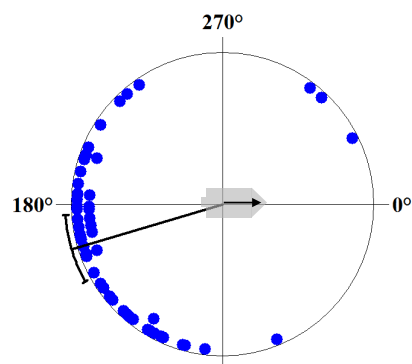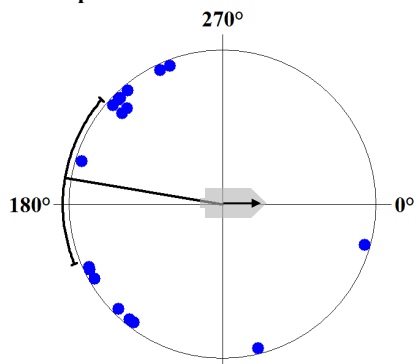
A

100 hp 16 km/hr

150 hp 16 km/hr

100 hp 24 km/hr

150 hp 24 km/hr

100 hp 32 km/hr

150 hp 32 km/hr

100 hp 40 km/hr

150 hp 40 km/hr

69

B

100 hp 16 km/hr

150 hp 16 km/hr
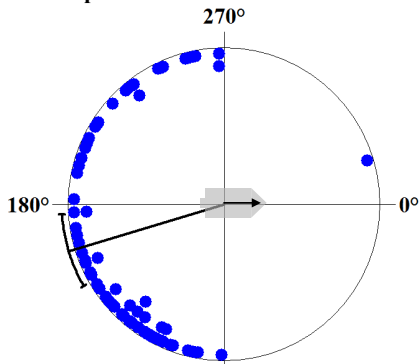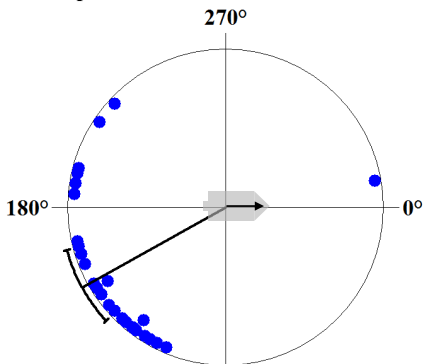

100 hp 24 km/hr

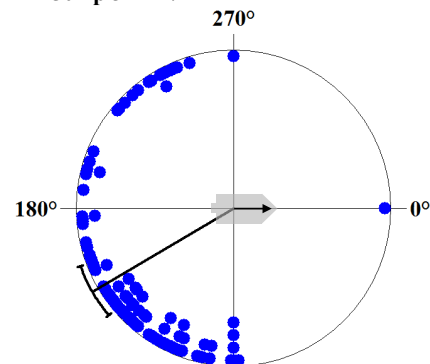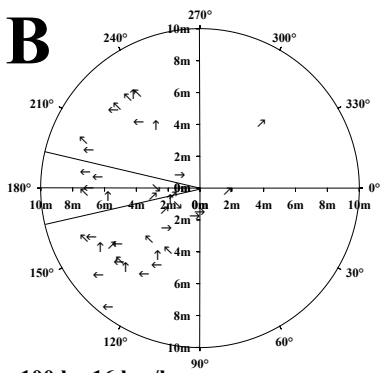150 hp 24 km/hr

100 hp 32 km/hr

150 hp 32 km/hr

100 hp 40 km/hr
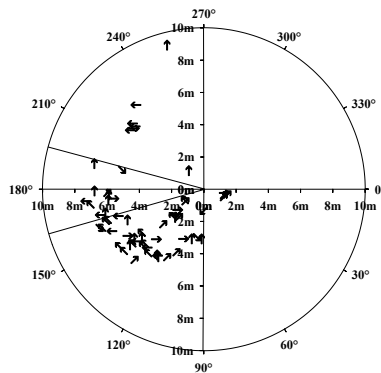

150 hp 40 km/hr

70
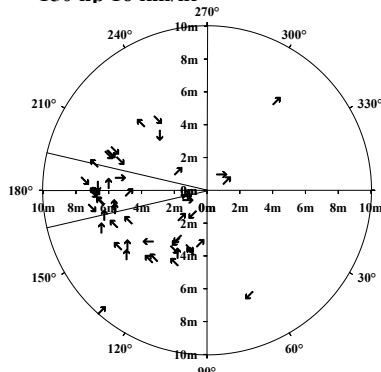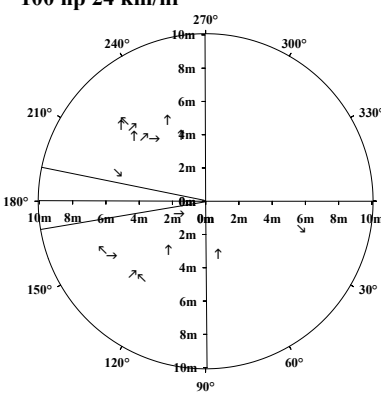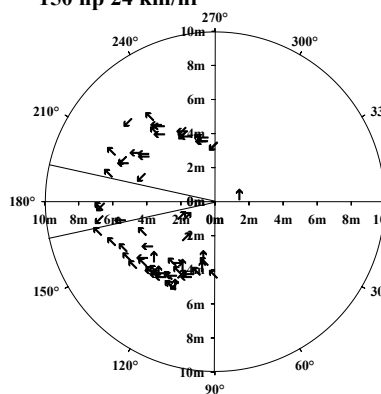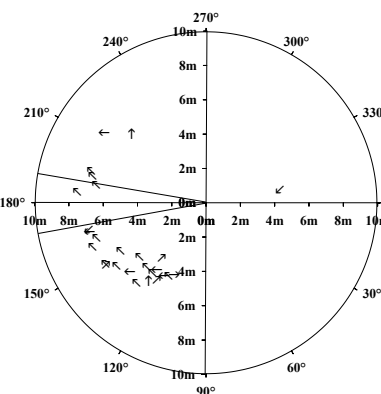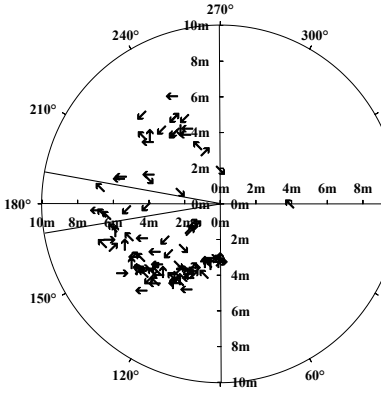
C
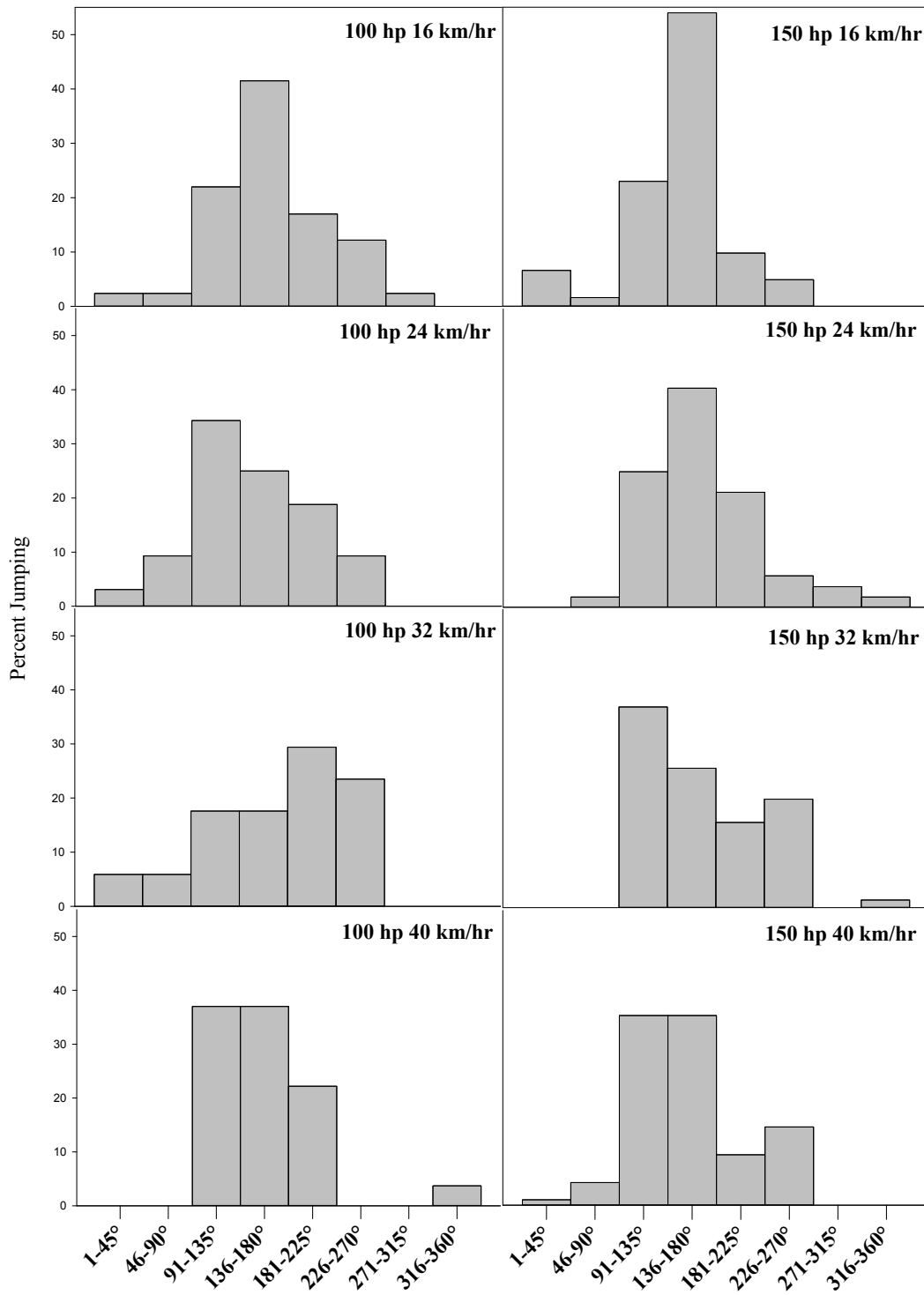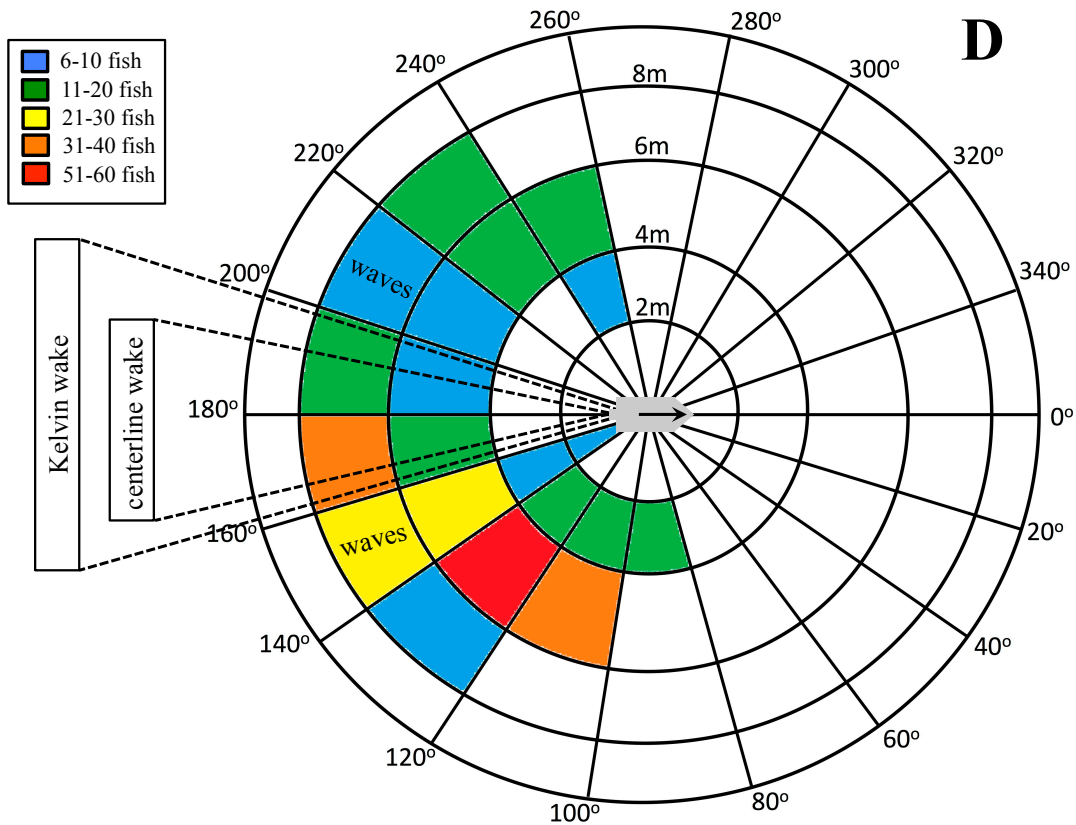
Figure 3: A) Circular plots displaying the angle from the boat in which the fish jumped. The blue circles mark the location of each fish and the thick black line represents the median angle and 95% confidence intervals for the eight motor/speed combinations. Boat position and direction of travel (0°) is indicated in the center of each plot (Rao's $P < 0.01$). **B)** Polar plots for all eight treatments. Each arrow represents one fish and marks the estimated distance from and jump initiation angle in relation to the boat and its direction of movement (0°). To assess the jumping vector, each fish was categorized in one of eight arcs ($0^{o} – 45^{o}$, $46^{o} – 90^{o}$, $91^{o} – 115^{o}$, $116^{o} – 135^{o}$, $136^{o} – 180^{o}$, $181^{o} – 270^{o}$, $271^{o} – 315^{o}$, and $316^{o} – 360^{o}$) and plotted with a corresponding arrow. The arrows point in the direction of each fish's trajectory (jumping vector). The solid lines represent the boat's wake **C)** Histograms assessing the percentage of fish that initiated jumping in one of eight angular arcs ($0^{o} – 45^{o}$, $46^{o} – 90^{o}$, $91^{o} – 115^{o}$, $116^{o} – 135^{o}$, $136^{o} – 180^{o}$, $181^{o} – 270^{o}$,

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271° – 315°, and 316° – 360°) in relation to the boat. **D)** Summary of all jump initiation locations. The area around the boat was divided into 2 m segments in 20° arcs, giving 90 total sections. The colors represent the number of jumping fish in each section, see legend inset. White segments represent areas where 5 or less fish jumped. There were no sections in which the total number of jumping fish was between 41 – 50.

Distance (m)

75
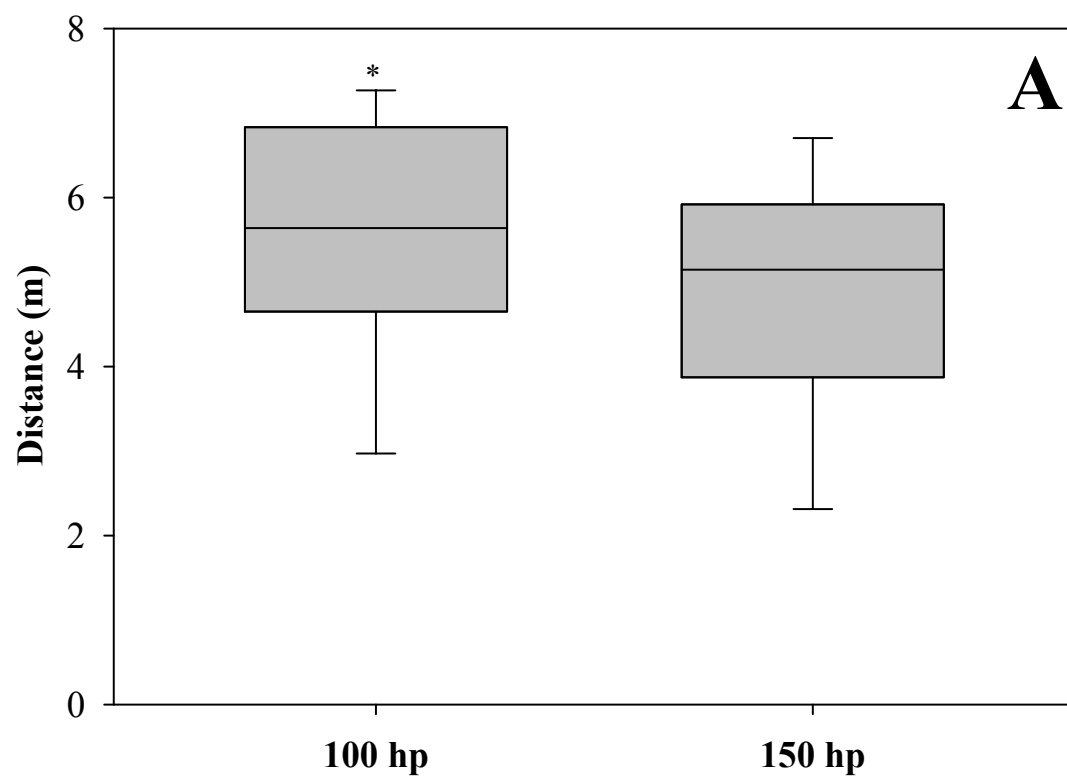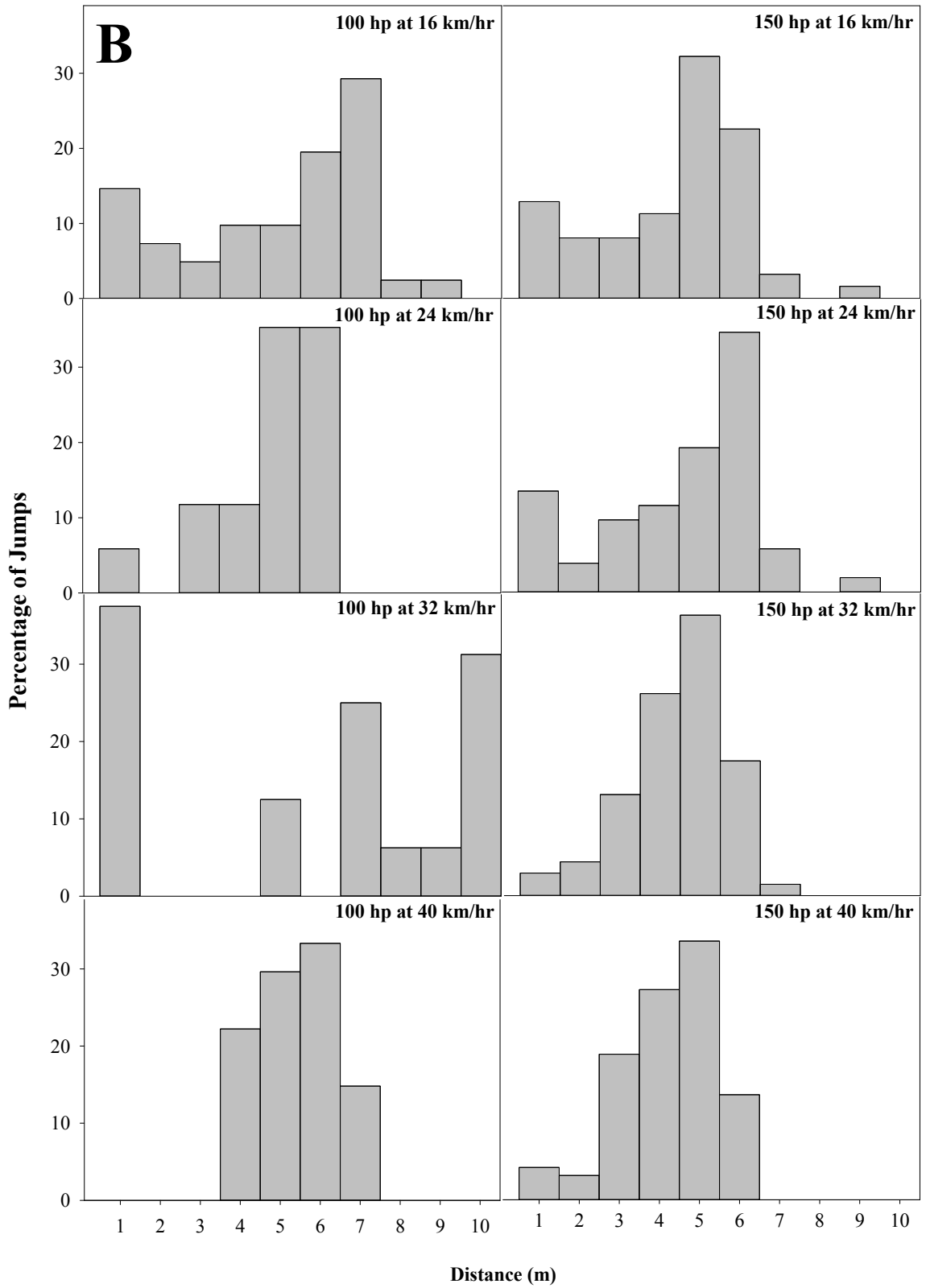
Figure 4: A) Median distance from the boat in which fish initiated jumping for the 100 and 150 hp motors. Each box represents the 24th and 75th quartiles with the median marked by the line within the box. The 10th and 90th percentiles are indicated by the whiskers (error bars). * indicates significantly different groups (Shapiro-Wilk P < 0.001) **B)** Histograms representing the percentage number of jumps that occurred at 1 m increments from the boat.
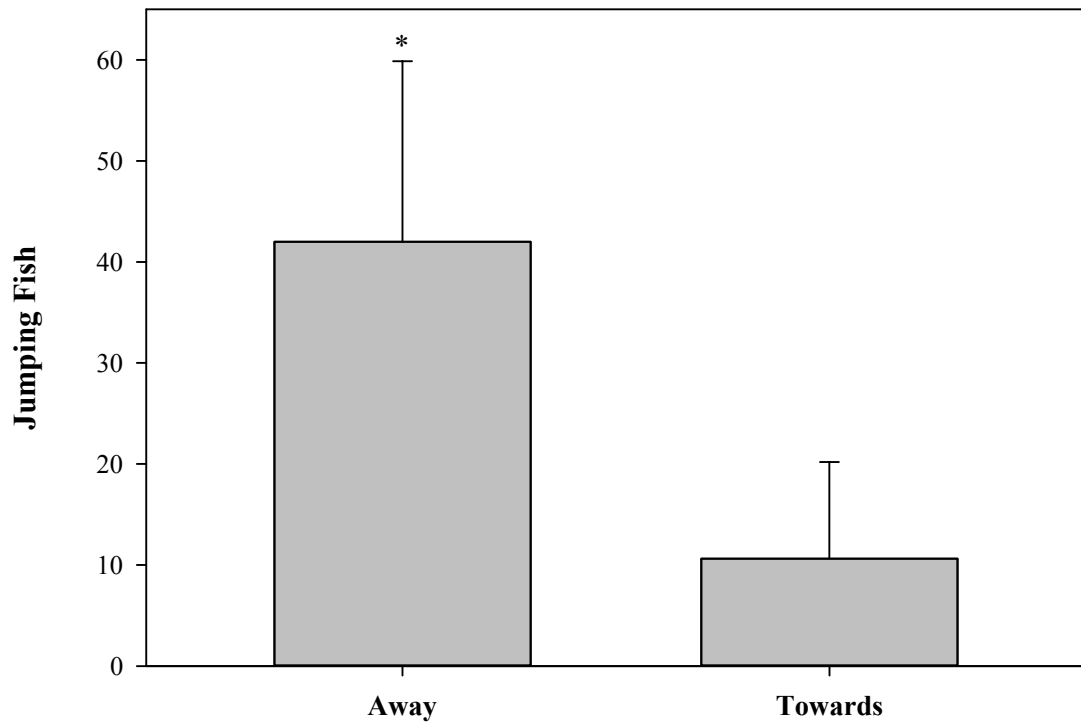
Figure 5: Average number of fish that jumped "away" from versus "towards" the boat. Averages were calculated based on pooling the total number of jumping fish in response to each outboard motor type at one of the four speeds (100 hp @ 16 km/hr, 100 hp @ 24 km/hr, 100 hp @ 32 km/hr, and 100 hp @ 40 km/hr, 150 hp @ 16 km/hr, 150 hp @ 24 km/hr, 150 hp @ 32 km/hr, and 150 hp @ 40 km/hr) * indicates significantly different groups (t-test P < 0.001). Error bars represent ± SE.

Chapter 5: Behavioral response of wild silver carp (*Hypophthalmichthys molitrix*) to broadband sound in the Spoon River, IL

Abstract

Invasive silver (*Hypophthalmichthys molitrix*) carp are notorious for their prolific and unusual jumping behavior. Juvenile and adult (~25 kg) carp can jump up to 3 m above the water surface; however, it is unclear what aspect of the moving boats elicits jumping. Studies examining wild silver carp jumping in response to fast moving (16 – 40 km/hr) boats (100 and 150 hp motors) and captive carp swimming behavior when exposed to broadband sound (0.06 – 10 kHz) recorded from an outboard motor (100 hp at 32 km/hr), suggest that fish react to both the boat wake and high sound pressure levels. In this study, broadband sound (0.06 – 10 kHz) broadcast at a high intensity (170 dB re 1 μPa) elicited jumping behavior in wild fish when played from a slow moving boat (> 5 km/hr) in the Spoon River near Havana, Illinois. Fish jumped in 100% of the sound trials, implying high intensity sound alone elicits jumping. Furthermore, the fish showed behavioral differences depending on the presence of partially submerged woody debris, as more carp jumped in river regions where debris was present. Finally, there was a decreasing trend of jumping over multiple exposures to broadband sound, implying that anthropogenic sound can be used to alter the behavior of silver carp and has implications for deterrent barriers or herding fish for removal.

Introduction

Silver carp (*Hypophthalmichthys molitrix*) were introduced to North America from Eastern China in the 1970's as a biological control to remove algae via filter feeding in aquaculture facilities and wastewater treatment plants (Kolar et al. 2007). They subsequently escaped into the southern regions of the Mississippi River Drainage and continue to expand their range. Silver carp are now present in waterways of 15 states including the northern reaches of the Illinois River, a tributary of the Mississippi River (Kolar et al. 2007). The Illinois River is

connected to Lake Michigan via the Chicago Ship and Sanitary Canal and silver carp egress into Lake Michigan could expose the entire Great Lakes system to invasion. Silver carp have high fecundity, fast growth rates, and opportunistically feed on both phytoplankton and zooplankton, altering in this lower trophic level (Kolar et al. 2007, Sass et al. 2014). These fish further threaten the food web as they compete with native species including bigmouth buffalo (*Ictiobus cyprinellus*; Irons et al. 2007), gizzard shad (*Dorosoma cepedianum*; Sampson et al. 2009), and paddlefish (*Polyodon spathula*; Schrank et al. 2003) for resources. Finally, they demonstrate an unusual jumping behavior in response to motorized watercraft. Airborne carp not only present a hazard to boaters, but this behavior is also detrimental to the fish, as they collide with boat hulls and partially submerged woody debris in rivers and land in boats or on shore.

V.K. Soldatov first recorded jumping silver carp in Russia in 1928 (Berg 1964) and this behavior has also been observed in other regions where silver carp have been introduced including Africa (Skelton 1993) and North America (Kolar et al. 2007). In North American rivers, where carp densities are high, motorized watercraft can stimulate hundreds of jumping carp. While this behavior has become the focus of news and social media outlets, there has been very little research to determine what triggers silver carp to jump.

Vetter et al. (2015) successfully repelled captive silver carp using a broadband (0.06 – 10 kHz) sound stimulus recorded from a 100 hp motor traveling 32 km/hr on the Illinois River. This finding suggests that carp are responsive to the sound emitted from motorized watercraft, but does not verify that this sound stimulates fish to jump. As sound travels approximately four times faster underwater than it does in the air, the sound emitted by an outboard motor reaches the fish well before an approaching boat. However, rather than jumping in front of fast (16 – 40 km/hr) moving watercraft, wild silver carp tend to jump after the boat has passed (Kolar et al. 2007, Vetter et al., unpublished). An evaluation of jumping patterns also suggests that this behavior is

influenced by the wake created (Vetter et al., unpublished). It is imperative that wild silver carp behavior in response to broadband sound be determined.

Although broadband sound has been shown to alter the swimming behavior of captive silver carp (Vetter et al. 2015), this response must be evaluated in the field to address issues with applying laboratory studies to a natural setting (Popper et al., 2014; Hawkins et al., 2015). Researchers have been evaluating acoustic deterrents for implementation in the Mississippi River and its tributaries as a way to prevent bigheaded (silver and bighead *H. nobilis*) carp egress through locks or for herding the fish for removal. Understanding silver carp's behavior in response to broadband sound will aid in developing these methods. Finally, an investigation into the relationship between sound and jumping could help identify the trigger for this behavior and allow fisheries managers to incorporate the jumping stimulus into control methods. One characteristic that has yet to be evaluated is sound pressure. The captive silver carp exhibited negative phonotaxis to broadband sound at approximately 150 dB re 1 μPa (Vetter et al. 2015) while the fast moving boats produced a lower intensity sound, around 130 dB re 1 μPa. This study investigated the relationship between wild silver carp jumping and high intensity broadband sound that was isolated from a moving boat.

Methods

Behavioral Observations

Silver carp jumping behavior was assessed in response to broadband sound in the Spoon River near Havana, IL. The broadband sound used as the stimulus was originally recorded in the Illinois River from a fast moving (32 km/hr) boat equipped with a 100 hp motor using a hydrophone (HTI-96-MIN High Tech, Inc., Long Beach, MS) positioned 10 m from the boat's path at a depth of 1 m. To deliver the broadband sound stimulus in this study, a 6 m aluminum boat was outfitted with two underwater speakers (LL916C-025; Lubell Labs, Columbus, OH) mounted to the railing of the boat via a 3 m aluminum pipe. The top of the speakers were placed

approximately 0.15 m underwater, 1 m apart, and oriented so that the sound would project along the longitudinal axis of the boat.

The Spoon River originates south of Kewanee, IL and is a 237 km tributary of the Illinois River, meeting the main channel near Havana, IL. Approximately 3.25 km from its terminus, a collapsed bridge has blocked upstream access. Five sites (45 m wide) were selected downstream from the barrier to assess silver carp behavior in response to broadband sound (Fig. 1A). The section of the Spoon River in which all five sites were located runs from west to east. Site 1 (100 m long) was located at the mouth of the river and had an average depth of 2.5 m. This site contained three distinct regions in which submerged logs and tree branches, which protruded from the water, were present (Fig. 1B). These regions were located along the north shore and had a width of about 6 m, with a total area of approximately 450 m^2. The second site was approximately 300 m from the river mouth, was 100 m long, and 1.8 m deep. This site also contained partially submerged woody debris, however, all three regions were located near the south bank. The first two sections were roughly 30 m from the upstream end of the site and were 15 m apart. They consisted of dead trees that extended into the river about 6 m from the shore. The third area was located at the downstream end of the site and consisted of branches and logs that were visible above the water extending about 5 m out into the river from shore. Site 3 was located at a large bend approximately 650 m upstream from the river mouth. This site contained a long (~ 60 m) stretch of partially submerged logs and branches extending 6 m into the river from the south shore. It was 140 m in length and had a depth of 2.5 m, except for a deep hole (~ 4.5 m) located half way through the site at the apex of the river bend. The fourth site was 1.1 km from the river mouth, had a length of 100 m, a depth of 1.5 m, and was relatively uniform, containing no visible woody debris or structure (Fig. 2C). The downstream end of the fifth site was located 1.5 km from the confluence of the river. This site was 2.3 m deep, 100 m long, and ended at a bridge on the upstream end. On the south shore, near the bridge, there was a 6 m stretch of rocks

and boulders that extended 1 m into the water. This site also contained two regions (each ~ 35 m^2) of partially submerged woody debris on either side of the downstream end of the site. All sites had an approximate area of 4,500 m^2 except for Site 3 (6,300 m^2) and turbidity readings were between 28 and 90 FTU (USGS 2016).

A run consisted of a downstream transit of the sites followed by a slow 180° turn and upstream return to the origin. In Sites 1 – 3, the boat was positioned closest to the shorelines containing woody debris, which were all located on the starboard side of the boat upon the initiation of each run (Fig. 2). The complete circuit in Sites 4 and 5 resembled an elongated ellipse rather than straight line bisecting the middle of the channel (Fig. 2). Sound was broadcast during the entire transit, with boat speed maintained between 3 – 6 km/hr. The boat was propelled using a 4-stroke 150 hp motor (Yamaha; Kennesaw, GA). Each trial consisted of at least three complete runs with the sound and one transit without sound broadcast. A 10 minute recovery period was allowed between each run. One trial in all five sites was conducted on September 15, 2015. On September 16, 2015, one trial was conducted in both Sites 1 and 5, for a total of seven trials over the two-day testing period. Controls consisted of one run conducted with no sound. The order of experimental and control runs was randomized. To record the silver carp's jumping behavior, four GoPro Hero3 (San Mateo, CA) cameras were mounted to the bow, port, stern, and starboard sides of the boat and positioned so there was no overlap between each camera's field of view (recording quality: 1080 pixels; 30 frames/second).

Video Footage Analysis

The GoPro video was analyzed frame-by-frame (30 frames per second) in Adobe Photoshop CS6 (version 13; San Jose, CA) and the number of observed jumping fish in each run was determined. To ensure each fish was counted once, only jumps that were initiated in a camera's field of view were quantified. All jumping fish in Sites 2 – 5 (Site 5 on both days) and 40% of the fish that jumped in Site 1 (on the first testing day) were further analyzed to assess the

silver carp's position in relation to the boat when responding to the broadband sound. These fish were assessed for their "jump origin" (when the fish first broke the water surface) in relation to the boat position (bow = 0°; starboard = 90°; stern = 180°; port = 270°). The approximate distance from the boat was estimated by comparing the number of pixels between two landmarks (i.e. distinct submerged logs or trees) with known distances apart to the number of pixels measured between the fish and the boat.

Data Analysis

For all trials, the number of fish that jumped in each experimental and control run were counted. Next, for all the sites, the areas of the regions that contained woody debris or rocks or were free from submerged debris were estimated based on the length and width of these regions. The length and width estimates were obtained using a Humminbird 998c HD 360° sonar unit (Eufaula, AL). All fish were grouped based on jumps occurring in areas where debris was visible, projecting above the water, or in areas free from visible debris. To assess the effect of river composition on jumping occurrence, the density (fish/m^2) of fish that jumped in areas containing submerged debris was compared with the densities of fish jumping in regions free from debris using a non-parametric Mann-Whitney Rank Sum Test performed in SigmaPlot for Windows (version 12.4). The medians are reported as (median; 1st Q, 3rd Q).

The jump initiation angles were analyzed in Oriana (version 4; Kovach Computing Systems; Wales, UK). Rao's Spacing Test and the circular variance test were implemented to determine whether or not the jump initiation angles were randomly distributed.

Results

The number of observed jumping carp varied greatly among the three sites, however, the initial run stimulated the most jumpers at all seven sites (Fig. 3). Site 1 (day 1) had the highest number of observed jumping fish (1268 total), with 638, 407, and 223 jumping during the respective runs (Fig. 3). On the second day of testing, there were less overall jumping fish (626)

in Site 1 (run 1: 402, run 2 149, run 3: 75). The number of observed jumping silver carp decreased over the three runs in all sites with the exception of Site 2 and Site 5 (day 2). In Site 2, 207 fish jumped during the first run, 72 in the second, and 106 in the third (Fig. 3). For Site 5 (day 2), 25 fish jumped in run 1, 10 in run 2, and 21 in run 3. This trial also had the lowest total jumping fish (56) of all trials. The most dramatic decrease (91.4%) from the first (409 jumping carp) to the third (35 jumping carp) run occurred in Site 3 (run two had 98 jumpers). Less carp responded in both Sites 4 (run 1: 39 jumping carp; run 2: 20; run 3: 7) and 5 (run 1: 33; run 2: 18; run 3: 11) than in the other sites on the first testing day (Fig. 3). Only the control runs in Site 1 (4 fish) and 3 (46 fish) elicited jumping from the carp (day 1). The density of fish jumping in regions containing partially submerged debris (0.43 fish/m^2; 0.20, 0.87) was significantly (P = 0.001) higher than the density of jumping fish in clear river areas (0.014 fish/m^2; 0.0027, 0.029) (Fig. 4).

Silver carp jumped in every 30° arc around the boat, however, there were some arcs in which more jumping fish were observed (Fig. 5A). The most carp jumped between the 30° – 60° (22.0% of all jump origins) and 120° – 150° (17.3%) arcs. Jumping was also concentrated between the 210° – 240° (9.0%) and 300° – 330° (9.4%) arcs. The mean jump initiation angle was 74.6^o ± 3.7^o and the both the Rao's test (P < 0.01) and circular variance (0.72) indicate that there was significant clustering (Fig. 5B).

Discussion

This is the first study to examine wild silver carp responses to a sound signal independent of stimulation from moving watercraft and the results indicate that high intensity (> 150 dB) broadband sound alone can elicit jumping. The broadband sound stimulus used in this experiment is comprised of high frequency components (0.06 – 10 kHz) that are at least partially within silver carp's hearing range (Lovell et al. 2006). Previous work has shown that captive silver carp

demonstrate consistent negative phonotaxis to the same broadband sound (Vetter et al. 2015) and these results together suggest that this stimulus is effective in altering silver carp behavior.

In all trials, there was a decrease in the number of jumping fish from the first to the third run. This suggests that the fish were either habituating to the stimulus or moving out of the area. As silver carp can be repeatedly deterred using broadband sound (Vetter et al. 2015, Murchy et al. 2016), it is possible that the broadband sound stimulus was causing fish to leave the sites. This has implications for sound as a tool to herd fish towards a net or shallow area for removal.

The jumping pattern observed in this study was different from that demonstrated by fish responding to fast moving boats (16 – 40 km/hr). When reacting to the fast moving watercraft, silver carp primarily jumped behind the boat but avoided the area directly astern (< 4.0 m), in the most turbulent part of the wake (Vetter et al., unpublished). When presented with high intensity broadband sound, the fish jumped around the boat. However, the jump initiation angles were clustered in the $30^{\circ} – 60^{\circ}$, $120^{\circ} – 150^{\circ}$, $210^{\circ} – 240^{\circ}$, and $300^{\circ} – 330^{\circ}$ arcs. The speakers were positioned on either end of the boat's bow so they projected sound underwater ahead of and behind the boat and it appears that the jumps were concentrated in the path of sound. Additionally, significantly more silver carp jumped in river areas where partially submerged woody debris was present. It is possible that the fish prefer these more protected and shaded regions, so the densities of carp are higher.

There was also a greater number of jumping fish on the starboard ($0^{\circ} – 180^{\circ}$) side of the boat. This is possibly related to the locations of submerged woody debris in the river. In order to elicit jumping from as many fish as possible during a run, the boat would remain on the side of the river in which submerged woody debris was present. The most fish jumped in Sites 1 and 3, particularly among the debris, and these areas were on the starboard side of the boat at the start of each run.

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It appears that fish responding to both the fast moving boats and high intensity sound are stimulated to jump at the interface between high and low stimuli. The wake created by a fast moving boat is most turbulent in the region directly behind the boat because of the viscous wake and propeller downwash within the centerline wake (Reed and Milgram 2002). The Kelvin wake, which extends along the outside of the centerline wake, creates waves. Most of the jumping occurred both in these waves and in the Kelvin wake, just outside of the whitewater and viscous wake (Vetter et al. unpublished). Similarly, in this study, the fish were primarily jumping in the regions where sound was projected.

Although the broadband sound used was recorded from a 100 hp motor moving at 32 km/hr in the Illinois River, the sound broadcast from the speakers was likely altered from the original motor noise. First of all, the hydrophone is only capable of detecting sound pressure, so the particle motion component of the sound output from the motor was not included in the recording. Secondly, the speakers used have a flat frequency output, so it is likely that they also modified the sound. However, the broadband sound used in this experiment was highly effective at stimulating jumping behavior and suggests that this kind of stimulus may be an important tool to manage silver carp.

Researchers are currently evaluating the efficacy of acoustic deterrents to prevent further range expansion of silver carp. This study not only implies that high intensity sound is capable of eliciting jumping behavior from carp, it also supports the use of broadband sound as a management tool. For instance, high intensity broadband sound could be used to herd fish or clear them from a region, such as a concrete lock, before allowing a ship to pass. It is imperative that field trials utilizing broadband sound to remove fish from a lock or herd them into a net or shallow area be conducted to evaluate these practical uses for the stimulus.

Figure 1: A) Aerial view of the five sites in the Spoon River. **B)** An example of the partially submerged woody debris located in Site 3. **C)** View of Site 4 from the bow camera; this site was free from debris.

Figure 2: Boat paths (dotted lines) utilized when stimulating carp to jump during runs in Sites 1 – 3 (Site 3 is example) and Sites 4 and 5 (Site 5 is example). Areas where submerged rocks and partially submerged woody debris are indicated. Green arrows signify the start of a run and the red arrows denote the end.
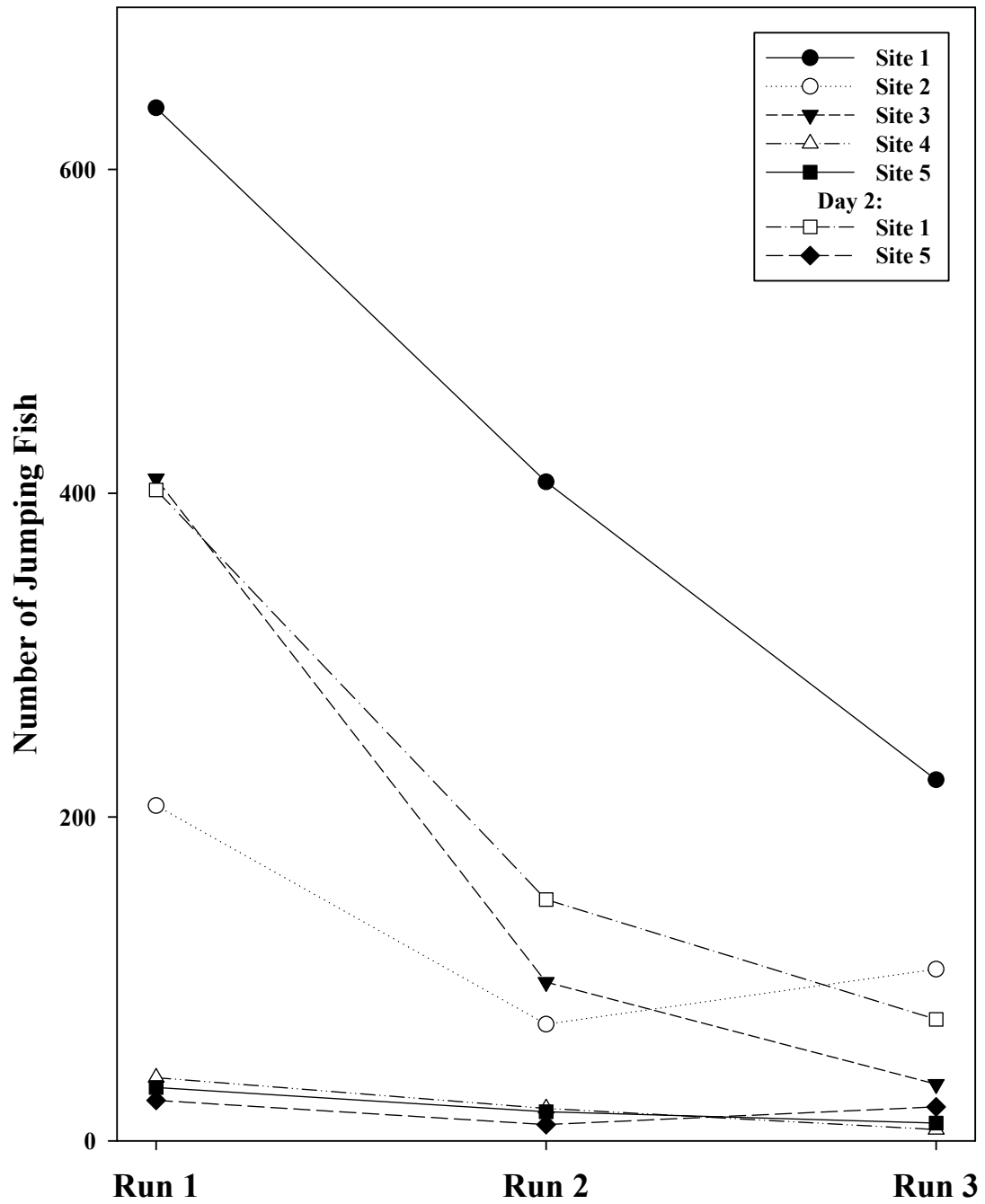
Figure 3: Number of jumping fish during each run at all five sites on the first testing day and Sites 1 and 5 on the second day of testing.
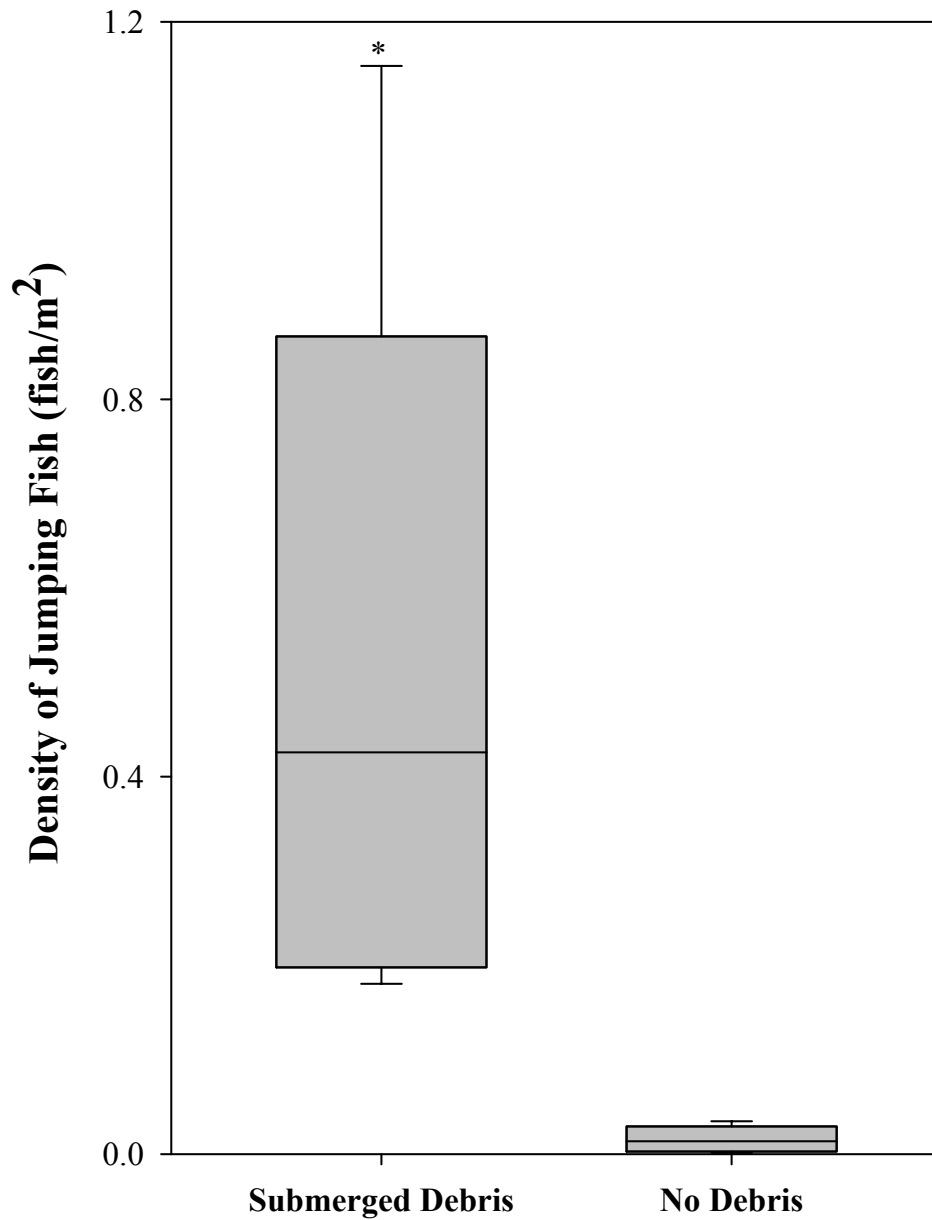
Figure 4: Median densities of jumping carp (number of observed jumping carp/m^2) in areas where partially submerged woody debris was present and areas where there was no debris during all seven trials. The bottom of the box represents the lower quartile while the top of the box is the upper quartile and the median is located within the box. The whiskers indicate the 10th and 90th percentiles. * represents significant difference (Mann-Whitney P = 0.001).
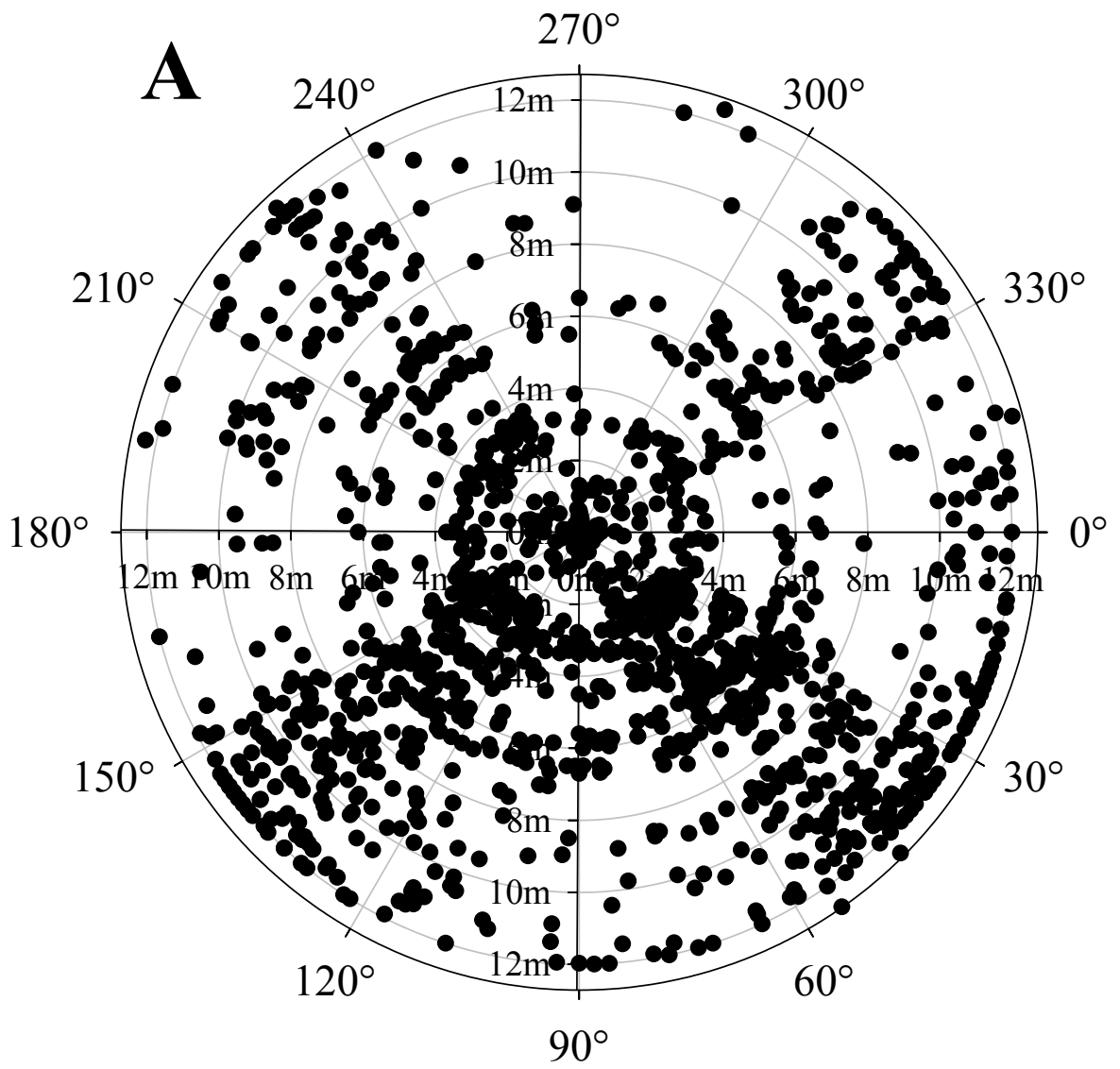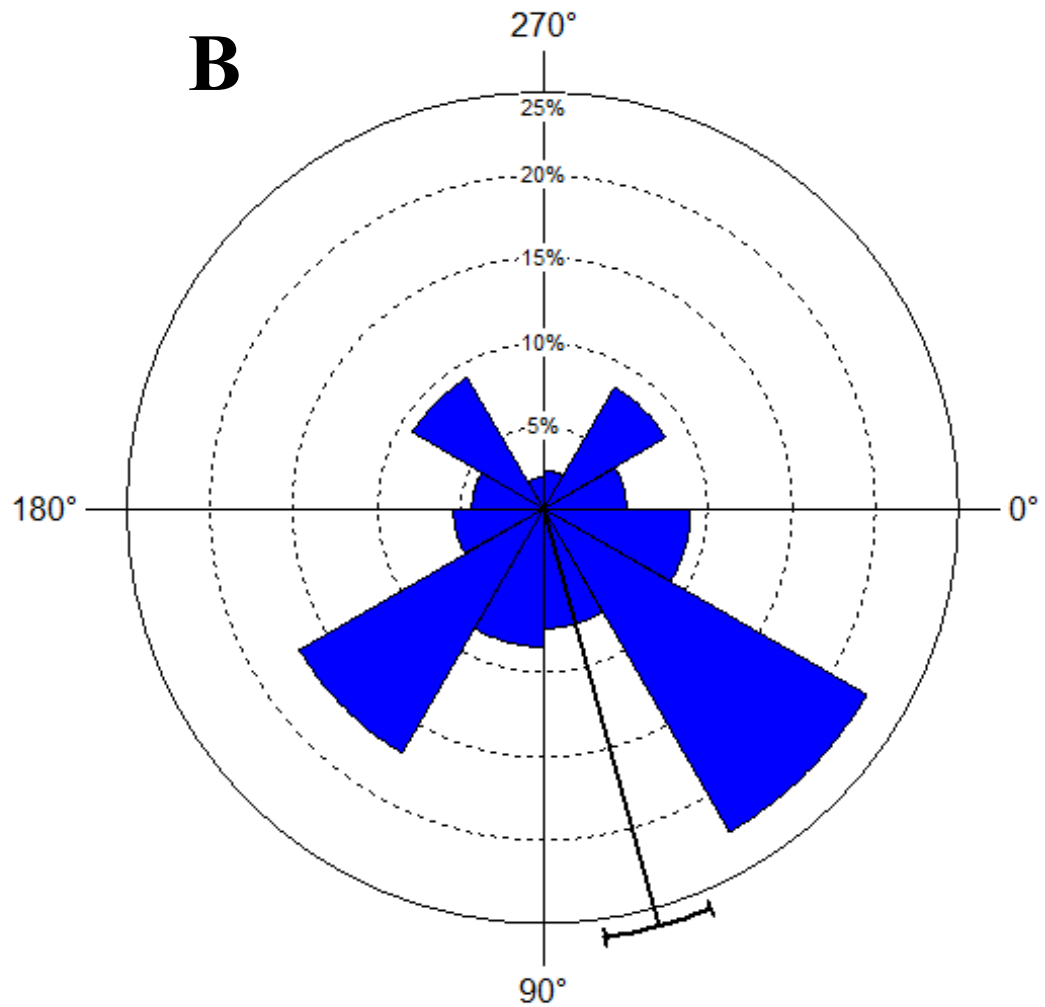
Figure 5: A) Polar plot indicating the jump initiation angle of all fish analyzed from the seven trials. The boat's bow and direction of movement are at $0°$, starboard site is $90°$, stern is $180°$, and port is $270°$. **B)** Circular plot representing the percentage of jumps initiated in twelve $30°$ arcs around the boat. Median jump initiation angle ($74.6°$) is indicated by the solid black radial line with 95% confidence interval brackets. Rao's $P < 0.05$ and the circular variance was 0.72, indicating significant clustering around the mean.

Chapter 6: Conclusions and potential uses for broadband sound

Silver and bighead carp possess high frequency hearing (up to at least 3 kHz) and they demonstrate consistent negative phonotaxis to broadband (0.06 – 10 kHz) sound stimuli (Chapters 2 and 3). Furthermore, wild silver carp jump in response to high intensity broadband sound (> 150 dB) (Chapter 5). There appears to be a link between silver carp jumping and the sound emitted from motorized watercraft, as the broadband sound stimulus used to elicit jumping was recorded from an outboard motor on the Illinois River. However, it seems that while jumping behavior can be provoked with sound alone, it is a multifactor response that is also related to the boat wake. When responding to fast moving boats (> 15 km/hr) silver carp may have been more influenced by the boat wake than the engine noise (Chapter 4), which is not as loud (130 dB) as the broadband sound used in Chapter 5 (> 150 dB).

The captive silver carp did not jump when presented with broadband sound (Chapter 2); however, there are some important differences between the environment in the concrete ponds and the Illinois and Spoon Rivers. First of all, the water in the concrete ponds was relatively clear while the turbidity is high in the Illinois and Spoon Rivers, with readings between 22 and 98 FTU (USGS 2016). Secondly, the densities of fish are much higher in the rivers, particularly in the Spoon River because it is narrower than the Illinois River. In addition, the rivers contain regions where there is extensive submerged woody debris or rocks. Therefore, it is possible that turbidity, high densities of fish, and presence of debris also contribute to jumping. In the Spoon River, for instance, carp are over-crowded in areas of low visibility and therefore jumping could be an escape mechanism when swimming away is not an option. The results from Chapter 4, which demonstrated that more fish jumped in the narrower river section (Sites 1 and 2) than the wide segment (Site 3), support this. As native silver carp have been observed to jump when captured in nets (Duane Chapman, personal communication), future studies on carp in China, especially their

behavioral response to high intensity broadband sound, would aid in a better understanding of the jumping trigger and its relationship to sound stimuli.

Characterizing silver and bighead carp behavioral responses to sound stimuli is intriguing from both an animal behavior and management perspective. First of all, as these fish are so closely related, it is interesting that silver carp jump response to moving watercraft while bighead carp do not. Furthermore, the results from this dissertation work, which showed that bighead carp exhibited more consecutive negative phonotaxic reactions (Chapter 3), suggests that the bigheads are more responsive than the silvers to broadband sound recorded from an outboard motor. This behavioral paradox could be explained through a better understanding of the individual hearing abilities for both fish. It is possible that variations in their frequency sensitivities are responsible for these behavioral differences.

An assessment of the complete frequency sensitivity for both species will allow for the development of a specific broadband sound to target these invasive carp and pose minimal impact on the native species. Furthermore, determining the optimal wavelength to vibrate the silver and bighead carp's swim bladders may also give insight on frequencies that would be most effective in altering these fishes' behavior. Many native species, including lake sturgeon, paddlefish, and a number of game fish, are non-ostariophysans and cannot hear higher frequencies. Therefore, it may be possible to develop a refined broadband stimulus that deters only silver and bighead carp. Such an acoustic deterrent could be useful in many practical applications. For instance, the research conducted in the concrete ponds suggests that underwater speakers placed in a lock chamber could be used to drive fish out of the area before allowing a ship to pass. Additionally, both the captive carp experiments and field studies from this dissertation suggest that carp could be herded with sound or fast moving boats. It is imperative that further field tests using broadband sound be conducted so this tool can be incorporated into an integrated pest management approach.

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