A field study testing the ability of a sound deterrent system to block the upstream migration of invasive carp through a Mississippi River Lock and Dam

A PLAN B THESIS
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Abstract

Preventing the spread of invasive species is one of the most pressing issues in fisheries management. Because of the hydrologic connectivity of North American waterways, there is an urgent need to develop behavioral deterrent systems to impede the spread of the invasive silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), and common carp (*Cyprinus carpio*). Although the sound of an outboard-motor has been shown to affect the behavior and movement of invasive carp in small-scale laboratory and field studies, these studies were performed under conditions that failed to imitate the conditions of a Mississippi River Lock and Dam (LD). Mississippi River LD's create permeable conditions that impede the natural movement of many fishes, including the upstream migration of invasive carp. It has been hypothesized that a sound deterrent system placed at the entrance of a lock could enhance the blocking ability of a LD. The overarching goal of this thesis was to determine if the sound of an outboard-motor (500 Hz – 1,500 Hz) would deter wild, free-ranging, adult invasive carp from entering the lock of a Mississippi River LD. This is the first full-scale field study to test the efficacy of a sound deterrent system to block the upstream movement of free-ranging invasive fish through a large river lock and dam. This study took place at Mississippi River LD8, where a sound deterrent system was installed on the face of the downstream lock chamber gates. Eight trials were conducted, with each trial monitoring the movements of 20 acoustically tagged common carp (8 trials, 20 fish per trial, n = 160) for 14 consecutive days. The eight trials alternated between sound off and sound on (sound off n = 4, sound on n = 4). The number of attempts, passages, and passage rates of fish through the sound deterrent system were analyzed for each trial, and then compared to determine if there were significant differences between treatments. There was no significant difference between treatment attempts (Mann-Whitney *U* Test: U=5, p value >0.05) A total of 15 common carp passed upstream through the sound deterrent system, nine when the sound was off, and six when the sound was on. There was no significant difference between the number of passages between treatments (Mann-Whitney *U* Test: U=5.5, p value >0.05). A two proportion z-test rejected the hypothesis that a lower overall passage rate would occur during sound-on trials (two proportion z-test, p value = 0.5).

In addition to the full-scale field study, a short laboratory study was performed. To avoid damaging the LD8 field speakers, the outboard-motor sound was truncated from 20 Hz – 10,000 Hz to 500 Hz – 1,500 Hz. Previous laboratory studies tested the 20 Hz – 10,000 Hz frequency range on common carp, with results showing significant negative phonotaxis. However, the truncated frequency range had not been tested before. The goal of this laboratory study was to test this truncated frequency range on common carp in the laboratory. Ten different groups of ten randomly selected juvenile common carp were exposed to eight trials (N= 80 observations). Each trial consisted of a one hour acclimation period, eight exposure periods that consisted of a six min pre-test period with no sound stimuli, followed by a six min test period with the sound stimuli active, and concluding with a ten min recovery period. We examined passage rates to estimate change in behavior during pre-test periods and behavior induced during test periods. When exposed to the truncated stimulus, common carp passage decreased significantly for the first two exposures ($p < 0.05$), resulting in an overall blockage efficiency of 26 ± 4% (mean ± SD) relative to the mean passage rate during the no-treatment control experiment ($p < 0.05$). While pre-test passage rates did not change ($p < 0.05$), test passage rates increased on average by 9% during each subsequent exposure, which is symptomatic of habituation ($p < 0.05$). Results indicate that lower (<500 Hz) and higher (>1,500 Hz) frequencies are likely needed to increase deterrent effectiveness, which in part, may explain the field study results.

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Chapter 1: General Introduction

The Invasive Carp Problem

Although liquid fresh water makes up only 0.01% of total global surface water (Stiassny 1996), it comprises an extremely important ecosystem. Freshwater ecosystems are home to at least 126,000 plant and animal species (Hawksworth et al. 1995), and provide humans with many services and benefits. Freshwater ecosystems provide water supply for drinking, household uses, manufacturing, industrial and power generation, irrigation, aquaculture, food and clothing, and non-extractive services such as flood control, transportation, wildlife habitat, and recreation (Daily 1997). Unfortunately, freshwater ecosystems may well be the most endangered type of ecosystems in the world, as the threat of overexploitation, water pollution, flow modification, destruction or degradation of habitat, and the introduction and spread of invasive species continues to increase worldwide (Dudgeon et al. 2006). One of the prevalent themes in freshwater ecosystem research and management is preventing the introduction and spread of aquatic invasive species.

Invasive species by definition are any kind of living organism that is not native to an ecosystem that does ecological and/or economic harm, or harm to human health (Clinton 1999). Invasive species can change the structure or function of the ecosystem, causing impacts at various levels of ecological organization (i.e., ecosystem, community, population, etc.). In extreme cases, invasive species have been cited as the sole cause of native species extinction. For example, Clavero and Garcia-Berthou (2005) analyzed IUCN Red List extinction records and determined that 34 cases (20%) were solely caused by invasive species. In the United States, direct damage estimates to the Laurentian Great Lakes caused by aquatic invasive species (AIS) is estimated to be $138 million per year, and as high as $800 million annually when secondary impacts (e.g., sport fishing losses) are included (Rothlisberger et al. 2012). In an effort to combat ecological and economic impacts and losses, the United States spends almost $120 billion per year trying to prevent the introduction and/or spread of invasive species (Pimentel et al. 2005). At least 182 non-native species have been introduced into the Laurentian Great Lakes and Mississippi River basins, and currently threaten an area with the highest diversity of freshwater fishes and mussels in North America (Rasmussen 2002; Ricciardi 2006; Patel et al. 2010; Rasmussen et al. 2011). Because of the hydrologic connectivity of North American waterways and the ecological and economic burdens associated with invasive species, stakeholders of the Laurentian Great Lakes and Mississippi River basins are particularly concerned by two invasive fishes: the silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*), hereafter collectively referred to as bigheaded carp.

Bigheaded carp are filter-feeding fish that were introduced to Arkansas from Asia in the early 1970s to keep commercial aquaculture ponds and wastewater treatment facility ponds clean (Freeze and Henderson 1982; Jennings 1988). Shortly thereafter, these carp escaped confinement during flood events and have been spreading northward throughout the Mississippi River Basin and towards the Laurentian Great Lakes via the Illinois River. A recent study by Larson et al. (2017) determined that the leading edge of reproducing bigheaded carp ("invasion front ") lies somewhere along the Iowa/Illinois border between Pool 14 (Clinton, IA), where no eggs or larvae were found, and Pool 16 (Muscatine, IA), where eggs and larvae have been found. It is important to note that Pool 16 is upstream of the Illinois River confluence. The bigheaded carp invasion front has progressed up the Illinois River to within 75 river kilometers (rKm) of Lake Michigan (Asian Carp Regional Coordinating Committee [ACRCC], 2016).

Like many invasive species, bigheaded carp possess characteristics that promote their ability to spread and thrive in new environments. For example, bigheaded carp are fast growing (i.e., high rates of consumption), prolific breeders, food generalists (i.e., in terms of plankton), have few natural predators, and can tolerate a broad range of environmental conditions (Kolar et al. 2007; DeGrandchamp et al. 2011). In terms of ecological impacts, the feeding tendencies of bigheaded carp are of concern to managers and scientists of the Laurentian Great Lakes and Mississippi River basins. Bigheaded carp filter-feed on phytoplankton and zooplankton, and because almost all fish forage on planktonic organisms during early life-history stages, they compete for food with every species of fish (Xie and Yang 2001; Kolar et al. 2007; Solomon et al. 2016). Bigheaded carp have also shown to compete with economically important adult native fishes with similar diets, such as the bigmouth buffalo (*Ictiobus cyprinellus*), American paddlefish (*Polyodon spathula*) and gizzard shad (*Dorosoma cepedianum*) (Irons et al. 2007; Sampson et al. 2009; Schrank et al. 2011; Wang et al. 2018). For example, in the La Grange reach of the Illinois River, a tributary to the Mississippi River, evidence from a long-term monitoring program (i.e., over 20 years) revealed evidence that silver carp abundance adversely affected the relative abundance of nineteen species of native sport fish, by likely reducing the abundance of zooplankton both through direct consumption of zooplankton and by competing with zooplankton for phytoplankton (Chick et al. 2019). In the same reach, Sass et al. (2010) analyzed Long Term Resource Monitoring Program (LTRMP) data that showed an exponential increase in silver carp catches since 1998, with an intrinsic population growth rate increase of 83.7% (i.e., reproduction rate minus the death rate). Further, the percentage of silver carp captured versus all other fishes increased from <0.1% in 1998 to 50.9% in 2008 (Sass et al.

2008). The effects of bigheaded carp is not limited to ecological disruptions, as there is great concern over their potential economic impacts.

The Laurentian Great Lakes and Mississippi River basins have valuable recreational and commercial fisheries that could be affected by the further spread of bigheaded carp (Carlson et al. 1995; Stern et al. 2014; Chick et al. 2019). Silver carp pose a direct threat to human safety because of their habit of jumping out of the water in response to acoustic stimuli (Vetter et al. 2017). This threat to human safety combined with the aforementioned ecological threats could be detrimental to the economic value of the Laurentian Great Lakes and Mississippi River basins. An estimated $7 billion annually is generated by fisheries of the Laurentian Great Lakes (Stern et al. 2014), and recreation on the Mississippi River was valued at $1.2 billion in 1990, which is equivalent to $2.2 billion in 2018 (Chick et al. 2019). These values could significantly decrease with the establishment of a breeding bigheaded carp population. Thus, finding a solution to prevent the further spread of these fish has intensified among freshwater ecosystem researches and managers.

In addition to bigheaded carp, common carp (*Cyprinus carpio*) were deliberately introduced to the United States from Eurasia in the 1800s for as a food source (Sorensen and Bajer 2011; Memis and Kohlmann 2006). In the United States , the common carp is the most frequently reported nuisance fish (Kohler and Stanley 1984), and one of only eight fish on the IUCN list of the world’s worst 100 invaders (Lowe et al. 2000). The common carp is highly invasive in many shallow freshwater ecosystems throughout the United States, including areas of the Laurentian Great Lakes and Mississippi River basins (Lougheed and Chow-Fraser 1998; Lubinski et al. 1986).

Common carp also have life history traits that likely explain their propensity to establish populations in many non-native habitats. For example, common carp are highly fecund, with females carry up to 3 million eggs (Swee and McCrimmon 1966, Sorensen and Bajer 2011), become sexual mature between ages 2 and 3 (Weber and Brown 2009), can make expansive reproductive migrations up to 200km (Stuart and Jones 2006b), have fast growth rates (Jackson et al. 2008; Weber et al. 2010), and live up to 64 years (Koch 2014). The feeding tendencies of common carp also cause ecosystem damage. Common carp are benthic omnivorous with a diet that includes, detritus, macroinvertebrates, zooplankton, and plants (Weber and Brown 2009). Common carp directly feed on macrophytes and invertebrates and indirectly uproot/damage the macrophyte community while foraging (Miller and Crowl 2006). This feeding behavior re-suspends sediments and nutrients that would otherwise remain in the benthos, which can lead to significant reductions in water quality via increased turbidity and eutrophication (Huser et al. 2016). Thus, freshwater ecosystem researchers and managers take costly measures to control and/or eradicate these fish, albeit with limited success (Sorensen and Bajer 2011).

Chemical toxins, barriers, physical removal, and predator management (e.g., increasing fish species that predate on common carp eggs and larval) are current techniques being deployed to manage established common carp populations. For example, Smith-Root (Vancouver, WA, USA; www.smith-root.com/barriers) develops electric barriers to block the movement of fish, including common carp. However, electric barriers are costly and pose safety concerns to humans and wildlife. With the exception of electric barriers, the aforementioned techniques fail to prevent the movement of common carp between nursey wetlands and larger lakes/rivers. Because adult common carp migrate from larger lakes/river into shallow nursery wetlands to spawn (Bajer and Sorensen, 2010), this behavior could be targeted to control/eradicate their populations. For example, developing and deploying cost-conscious technology that prevents common carp from gaining access to these spawning habitats could reduce recruitment. In sum, developing a solution that impedes the movement of both common carp and bigheaded carp is critically needed.

Mississippi River Locks and Dams

The flows of the upper Mississippi River, from Minneapolis, MN to St. Louis, MO, are regulated by 29 dams that span the entire width of the river and regulate water depths to maintain a 9 foot channel for commercial navigation. Dams create permeable conditions that impede the natural movement of many fishes (Argent and Kimmel 2011; Liermann et al. 2012, Anderson et al. 2019), including the upstream migration of common and bigheaded carps (Kolar et al. 2007; Tripp et al. 2014; Lubejko et al. 2017; Finger et al. 2020). Therefore, strategies and technologies could be deployed at dams to further enhance their blocking ability.

Most dams on the Mississippi River offer two pathways for the upstream movement of fishes: navigational locks and gated spillways (i.e., the actual dam), hereafter referred to as a lock and dam (LD). A navigational lock is a chamber that enables a vessel (e.g., large ship or barge) to move through a dam while maintaining water depths both above and below the dam. Water velocities around and within lock chambers are negligible and telemetry studies have shown that fish, including bigheaded and common carp, often use locks as an avenue to migrate upstream (Tripp et al. 2014; Lubejko et al. 2017, Finger et al. 2020). A lock and dam is largely comprised of spillway gates, which are raised and lowered to maintain sufficient water levels for navigation. When spillway gates are lowered into the water to control the water depth the condition is termed “controlled-river.” When the gates are raised completely out of the water to allow unrestricted flows the condition is termed “open-river.” As the spillway gates become more controlled, lowered further into the water column, water velocities flowing through the spillway gates increase. Conversely, water velocities decrease as the gates become less controlled and are lowest when in open-river. Recent telemetry studies have shown that fish passage through spillway gates tends to coincide with gates being in or near open-river conditions (Tripp et al. 2014; Lubejko et al. 2017; Finger et al. 2020). For example, Lubejko et al. (2017) tracked more than two hundred bigheaded carp and found that only 3 were able to pass through spillway gates during controlled-river conditions at Starved Rock Lock and Dam on the Illinois River. The lack of bigheaded carp passage through spillway gates during controlled-river conditions results from water velocities that exceed the physiological swimming capability of these fish (Zielinski et al. 2018). Spillway gate operations are highly site specific. For example, 10 out of the 29 LDs on the Mississippi River are in open-river less than 5% of the time (Fishpro 2004). In contrast, 11 of the 29 LDs are in open-river greater than 15% of the time (Fishpro 2004). Unlike the other 26 LDs, Upper St. Anthony Falls Dam, Lower St. Anthony Falls Dam, Lock and Dam 1, and Lock and Dam 19 (LD19) have fixed-crest spillways that are completely impassable to upstream fish movement (Wilcox et al. 2004). Upstream passage through these dams is only possible through the lock chambers (Wilcox et al. 2004). Therefore, strategies/technologies to block the upstream migration of bigheaded carp at LDs should be implemented at LDs that are rarely in open-river or have spillways that are completely impassable.

In instances where bigheaded carp are able to pass upstream through spillway gates during controlled-river conditions (e.g., Lubejko et al. (2017)), Zielinski et al. (2018) developed a physiologically inspired model based on swimming performance and fatigue that determines whether bigheaded carp passage could be reduced by altering spillway gate operations. Model results suggest that passage is low under current controlled-river gate operations, but could be further reduced by 50% through small adjustments to spillway gate operations. For example, the model can expose areas of lower flow velocities that bigheaded carp could exploit to gain upstream passage. With this information, slight changes to spillway gate operations (e.g., raise or lower gates) can be made to create water velocities that are always greater than the swimming performance of bigheaded carp. Implementing spillway gate operations based on this model at LDs that are rarely in open-river conditions essentially eliminate upstream passage of bigheaded carp through the spillway portion of the dam. However, passage would still be possible through the lock chamber. To address this issue, non-physical barriers (e.g., behavioral deterrents) have been suggested as a means of deterring bigheaded carp from entering the lock chamber.

Sound as an Invasive Carp Deterrent

Fish detect light, chemical, magnetic, tactile, and sound cues as they navigate the waters they inhabit. Behavioral deterrent systems that utilize aversive stimuli such as sound, electricity, light, bubbles, and carbon dioxide have been suggested as a means to impede the movements of bigheaded and common carp (Fishpro 2004; Noatch and Suski 2012). However, sound has emerged as having special promise. Sound propagates underwater as travelling pressure with accompanying particle motion that fish use to perform various functions including; navigation, obstacle and prey detection and avoidance, communication, and mating (Popper and Carlson 1998). Carps, like all ostariophysian fishes, have a better sense of hearing compared to many non-ostariophysian fishes. Non-ostariophysian fishes primarily detect and use the particle motion component of sound, whereas ostariophysian fishes are capable of detecting sound particle motion and pressure with notable sensitivity (Lovell et al. 2005, 2006; Mann et al. 2007). For example, silver, bighead, and common carp can hear sound frequencies up to 5000 Hz

(Vetter et al. 2018), whereas non-ostariophysian fishes like the lake sturgeon (*Acipenser fulvescens*) and American paddlefish (*Polyodon spathula*) can only hear sound to approximately 400 Hz (Lovell et al. 2006). This additional sensitivity is possible because ostariophysian fishes possess an anatomical connection between their inner ear (i.e., otoliths) and swim bladder (i.e., internal gas-filled organ that assists in buoyancy) called a Weberian apparatus (Higgs et al. 2006; Braun and Grande 2008). This anatomical feature combined with physiological/behavior studies (Putland and Mesinger 2019) and field observations of bigheaded carp displaying sensitivity (e.g., silver carp jumping behavior) to loud noises, like outboard-motors (Kolar et al. 2007; Vetter et al. 2017, Wamboldt et al. 2019), has led researchers to study whether sound can be used to manipulate the movements (e.g., active avoidance) of invasive carp species (Taylor et al. 2005; Ruebush et al. 2012; Vetter et al. 2015, 2017; Murchy et al. 2016, 2017; Zielinski and Sorensen 2017; Dennis et al. 2019). Additionally, the use of higher frequency sounds (e.g., >400 Hz) has the potential to manipulate carp behavior with minimal effects on native species without hearing specialization.

The complex sound (i.e., a sound composed of a number of sounds of different frequencies) of an outboard-motor has been shown to alter the movement patterns of bigheaded and common carp, causing active avoidance (Vetter et al. 2015, 2017; Murchy et al. 2016, 2017, Zielinski and Sorensen 2017; Dennis et al. 2019; Wamboldt et al. 2019). Several studies in well-lit outdoor concrete ponds demonstrated that bigheaded carp consistently and repeatedly turned away and did not cross a barrier (underwater speakers) playing complex tones (0 Hz - 10,000 Hz) derived from underwater recordings of a 100 horsepower outboard-motor (Vetter et al. 2015, 2017; Murchy et al. 2016). Results showed a significant decrease in the number of crossing attempts during sound projection, with repulsion rates of 82.5% for silver carp and 93.7% for bighead carp (Murchy et al. 2017). When a similar 40 horsepower complex outboard-motor sound (20 Hz - 10,000 Hz) was tested by Zielinski and Sorensen (2017) in a darkened laboratory maze, they found that this sound repelled about 75% of bigheaded and common carp. However, after two sound exposures, all three carp species ceased negative phonotaxis, suggesting habituation to the sound stimulus. Dennis et al. (2019) exposed bighead carp and common carp to the same outboard-motor sound employed and described by Zielinski and Sorensen (2017). Trials were performed in a large indoor elliptical fiberglass flume in complete darkness, where the sound barrier played on one side of the flume while the other side provided a place of refuge (i.e., background noise levels). Blockage efficiencies were calculated for both species, with bighead carp and common carp being blocked 76% and 42% of the time, respectively. Contrary to Zielinski and Sorensen (2017), habituation was not observed. Finally, in a small shallow (0.38-0.55 m) water field application, Wamboldt et al. (2019) tested the same aforementioned 100 horsepower outboard-motor sound on various wild fish species, including silver carp. This study took place at a water control structure (2 concrete culverts) that connect/control flows between the Emiquon Preserve floodplains and the Illinois River, near Lewiston, Illinois. Two underwater speakers were placed within each culvert and fish passage through this sound deterrent system was monitored using a passive integrated transponder (PIT) antenna array. Over 2 days of periodic sound stimuli exposure, passage of silver carp was not significantly reduced, as 29% of fish detected passed through the culvert. However, the authors speculate that the shallow water depth and reverberation (i.e., reflection and refraction) off the concrete culverts, degraded the coherency of the sound waves as they attenuated from the source, which likely reduced the effective range of the sound deterrent.

While these studies provide evidence that a complex outboard-motor sound can affect invasive carp behavior and movement, they were performed under conditions that fail to imitate the conditions of a Mississippi River Lock and Dam (LD). The laboratory studies were performed on commercially farm raised juvenile fish (< 300 mm) in small containments (e.g., concrete pond, fiberglass tank), with shallow water depths (< 2 m). Although the Wamboldt et al. (2019) field study was performed on wild adult silver carp, it took place in an extremely shallow water environment (< 0.5 m). The propagation of sound in shallow water is very different than in deeper waters (> 3 m) of a Mississippi River lock chamber. Additionally, the methods and lengths of sound exposure were highly variable among these studies, with none imitating a typical Mississippi River barge lockage (i.e., 1.5 h - 3.5 h). Lastly, abiotic factors such as water depth, flow, temperature, and clarity through and around a Mississippi River LD are continually in flux and impossible to replicate in small-scale laboratory/field studies. Therefore, there is a need to test this complex outboard-motor sound on wild, free-ranging, adult carps in a full-scale (Mississippi River LD) field study that accounts for the highly variable environmental conditions. In closing, it is important to note that behavioral deterrents, including sound, are not expected to be 100% effective. However, they are still extremely important because the goal of invasive carp management is to prevent a critical number of fish from occupying a new area (i.e., the number required to establish a breeding population) (Cudmore et al. 2012).

Stakeholder Analysis of the Field Study (Chapter 2)

During the initial planning stage of the field study (see chapter 2), it was important to analyze potential stakeholders and determine which ones to engage. Gaining support from various governmental agencies and a local business owner was essential for the success of this study. Government agencies that needed to be involved included the United States Army Corps of Engineers (USACE), United States Fish and Wildlife Service (USFWS), and the Minnesota and Wisconsin Department of Natural Resources (MNDNR, WDNR). The USACE owns and operates the various LDs along the length of the Mississippi River. Therefore, their permission and assistance was necessary to deploy and broadcast a sound deterrent system from the lock at LD8. The USACE also needed to approve the fish tracking equipment that was mounted on the LD. Additionally, the USACE records daily flow discharge and water temperatures that were important for result inferences. The USFWS is dedicated to the management of fish, wildlife, and natural habitats. Because bigheaded carp have shown to negatively affect native fishes and their natural habitats, the USFWS is heavily involved in the implementation and testing of deterrents to stop their spread. In fact, the USFWS's La Crosse Fish and Wildlife Conservation Office assisted with my chapter 2 field study. The MNDNR funded this project through an allocation from the Minnesota Outdoor Heritage Fund. The Mississippi River partially denotes the border between the states of Minnesota and Wisconsin, but jurisdiction is shared. Therefore, permits to collect fish for this study had to be granted by both the MNDNR and WDNR. Lastly, Clements Fishing Barge, Genoa, WI, needed to be informed of this study, as their stationary fishing barge is in close proximity to LD8. Although the lock chamber and the fishing barge are on opposite banks of the river and the sound deterrent system was unlikely to interfere with their operations, it was important to gain their support, as they are highly influential within the surrounding community.

Conclusion

The overall goal of my thesis was to determine if an outboard-motor sound could be used to impede the movement of bigheaded and common carp. In order to achieve this goal, two separate, yet complimentary studies, were performed. The first study (Chapter 2) was a full-scale field study at Mississippi River Lock and Dam 8 (LD8) that examined the efficacy of the outboard-motor sound to deter common carp from entering the navigational lock chamber. This study demonstrated that a complex sound deterrent system can be installed and tested at a Mississippi River LD. Chapter 2 was written as a management brief, with the intent of submitting this manuscript to the North American Journal of Fisheries Management. The second study (Appendix A) took place in the laboratory and examined the efficacy of the same outboard-motor sound to block common carp passage. We sought to compare the response of the wild, free-ranging, adult common carp in Chapter 2 to the response of juvenile common carp in the laboratory, to determine whether our laboratory results are applicable to the field. Unfortunately, the two studies were not comparable because the production and propagation of sound between the study sites were very different. Appendix A contains only the methods, results, and a short discussion of this laboratory study, which may be valuable to future studies.

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Chapter 2: A field study testing the ability of an outboard-motor sound to block invasive carp from entering the lock of a Mississippi River Lock and Dam

Abstract

Preventing the spread of invasive species is one of the most pressing issues in fisheries management. Because of the hydrologic connectivity of North American waterways, there is an urgent need to develop behavioral deterrent systems to impede the spread of the invasive silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), and common carp (*Cyprinus carpio*). Because carps have a better sense of hearing compared too many native non-hearing specialist fishes, sound deterrent systems strategically placed at Mississippi River locks and dams have been suggested as a promising technology to impede the upstream movement of these fish. Although the sound of an outboard-motor has been shown to affect the behavior and movement of invasive carp in small-scale laboratory and field studies, these studies were performed under conditions that failed to imitate the conditions of a Mississippi River Lock and Dam (LD). The overarching objective of this study was to determine if the sound of an outboard-motor would block wild, free-ranging, adult invasive carp from entering the lock of Mississippi River LD. This study took place at Mississippi River LD8, where a sound deterrent system was installed on the face of the downstream lock chamber gates. Eight trials were conducted, with each trial monitoring the movements of 20 acoustically tagged common carp (8 trials, 20 fish per trial, n = 160) for 14 consecutive days. The eight trials alternated between sound off and sound on (sound off n = 4, sound on n = 4). The number of attempts, passages, and passage rates of fish through the sound deterrent system were analyzed for each trial, and then compared to determine if there were significant differences between treatments. There was no significant difference between treatment attempts (Mann-Whitney *U* Test: U=5, p value >0.05). A total of 15 common carp passed upstream through the sound deterrent system, nine when the sound was off and six when the sound was on. There was also no significant difference between treatment passages (Mann-Whitney *U* Test: U=5.5, p value >0.05). A two proportion z-test rejected the hypothesis that a lower overall passage rate would occur during sound on trials (two proportion z-test, p value = 0.5). There are a number of potential reasons for the lack of response including; the chosen frequency spectrum, targeted sound pressure levels (dB ref. 1 µPa), the location of the speakers, the onset of sound activation, and fish habituation to the sound.

Introduction

A prevalent theme in freshwater ecosystem research and management is the prevention of aquatic invasive species. Because of the hydrologic connectivity of North American waterways, there is an urgent need to develop behavioral deterrent systems to impede the spread of the invasive silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), and common carp (*Cyprinus carpio*). Silver carp and bighead carp, hereafter referred to as bigheaded carp, are filter-feeding fish that were introduced to Arkansas from Asia in the early 1970s to keep commercial aquaculture ponds and wastewater treatment facility ponds clean (Freeze and Henderson 1982; Jennings 1988). Shortly thereafter, these carp escaped confinement during flood events and have been spreading northward throughout the Mississippi River Basin and towards the Laurentian Great Lakes via the Illinois River. A recent study by Larson et al. (2017) determined that the leading edge of reproducing bigheaded carp (“invasion front “) lies somewhere along the Iowa/Illinois border between Pool 14 (Clinton, IA), where no eggs or larvae were found, and Pool 16 (Muscatine, IA), where eggs and larvae were found. In is important to note that Pool 16 is upstream of the Illinois River confluence. The bigheaded carp invasion front has progressed up the Illinois River to within 75 river kilometers (rKm) of Lake Michigan (Asian Carp Regional Coordinating Committee [ACRCC], 2016).

Like many invasive species, bigheaded carp possess characteristics that promote their ability to spread and thrive in new environments. For example, bigheaded carp are fast growing (i.e., high rates of consumption), prolific breeders, food generalists (i.e., in terms of plankton), have no natural predators, and can tolerate a broad range of environmental conditions (Kolar et al. 2007; DeGrandchamp et al. 2011). In terms of ecological impacts, the feeding tendencies of bigheaded carp are a concern to managers and scientists of the Laurentian Great Lakes and

Mississippi River basins. Bigheaded carp filter-feed on phytoplankton and zooplankton, and because almost all fish forage on planktonic organisms during early life-history stages, they compete for food with every species of fish (Xie and Yang 2001; Kolar et al. 2007; Solomon et al. 2016). Bigheaded carp have shown to compete with economically important adult native fishes with similar diets, such as the bigmouth buffalo (*Ictiobus cyprinellus*), American paddlefish (*Polyodon spathula*) and gizzard shad (*Dorosoma cepedianum*) (Irons et al. 2007; Sampson et al. 2009; Schrank et al. 2011; Wang et al. 2018). For example, in the La Grange reach of the Illinois River, a tributary to the Mississippi River, evidence from a long-term monitoring program (i.e., over 20 years) revealed evidence that silver carp abundance adversely affected the relative abundance of nineteen species of native sport fish, by likely reducing the abundance of zooplankton both through direct consumption of zooplankton and by competing with zooplankton for phytoplankton (Chick et al. 2019). In the same reach, Sass et al. (2010) analyzed Long Term Resource Monitoring Program (LTRMP) data that showed an exponential increase in silver carp catches since 1998, with an intrinsic population growth rate increase of 83.7% (i.e., reproduction rate minus the death rate). Further, the percent of silver carp captured versus all other fishes increased from <0.1% in 1998 to 50.9% in 2008 (Sass et al. 2008).

In addition to bigheaded carp, the invasive common carp (*Cyprinus carpio*) was deliberately introduced to the United States from Eurasia in the 1800s as a food source (Sorensen and Bajer 2011; Memis and Kohlmann 2006). In the United Stated, the common carp is the most frequently reported nuisance fish (Kohler and Stanley 1984), and one of only eight fish on the IUCN list of the world's worst 100 invaders (Lowe et al. 2000). The common carp is highly invasive in many shallow freshwater ecosystems throughout the United States, including areas of the Laurentian Great Lakes and Mississippi River basins (Lougheed and Chow-Fraser 1998;

Lubinski et al. 1986). The common carp has life history traits that likely explain their propensity to establish populations in many non-native habitats. For example, the common carp is highly fecund, with females carry up to 3 million eggs (Swee and McCrimmon 1966, Sorensen and Bajer 2011), become sexually mature between ages 2 and 3 (Weber and Brown 2009), can have expansive reproductive migrations up to 200km (Stuart and Jones 2006b), have fast growth rates (Jackson et al. 2008; Weber et al. 2010), and can live up to 64 years (Koch 2014). Similar to bigheaded carp, the feeding tendencies of common carp also cause undesirable environmental consequences. Common carp are benthic omnivorous with a diet that includes, detritus, macroinvertebrates, zooplankton, and plants (Weber and Brown 2009). Common carp directly feed on macrophytes and invertebrates and indirectly uproot/damage the macrophyte community while foraging (Miller and Crowl 2006). This feeding behavior re-suspends sediments and nutrients that would otherwise remain in the benthos, which can lead to significant reductions in water quality via increased turbidity and eutrophication (Huser et al. 2016).

Freshwater ecosystem researchers and managers take costly measures to eradicate and/or control the spread bigheaded and common carp, albeit with limited success (Sorensen and Bajer 2011). Thus, development of behavioral deterrent systems that specifically impede the movements of bigheaded and common carp is urgently needed. Behavioral deterrent systems that utilize aversive stimuli like sound, electricity, light, bubbles, and carbon dioxide have been suggested as a means to impede the movements of bigheaded and common carp (Fishpro 2004; Noatch and Suski 2012). However, sound has emerged as having special promise. Sound propagates underwater as a travelling pressure wave with accompanying particle motion that all fish detect via otoliths within the inner ear (Popper and Carlson 1998). Carps, like all ostariophysian fishes, have a sense of hearing that is superior to that of many non-ostariophysian fishes. Non-ostariophysian fishes primarily detect and use the particle motion component of sound, whereas ostariophysian fishes are capable of detecting sound particle motion and pressure with notable sensitivity (Lovell et al. 2005, 2006; Mann et al. 2007). For example, silver carp, bighead carp, and common carp hear sound frequencies up to 5000 Hz (Vetter et al. 2018), whereas non-ostariophysian fishes like the lake sturgeon (*Acipenser fulvescens*) and American paddlefish (*Polyodon spathula*) can only hear sound to approximately 400 Hz (Lovell et al. 2006). This additional sensitivity is possible because ostariophysian fishes possess an anatomical connection between their inner ear (i.e., otoliths) and swim bladder (i.e., internal gas-filled organ that assists in buoyancy) called a Weberian apparatus (Higgs et al. 2006; Braun and Grande 2008). This anatomical feature combined with physiological/behavior studies (Putland and Mesinger 2019) and field observations of bigheaded carp displaying sensitivity (e.g., silver carp jumping behavior) to loud noises, like outboard-motors (Kolar et al. 2007; Vetter et al. 2017, Wamboldt et al. 2019), has led researchers to study whether sound can be used to influence the movements (e.g., active avoidance) of invasive carp species (Taylor et al. 2005; Ruebush et al. 2012; Vetter et al. 2015, 2017; Murchy et al. 2016, 2017; Zielinski and Sorensen 2017; Dennis et al. 2019). Additionally, the use of higher frequency sounds (e.g., >400 Hz) has the potential to manipulate carp behavior while minimalizing the effects on native fishes.

The complex sound (i.e., a sound composed of a number of sounds of different frequencies) of an outboard-motor has been shown to alter the movement patterns of bigheaded and common carp, causing active avoidance (Vetter et al. 2015, 2017; Murchy et al. 2016, 2017, Zielinski and Sorensen 2017; Dennis et al 2019; Wamboldt et al. 2019). Several studies in well-lit outdoor concrete ponds demonstrated that bigheaded carp consistently and repeatedly turned away and did not cross a barrier (underwater speakers) playing complex tones (0 Hz - 10,000

Hz) derived from underwater recordings of a 100 horsepower outboard-motor (Vetter et al. 2015, 2017; Murchy 2016). Results showed a significant decrease in the number of crossing attempts during sound projection, with repulsion rates of 82.5% for silver carp and 93.7% for bighead carp (Murchy 2017). When a similar 40 horsepower complex outboard-motor sound (20 Hz - 10,000 Hz) was tested by Zielinski and Sorensen (2017) in a darkened laboratory maze, they found that this sound repelled about 75% of bigheaded and common carp. However, after two sound exposures, all three carp species ceased negative phonotaxis, suggesting habituation to the sound. Dennis et al. (2019) exposed bighead carp and common carp to the same outboard-motor sound employed and described by Zielinski and Sorensen (2017). Their trials were performed in a large indoor elliptical fiberglass flume in complete darkness, where the sound barrier played on one side of the flume while the other side provided a place of refuge (i.e., background noise levels). Blockage efficiencies showed bighead carp and common carp being blocked 76% and 42% of the time, respectively. Contrary to Zielinski and Sorensen (2017), habituation was not observed. Finally, in a small shallow (0.38-0.55 m) water field application, Wamboldt et al. (2019) tested the same aforementioned 100 horsepower outboard-motor sound on various wild fish species, including silver carp. This study took place at a water control structure (2 concrete culverts) that connect/control flows between the Emiquon Preserve floodplains and the Illinois River, near Lewiston, Illinois. Two underwater speakers were placed within each culvert and fish passage through this sound deterrent system was monitored using a passive integrated transponder (PIT) antenna array. Over two days of periodic sound stimuli exposure lasting 6-hr, passage of silver carp through the culvert was not significantly reduced, as 29% of fish detected passed through the culvert. However, the authors speculate that the shallow water depth and reverberation (i.e., reflection and refraction) off the concrete culverts, degraded the coherency of the sound waves as they attenuated from the source, which likely reduced the effective range of the sound deterrent.

While these studies provide evidence that a complex outboard-motor sound can affect invasive carp behavior and movement, they were either performed on juvenile fish in the laboratory or in a small-scale shallow water field setting. The laboratory studies were performed on commercially farm raised juvenile fish (< 300 mm) in small containments (e.g., concrete pond, fiberglass tank), with shallow water depths (< 2 m). Although the Wamboldt et al. (2019) field study was performed on wild adult silver carp, it took place in an extremely shallow water environment (< 0.5 m) under semi-controlled conditions. Therefore, there is a need to test this complex outboard-motor sound on wild, free-ranging, adult carps in a full-scale natural environment (e.g., Mississippi River) where abiotic conditions are variable.

The flows of the upper Mississippi River, from Minneapolis, MN to St. Louis, MO, are regulated by 29 dams that span the entire width of the river and regulate water depths to maintain a 9 foot channel for commercial navigation. Dams create semi-permeable conditions that impede the natural movement of many fishes (Argent and Kimmel 2011; Liermann et al. 2012, Anderson et al. 2019), including the upstream migration of common and bigheaded carps (Kolar et al. 2007; Tripp et al. 2014; Lubejko et al. 2017; Finger et al. 2020), and a sound deterrent system could be deployed at these structures to further enhance their blocking ability.

Most Mississippi River dams offer two pathways for the upstream movement of fishes: gated spillways (i.e., the actual dam) and a navigational locks. Dams are largely composed of spillway gates that are raised and lowered daily to maintain sufficient water levels for navigation and/or flood control. Zielinski et al. (2018) developed a physiological model based on swimming performance and fatigue that tests whether bigheaded carp passage could be reduced by altering spillway gate operations. Model results suggest that passage is low under current spillway gate operations, but could be further reduced by 50% through small adjustments to operations. However, passage would still be possible through the navigational lock, which enables a vessel (e.g., large ship or barge) to move through a dam while maintaining water depths. Water velocities around and within lock chambers are negligible and telemetry studies have shown that fish, including bigheaded and common carp, often use locks as an avenue to migrate upstream (Tripp et al. 2014; Lubejko et al. 2017, Finger et al. 2020). The overarching goal of my study was to determine if the sound of an outboard-motor would block wild, free-ranging, adult invasive carp from entering the navigational lock of Mississippi River Lock and Dam 8.

Methods

Study Site

This study took place at Mississippi River Lock and Dam 8 (LD8), Genoa, Wisconsin, USA (43°34’12” N 91°13’54” W. LD8 is Minnesota’s southernmost lock and dam (Figure 2-1a) and approximately 250 km north of the leading edge of bigheaded carp reproduction (Larson et al. 2017). LD8 stretches the entire width of the river (370 m) and consists of 5 roller gates, 10 tainter gates (together referred to as spillway gates), one inoperable auxiliary lock, and one active lock chamber (Figure 2-1b). River discharge and water temperature through LD8 were collected for all trial days by LD8 staff (Table 2-1).

Experimental Design

The objective of this experiment was to test the ability of an outboard-motor sound to block locally caught and displaced common carp from entering the lock of a Mississippi River lock and dam. This required catching, tagging, and tracking groups of common carp in the vicinity of the LD8 lock, which was equipped with a sound deterrent system. We conducted eight trials between the months of June and October, with each trial monitoring the passage of 20 acoustically tagged common carp (8 trials, 20 fish per trial, n = 160) for 14 consecutive days. To account for variation in abiotic factors (e.g., water temperature, river stage, etc.) the eight trials alternated between sound off and sound on (sound off n = 4, sound on n = 4). The number of attempts, passages, and passage rates were analyzed for each trial and then compared to determine if there were significant differences in the presence or absence of the outboard-motor sound.

Sound Stimulus and Sound Deterrent System

The sound stimulus is a complex signal (containing multiple frequencies) that was derived from and underwater recording of a 40 horsepower outboard-motor containing frequencies between 20 Hz and 10,000 Hz (Zielinski and Sorensen 2017). We used this outboard-motor sound because it was previously tested in the laboratory on bigheaded and common carps by Zielinski and Sorensen (2017) and Dennis et al. (2019). Zielinski and Sorensen (2017) found that this stimulus repelled 75% of silver carp, bighead carp, and common carp, while Dennis et al. (2019) found that 76% of bighead carp and 42% of common carp were repelled. Due to the limitations of the speakers being used in this field experiment, we had to truncate the 20 Hz – 10,000 Hz frequency range to between 500 Hz and 1,500 Hz to avoid speaker damage. The most sensitive part of the hearing range of common and bigheaded carps is between 300 Hz and 1,500 Hz (Popper 1972; Lovell et al. 2006; Vetter et al. 2018), which is largely within this truncated frequency range, yet above the hearing range of many native fish without similar hearing specializations (Ladich and Fay 2013).

The sound deterrent system composed of five underwater speakers (LL-1424HP, Lubell labs, OH) that emitted the outboard-motor stimulus when the lock gates were in active use (i.e., opening, open, and closing). The speakers were evenly mounted at a distance of 5 m apart to the southern face of the downstream lock gates using commercial divers (Figure 2-1c; Figure 2-2). Each speaker was connected to a bridge transformer (AC1424HP, Lubell Labs, OH) and power amplifier (CDi2000, Crown Audio, IN). A transmission signal was sent to each amplifier from a signal splitter (Ultralink Pro MX882, Behringer, BVI), that was generated from a custom-built microprocessor control unit. The control unit was programed to play the outboard-motor stimulus (stored on microSD card) while the lock gates were in active use. Opening and closing of the lock gates was detected via a magnetic reed switch that was attached to the lock gates and turned on when the lock gates were in use and turned off when the gates were closed and not in use.

Sound Mapping

Sound measurements were acquired using a similar protocol to Dennis et al. (2019). Measurements were obtained via a CR1 hydrophone [sensitivity: -198.0 dB ref 1V/µPa; usable frequency range: 0.016-68 kHz] (Cetacean Research, Seattle, WA), sampled at 44.1 kHz and digitized using a TASCAM US-122mkII (TEAC, Montebello, CA) USB audio interface. The hydrophone was placed at a depth of 3 m (mid-depth) and measurements were taken at 2.5 m intervals across the width of the lock channel, at distances of 0, 5, 10, 17, 24, 30, and 60 m downstream of the open lock gates. Background measurements were taken at mid-depth, 5 m downstream of the lock gates at the channel width midpoint. The acoustic playback signal was recorded for 5 seconds at each measurement location and then split into ten 0.5 s signal batches and averaged to improve the signal-to-noise ratio. A custom Matlab graphical user interface, previously used by Zielinski and Sorensen (2017) and Dennis et al. (2019) was then used to analyze and transform the pressure waveforms into the frequency domain (Figures 2-3, 2-4).

Fish Capture, Tagging, Release, and Monitoring

Since bigheaded carp are not routinely found at our study site, we used common carp. Zielinski and Sorensen (2017) showed that common carp can be used as a conservative surrogate species for bigheaded carp, as their avoidance to sound in the laboratory is very similar. Additionally, common carp were abundant at the study site and like all ostariophysan fishes are sensitive to sound. Homing behavior (defined here as the inherent ability to navigate towards an original location when displaced from it, often over great distance) has been demonstrated in numerous freshwater fishes (Lucas and Baras, 2003), including common carp (Crook, 2004). A recent study at Mississippi River Lock and Dam 2 (LD2) showed 13 of 56 (23%) adult common carp displaced from Pool 2 (above LD2) to Pool 3 (below LD2) swam back upstream through the lock (Finger et al. 2020). Conversely, 4 of 56 (7%) of adult common carp that were not displaced (i.e., Pool 3 fish) swam upstream through the lock (Finger et al. 2020). For this reason, we captured adult common in Pool 8 (above LD8) and displaced them to Pool 9 (below LD8) in hopes of increasing the likelihood of fish challenging the sound deterrent system.

Adult common carp (total length 67.1 ± 7.03 cm (mean ± SD)) were captured via boat electrofishing (5-12 A, 150-250 V, 25-30% duty cycle, and 60 pulse frequency) in Pool 8 (13 km upstream of LD8). Fish were transported in a 400 L holding tank with recirculating water to the Pool 9 surgery site, located 250 m downstream of LD8 (Figure 2-1b) for acoustic tag insertion. Only fish larger than 30 cm (TL) were kept and tagged. Once at the surgery site, fish were moved from the holding tank to a 1.6 x 1.6 m net pen in the river to await tag insertion. Fish were anesthetized in a 1:7000 solution of eugenol (Sigma, St. Louis, MI) following established procedures (Hajek et al. 2006; Finger et al. 2020). A 3.0 cm incision was made along the dorsal fin of the anaesthetized fish and an acoustic tag was inserted into the muscle tissue. We used individually coded SS300 acoustic tags (Advanced Telemetry Systems, Isanti, MN) with a 5 s pulse rate at 416 kHz and a battery life of roughly 37 days. Once a tag had been inserted, the incision was closed using 1 to 2 interrupted re-absorbable sutures (2-0, Ethicon PDS II). Fish were then placed in the river in a 1.3 x 1.3 m net pen until recovered (approximately 20 minutes) before being released. There were no known mortalities, and 91% (145/160) of tagged fish were detected by receivers upstream of the surgery site. Protocols were approved by the University of Minnesota institutional Animal Care and Use Committee (#1605-33753A).

Fish attempts and passage through the sound deterrent system and into the lock chamber were monitored using an array of three submersible SR3000 acoustic receivers (Advanced Telemetry Systems, Isanti, MN; continuous scan). Receivers were fastened to custom-built mounts that were attached to the ladder rungs found within recessed ladder wells along the lock chamber (Figure 2-1c).

Analysis of Fish Data

Receiver data was downloaded and analyzed after each trial to determine the number of attempts and number of passages through the sound deterrent system and into the lock chamber. Data were filtered to remove uncertain detections (i.e., single detections that were not followed by another within 3 s or multiples thereof within 18 s). Range tests revealed that when the downstream lock gates were in active use (i.e., opening, open, or closing), receiver 3 could only detect a tag if its position was within the lock chamber and upstream of the sound deterrent system (Figure 2-1c). It is important to note that it was possible for a fish to get detected by receiver 1, pass through the LD by swimming through the spillway gates, and then get detected by receiver 3 (i.e., if the upstream lock gates were open at the time the fish was in the area), without ever going through the sound deterrent system. Therefore, a passage through the sound deterrent system was only considered valid when the fish was first detected at receiver 1, followed by detection at receiver 2, and finally detection at receiver 3 (Figure 2-1c).

To determine a passage rate for each trial, we also had to define an attempt. If a fish was detected by receiver 1 (Figure 2-1c) it was counted as an attempt, regardless of detection duration. For another attempt to be counted, the same fish had to leave the immediate area (i.e., no longer being detected by receiver 1) for a minimum of 30 minutes (based on the resolution of the data) before being detected again. For each trial, attempts for each fish detected were summed to obtain total attempts (Table 2-1). The total number of passages for each trial were then divided by total attempts for each trial and multiplied by 100 to get a passage rate (Table 2-1). A two sample test for equality of proportions with continuity correction (two proportion z-test) was used to test the hypothesis that a lower overall passage rate would occur during sound on trials (Table 2-1).

Results

Sound Stimulus Mapping

The background sound pressure level (SPL) was 98 dB (ref. 1 µPa) (Figure 2-3). During sound stimulus playback, the SPL power spectrum showed a distinct frequency range between 500 Hz and 1,500 Hz (Figure 2-3). Speaker output was initially programmed to produce a SPL >150 dB (ref. 1 µPa), but concerns over interference with barge operations forced the reduction of the sound stimulus amplitude. As a result, speaker output was adjusted to produce a peak SPL of 139 dB (ref. 1 µPa), measured 1 m from the lock gates that attenuated to 108 dB (ref. 1 µPa), 60 m downstream (ref. 1 µPa; Figure 2-4). This reduction in amplitude was not concerning because Zielinski and Sorensen (2017) found that silver carp, bighead carp, and common carp did not enter outboard-motor sound fields greater than 140 dB (ref. 1 μPa).

Fish Passage

Of the 160 common carp tested, 145 (91%) swam upstream from the release point and were detected by a receiver at least once, indicating a motivation to challenge the lock and dam. A total of 15 common carp passed upstream through the sound deterrent system; nine when the sound was off and six when the sound was on (Table 2-1). There was no significant difference between treatment passages (Mann-Whitney *U* Test: U=5.5, p value >0.05; Table 2-1). There was also no significant difference between treatment attempts, with 388 attempts occurring with the sound off and 297 attempts occurring with the sound on (Mann-Whitney *U* Test: U=5, p value >0.05; Table 2-1). The two proportion z-test rejected the hypothesis that a lower overall passage rate would occur during sound on trials (two proportion z-test, p value = 0.5; Table 2-1), with a sound off passage rate of 2.3% and a sound on passage rate of 2.0%.

Environmental Conditions

When comparing environmental conditions between sound off and sound on trials (Table 2-2), water discharge was not significantly different (Mann-Whitney *U* Test: U=1386.5, p-value >0.05) and water temperature was significantly different (Mann-Whitney *U* Test: U=1300.5, p-value <0.05 Table 2-2). The median discharge was 42650 cubic feet per second (cfs) during sound off trials and 48550 cfs during sound on trials (Table 2-2). The median water temperature during sound off and sound on was 23.9 °C and 22.5 °C, respectively (Table 2-2).

Discussion

This is the first full-scale field study in a large river lock and dam to test the efficacy of a sound deterrent system to block upstream movements of wild, free-ranging adult invasive carp. This study clearly demonstrates that a complex sound deterrent system can be installed at the navigational lock of a Mississippi River Lock and Dam. The most important finding from this study is that the sound of an outboard-motor did not significantly deter wild, free-ranging, adult common carp from entering the navigation lock. Of the 160 locally caught and displaced common carp, 15 passed upstream through the sound deterrent system, 9 when the sound was off and 6 when the sound was on. There were no significant differences between the number of attempts, passages (i.e., through the sound deterrent system and into the lock entrance), and passage rates between sound off and sound on trials.

It is unclear what caused the lack of response, but there are a number of potential reasons. A major constraint of the sound deterrent system was the number of speakers and their mounted location. The sound deterrent system was composed of only 5 speakers that were attached directly to the lock gates (Figure 2-2). As a result, the system failed to deliver a uniform sound pressure field across the width of the lock chamber entrance (Figure 2-4). Therefore, fish may have exploited areas with lower sound pressure levels to pass through the sound deterrent system. The sound was only active throughout the duration of a lockage (i.e., opening, open, and closing). This is likely not optimal because fish were not given a good opportunity (i.e., time) to swim away from the lock prior to opening. As previously suggested (Vetter et al. 2015, 2017; Murchy et al. 2017; Zielinski and Sorensen 2017; Wamboldt et al. 2019), fish may have habituated to this outboard-motor sound, a possibility that might have been enhanced by boat activity in the area. Additionally, we used wild, free-ranging, adult fish that have likely heard the sound of an outboard-motor before, especially given that this particular stretch of river is popular amongst fishers. Therefore, it is possible that these common carp do not display aversive behavior when encountered with this specific sound.

Due to potential speaker damage, we had to truncate the outboard-motor frequency range. This eliminated both low (<500 Hz) and high (>1,500 Hz) frequencies, which may have reduced efficacy. For example, Zielinski and Sorensen (2017) and Dennis et al. (2019) found that common carp exhibited significant negative phonotaxis to the full outboard-motor frequency spectrum (20 Hz – 10,000 Hz), which suggests the importance of lower and higher frequencies. The ambient sound pressure level at our field site was approximately 100 dB (ref. 1 µPa), whereas the ambient sound pressure level in both Zielinski and Sorensen (2017) and Dennis et al. (2019) was approximately 80 dB (ref. 1 µPa). The difference between the ambient and outboard-motor sound pressure levels in both Zielinski and Sorensen (2017) and Dennis et al. (2019) was approximately 60 dB (ref. 1 µPa). However, in this study, the difference between the ambient and outboard-motor sound pressure levels was approximately 40 dB (ref. 1 µPa), which is 20 dB (ref. 1 µPa) lower than the aforementioned lab studies. It is possible that this decrease in amplitude above the ambient environment resulted in decreased blockage effectiveness. Although, this sound deterrent system and associated outboard-motor sound failed to deter common carp from entering the lock of a Mississippi River Lock and Dam, the possibility of using a different sound paired with other aversive stimuli (e.g., bubbles, strobing lights) is currently being tested.

In addition to sound, laboratory tests have shown that air curtains (streams of bubbles) can repeatedly block up to 80% of both bigheaded and common carps (Zielinski et al. 2014; Zielinski and Sorensen 2016). A commercially available fish deterrent system that couples a proprietary complex cyclic sound (20 Hz and 2,000 Hz) with an air curtain, known as a “Bio-Acoustic Fish Fence” or “BAFF” (Fish Guidance Systems Ltd; Southhampton, UK; http://www. fish-guide.com/), enhances the efficacy of sound as a fish deterrent by encapsulating the sound within the air curtain, which creates a “wall of sound” (Welton et al. 2002). The BAFF has shown to block at least 95% of bigheaded and common carps in the laboratory (Taylor et al. 2005; Dennis et al. 2019) and a small creek (Ruebush et al. 2012). Most notably, Dennis et al. (2019) investigated whether bighead carp and common carp were more sensitive to the outboard-motor sound or the propriety-cyclic sound, and whether coupling an air curtain to the latter (BAFF) makes it more effective at blocking these fish. Results showed that the proprietary-cyclic sound performed better than the outboard-motor sound at blocking both bighead and common carp. Additionally, when the proprietary-cyclic sound was coupled with the air curtain (BAFF) blocking blockage efficiencies for bighead carp and common carp were 97% and 100%, respectively, which was 20% higher than the proprietary-cyclic sound alone. The next step is to test the BAFF system in a full-scale field setting on wild, free-ranging, adult carps that includes variable abiotic conditions.

Tables and Figures

Table 2-1. Number of common carp that passed through the sound deterrent system at LD8 during sound off and sound on trials. There was no significant difference between treatment passages (Mann-Whitney U Test: U=5.5, p-value >0.05) or treatment attempts (Mann-Whitney U Test: U=5, p-value >0.05). A two sample test for equality of proportions with continuity correction, rejected the hypothesis that a lower overall passage rate would occur during sound on trials (two proportion z-test, p value = 0.5).

	Trial	Passages	Attempts	Passage Rate (%)
Sound Off	1	2	97	2.1 %
	3	3	141	2.1 %
	5	0	104	-
	7	4	46	8.7 %
	Total	**9**	**388**	**2.3 %**
Sound On	2	3	103	2.9 %
	4	2	61	3.3 %
	6	0	60	-
	8	1	73	1.4 %
	Total	**6**	**297**	**2.0 %**

Table 2-2. Environmental conditions at LD8 during each of the eight trials. When treatments were compared, discharge was not significantly different (Mann-Whitney U Test: U=1386.5, p-value >0.05) and water temperature was significantly different (Mann-Whitney U Test: U=1300.5, p-value <0.05).

	Trial	Discharge (cfs) Min - Max	Discharge (cfs) Median	Temperature (°C) Min - Max	Temperature (°C) Median
Sound Off	1	39900 - 75300	42650	24.4 - 26.1	23.9
	3	25700 - 54600		22.2 - 23.3	
	5	26700 - 45000		18.3 – 22.8	
	7	62600 - 84100		24.4 – 27.2	
Sound On	2	16500 – 42700	48550	22.8 – 26.7	22.5
	4	38200 – 58900		18.9 – 22.2	
	6	47700 - 89400		13.3 – 18.3	
	8	34000 - 57700		23.9 – 27.2	

Figure 2-1. (a) Location of Mississippi River Lock and Dam 8 (LD8), Genoa, Wisconsin, USA. (b) Schematic of LD8 including the navigational lock chamber, spillway gates, and location of the surgery/release site (*). (c) Enlargement of the LD8 lock chamber showing the locations of the acoustic tracking receivers (1-3) and sound deterrent system. Figure created in part by Dr. Jean Finger.

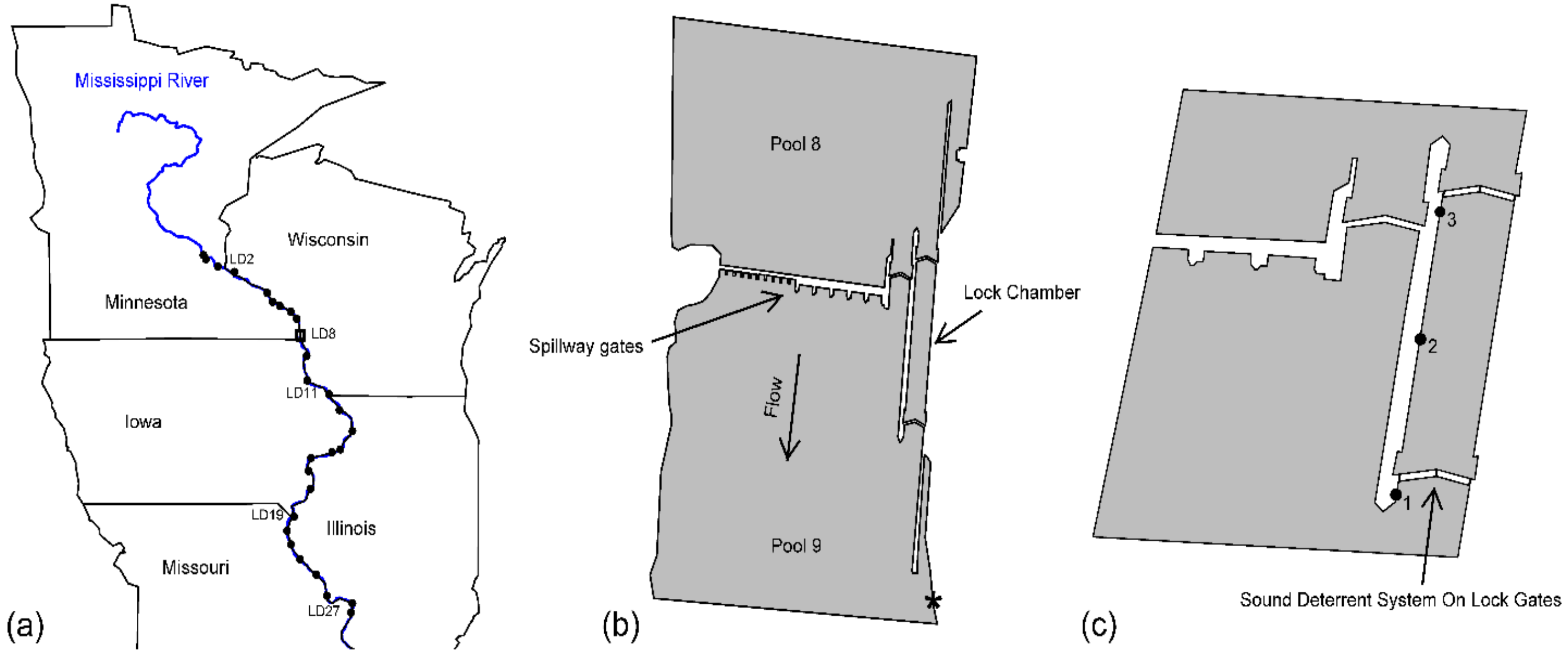


Figure 2-2. Rendering of the sound deterrent system installed on the face of the downstream lock gates. The system was comprised of five evenly staggered speakers (Transducers A-E) mounted 22 feet from each other. Transducers B and D are mounted 5ft from the river bottom and Transducers A, C, and E are mounted 10 ft from the river bottom. The strategic spacing of the transducers ensured a uniform sound field throughout the width and depth of the water column. Figure created by Dr. Daniel Zielinski.

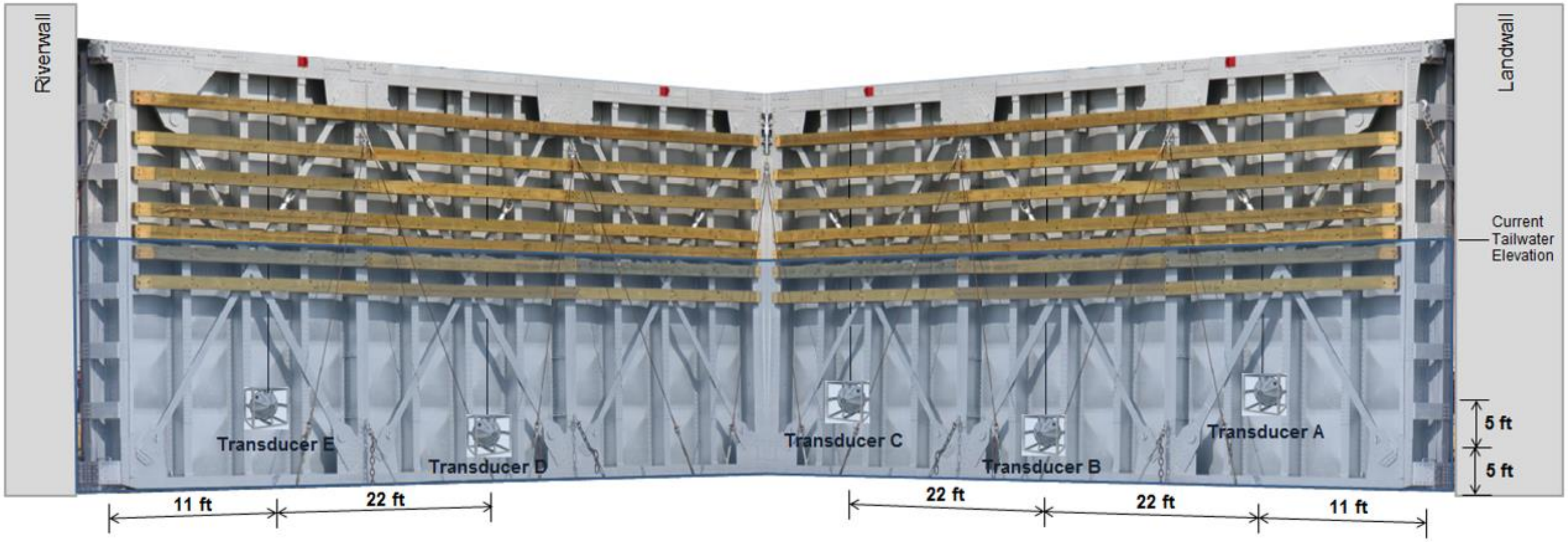


Figure 2-3. Sound pressure level power spectrum of the outboard-motor sound measured 10 m from the speakers (green line). Background noise is presented as a reference (black line). Sound pressure level measurements are provided at a 2 Hz bandwidth. Figure created by Dr. Clark Dennis.

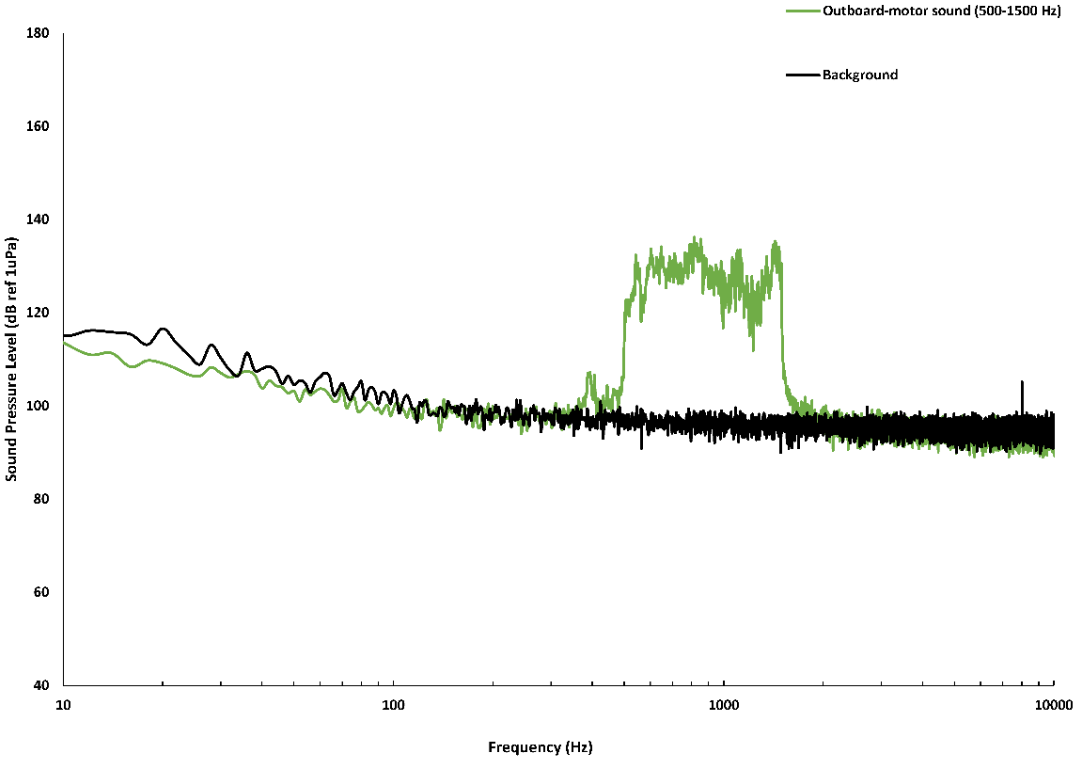
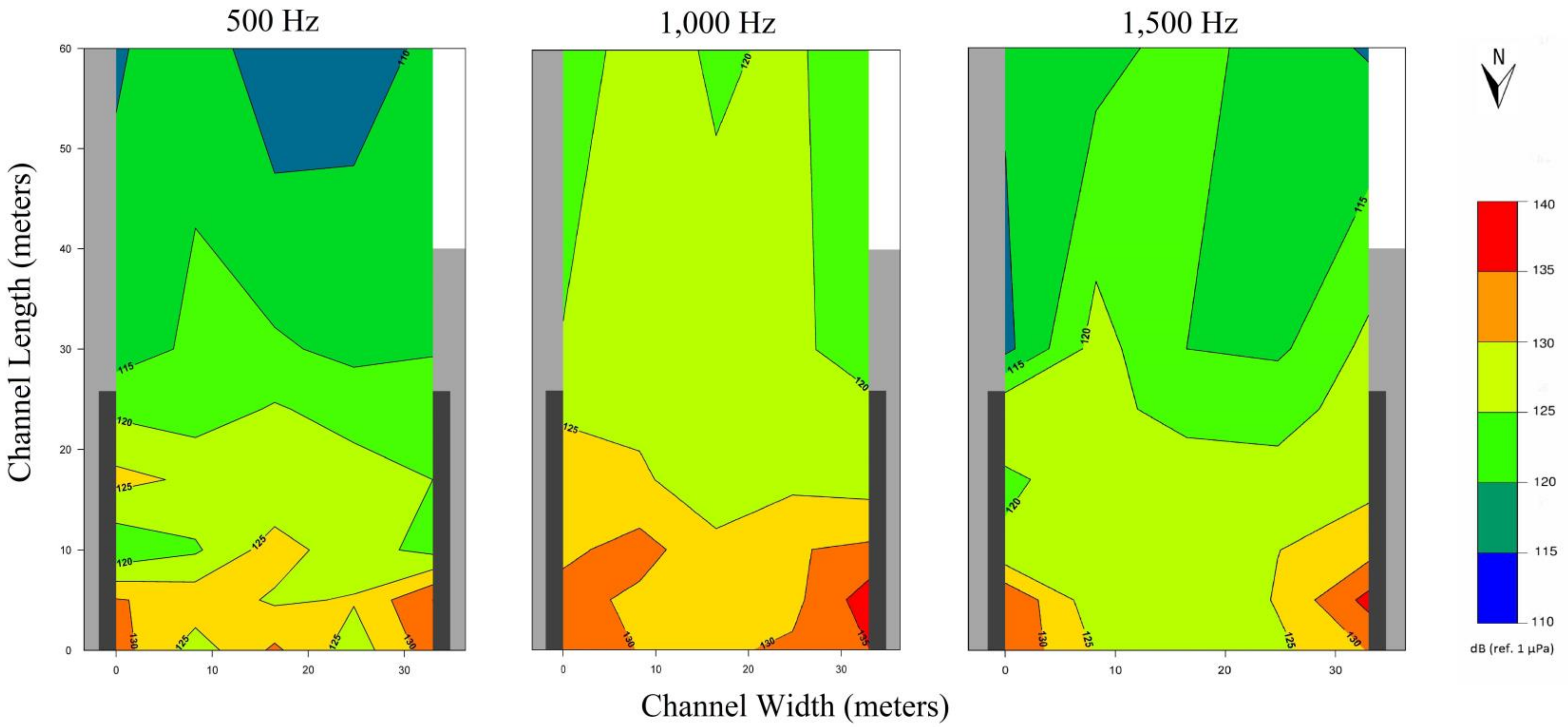


Figure 2-4. Sound pressure levels (SPL) of the sound deterrent system when the lock gates are in the open position and the sound is on. SPL are depicted at 500 Hz, 1,500 Hz, and 1,500Hz. The peak SPL was 139 dB (ref. 1 µPa), measured 1 m from the lock gates, and decrease to 108 dB (ref. 1 µPa) 60 m downstream of the lock gates. The gray rectangles represent the lock guide walls and the dark gray rectangles represent the lock gates in the open position. Figure created in part by Dr. Clark Dennis.



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Appendix A: A laboratory study testing the ability of a truncated outboard-motor sound to block common carp passage in an indoor flume

Methods

Experimental Design

Laboratory experiments were performed in the Minnesota Aquatic Invasive Species Research Center (MAISRC) Containment Laboratory. Because we had to truncate the outboard-motor sound in the chapter 2 field study from 20 Hz - 10,000 Hz to 500 Hz - 1,500 Hz, we tested this truncated frequency range in the laboratory. We wanted to determine if the truncated frequency range would block common carp passage because the results could provide inferences for the results obtained from the chapter 2 field experiment. We accomplished this in three steps, similar to Dennis et al. (2019). First, a series of control trials examined the swimming behavior of common carp within the laboratory flume in the absence of a sound stimulus, which allowed us to confirm that fish behavior did not change over time. Second, we examined the effect of exposing groups of fish to the outboard-motor sound stimulus to determine if fish swimming behavior changed with sound exposure. Third, we calculated a blockage efficiency, defined as the ability of the sound stimulus to prevent fish from passing through the sound field.

The common cap used in this experiment were considered non-naïve because the fish had been previously exposed to sound deterrent experiments one year prior. Therefore, we also examined the effect of exposing groups of these fish to the full frequency spectrum (20 Hz - 10,000 Hz) because it allowed us to compare our results to that of Dennis et al. (2019), who tested naïve common carp on this same outboard-motor frequency spectrum under identical conditions. If our results are similar, we can postulate that results obtained from the truncated frequency spectrum experiment are a result of the sound stimulus and not caused by the fish being non-naïve.

Laboratory Flume

Trials were executed in the same custom-built indoor fiberglass flume (8 m long x 1 m wide x 1 m height) that Dennis et al. (2019) used in their study (Figure A-1). Within the flume, two speakers (FGS MkII 15-100; Fish Guidance System Ltd.; Southampton, UK) were placed in the center of each channel straightaway. Concrete blocks with foam backing were secured to the exterior sides and along the interior floor of the flume to minimize sound reverberation, which ensured that sound levels eventually dropped to background levels (60-80 dB ref 1 µPa; Figure A-2), where fish could find refuge from the sound stimulus. Because the trials were conducted in complete darkness, fish movements were monitored using overhead cameras paired with infrared illuminators (VT-IR1 and VT-IR2; Vitek; Valencia, CA; 840nm wavelength, < 1 lx). Water in the flume was maintained at a constant temperature (18 ˚C) and depth (0.3 m).

Sound Stimulus

We used the same 40 horsepower outboard-motor sound described and employed in Chapter 2. We tested two frequency spectrums, 500 Hz - 1,500 Hz (truncated spectrum) and 20 Hz - 10,000 Hz (full spectrum), that played continuously throughout each test period. Speaker output was adjusted to produce a target peak sound pressure level of 140 dB (ref. 1 µPa) directly in front of each speaker for both frequency spectrums (Figure A-2). This is the peak sound pressure level used in the chapter 2 field experiment.

Sound pressure levels (dB ref 1 µPa) were mapped following protocols established by Dennis et al. (2019). Sound pressure levels were acquired using a C55 hydrophone (sensitivity: -

163.5 dB ref 1 V/µPa; frequency range: 0.01–100 kHz) with an integral power amplifier (Cetacean Research, Seattle, WA), sampled at 44.1 kHz and digitized using a TASCAM US-122mkII (TEAC, Montebello, CA) USB audio interface. The hydrophone was mounted on a PVC probe and placed at a depth of 0.15 m. Measurements were taken at 0.25 m intervals across the width of the flume channel, at: 0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.80, 1.00, 1.20, 1.60, 2.00, and 2.40 m away from the speaker system. At each location, the sound stimulus was recorded for 5-s snd then split into ten 0.5 s batches and averaged. A custom Matlab graphical user interface developed by Zielinski and Sorensen (2017) was used to analyze and transform the pressure waveform into the frequency domain.

Fish

Juvenile common carp (TL: 129 ± 21 mm; TW: 33 ± 16 g; mean ± SD) were obtained from Osage Catfisheries; Osage Beach, MO. Fish care followed the same procedures as Dennis et al. (2019). Fish were held in flow-through circular tanks (300 L: 1 m diameter) that were supplied with 18 ˚C well water, aerated with air stones, dimly lit (5 lx; 16 h day; 8 h night) and relatively quiet (80-100 dB ref. 1 µPa). Fish were fed 2.5 mm floating pellets once per day (Skretting, Tooele, Utah) until sated. We consider these fish non-naïve, as they were exposed to a similar sound deterrent experiment approximately one year prior to this experiment. All procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol: 1712-35381A) and all state and federal permits were obtained.

Experimental Protocol

We conducted three experiments, no stimuli (control), 500 Hz - 1,500 Hz (truncated spectrum), and 20 Hz - 10,000 Hz (full spectrum) each containing 10 different groups of 10 randomly selected common carp, with each group randomly allocated to an experiment. Each experiment consisted of 8 trials that began with a 1 h acclimation period (no stimuli), followed by 8 sets of exposure periods. Each exposure period started with a 6 min pre-test period (no stimuli), followed by a 6 min test period (stimuli on [or not in the control experiment]), and concluding with a 10 min recovery period (no stimuli). The set of speakers (Figure A-1) that emitted sound during sound stimuli tests was randomly chosen for each trial. All trials were conducted at night between 2100 and 0400 h in near-darkness (< 1 lx) because this was the only time that the flume was available. Fish position was continually recorded using low-light cameras and analyzed after trials by quantifying the number of times fish crossed through the sound deterrent/speaker system (i.e., passage rates). We examined passage rates during both pre-test and test periods to provide estimates of change in behavior during pre-test periods and behavior induced during test periods.

Statistical Analysis

We used a generalized linear mixed model (GLMM) that was designed and implemented by Dennis et al. (In Review) that used a Poisson distribution to analyze passage rate count data. Custom designed matrices were entered into the GLMM to perform comparisons of interest (e.g., comparison of passage rates between sound stimuli and control experiments), with much greater power than a three-way ANOVA. This custom model also allowed us to account for changes in passage rates due to time spent in the flume. “Fish group number” was entered as a random effect to account for repeated measurements taken from the same group of fish (i.e., each group was exposed to a stimulus eight times and passage rates were taken during each exposure). Assumptions for the GLMM were checked visually using a fitted residual plot, and the variance of the residuals needed to be approximately 1 (McCullagn and Nelder 1989). If the variance of the residuals was greater than 2, we corrected the hyper-variability by dividing our test statistic by the square root of the dispersion parameter (McCullagn and Nelder 1989) and calculated a new p-value for this comparison.

To answer our question (i.e., does a complex outboard-motor sound block fish passage?), we analyzed each stimulus separately and then used specific contrasts in each GLMM to directly compare mean passage rates during the no-treatment control experiments (n=160 observations [80 pre-test and 80 test passage rates] per experiment) with the mean passage rates during a specific sound stimulus experiment (n=80 observations [80 test passage rates] per experiment) ($p < 0.05$ corrected for multiple comparisons). Because we wanted to know if fish passage rates changed with repeated exposure, we examined temporal variation in passage rates over the course of the eight trial periods by fitting a curve (e.g., linear, quadratic, etc.). To determine which trial(s) had passage rates during test periods that differed from their matched pre-test period, we then directly compared mean passage rates between matched pre-test and test periods within each experiment ($p < 0.05$ corrected for multiple comparisons; n=10 observations [passage rates] per trial period). All analyses were performed using R and at an α value of 0.05.

Results

Sound Stimulus

Sound pressure levels (SPL) between 500 Hz and 1,500 Hz for the truncated spectrum and full spectrum were nearly identical, which should be the case because the truncated spectrum was derived from the full spectrum (Figure A-2). Outside of the 500 Hz to 1,500 Hz range (i.e., the lower and higher frequencies), the truncated spectrum SPL were approximately 10 dB (ref. 1 μPa) lower than the full spectrum SPL (Figure A-2). Both the truncated and full spectrums were well above background SPL.

Fish Passage

Each group of common carp averaged 17 ± 9 (mean ± SD) passages across the inactive sound deterrent system during each six minute period of the no-treatment control experiment (Figure A-3A). This passage rate decreased significantly over time ($p < 0.05$; Table A-1). When exposed to the truncated spectrum stimuli, common carp passage decreased significantly for the first two exposures ($p < 0.05$; Figure A-3B; Table A-1), resulting in an overall blockage efficiency of 26 ± 4% (mean ± SD) relative to the mean passage rate during the no-treatment control experiment ($p < 0.05$; Table A-1). While pre-test passage rates did not change ($p < 0.05$; Table A-1), passage rates increased on average by 9% during each subsequent exposure which is systematic of habituation ($p < 0.05$; Table A-1). When exposed to the full spectrum, common carp passage was significantly blocked at an overall efficiency rate of 47 ± 2% compared to the no-treatment control experiment ($p < 0.05$; Table A-1), with significant reductions observed during all eight trials ($p < 0.05$; Figure A-3C; Table A-1). Pre-test and test passage rates during the full spectrum experiment did not significantly decline with repeated exposure ($p > 0.05$; Table A-1).

Discussion

The blockage efficiency of the full frequency spectrum was very similar to the blockage efficiency obtained by Dennis et al. (2019). Therefore, the truncated frequency spectrum results were likely an artifact of the sound stimulus and not due to the fish being non-naïve. When the truncated spectrum sound properties were mapped (Figure A-2), frequencies outside of the 500 Hz to 1,500 Hz range should have matched the background noise frequencies. However, the test flume created additional lower and higher frequencies (i.e., <500 Hz and >1,500 Hz), that were similar to the full 20 Hz – 10,000 Hz spectrum (Figure A-2). This unforeseen result demonstrates the importance of sound pressure levels (dB ref. 1 μPa). Outside of the 500 Hz – 1,500 Hz range the sound pressure levels for the truncated spectrum were approximately 10 dB (ref. 1 μPa) lowered than the full spectrum sound pressure levels (Figure A-2). This relatively minor difference likely contributed to the differing passage rate/blockage efficiency results. When exposed to the truncated spectrum stimulus, common carp passage decreased significantly for the first two exposures and passage rates increased an average of 9% during each subsequent exposure, which is symptomatic of habituation ($p < 0.05$; Figure A-3B; Table A-1). The overall blockage efficiency was 26 ± 4% (mean ± SD). Conversely, when exposed to the full spectrum stimulus, common carp passage decreased significantly for all eight exposures with no decline in passage rates ($p < 0.05$; Figure A-3B; Table A-1). The overall blockage efficiency was 47 ± 2% (mean ± SD). This short laboratory study demonstrates that sound frequencies outside of 500 Hz – 1,500 Hz are important when using sound as a carp deterrent, and to some extent, may explain the results obtained in the chapter 2 field study.

Tables and Figures

Table A-1. Generalized linear mixed model (GLMM) results for common carp exposed to an outboard-motor sound at a full spectrum (20 Hz – 10,000 Hz) and a truncated spectrum (500 Hz – 1,500 Hz) frequency range. Bold text denotes statistically significant results. Table created in part by Dr. Clark Dennis.

Fixed Effects	**Estimate**	**Std. Error**	**Z value**	**Z value (corrected)**	**Pr (>|z|)**
Does sound exposure reduce mean passage of fish? (α = 0.05/4 = 0.0125)					
Pre-Test (20 Hz – 10,000 Hz) vs No Treatment Control	0.1129	0.0498	2.2680	1.4903	0.1361
Test (20 Hz – 10,000 Hz) vs No Treatment Control	**-0.6226**	**0.0555**	**-11.221**	**-7.3733**	**< 0.0001**
Pre-Test (500 Hz – 1,500 Hz) vs No Treatment Control	0.1862	0.0565	3.2970	2.1665	0.0303
Test (500 Hz – 1,500 Hz) vs No Treatment Control	**-0.3015**	**0.0611**	**-4.9330**	**-3.2415**	**0.0019**
Do passage rates change with repeated exposure to sound (or with time in the flume)? (α = 0.05/7 = 0.0071)					
No Treatment Control					
Linear	**-0.0510**	**0.0084**	**-6.055**	**-3.9787**	**0.0001**
Quadratic	0.0034	0.0549	0.0630	0.0414	0.9670
Cubic	-0.0122	0.0544	-0.2250	-0.1478	0.8825

Quartic	-0.0518	0.0544	-0.9510	-0.6249	0.5320
Quintic	0.0226	0.0542	0.4160	0.2734	0.7845
Sextic	0.0289	0.0541	0.5350	0.3515	0.7252
Septic	-0.0293	0.0547	-0.5360	-0.3522	0.7247
20 Hz – 10,000 Hz: Pre-Test Passage Rates					
Linear	-0.0002	0.0103	-0.0170	-0.0112	0.9911
Quadratic	-0.0813	0.0677	-1.2000	-0.7885	0.4304
Cubic	-0.0220	0.0661	-0.3330	-0.2188	0.8268
Quartic	-0.1679	0.0650	-2.5830	-1.6973	0.0896
Quintic	0.0870	0.0645	1.3490	0.8864	0.3754
Sextic	-0.0122	0.0648	-0.1880	-0.1235	0.9017
Septic	-0.1724	0.0662	-2.6040	-1.7111	0.0871
20 Hz – 10,000 Hz: Test Passage Rates					
Linear	0.0555	0.0154	3.5930	2.3610	0.0182
Quadratic	-0.2911	0.0968	-3.0080	-1.9766	0.0481
Cubic	-0.0211	0.0964	-0.2190	-0.1439	0.8856
Quartic	0.1216	0.0981	1.2390	0.8141	0.4156
Quintic	0.0082	0.0960	0.0850	0.0559	0.9554
Sextic	0.1210	0.0920	1.3150	0.8641	0.3875
Septic	-0.0096	0.0899	-0.1070	-0.0703	0.9440
500 Hz – 1,500 Hz: Pre-Test Passage Rates					

Linear	-0.0263	0.0125	-2.1090	-1.3858	0.1658
Quadratic	0.0039	0.0829	0.0470	0.0309	0.9753
Cubic	0.0588	0.0807	0.7280	0.4784	0.6324
Quartic	-0.2151	0.0795	-2.7060	-1.7781	0.0754
Quintic	0.0260	0.0791	0.3280	0.2155	0.8294
Sextic	-0.0128	0.0800	-0.1600	-0.1051	0.9163
Septic	0.0384	0.0826	0.4650	0.3056	0.7599
500 Hz – 1,500 Hz: Test Passage Rates					
Linear	**0.0838**	**0.0166**	**5.0450**	**3.3151**	**0.0009**
Quadratic	-0.1711	0.1029	-1.6630	-1.0928	0.2745
Cubic	0.0325	0.1049	0.3100	0.2037	0.8386
Quartic	0.2460	0.1076	2.2850	1.5015	0.1332
Quintic	-0.0728	0.1065	-0.6840	-0.4495	0.6531
Sextic	0.0606	0.1025	0.5920	0.3890	0.6973
Septic	-0.1952	0.0981	-1.9900	-1.3076	0.1910
For a specific treatment, during which trial (1-8) does exposure to sound change passage rates? (α = 0.05/8 = 0.0063)					
20 Hz – 10,000 Hz (Full Spectrum)					
Pre-Test vs Test Trial 1	**-0.9452**	**0.1301**	**-7.2670**	**-4.7751**	**< 0.0001**
Pre-Test vs Test Trial 2	**-1.1026**	**0.1251**	**-8.8130**	**-5.7910**	**< 0.0001**
Pre-Test vs Test Trial 3	**-0.7610**	**0.1134**	**-6.7120**	**-4.4104**	**< 0.0001**

Pre-Test vs Test Trial 4	**-0.4958**	**0.1157**	**-4.2840**	**-2.8150**	**0.0049**
Pre-Test vs Test Trial 5	**-0.6853**	**0.1075**	**-6.3750**	**-4.1890**	**< 0.0001**
Pre-Test vs Test Trial 6	**-0.5353**	**0.1075**	**-4.9790**	**-3.2717**	**0.0011**
Pre-Test vs Test Trial 7	**-0.7861**	**0.1134**	**-6.9300**	**-4.5537**	**< 0.0001**
Pre-Test vs Test Trial 8	**-0.5726**	**0.1146**	**-4.9980**	**-3.2842**	**0.0010**
500 Hz – 1,500 Hz (Truncated Spectrum)					
Pre-Test vs Test Trial 1	**-0.8029**	**0.1447**	**-5.5470**	**-3.6449**	**0.0003**
Pre-Test vs Test Trial 2	**-1.1251**	**0.1461**	**-7.7030**	**-5.0616**	**< 0.0001**
Pre-Test vs Test Trial 3	-0.5190	0.1281	-4.0510	-2.6619	0.0078
Pre-Test vs Test Trial 4	-0.4433	0.1214	-3.3750	-2.2177	0.0266
Pre-Test vs Test Trial 5	-0.1077	0.1241	-0.8670	-0.5697	0.5689
Pre-Test vs Test Trial 6	-0.4057	0.1290	-3.1450	-2.0666	0.0388
Pre-Test vs Test Trial 7	-0.4055	0.1265	-3.2060	-2.1067	0.0351
Pre-Test vs Test Trial 8	-0.0925	0.1241	-0.7450	-0.4895	0.6245

Figure A-1. Schematic drawing of an overhead and cross-sectional view of the fiberglass flume. Two underwater speakers were placed in each of the two 4.9 m channels. The grey box around the speakers denotes a 1 m area away from the deterrent system. Standpipes ensured that water depth was maintained at 0.3 m. Modified from Dennis et al. (2019).

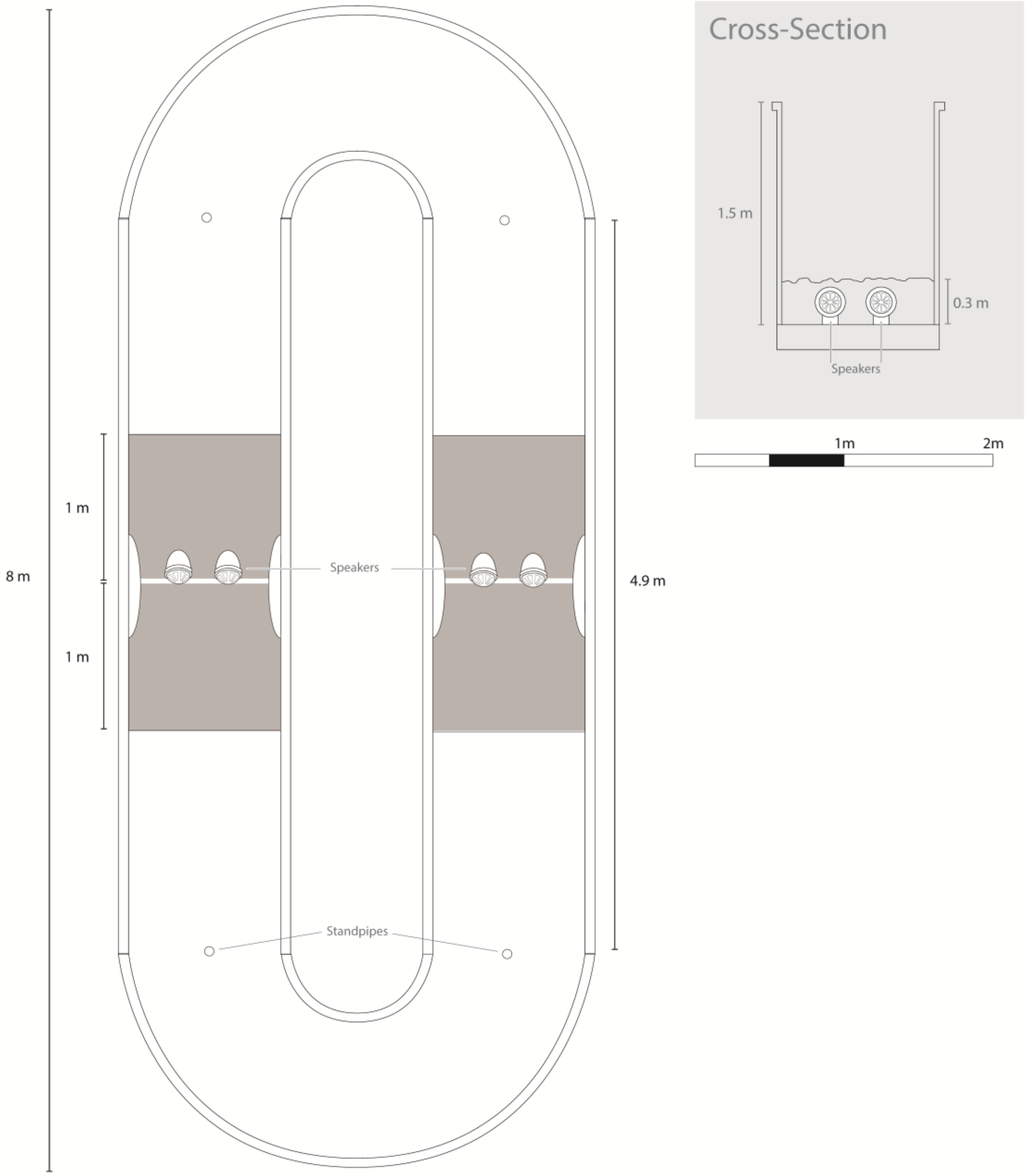


Figure A-2. Sound pressure level power spectrum of the outboard-motor sound measured 5 cm from the speakers in the laboratory flume. The truncated frequency (green line), full spectrum frequency (purple line), and background (black line) sound pressure levels are provided at a 2 Hz bandwidth. The background is presented as a reference. Figure created by Dr. Clark Dennis.

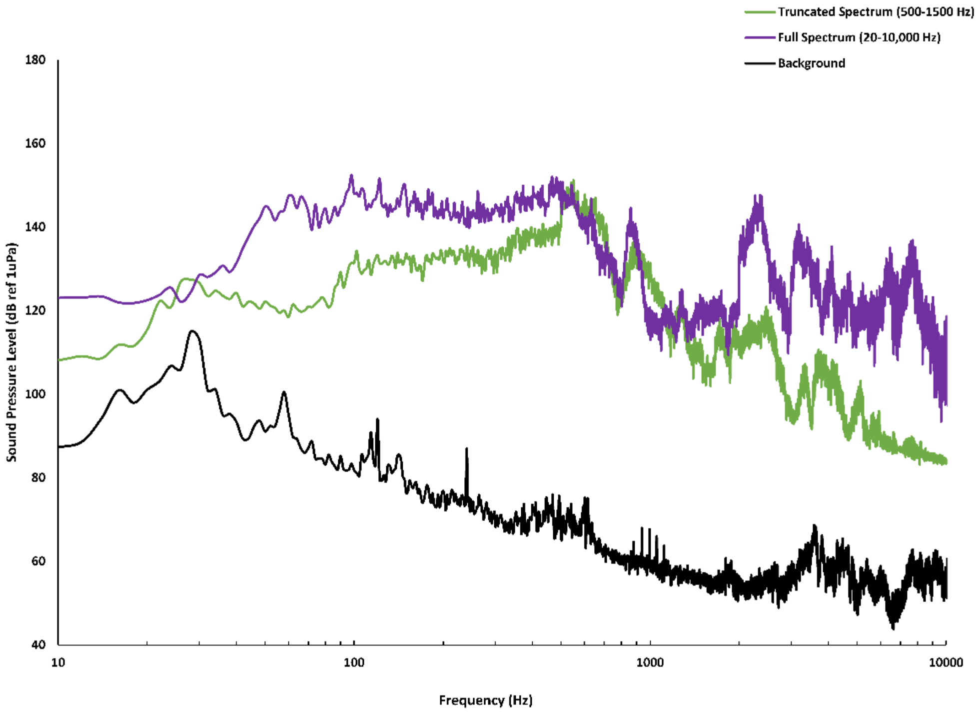
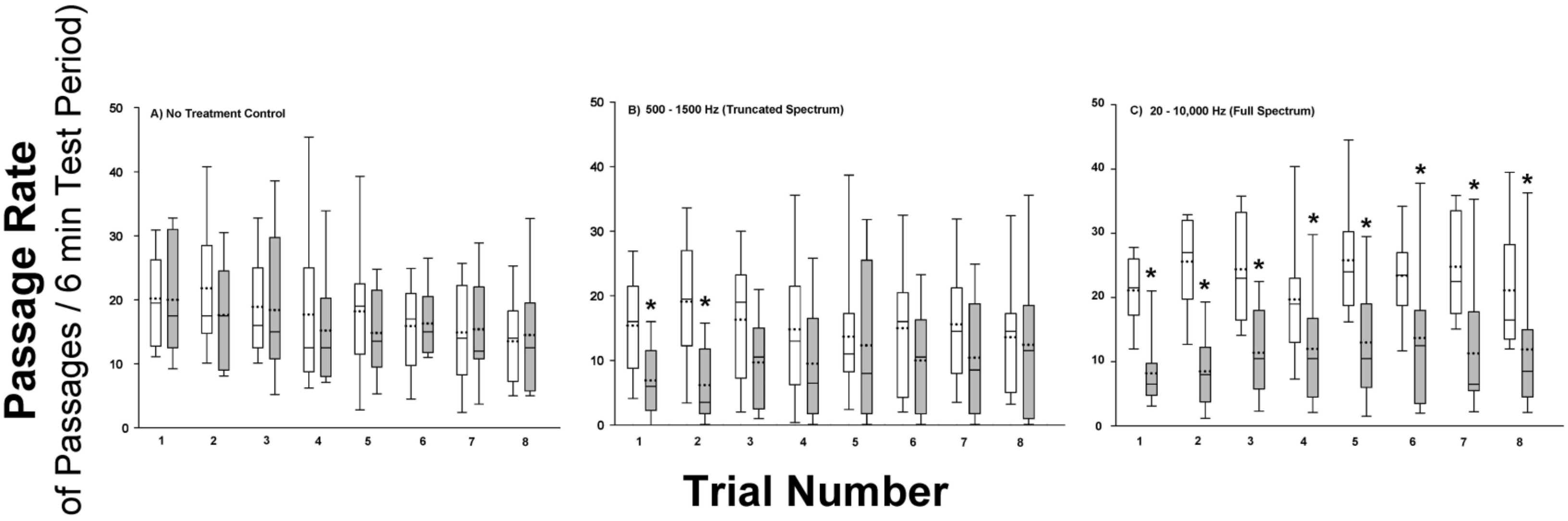


Figure A-3. Passage rates (i.e., the number of passages per 6-min pre-test or test period) of common carp exposed to 2 outboard-motor sound stimuli across time: A) no-treatment control; B) 500 Hz – 1,500 Hz; C) 20 Hz – 10,000 Hz. Box-and-whisker plots in each panel show the lower bound, 25th percentile, median (solid line), mean (dotted line), 75th percentile, and upper bound values for passage rates during pre-test periods (white bars) and test periods (gray bars) over the course of eight consecutive periods (i.e., trial numbers). Asterisks denote differences between pre-test and test passage rates for that test ($p < 0.05$ [corrected for multiple comparison]). Blockage efficiency (mean ± SD) denotes a reduction in mean passage rates and are shown below their respective panel. Figure created in part by Dr. Clark Dennis.



Blockage efficiency (mean ± SD): 47% ± 2

Blockage efficiency (mean ± SD): 26% ± 4

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