The ability of a cyclic sound on its own, and when coupled with an air curtain, to block ten species of fish including carp in a laboratory flume

A Thesis

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Abstract

There is a critical need to stop the upstream movement of invasive Asian carps in the Upper Mississippi River and its tributaries where they could cause ecological harm. One possible strategy to stop this would be to install sensory deterrent systems in navigation locks. Ideally, such systems would not impede the movement of migratory native fishes. Because carps have an excellent sense of hearing compared to many other fishes, sound is being considered. In previous work, a cyclic sound has been shown to have promise in the laboratory where it blocks 78-79% of both bighead and common carp, and largemouth bass to a lesser extent (~50%). These blockage rates increase to about 97% for all three species when this sound is coupled with an air curtain. However, the effects of sound and sound coupled with air have not been tested on other species of fish, including other carp species. My thesis examined the ability of sound and sound coupled with air to block a range of fish species, including four species of carp, in the laboratory. Several important findings emerged. Responses to the cyclic sound alone varied between -41+/-15% and 86 +/- 48%, with catfish being attracted, and response across taxa did not appear to be related to the possession of hearing specializations. In particular, although bighead and common carp were strongly repelled (86% and 83% blocked, respectively), silver and grass carp were relatively unaffected (31% and 21%). Second, coupling this sound with an air curtain consistently increased its efficacy at repelling all fish species (average increase 38%), in a manner which generally corresponded with the presence of hearing specializations. Thus, all four carp species were blocked between 92-87% by this complex stimulus. In conclusion, this laboratory study suggests that although a cyclic sound has little potential to block all carp species, coupling the sound to an air curtain has much greater potential, but the response is not highly specific.

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Invasive Fish and Nonphysical Sensory Deterrents

Presidential Executive Order 13112 defines invasive species as “an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health” (Beck et al. 2008). Many species of invasive carp, including grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), black carp (*Mylopharyngodon piceus*), and common carp (*Cyprinus carpio*), are of particular concern to North American and other global aquatic ecosystems due to the ecological and economic threats they may inflict. Controlling the spread of these species is vital for maintaining the health of these ecosystems.

Bighead and silver carp (collectively known as bigheaded carp) were introduced to Arkansas, USA during the 1970s for the purposes of controlling excess plankton and food in aquaculture farms. They were able to establish themselves into the Mississippi River, possibly from flooding events or from having escaped their hatcheries (Reeves 2019). Silver carp were caught in Arkansas’s White River in 1980 and 1981 (Freeze and Henderson 1982). Currently, adult bigheaded carp can commonly be found as far north as Burlington, Iowa, and they threaten to spread into the Upper Mississippi River and upper Illinois River (Hoover et al. 2016; Nissen et al. 2019). Bigheaded carp are filter-feeders, meaning they consume planktonic organisms such as phytoplankton and zooplankton using very fine gill rakers. They share this diet with native filter-feeders, such as the gizzard shad (*Dorosoma cepedianum*) and bigmouth buffalo (*Ictiobus cyprinellus*), leading to concerns that bigheaded carp could outcompete native fishes and destabilize native ecosystems (Minder and Pyron 2017; Wang et al. 2018). Were bigheaded carp to invade the Great Lakes, they would likely cause a negative economic impact to its fisheries, which have been calculated to generate $7 billion annually, although the extent of this disruption is difficult to estimate (Buck et al. 2010). Their ecological impact, as well as their potential economic impact on fisheries, has made controlling their spread northwards a priority.

Another species of Asian carp, the grass carp, was introduced to Arkansas in 1963 for the purpose of controlling aquatic vegetation (Reeves 2019). The first free-ranging grass carp was caught by fishers in 1970 (Reeves 2019). Grass carp are sometimes stocked as fertile diploids or sterile triploids for the purposes of vegetation control, although 12 states entirely prohibit stocking grass carp (Embke et al. 2016; Kinter et al. 2018; Jones et al. 2017). Because of their size and consumption rates, grass carp are capable of negatively impacting native macrophytes and filamentous algal feeders (Wittman et al. 2014). Their voracious feeding also threatens wetlands that are important spawning habitats (Chapman et al. 2013). Evidence of grass carp reproduction has been observed within Lake Erie (George et al. 2018).

The black carp invaded the Mississippi River basin after flood events allowed them to escape an Arkansas farm in the 1970s (Chapman 2018). Black carp are molluscivores, which means they pose a threat to the native mollusks and mussels within the Mississippi River, many of which already exist in a critical state (Chapman 2018). Similar to grass carp, black carp have been stocked as triploids for the purpose of controlling pond snails (Hunter and Nico 2015). Black carp have been caught in the Ohio River Basin and Barkley Lake, and there is concern that they will swim further upstream on the Mississippi River, similar to silver carp (Chapman 2018).

The common carp was brought to Washington, D.C. from Germany in the 1880s by the United States Fish Commission and was soon after bred and distributed throughout the country (Sorensen and Bajer 2011). Common carp were also able to spread into new habitats using interconnected rivers. Failure to remove them led to most concerted control efforts ending by the 1950s (Sorensen and Bajer 2011). The feeding habits of common carp, which lead them to uproot vegetation and release sediment, increase water turbidity and decrease water quality (Sorensen and Bajer 2011). Ultimately, managing the upstream movement of carp, or the movement of carps into certain interconnected lakes, is necessary for preserving the quality of these aquatic ecosystems.

Numerous aquatic ecosystems outside of the Mississippi River Basin have been impacted by the spread of a variety of invasive fish across North America and the world. For example, devil firefish (*Pterois miles*) and red lionfish (*P. volitans*) threaten biodiversity in the Atlantic waters off of the southeastern United States due to their high fecundity and lack of predators (Ballew et al. 2016). Oriental weatherfish (*Misgurnus anguillicaudatus*) have become established in several states due to aquaculture escape or releases, and some issues associated with their presence include the predation of eggs and induced competition between it and natural fishes (Kirsch 2018). The northern snakehead (*Channa argus*), a piscivorous fish from east Asia, has been introduced to the eastern United States. It has been predicted that their presence would lead to competition between their species and largemouth bass (*Micropterus* *salmoides*), and they have negatively impacted the biodiversity of freshwater fish around the world (Saylor et al. 2012).

One way to control the spread of invasive fishes, carp in particular, is to block mature adults from traveling up rivers and streams including the Mississippi River. To accomplish this, deterrent systems could be installed within the lock chambers in the Mississippi River. Lock chambers are part of the lock and dam systems present found in the upper third of the Mississippi River, the other part being gated spillways. For lock and dam systems that rarely go into "open river" conditions (i.e., having their spillway gates completely out of the water), such as Lock and Dams 2, 4, 5, and 8, slightly modifying the opening of the gates can reduce the potential of invasive and native fish passages (Zielinsi et al. 2018). Lock chambers are a viable route for invasive species traveling upstream. In one study on fish passage through locks and dams, 20 silver carp passages (18% of all silver carp tagged in the study) were detected to pass through the lock chamber over a four-year period. Other species in the same study appeared less successful in using the lock chambers to pass through: for instance, only three lake sturgeon passages were detected over four years (3.5% of total lake sturgeon tagged) (Tripp et al. 2014).

Nonphysical Deterrents

Non-physical deterrents (sensory stimuli) could be used to block invasive fish from entering locks . Examples of non-physical stimuli include aversive chemicals, electricity, sound, and/or air (Noatch and Suski 2011). A series of electrical barriers have been constructed in the Chicago Area Waterway System (CAWS). There is a significant cost associated with the creation of these barriers: two arrays of one such barrier were estimated to cost $10 million and $13 million, respectively (Buck et al. 2010). The effectiveness of electrical barriers has also been shown to be influenced by the presence of metal-hull barges as the electrical field can be distorted by conductive materials, allowing fish to swimming farther into the barrier (Parker et al. 2014). Ultimately, the cost of maintaining these barriers, as well as their lack of specificity, limits their potential use. Similarly, although toxins such as lampricides have been used in the Great Lakes tributaries to target larval lamprey, there is concern about the danger these chemicals may impose on the health of non-target species. (Noatch and Suski 2012; Katopodis et al. 1994). Because not all fish have sensitive hearing, sound has the potential to be a cost-effective, species-specific deterrent. For the purposes of my thesis, I will focus on sound and air deterrents.

Underwater Sound

Sound energy travels through water as a pressure wave with accompanying oscillatory particle motion (Popper and Carlson 1998). The speed of the oscillations of the acoustic wave is influenced by the properties of the medium, such as its density. As the acoustic wave travels through water at approximately 1,500 m/s, its energy gradually diminishes in intensity (Popper and Carlson 1998). Because of the density of water, sound is able to travel faster and propagate longer compared to air, meaning that water functions extremely well as a medium for sound transmission. A sound is detected and measured in two ways: by the oscillatory motion of water particles caused by the energy generated by the source of the sound (particle motion) and by the intensity of the sound pressure (measured in decibels) caused by the compression and rarefaction of particles as the acoustic wave passes through water (Popper and Carlson 1998; Wahlberg and Westerberg 2005). Particle motion is the primary component of sound detection closer to the source of the sound, while pressure is the primary component farther away from the sound source (Popper and Carlson 1998). Closer to the source of the sound (the "nearfield"), particle motion consists of both the oscillatory motion of the water particles, as well as the hydrodynamic cues generated by the displacement of water (Popper and Carlson 1998). While sound pressure has a scalar quantity, particle motion is a vector quantity and can provide directional cues to fish (Zielinski and Sorensen 2017). Sound can be measured with a hydrophone, which measures sound pressure, and geophones, which measure particle motion (Rogers and Cox 1988; Hawkins 1986). The wavelength of a sound is inversely proportional with its frequency, meaning lower frequency sounds have longer wavelengths (Nummela et al. 2007). Lower frequency sound travels better and further than high frequency sound in shallow waters. Most fish are capable of detecting sounds within the range of 50 and 2000 Hz (Popper and Carlson 1998).

Hearing in Fish

Fish use their inner ear to detect sound pressure and particle motion. Most fish have three semicircular canals in their inner ear, which contain three otolith organs (the saccule, utricle, and laguna) (Pitcher 1986). At the base of each semicircular canal there is a cluster of sensory cells referred to as an ampulla, which is used to detect balance information. The sensory epithelia of the otolith organs are vital to a fish's hearing. The hair cells of the epithelia have both stereocilia and kinocilia on their surface. These cells are innervated by afferent and efferent neurons and are surrounded by microvillar supporting cells. The hair cells of the sensory epithelium are surrounded by microvillar supporting cells, and they can be displaced by acoustic waves, causing stimulation that is interpreted as sound (Pitcher 1986). The inner ear system is capable of detecting sounds up to hundreds or thousands of Hz (Slabbekoorn et al. 2010).

The hearing capabilities of fish are often measured by the approximate threshold of a sound, or the minimum pressure level of a sound required for them to detect it using the auditory brain-stem response method (ABR), which involves using electrodes to measure a fish's neural response to various sounds (Vetter et al. 2018). The threshold varies depending on the duration of the sound stimulus and its frequency. This information can be used to classify fish based on their hearing capabilities (Hawkins 1981).

Ostariophysans (a superorder of freshwater fish) have hearing specializations known as Weberian ossicles, which are bones that connect their swim bladder to their inner ear and result in an amplification of sound (Melotte et al. 2018). There are four ossicles: the claustrum, scaphium, intercalarium, and tripus. These ossicles are connected to each other by ligaments. Oscillations from acoustic waves are transmitted via the swim bladder, through these ossicles, to the perilymphatic space of the ear, allowing fish with hearing specializations to detect a wider range of frequencies with greater sensitivity (Fay et al. 2004). The auditory brainstem response (ABR) technique tests a fish's neural responses to sounds of varying frequencies and pressure levels by attaching cutaneous electrodes to the fish's head. Although this technique can indicate relative thresholds of a fish's hearing ability, the information obtained from this technique cannot be considered as precise because it is testing for integrated peripheral neural activity and is not necessarily indicative of higher order acoustical processing. To completely assess fish hearing capabilities, behavioral tests need to be performed (Vetter et al. 2018). One study found using ABR that common carp are most sensitive to around 500 Hz, although they can detect above 1000 Hz (Kojima et al. 2005). Similar results were found using behavioral conditioning (Popper and Fay 2011). Lake sturgeon (*Acipenser fulvescens*) have been shown to be most sensitive to sounds between 100-500 Hz through ABR (Lovell et al. 2005, Ladich and Fay 2013). Through behavioral tests, channel catfish (*Ictalurus punctatus*), an ostariophysan native to the United States, have been shown to be capable of detecting frequencies up to several thousand hertz (Popper and Fay 2011). Bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*), two species which lack hearing specializations, are more sensitive to sounds below 1000 Hz according to ABR results (Ladich and Fay 2013). Using the ABR technique, bighead and silver carp were found to be capable of detecting sounds ranging from 100-5000 Hz, with their lowest sound pressure threshold at around 500 Hz (e.g., silver carp threshold was at 80.6 ± 3.29 dB re 1 μPa SPLrms) (Vetter et al. 2018). Because bighead carp, common carp, grass carp, and silver carp are fishes with hearing specializations, sound is being considered as a potential deterrent for these species. Since these carp species have hearing specializations, it is possible they would be more affected by a sound deterrent than non-native species without hearing specializations.

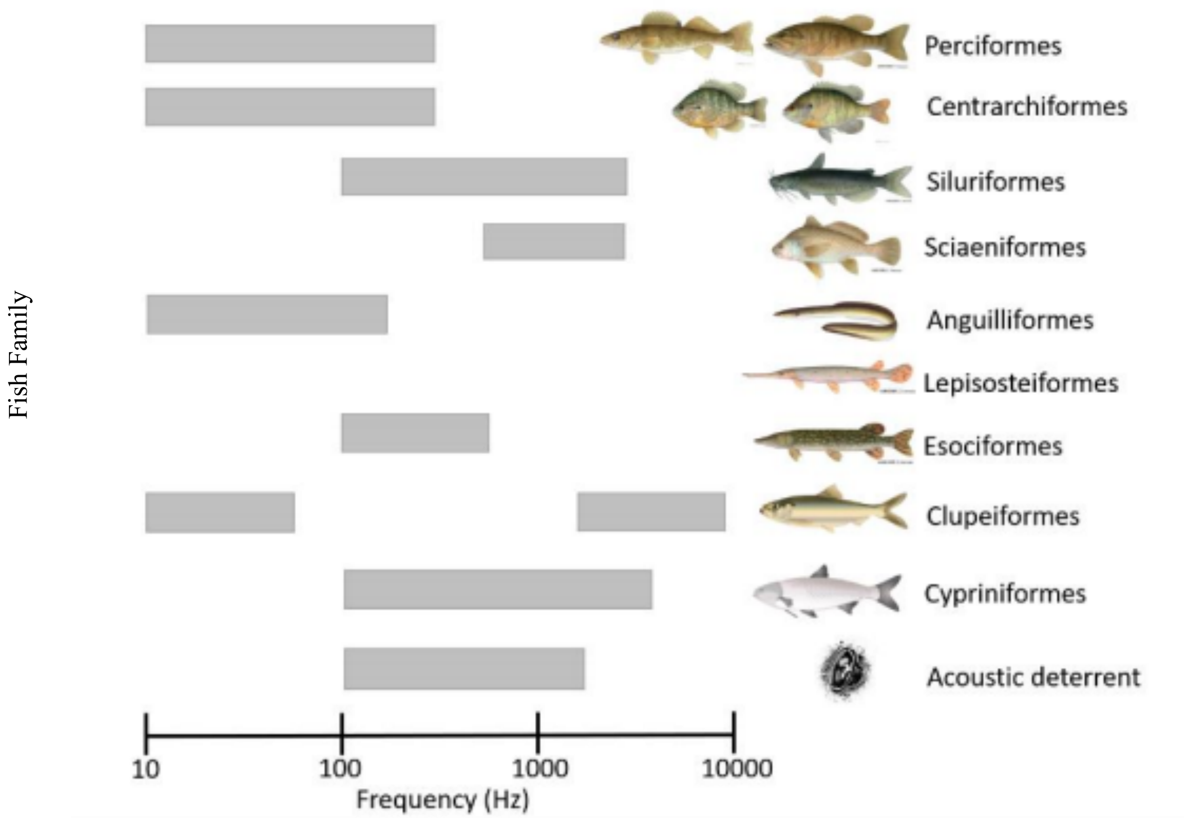


Figure 1-1: Hearing ranges for several families of fish using ABR. Y-axis includes the scientific name of the fish family. The x-axis indicates a range of frequencies from 10-10,000 in Hz. Black bars indicate the lowest and highest frequencies the fish family can detect. Data compiled from This figure is copied from Putland and Mensinger (2019) with their consent. The frequency range of the acoustic deterrent is based on the frequencies commonly used in previous studies (100-2000 Hz).

Fish also have a mechanosensory system called the lateral line system (Popper and Carlson 1998). Fishes' lateral line systems consist of hair cells bundled into neuromasts that either exist within canals or on the surface of a fish's body (Popper and Carlson 1998). The displacement of these hair cells causes stimulation that primarily supplies information on hydrodynamic cues occurring within short distance from the fish, as well as sounds lower than 100 Hz (Popper and Carlson 1998). Fish may use these hydrodynamic cues to orient away from the source of an aversive stimulus (Zielinski and Sorensen 2016).

Notably, over 50 families of fish species use sound as a method of communication (Hawkins 1986). Gadiforms such as the cod (*Gadus morhua*) and the haddock (*Melanogramus aeglfinus*) can contract muscles attached to their swim bladder to produce grunts or knocks. Sounds are produced by these species for specific reasons, such as males emitting sounds during courtship, or communicating sounds to male reproductive competitors. Toadfish, such as the oyster toadfish (*Halobatrachus didactylus*) emit a whistling noise near a frequency of 100 Hz for the purpose of attracting mates. Several cichlid species use their jaw muscles to emit purrs in the range of 250-1250 Hz in order to demonstrate aggression or courtship behaviors (Amorim 2006). Sound can be an important component of social behavior for certain fish, and these sounds can be used to deter or attract other fish depending on the type of noise and the context (Ladich 2015).

Sound as a Deterrent

Sound can be used as a nonphysical, underwater deterrent against fish. This approach can involve placing speakers underwater that play a noise that elicits an avoidance response in targeted fish species. Bigheaded carp, like other ostariophysans, are especially sensitive to sound because they are fish species with hearing specializations. In one laboratory study, silver carp avoided the source of a complex (multiple frequency) sound signal 100% of the time (Vetter et al. 2015). Their avoidance responses were monitored by playing the sound signal once the carp had reached the end of a 10 m x 5 m x 1.2 m tank. If the carp swam to the opposite side of the tank, the sound signal was delivered from the speakers located in that end of the tank. Notably, silver carp did not demonstrate an avoidance response to a simple (single frequency) sound signal. Silver carp were considered to have demonstrated an avoidance response if they were exposed to a sound signal, then swam to the opposite end of the tank (Vetter et al. 2015). The initial responses to the sound signal were characterized as indicative of startle responses, although similar exposures did not elicit the same behavior (contraction of the axial muscles followed by quickly moving away from the source). However, the tests did not occur in darkness and speakers were visible, so it is uncertain to what extent visual cues may have influenced the avoidance response seen in the carp (Vetter et al. 2015).

In another laboratory study, carp, silver carp, and common carp were shown to orient away from an aversive sound signal in a manner that correlated with the axes of particle motion. When tested in a laboratory tank and exposed to a complex sound signal, the three carp species swam away from the sound on a curvilinear trajectory that was parallel to the axes of local particle acceleration. The results of the study indicated that while these fishes likely become aware of an aversive signal due to the change in sound pressure, they possibly use particle motion to guide their path away from the noise, since the test occurred in darkened conditions that removed the possibility of visual cues having an influence (Zielinski and Sorensen 2017).

Another laboratory study examined the avoidance response of bighead and common carp to two different complex sound signals, an outboard motor signal and a proprietary cyclic signal created by the Fish Guidance System Ltd. (FGS). The cyclic signal contained frequencies between 20 and 2000 Hz (Dennis et al. 2019). This study found that bighead and common carp were significantly more affected by the cyclic sound than the outboard motor sound, even though the amplitudes of the different sounds were balanced. In particular, while 42% of common carp were blocked by the outboard motor sound, 79% were blocked by the cyclic sound. The variation in responses indicates that there is a cognitive aspect to the avoidance response to these sound signals and that some species of fish are capable of discriminating between complex sound signals (Dennis et al. 2019).

Fish without hearing specializations have also been studied for their responses to complex sound signals. Atlantic salmon (*Salmo salar*) avoided the source of a complex sound signal played at low frequencies in an artificial pool (Knudsen et al. 1991). These low frequency sounds (5-10 Hz) were also capable of deterring Atlantic salmon when tested in a field experiment at a small stream. Notably, Atlantic salmon's hearing sensitivity is limited to around 380 Hz (Knudsen et al. 1993). Another field study showed that a 20-600 Hz signal meant to guide fish away from a power station cooling water inlet had varying success depending on the hearing capabilities of the fish. Clupeiformes, such as herring (*Clupea harengus*), have hearing specializations and were more deterred by the sound signal, whereas species without hearing specializations such as the river lamprey (*Lampetra fluviatilis*) were less affected (Maes et al. 2004). While this study indicated that hearing sensitivity influences the sensitivity to sound deterrents, there were cyprinids in this study that showed little or no response to the sound deterrent (Maes et al. 2004). Sound has been used to direct the movement of fish with and without hearing specializations, but sound's effectiveness may vary based on the frequencies used in the sound signal, as well as the hearing sensitivity of the species that is targeted.

Sound stimuli can be used without causing lasting damage to fishes' hearing ability, meaning a sound deterrent will not quickly lose its effectiveness by causing deafness in the targeted fish. In one experiment studying the deterrence of bigheaded carp exposed to a broadband sound, sound pressure levels reached a maximum of 156 decibels, and the fishes' exposure to these sound levels did not appear to have a significant impact on their hearing capabilities. However, the bighead carp exhibited avoidance responses when the noise was played underwater (Vetter et al. 2017). A sound signal utilized for the purposes of guiding or blocking fish can be implemented without implementing sound pressure levels that cause physical damage to the fish.

Sound as an Attractant

Other sound tests have shown that sound cues attract fish, and can be deployed in this manner. Speakers that played a reef noise were able to attract reef fish larvae (Mann et al. 2007). Another test found similar results: 67.0% of 40,191 tested reef fishes appeared in a trap that utilized reef noises (which consisted of fish pops ranging from 600-800 Hz overlaid a background crackle of 2.5-200 kHz) (Simpson et al. 2004). Another study found that fish such as the cod (*Gadus morhua*) were attracted to the noise of exhaled air (30-110 Hz), likely because of fish associating the arrival of the divers with a surplus of invertebrates disturbed from the seabed because of the divers' presence (Chapman et al. 1974). These results demonstrate that sounds can be utilized to attract fish as well as deter, although notably, the sounds used in these experiments are either biological in origin (reef noises) or were learned by fishes to be associated with a positive outcome (divers' exhaled air).

Knowns and Unknowns about Sound as a Deterrent

While a multitude of sound deterrents have been tested against various species of fish, a single sound deterrent has not been tested against a wide array of fish species. Furthermore, tests that have demonstrated the potential success of a sound deterrent often did not consider habituation, report the efficacy of the deterrent, or record the sound pressure levels used to achieve a significant level of blockage (Putland and Mensinger 2019). One study demonstrated that a sound deterrent utilizing frequencies between 20-600 Hz blocked Atlantic herring (*Clupea harengus*) by 94.7%, but did not examine whether these fish would habituate to the deterrent (Putland and Mensinger 2019). Because of this, the long-term effectiveness of the 20-600 Hz sound signal is uncertain. There has been relatively little success in utilizing sound deterrents to block gadids and perciformes, with 14% and 19.2% (respectively) of literature reporting sound deterrent efficacy greater than 50% (Putland and Mensinger 2019). A low frequency pure tone (5-10 Hz) has shown promise in deterring the movement of Atlantic salmon, but single-frequency sound deterrents have had little success in deterring cyprinids such as silver carp or Iberian barbel (*Luciobarbus bocagei*) (Putland and Mensinger 2019). One broadband motor sound was successful in directing bighead carp from one side of the testing area to another, although it is uncertain to what extent visual cues affected this experiment (Vetter et al. 2017). Largemouth bass, bighead carp, and common carp were tested against a cyclic (20-2000 Hz) sound signal in a darkened laboratory flume and were all significantly deterred (Dennis et al. 2019). This study did not find evidence of habituation to the cyclic sound signal, demonstrating that it is currently the most promising sound deterrent that could be installed to block carp (Dennis et al. 2019).

The response of a wide array of fish species with and without hearing specializations to this promising cyclic sound signal has not been tested before in the laboratory. Although it has been demonstrated that fishes such as bighead carp can distinguish complex sounds and demonstrate varying levels of avoidance, there is no clear indication on why one complex sound is more effective than another (Dennis et al. 2019). Furthermore, it should be noted that these sound deterrent tests utilizing the promising cyclic sound signal have been performed in varying settings, from a laboratory flume (Dennis et al. 2019) to a small river in the field (Knudsen et al. 1993), so the results between these studies cannot be directly compared.

Air Curtains as Deterrents

Air curtains (a wall of bubbles) have also been tested in lab and field studies for their potential to deter the movement of bigheaded and common carp (Zielinski et al. 2014). This system generates sound cues (< 300 Hz) that are above the hearing threshold of carp, and with an amplitude great enough that they are not masked by background noise (Zielinski et al. 2014). They also generate hydrodynamic cues through altering the fluid flow around the air curtain, which could be detected by a fish's lateral line system (Zielinski et al. 2014). In a laboratory setting, 75-85% of common carp passages were reduced by the presence of the air curtain (Zielinski et al. 2014). The results indicated that common carp were responding to the sound and hydrodynamic cues, as opposed to any visual cues generated by the presence of the air curtain, since the experiments were performed with the lights turned off in the laboratory and a black tarp covering the experimental tank (Zielinski et al. 2014). However, it is not clear to what extent sound versus hydrodynamic cues contribute to the avoidance response (Zielinski et al. 2014). Laboratory-raised silver carp and bighead carp have demonstrated a similar level of avoidance when exposed to air curtains in a laboratory setting, with 73-80% of their passages blocked due to the presence of the air curtain (Zielinski et al. 2016). When tested within a small stream, the response of wild common carp to the air curtain was notably lower: approximately 59 ± 14% of downstream passages were blocked, and 16 ± 11% of upstream passages were blocked (Zielinski and Sorensen 2015). Common carp in this field study were monitored over a period of twenty-four hours (Zielinski and Sorensen 2015). These results indicate the potential of air curtains to be implemented in order to reduce invasive carp passage (Zielinski and Sorensen 2015). However, to improve the level of deterrence, the air curtain could be supplemented with additional nonphysical deterrents such as sound.

Air Curtains Coupled with Sound

Sound can be coupled with an air curtain to create a more effective deterrent. When a sound signal is coupled with an air curtain, it means the sound is playing directly within the air stream. The sound wave transitions between two different media (air and water), creating a density gradient that causes the sound to be louder than it would be without the air curtain. An ensonified air curtain has a sharper pressure gradient compared to the same sound signal played by itself (Dennis et al. 2019). In one study, sound coupled with air was shown to deflect upwards of 70% of Atlantic salmon (*Salmo salar*) smolts, which showed promise for diverting these fish away from dangerous areas such as hydropower plants (Welton et al. 2002). The sound coupled with air has been tested for its potential to block bighead carp, where it was demonstrated to block 95% of their passages (Taylor et al. 2005). A study of sound coupled with air used in a small creek seemed to demonstrate a similar level of deterrence against bigheaded carp (Ruebush et al. 2012).

A recent laboratory study on sound coupled with air confirmed that the air curtain contributed significantly to the deterrence of bigheaded carp, and that the proprietary sound signal developed by the Fish Guidance System (FGS) company was more effective than other complex sound signals. This study was performed by placing a group of ten fish of a single species (either common carp, bighead carp, or largemouth bass) into a darkened laboratory flume approximately 8 m long and 1 m high, then monitoring their movement across deterrent systems as sound, air curtain, or sound and air was turned on and off. While 42 ± 28% of common carp passages were blocked when exposed to a motor sound, 79 ± 19% of their passages were blocked by the FGS sound signal (Dennis et al. 2019). Furthermore, the blockage efficiency increased from 79 ± 19% to nearly 99 ± 1% when the air curtain was added (Dennis et al. 2019). The cyclic sound coupled with air also blocked common carp more effectively than the outboard motor sound coupled with air (88 ± 18% blocked when the outboard motor sound was coupled with air). Bighead and common carp exhibited no habituation to the presence of the cyclic sound coupled with air, meaning the magnitude of their responses did not lessen with repeated exposure (Dennis et al. 2019). The ability of the cyclic sound coupled with air to block 97% of bighead carp and 99% of common carp with no observed habituation demonstrates that it is the most promising deterrent that could be installed.

Sound mapping of the sound coupled with air combined indicate that the air curtain coupled with sound results in a sharper gradient of sound pressure compared to the pressure levels of sound alone, which is a possible explanation for the greater efficacy of the coupled deterrents (Dennis et al. 2019). Another explanation is that particle acceleration also had a steeper gradient when sound and air were coupled, and bigheaded and common carp are possibly able to use this nonscalar vector to orient themselves away from the source of the stimuli (Dennis et al. 2019; Zielinski and Sorensen 2017). Also notable in this laboratory study, largemouth bass were also significantly blocked by sound and air combined, at 87 ± 24% (Dennis et al. 2019). This demonstrates that sound coupled with air is a multimodal deterrent, and species without hearing specializations may also be susceptible to both the sound and hydrodynamic cues generated by the air curtain (Dennis et al. 2019).

Objectives of this Study

Because only four species of fish have been tested against the promising cyclic sound coupled with air (bighead carp, common carp, Atlantic salmon, largemouth bass), the primary objective of this study was to determine if this deterrent can block invasive carp without significantly impacting the movement of fishes native to the Mississippi River. We were also interested in determining to what extent hearing specializations influences the responses to these deterrents. To accomplish this, we asked three questions. Firstly, how well does a cyclic sound block a range of fish species, and how does its efficacy compare among species in particular when the presence of hearing specializations is considered? Secondly, does coupling a cyclic sound with an air curtain make it more effective at blocking carps, without having similar effects on other fishes, including those lacking hearing specializations? Finally, is there any evidence of habituation to sound alone or the cyclic sound coupled with an air curtain? The second chapter of my thesis was written in accordance with the style of the Canadian Journal of Fisheries and Aquatic Sciences. The authors will be Jane Feely and Peter Sorensen, and I am hoping to submit the manuscript in the fall of 2020.

Chapter 2: The ability of a cyclic sound both on its own, and when coupled with an air curtain, to block ten species of fish including carp in a laboratory flume

ABSTRACT

There is a critical need to stop the upstream movement of invasive Asian carps in the Upper Mississippi River and its tributaries where they could cause ecological harm. One possible strategy to stop them would be to install sensory deterrent systems in key lock chambers. Ideally, such systems would not impede the movement of migratory native fishes. Because carps have an excellent sense of hearing compared to many other fishes, sound (on its own or in conjunction with other sensory cues) is being considered. A cyclic sound has been shown to have promise in the laboratory where it blocks 78-79% of both bighead and common carp movement, and largemouth bass to a lesser extent (50%). These blockage rates increased to 97% when this sound was coupled with an air curtain. However, the effects of sound, and sound coupled with air, have not been tested on other species of fish, including other carp species. My study examined the ability of sound and sound coupled with air to block a range of fish species both with and without hearing specializations: grass carp, silver carp, common carp, bighead carp, channel catfish, golden shiners, largemouth bass, rainbow trout, lake sturgeon, and bluegill sunfish. Several important findings emerged. Firstly, we found that responses to the cyclic sound alone varied greatly by species and did not appear to be related to the possession of hearing specializations. Whereas bighead and common carp were strongly repelled (86% and 83% blocked, respectively), silver and grass carp were relatively unaffected (31% and 21%), channel catfish were attracted to the sound, and four non-hearing specialists were moderately affected (60-34%). Secondly, we discovered that coupling this sound with an air curtain consistently increased its efficacy at repelling fishes (average increase 38%), and in a highly variable manner which generally corresponded with the possession of hearing specializations. Thus, the group of 4 carps were the most strongly repelled with blockage rates ranging from 97-92%, followed by the golden shiners (89%), largemouth bass, lake sturgeon, rainbow trout, bluegill sunfish, and channel catfish. This laboratory study suggests that although a cyclic sound alone has the potential to block some fishes (bighead and common carp), this ability is highly species-specific and not restricted to exotic hearing specialists. Coupling the cyclic sound to an air curtain has much greater potential, but some of the possible specificity might be lost because this combined system is a strong repellent for native fishes.

INTRODUCTION

Silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*), grass carp (*Ctenopharyngodon idella*), black carp (*Mylopharyngodon piceus*), and common carp (*Cyprinus carpio*) are five fish species widely regarded as undesirable outside of Asia (Kolar et al. 2007). However, many species have been introduced into multiple countries both intentionally and unintentionally; for example, bighead carp are reproducing in 19 countries, and silver carp are reproducing in 23 (Kolar et al. 2007). In the United States, silver carp and bighead carp were imported from Asia, escaped into the Mississippi River basin, and now threaten to spread into the upper reaches of the Mississippi River and the Laurentian Great Lakes (Kolar et al. 2007). These fish can alter food webs due to their voracious consumption of zooplankton and phytoplankton, possibly allowing them to outcompete native filter-feeders such as the gizzard shad (*Dorosoma cepedianum*) (Minder and Pyron 2017; Wang et al. 2018). Grass carp were introduced into Arkansas in 1963, and their consumption of vegetation can threaten important fish spawning habitats (Reeves et al. 2019). Black carp were introduced into Arkansas in the 1970's, and their continued spread would threaten the native mollusks of the Mississippi River, many of which already exist in critical condition (Reeves et al. 2019; Chapman 2018). Common carp are established throughout many countries and are associated with poor water quality (Sorensen and Bajer 2011). Ultimately, managing the upstream movement of carp species, or their movement into certain interconnected lakes, is necessary for preserving native biodiversity and water quality.

One way of controlling the spread of these invasive fishes would be to install deterrent systems that block or deter their movement. To block carp from traveling up the Mississippi River, these systems could be placed in front of, or within navigation locks. Locks are one part of all lock and dam systems that are found in the upper third of the Mississippi River. Non-physical barriers, which utilize sensory stimuli such as sound, light, or air, could be used to block or deter the passage of invasive carp or other invasive fish species (Noatch and Suski 2012). Because ostariophysans (a superorder of fishes including carp) have an apparatus called a Weberian ossicle that increases their hearing sensitivity (a hearing specialization), sound is of particular interest in this regard.

Sound is generated by particle displacement as a longitudinal pressure wave accompanied by particle motion (Popper and Carlson 1998). Because of the density of water, sound can travel faster and propagate further in water compared to air, meaning that water functions extremely well as a medium for sound transmission. A fish can detect sound in two ways: by the oscillatory motion of water particles caused by the energy generated by the source of the sound and by the intensity of the sound pressure (measured in decibels) caused by the compression and rarefaction of particles as the acoustic wave passes through water (Popper and Carlson 1998; Wahlberg and Westerberg 2005).

Several studies have assessed the effectiveness of sound as a deterrent in blocking carp movement. In one laboratory study, silver carp avoided the source of a complex (multiple frequency) outboard motor sound nearly 100% of the time (Vetter et al. 2015). These tests took place in well-lit tanks, so it is uncertain if silver carp were responding to the sound itself, or if they were responding to the visual presence of the speakers (Vetter et al. 2015). Another laboratory test demonstrated that silver, bighead, and common carp exhibited an avoidance response to an outboard motor sound in a darkened arena, but they habituated to the sound signal after two exposures (Zielinski and Sorensen 2017).

In another experiment, bighead and common carp were blocked (about 75-80%) by a proprietary cyclic sound signal containing frequencies between 20 and 2000 Hz (Fish Guidance Ltd) in a darkened laboratory flume. However, largemouth bass (*Micropterus salmoides*), were also significantly blocked, albeit to a lesser extent (about 50% of passages blocked) (Dennis et al. 2019). The same outboard motor sound used in the Vetter et al. (2015) study was also tested in this experiment, but the fish guidance system (FGS) cyclic sound was found to block higher percentages of common carp (42% compared to 79%) (Dennis et al. 2019). The cyclic sound also consistently blocked bighead and common carp without habituation, meaning the magnitude of their responses did not lessen with repeated exposures to the cyclic sound signal (Dennis et al. 2019; Rankin et al. 2010). The lack of habituation is an important component to study, since a deterrent would prove ineffective over time if fish were observed to habituate to its presence. Dennis et al. (2019) demonstrated that the cyclic sound is the most effective sound deterrent that has been tested against invasive carp. In the same study, a cyclic sound coupled with air (meaning the sound was played within the air curtain) blocked 97% of bighead common carp passage through the stimuli without habituation, significantly more than the 75% blocked by sound alone. The velocity of sound in an ensonified air curtain is slower than the surrounding water and air, therefore ‘trapping’ the sound within the column of bubbles. Sound mapping of the sound and air combined indicate that the air curtain coupled with sound results in a sharper gradient of particle motion at higher frequencies compared to sound alone, which is a possible explanation for the greater efficacy of the coupled deterrents (Dennis et al. 2019). As shown in Zielinski and Sorensen (2017), bighead and common carp may use this vector to orient themselves away from the source of a sound stimuli they found aversive (Dennis et al. 2019; Zielinski and Sorensen 2017). Aside from sound and air's high efficacy, there was no evidence of habituation to these stimuli, so the level of blockage was maintained over a period of several hours as the deterrent was turned on and off (Dennis et al. 2019). Other tests of the cyclic sound coupled with air have shown similar levels of deterrence; for instance, one field study demonstrated that 95% of bighead carp passages were blocked by the same stimuli (Taylor et al. 2005). Ruebush et al. (2012) tested the cyclic sound coupled with air in a small creek and demonstrated similar results. The high efficacy of sound and air and the lack of habituation to their presence have shown that these stimuli have special promise in blocking the movement of invasive carp. However, largemouth bass were also significantly blocked by the cyclic sound coupled with air (87% of passages blocked), and there was evidence that they because sensitized to the stimuli over time (i.e., they crossed the barrier of sound and air less frequently with repeated exposures) (Dennis et al. 2019). The response of largemouth bass to sound coupled with air possibly indicates that it is a multimodal deterrent, since their susceptibility may have been due to the addition of hydrodynamic cues (Dennis et al. 2019). In another study, 70% of Atlantic salmon (*Salmo salar*), another species that lacks hearing specializations, were blocked in the field by the same stimuli (Welton 2002). However, it is difficult to directly compare these two results due to the different testing conditions (i.e., lab versus field).

The potential impact of this promising cyclic sound (or sound coupled with air) to the native fishes of the Mississippi River has not been studied. Although the cyclic sound signal was effective against bighead carp and common carp, it is unclear what component of the sound made it aversive to these fish, and why it was aversive to largemouth bass, which lacks hearing specializations. Largemouth bass and Atlantic salmon have demonstrated aversion to sound coupled with air, although it is uncertain if their response is due to hydrodynamic or sound cues (Dennis et al. 2019; Welton 2002). Directly comparing the results of a wider array of species, including those with hearing specializations and those without, would offer a clearer understanding of how the implementation of these stimuli could impact certain fish species. Ideally, a deterrent system could block the spread of invasive carp while minimally impacting non-target species.

The overarching goal of our study was to test and identify a set of sound and air cues that might block carp species while allowing other fish, particularly native species without hearing specializations, to pass. To achieve this, we asked three questions. Firstly, how well does a cyclic sound block a range of fish species, and how does its efficacy compare among species in particular when the presence of hearing specializations is considered? Secondly, does coupling a cyclic sound with an air curtain make it more effective at blocking carps without having similar effects on other fishes, including those lacking hearing specializations? Finally, is there any evidence of habituation to sound alone or the cyclic sound coupled with an air curtain? To answer these questions, we tested 10 species of fish with different hearing capabilities in a laboratory flume against the FGS cyclic sound alone and then to this sound coupled with an air curtain.

Materials and Methods

Experimental Design

To assess how fish were impacted by the sound and air stimuli, we tested groups of fish of the ten fish species in three steps. Different individual fish were used in each experiment that had not been tested before (i.e. they were naive). First, we tested fish passage in the absence of any stimuli ("no-treatment control") to monitor their basal level of activity. Performing this step allowed us to be sure that any change in passage rate when exposed to a stimulus was due to that stimulus, not simply the passage of time. Second, we tested fish passage in the presence of the cyclic sound using another set of naive fish in a matched experiment ("cyclic sound experiment"). Third, we tested fish passage to the cyclic sound coupled with the air curtain using another set of naive fish ("sound and air curtain experiment"). Each experiment was repeated 10 times using different groups of 10 fish (i.e.10 trials, each with 10 fish) for each species (N=30). For each trial, 10 naive fish of a single species were added into a darkened flume (Figure 2-1). Each trial started with an hour of acclimation, followed by a control period (one hour long). The control period allowed us to observe the basal level activity for the group, and was followed by a testing interval, which contained ten sets of exposures. Each set began with a six-minute "pre-test period" (no stimuli used), followed by a six-minute "test period" (stimuli used, except for no-treatment control trials). Exposure periods were separated by 10-minute gaps or "recovery periods" (no stimuli used). Fish distribution was monitored using overhead cameras, and trials were analyzed by tallying the number of times fish crossed over the deterrent system which extended across the flume and contained both speaker and air. Analyzing the movements of fish during these periods allowed us to determine the percent of fish blocked by the stimuli's presence. For those trials that involved the air curtain, underwater cameras were used to observe fish passage rates. With few exceptions (instances in which we did not have enough fish because of mortalities), all fish were previously untested or naive. When fish had to be reused (common carp and largemouth bass), we were able to use fish that had only been tested once before and gave them at least 3 weeks to recover, a treatment that worked for Zielinski and Sorensen (2017) and which we confirmed by first testing against no-stimulus control to ensure that baseline activity was unchanged (it did not, see supplemental data)

Fish

Species selected for use in this study were identified based on availability from local hatcheries, taxonomic variety and whether they had hearing specializations. Channel catfish (*Ictalurus punctatus*) and lake sturgeon (*Acipenser fulvescens*) were obtained from the Genoa National Fish Hatchery (Genoa, WI). Bluegill sunfish (*Lepomis macrochirus*), largemouth bass, golden shiners (*Notemigonus crysoleucas*), common carp, rainbow trout (*Oncorhynchus mykiss*), grass carp, bighead carp, and silver carp were obtained from Osage Catfisheries (Osage Beach, MO). All fish were held in flow-through circular tanks prior to testing for at least 3 months. Bluegill sunfish, channel catfish, largemouth bass, golden shiners, common carp, rainbow trout, and grass carp were fed 2.5 mm floating pellets manufactured by Skretting USA (Tooele, UT). Silver carp and bighead carp were fed a mixture of spirulina and chlorella. Lake sturgeon were fed brine shrimp (Hikari, Japan). Tanks were supplied with well water and aerated by air stones. Procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol: 1712-35381A) and all necessary federal and state permits were obtained.

Laboratory Flume

Experiments were performed in the custom-built indoor elliptical flume (8 m long x 1 m wide channel x 0.3 m water depth; 1.0 m wall height) used and further described by Dennis et al. (2019). At the center of each long side of the flume two speakers (FGS MkII 15-100; Fish Guidance System Ltd.; Southampton, UK) were positioned along with two porous pipes (AD100T; PentairAES; Apopka, FL).

Sound reverberation was minimized using concrete blocks and foam pads on the exterior of the flume, which allowed fish a refuge from the noise of a deterrent on the opposite side of the flume. Fish were monitored using both overhead cameras, underwater cameras (when the air curtain was in use), and infrared lights (VT-IR1 and VT-IR2; Vitek; Valencia, CA; 840 nm wavelength, <1 lux) (Figure 2-1).

Sound Stimuli

The sound signal used for this study was previously used and described in the Dennis et al. (2019) study. To produce the air curtain, the porous pipes placed in the flume were supplied with a flow rate of 1.2 L s-1, as done previously by Zielinski et al. (2014) and Dennis et al. (2019). The air curtain was placed in front of the speakers in order to mimic a bio-acoustic fish fence (BAFF system). Sound pressure and particle acceleration levels were mapped for the FGS proprietary sound signal using the same equipment and measuring protocol as given by Dennis et al. (2019).

Statistical Analyses

To analyze fish passage data, we used the generalized linear mixed model (GLMM) with a Poisson distribution described by Dennis et al. (2019) with minor modifications. This model used custom-built matrices to facilitate comparisons of interest (for instance, comparing passage rates during control experiments to passage rates during experiments with sound or with sound coupled with air). Using these matrices rather than a three-way ANOVA allowed us to directly answer our statistical questions with greater power and simplicity (Dr. Gary Oelhert, Statistical Consulting Center, University of Minnesota, Minneapolis, MN; personal communication). "Fish Group Number" (i.e., the group of ten fish used in a single trial) was used as a random effect in this model. Assumptions of normality were tested using a fitted residual plot where the variance of the residuals needed to be less than 2. For data with a residual greater than 2, the hyper-variability was corrected by dividing the test statistic by the square root of the dispersion parameter (McCullagh and Nelder 1989). This model allowed us to directly account for possible trends of passage rate (linear, quadratic, cubic, etc.) data during the eight trial periods for each experiment ($p < 0.05$, corrected for multiple comparisons). The GLMM also allowed us to analyze if there was any change in passage rates between pre-test and test periods ($N = 8$, $p < 0.05$, corrected for multiple comparisons) in an initial assessment of possible habituation. Analyses were performed in R with an α of 0.05. Each fish species was analyzed individually.

First, we tested whether either of the stimuli (sound alone or sound coupled with air) significantly altered the passage rate of fish (defined as the number of times a fish swam across the deterrent system during a six-minute pre-test period or test-period). We used the GLMM to compare mean passage rates of a single species during all 8 periods of each no-treatment control experiments (n = 160 passage rates [80 pre-test and 80 test passage rates]) to passage rates during sound experiments for that species (n = 80 observations [80 test passage rates]). If the model determined significance ($p < 0.05$, Bonferroni-corrected for multiple comparisons), we calculated the blockage efficiency of the deterrent. This was accomplished by dividing each of the test passage rates for a sound experiment or sound coupled with air experiment (80 observations) by the mean passage rate during the no-treatment control experiment (160 observations). The result was averaged across all 80 test passage values and then multiplied by 100. Overall blockage efficiencies were used to account for any change in passage rate during no-treatment control trials, as well as to account for any variation in basal passage rate across different species.

Second, we analyzed for habituation within each set of experiments for each species. We did this in a slightly different way than Dennis et al. (2020) whose analysis was simplified by the lack of an effect of time in the pre-stimulus control experiments, thereby allowing for direct comparisons of passage rate trends (i.e., comparing the trend in test passages against the trend of passages during no-treatment control experiments). Instead, we compared the difference in passage rates between pre-test and test periods for individual experiments (80 test passage rates were divided by their preceding pre-test passage rates, subtracted from 1, and multiplied by 100). We then tested for changes in pre-test and test periods over time using a 1-way repeated measures ANOVAs, with trial number (1-8) being the main effect, and fish group number (1-10) as a random effect. If the test was significant ($p < 0.05$, corrected for multiple comparisons), we performed a linear regression analysis to determine if the difference between pre-test and test passage rates was increasing over time (sensitization) or decreasing over time (habituation). Analysis was performed using JMP Pro 13. Experiments (individual species/stimuli combinations) were analyzed separately.

Third, having characterized responses of each fish species to each of the two stimuli, we next sought to determine whether coupling sound with air made it more effective. We did this by performing a series of

Bonferroni-corrected z-tests ($p < 0.05$, $n = 10$) to compare blockage efficiency of sound versus sound coupled with air for each fish species. Finally, we sought to determine which fish species were most responsive to each stimulus. To accomplish this, we performed two series of Bonferroni-corrected z-tests ($p < 0.05$, corrected for multiple comparisons). The first compared the blockage efficiencies of sound among the ten tested species ($N = 45$ comparisons), and the second compared the blockage efficiencies of sound coupled with air among the ten tested species ($N = 45$ comparisons). The compiled were ordered below by species and by stimulus. Questions about habituation and sensitization were answered for each species, followed by the relative efficacy of each stimulus. Blockage efficiencies have been back-transformed into a linear scale for the results.

Results

Question 1: How well does a cyclic sound block a range of fish species?

Bighead carp

Bighead Carp averaged 14 ± 8 (mean ± standard deviation) passages per six-minute period during no-treatment control trials, and the number of fish passing per 6- min period (passage rate) during those trials did not change significantly over time ($p > 0.05$, Table 2-S1). The mean passage rate was significantly reduced by the FGS cyclic sound, with an overall average blockage efficiency of 86% ± 48 ($p < 0.05$, Table 2-2). All 8 test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2A). There was no indication of habituation or sensitization to the sound deterrent ($p > 0.05$, Table 2-2).

Common carp

Common Carp averaged 16 ± 9 passages per six-minute period during no-treatment control trials, and the number of passages per period did not change significantly over time ($p > 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 83% ± 9 ($p < 0.05$, Table 2-2). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2A). There was no indication of habituation or sensitization to the sound deterrent ($p > 0.05$, Table 2-2).

Grass carp

Grass Carp averaged 57 ± 14 passages per six-minute period during no-treatment control trials, and the number of passages per period increased significantly in a linear manner over time ($p < 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 21% ± 3 ($p < 0.05$, Table 2-2). Five test periods during the sound experiment had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). The difference between pre-test passages and test passages decreased significantly over time, indicating habituation to the sound signal ($p < 0.05$, Table 2-2).

Silver carp

Silver Carp averaged 30 ± 20 passages per six-minute period during no-treatment control trials, and the number of passages per period during no-treatment control trials increased significantly in a linear manner over time ($p < 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 31% ± 4 ($p < 0.05$, Table 2-2). One test period had significantly fewer passages than its preceding pre-test period ($p < 0.05$, Table 2-2B). The difference between pre-test passages and test-passages decreased significantly over time over time ($p < 0.05$, Table 2-2), indicating habituation to the sound signal. Two extra trials were performed for this experiment, and there was no indication in those trails that sound significantly deterred silver carp (Figure 2-S2).

Channel catfish

Channel Catfish averaged 11 ± 5 passages per six-minute period during no-treatment control trials, and he number of passages per period decreased in a linear manner over time ($p < 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with a blockage efficiency of -41% ± 5 ($p < 0.05$, Table 2-2). Four test periods had significantly higher passage rates compared to their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no evidence of habituation or sensitization to the sound signal ($p > 0.05$, Table 2-2).

Golden shiners

Golden Shiners averaged 10 ± 5 passages per six-minute period during no-treatment control trials, and the number of passages per period did not change significantly over time ($p > 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 77% ± 9 ($p < 0.05$, Table 2-2). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2A). There was no evidence of habituation or sensitization to the sound signal ($p > 0.05$, Table 2-2).

Lake sturgeon

Lake sturgeon averaged 11 ± 6 passages per six-minute period during no-treatment control trials, and the number of passages per period during no-treatment control trials decreased significantly over time ($p < 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 47 ± 8% ($p < 0.05$, Table 2-2). Out of eight test periods, two of them had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no evidence of habituation or sensitization to the sound signal ($p > 0.05$, Table 2-2).

Rainbow trout

Rainbow trout averaged 7 ± 5 passages per six-minute period during no-treatment control trials, and the number of passages per period did not change significantly over time ($p > 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 45% ± 9 ($p < 0.05$, Table 2-2). Three test periods had significantly lower passage rates than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no evidence of habituation or sensitization to the sound signal ($p > 0.05$, Table 2-2).

Largemouth bass

Largemouth Bass averaged 11 ± 10 passages per six-minute period during no-treatment control trials, and the number of passages per period decreased significantly over time in a linear manner ($p < 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 34% ± 9 ($p < 0.05$, Table 2-2). Five test periods had significantly fewer passages than their preceding pre-test period ($p < 0.05$, Figure 2-2B). The difference between pre-test passages and test passages decreased significantly over time, indicating habituation to the sound signal ($p < 0.05$, Table 2-2).

Bluegill sunfish

Bluegill Sunfish averaged 8 ± 3 passages per six-minute period during no-treatment control trials, and the number of passages per period during the no-treatment control trials decreased significantly in a linear manner over time ($p < 0.05$, Table 2-S1). Their mean passage rate was significantly reduced by the sound signal alone, with an overall blockage efficiency of 60% ± 8 ($p < 0.05$, Table 2-2). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2A). There was no evidence of habituation or sensitization to the sound signal ($p > 0.05$, Table 2-2).

Which species are most influenced by the cyclic sound and does the presence of hearing specialization have a role?

Bighead carp (86 +/- 48% overall blockage efficiency), common carp (83 +/- 27%), and golden shiners (77 +/- 27%) were blocked to a greater extent than all other species and did not differ significantly from each other ($p < 0.05$; Table 2-2). Bluegill sunfish (60 +/- 24%) were significantly more affected than grass carp, silver carp, channel catfish, and largemouth bass ($p < 0.05$, Table 2-2). Their response did not differ significantly from lake sturgeon or rainbow trout ($p > 0.05$, Table 2-2). Lake sturgeon (47 +/- 24%) were impacted to a significantly greater extent than silver carp, grass carp, and channel catfish ($p < 0.05$, Table 2-2). Their response did not differ significantly from bluegill sunfish, rainbow trout, and largemouth bass ($p > 0.05$, Table 2-2). Rainbow trout (45 +/- 27%) were significantly more affected than grass carp and channel catfish ($p < 0.05$, Table 2-2). Their response did not differ significantly from bluegill sunfish, lake sturgeon, largemouth bass, or silver carp ($p > 0.05$, Table 2-2). Largemouth bass (34 +/- 27%), silver carp (31 +/- 12%), and grass carp (21 +/- 9%) were significantly more affected than channel catfish (-41 +/- 15%) ($p < 0.05$, Table 2-2). Given that silver carp, grass carp, and channel catfish were the least impacted by the sound stimulus and they are all ostariophysi, it does not appear that the ability of sound to block fish is related to their possessing hearing specializations (Figure 2-3).

Question 2: How well does a cyclic sound coupled with air block a range of fish species, and is it more effective than sound alone?

Bighead carp

When the air curtain was coupled with the cyclic sound, bighead carp were significantly blocked (compared to the no-stimulus control), with an overall blockage efficiency of 92+/-53% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Common carp

When the air curtain was coupled with the cyclic sound, common carp were significantly blocked, with an overall blockage efficiency of 95+/- 14% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Grass carp

When the air curtain was coupled with the cyclic sound, grass carp were significantly blocked, with an overall blockage efficiency of 95+/- 8% ($p < 0.05$, Fig 2-2B; Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Silver carp

When the air curtain was coupled with the cyclic sound, silver carp were significantly blocked, with an overall blockage efficiency of 97+/- 12% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Channel catfish

When the air curtain was coupled with the cyclic sound, channel catfish were significantly blocked, with an overall blockage efficiency of 19+/- 6% ($p < 0.05$, Table 2-3). Five test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Golden shiners

When the air curtain was coupled with the cyclic sound, golden shiners were significantly blocked, with an overall blockage efficiency of 89+/- 12% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Lake sturgeon

When the air curtain was coupled with the cyclic sound, lake sturgeon were significantly blocked, with an overall blockage efficiency of 84 +/- 13% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Rainbow trout

When the air curtain was coupled with the cyclic sound, Rainbow Trout were significantly blocked, with an overall blockage efficiency of 81+/- 13% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Largemouth bass

When the air curtain was coupled with the cyclic sound, largemouth bass were significantly blocked, with an overall blockage efficiency of 88+/- 16% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added compared to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Bluegill sunfish

When the air curtain was coupled with the cyclic sound, bluegill sunfish were significantly blocked, with an overall blockage efficiency of 83+/-10% ($p < 0.05$, Table 2-3). All eight test periods had significantly fewer passages than their preceding pre-test periods ($p < 0.05$, Figure 2-2B). There was no indication of habituation or sensitization to sound coupled with air ($p > 0.05$, Table 2-3). Comparisons showed that they were significantly more deterred when the air curtain was added than to sound alone ($p < 0.05$, Fig 2-2B; Table 2-3).

Which species are most influenced by the cyclic sound coupled with an air curtain and does the presence of hearing specializations have a role?

Silver carp (97 +/- 36%), were significantly more impacted than all other species to the cyclic sound coupled with the air curtain ($p < 0.05$, Table 2-3). Grass carp (95+/- 24%) and common carp (95 +/ -42%) were significantly more affected than channel catfish, golden shiners, lake sturgeon, rainbow trout, largemouth bass, and bluegill sunfish ($p < 0.05$, Table 2-3). Bighead carp (92 +/- 53%) were significantly more affected than channel catfish and rainbow trout ($p < 0.05$, Table 2-3). Golden shiners (89 +/- 36%), bluegill sunfish (83 +/- 30%), lake sturgeon (84 +/- 39%), rainbow trout (81 +/- 39%), and largemouth bass (88 +/- 48%), were significantly more impacted than channel catfish and significantly less impacted than common carp, grass carp, and silver carp ($p < 0.05$, Table 2-3). Channel catfish (19 +/- 18%) were significantly less affected than all species ($p < 0.05$, Table 2-3). Overall, the response to sound and air had a stronger correlation to the possession of hearing specializations than the responses observed from testing sound alone. Silver carp, grass carp, common carp, bighead carp, and golden shiners (all species with hearing specializations) were the five species most affected by the stimuli. However, channel catfish (a species with hearing specializations) were again the least impacted by the stimuli (Figure 2-3).

Discussion

The objective of this study was to determine whether a cyclic sound coupled with an air curtain was more effective deterrent for invasive carps versus native fishes than sound alone and why. We found that the sound coupled with air curtain was much stronger overall than sound alone, including for carp, but that it was also relatively nonspecific. While the cyclic sound alone strongly deterred bighead and common carp, it also was effective against many fishes without hearing specializations, and silver and grass carp were relatively unaffected. Coupling sound with air consistently increased the efficacy of the deterrent and most effectively blocked invasive carp. However, fishes without hearing specializations were still significantly impacted. While sound and air show great promise in deterring carp, there is no indication that it is sufficient as a species-specific deterrent.

Our most important finding is that a cyclic sound coupled with air is an extremely promising for deterring invasive carp and blocks them without any evidence of habituation. The four carp species were blocked by this set of stimuli to the greatest extent, followed by golden shiners, then the species without hearing specializations, and finally channel catfish. Carp responses in this study align with the results previously observed for bighead and common carp tested against sound coupled with air (approximately

95-99% of passages blocked) (Dennis et al. 2019). Sound mapping of sound coupled with air has demonstrated that sound pressure and particle motion have sharper gradients at higher frequencies compared to sound alone (Dennis et al. 2019). The steeper gradient of sound pressure and particle motion may be a factor that drove the response of fishes with hearing specializations. Notably, fishes without hearing specializations were significantly impacted, albeit to a lesser extent than sound alone. This may be due, in part, to the fact that our testing apparatus largely involves the nearfield region of sound. The nearfield refers to the region closest to the source of the sound, where sound pressure and particle motion are out of phase with each other. When particle motion and sound pressure are less than 40 degrees out of phase, this is known as the farfield (approximately two wavelengths away from the source of the sound) (Putland and Mensinger 2019). When approaching the source of the sound, particle motion increases at a faster rate compared to sound pressure (Siler 1968; Popper and Fay 1973). Because our testing apparatus involves solely the nearfield region of sound, and particle motion plays a larger role in sound localization in this region, this may have contributed to the responses seen by these fishes (Lu et al. 1996). A previous study demonstrated that bighead, common, and silver carp oriented away from an aversive sound stimulus in a manner that correlated with the axes of local particle motion (Zielinski and Sorensen 2017). Since all tested fish likely have the same capacity to detect particle motion, it is possible that fishes without hearing specializations were influenced by the sharper gradient of particle motion caused by the coupling of sound and air (Popper and Hawkins 2018; Dennis et al. 2019; Zielinski and Sorensen 2017).

Cyclic sound coupled with air did not deter fish species in a manner that correlated with the presence or absence of hearing specializations. While carp and golden shiners were the most impacted, channel catfish were impacted the least. It is unclear what, precisely, caused the variation in responses between species with hearing specializations. Channel catfish demonstrated an attraction to sound alone (-41% of passages blocked), which may have influenced their weaker response to sound coupled with air. Channel catfish are known to use sound production for social communication purposes, which also may have influenced their responses to sound alone and sound coupled with air (Fine et al. 1995). Ultimately, coupling sound with air is necessary to significantly deter invasive carp species. However, although these stimuli target carp to the greatest extent, there is little species-specificity compared to sound alone.

Another major finding in this study was that sound blocked silver and grass carp significantly less than bighead and common carp, and they habituated to the sound signal over time. Previous studies had hypothesized that sound had the potential of being a taxon-specific deterrent due to carp's hearing specializations, which would possibly make them more susceptible to sound compared to species that lacked such anatomical features (Zielinski and Sorensen 2015). The previous laboratory study on the cyclic sound supported this notion by demonstrating that largemouth bass were deterred significantly less than bighead and common carp (Dennis et al. 2019). It was unexpected that silver and grass carp would be minimally deterred by the sound signal, given its high efficacy in deterring the other two carp species. Due to the carp species all having a Weberian apparatus, the results of this study indicate that there is possibly a cognitive component involved in the avoidance response to the sound signal. Previous research has indicated that bighead and common carp discriminated between an outboard motor sound and the cyclic sound signal, indicating that there is a cognitive component to their aversion response, although it remains unclear what drives the variation in responses between species and sound signals (Dennis et al. 2019). One other possible explanation for the discrepancy between the bighead and common carp response versus silver and grass carp is the species' activity in the laboratory flume. Silver and grass carp consistently exhibited more activity in the flume compared to other species; their passage rate ranged between 30- 60 passages per 6-min on average, whereas other species typically had a passage rate of 20 passages or less (Table 2-S1). It is uncertain what drove silver and grass carp's higher level of activity, but their frequent approaches towards the stimuli may have influenced their responses.

Our study had some significant strengths and weaknesses. Among the strengths was the fact that we tested nearly 3000 naive fish under carefully controlled conditions; the laboratory flume was darkened, and the fish were almost always naive (barring seven groups of fish tested once more after several months of holding). The measures taken in this study to keep control variables constant ensure that any response seen by the tested fish is likely due to the presence of the stimuli, rather than an extraneous factor. However, the laboratory flume was not able to perfectly replicate conditions that will affect fishes' response in the field. Factors such as water depth, potential behavioral differences between hatchery-raised and free-range fish, and specific motivation to cross the barrier (feeding needs, reproduction, etc.) will need to be considered when this system is implemented.

Taken together, these results indicate that sound coupled with air is the most promising set of stimuli to deter all species of invasive carp. However, the implementation of a bio-acoustic fish fence system or BAFF system (the term given to the sound linked with air) may have a significant impact on the movement of fishes native to the Mississippi River, regardless of whether they have a hearing specialization or not. Field studies of these stimuli in front of lock chambers will be necessary to confirm these results. Another avenue that will need to be studied is the potential impact of strobe lights (a common component of the bio-acoustic fish fence) on native fishes.

Table 2-1: Species tested, their taxonomy, size, origin and hearing ability.

Fish Species	Family	Native or Exotic	Length (mean ± SD, n = 300) cm	Weight (mean ± SD, n = 300) g	Hearing Specialization (Yes or No)
Bighead carp (BC) (*Hypophthalmichthys nobilis*)	Cyprinidae	Exotic	142 ± 15	33.06 ± 8.48	Yes
Silver carp (SC) (*Hypophthalmichthys molitrix)*	Cyprinidae	Exotic	85 ± 11	5.58 ± 1.55	Yes
Common carp (CC) (*Cyprinus carpio*)	Cyprinidae	Exotic	110 ± 19	20.22 ± 10.59	Yes
Grass carp (GC) (*Ctenopharyngodon idella)*	Cyprinidae	Exotic	133 ± 18	24.34 ± 9.78	Yes
Golden shiners (GS) (*Notemigonus crysoleucas)*	Cyprinidae	Native	88 ± 10	6.52 ± 3.18	Yes
Channel catfish (CH) (*Ictalurus punctatus)*	Ictaluridae	Native	138 ± 19	23.62 ± 8.89	Yes
Largemouth bass (LB) (*Micropterus salmoides)*	Centarchidae	Native	124 ± 23	24.76 ± 13.54	No
Bluegill sunfish (BS) (*Lepomis macrochirus)*	Centrarchidae	Native	106 ± 15	20.75 ± 10.19	No
Rainbow trout (RT) (*Oncorhynchus mykiss)*	Salmonidae	Native	130 ± 13	24.50 ± 5.24	No
Lake sturgeon (LS) (*Acipenser fulvescens)*	Acipenseridae	Native	162 ± 18	12.75 ± 3.14	No

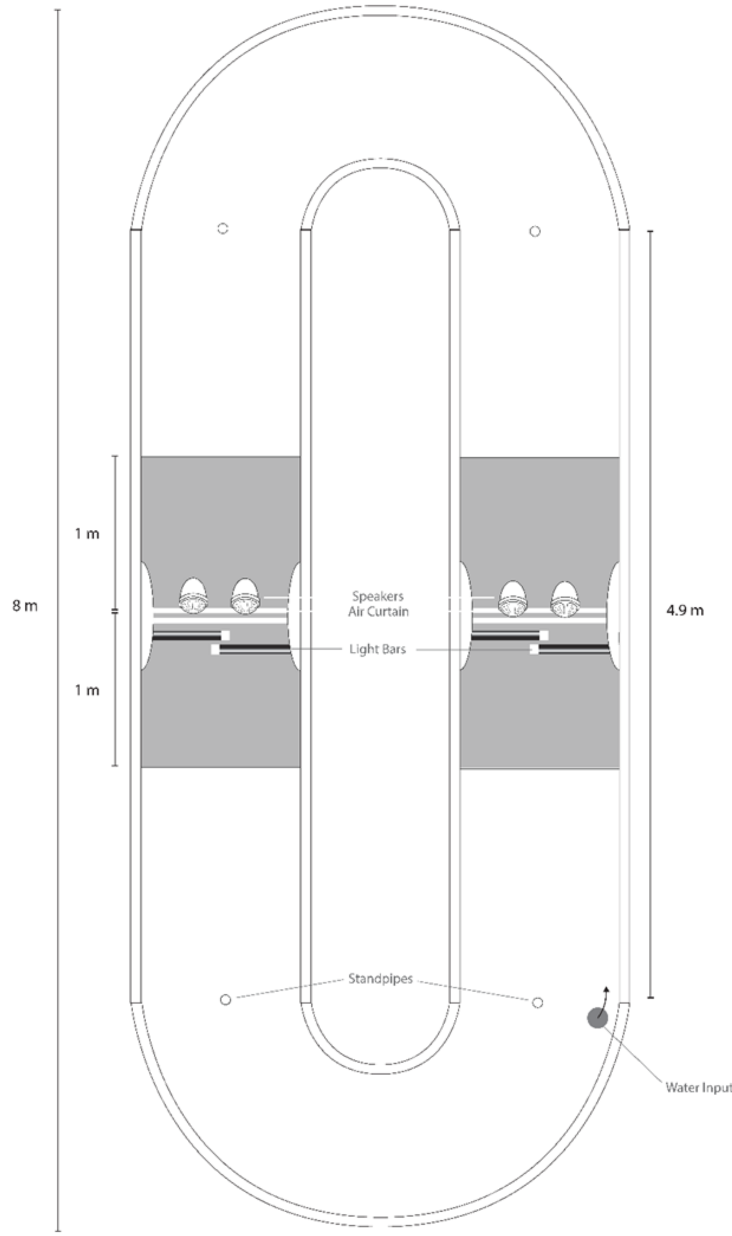
Table 2-2: Compilation of results obtained from each species tested with the cyclic sound. Average blockage efficiency (see methods), compared with other species and whether (or not) responses changed with time is noted. Species are ordered by relative blockage efficiency. Results between species were analyzed using z-tests that compared estimates of blockage efficiencies (comparisons are significant if $p < 0.05$, corrected for multiple comparisons).

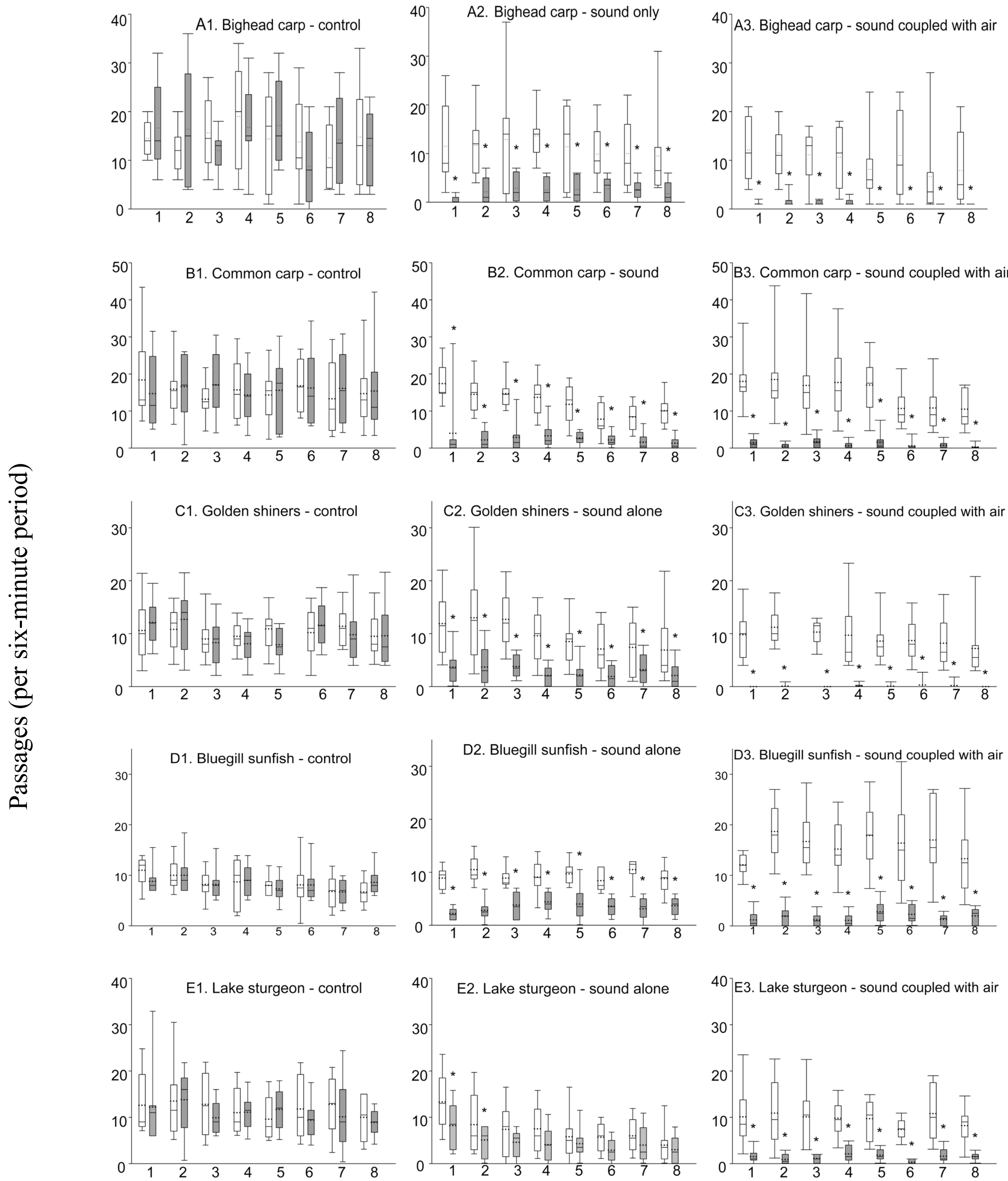
Species	Mean Sound Blockage Efficiency (± SD)	Significantly more affected than:	Significantly less affected than:	Habituation or sensitization?
Bighead carp (BC)	86 +/- 48%	GC, SC, CH, LS, RT, LB, BS	N/A	None measured
Common carp (CC)	83 +/- 27%	GC, SC, CH, LS, RT, LB, BS	N/A	None measured
Golden shiners (GS)	77 +/- 27%	GC, SC, CH, LS, RT, LB, BS	N/A	None measured
Bluegill sunfish (BS)	60 +/- 24%	GC, SC, CH, LB	BC, CC, GS	None measured
Lake sturgeon (LS)	47 +/- 24%	GC, SC, CH	BC, CC, GS	None measured
Rainbow trout (RT)	45 +/- 27%	GC, CH	BC, CC, GS	None measured
Largemouth bass (LB)	34 +/- 27%	CH	BC, CC, GS, BS	Habituation
Silver carp (SC)	31 +/- 12%	CH	BC, CC, GS, BS	Habituation
Grass carp (GC)	21 +/- 9%	CH	BC, CC, GS, LS, RT, LB, BS	Habituation
Channel catfish (CH)	-41 +/- 15%	N/A	BC, CC, GC, SC, GS, LS, RT, LB, BS	None measured

Table 2-3: Compilation of results obtained from the cyclic sound coupled with air experiments. Blockage efficiencies were obtained by comparing the average of pre-test periods' passage rates to test periods' passage rates. Results between species were analyzed using z-tests that compared estimates of blockage efficiencies (comparisons are significant if p < 0.05, corrected for multiple comparisons). All increases in blockage efficiency compared to sound alone were statistically significant (p < 0.05, corrected for multiple comparisons).

Species	Mean Blockage Efficiency (± SD) of Sound + Air	Significantly more affected than:	Significantly less affected than:	Habituation or Sensitization?	% increase blockage efficiency from sound alone?
Bighead carp (BC)	92+/-53%	CH, RT	SC	None measured	+6%
Common carp (CC)	95+/- 42%	CH, GS, LS, RT, LB, BS	SC	None measured	+12%
Golden shiners (GS)	89+/- 36%	CH	CC, GC, SC	None measured	+12%
Bluegill sunfish (BS)	83+/- 30%	CH	CC, GC, SC	None measured	+23%
Lake sturgeon (LS)	84+/- 39%	CH	CC, GC, SC	None measured	+37%
Rainbow trout (RT)	81+/- 39%	CH	CC, GC, SC	None measured	+36%
Largemouth bass (LB)	88+/- 48%	CH	CC, GC, SC	None measured	+54%
Silver carp (SC)	97+/- 36%	BC, CC, GS, BS, LS, RT, LB, GC, CH	N/A	None measured	+66%
Grass carp (GC)	95+/- 24%	CH, GS, LS, RT, LB, BS	SC	None measured	+74%
Channel catfish (CH)	19+/-18%	N/A	BC, CC, GS, BS, LS, RT, LB, SC, GC	None measured	+60%

Figure 2-1: Schematic of the laboratory flume from an overhead view. Two speakers and an air curtain are present at the middle of each of the flume's channels. Figure modified from Dennis et al. 2019 who also shows the sound fields




Passages (per six-minute period)
A1. Bighead carp - control
A2. Bighead carp - sound only
A3. Bighead carp - sound coupled with air
B1. Common carp - control
B2. Common carp - sound
B3. Common carp - sound coupled with air
C1. Golden shiners - control
C2. Golden shiners - sound alone
C3. Golden shiners - sound coupled with air
D1. Bluegill sunfish - control
D2. Bluegill sunfish - sound alone
D3. Bluegill sunfish - sound coupled with air
E1. Lake sturgeon - control
E2. Lake sturgeon - sound alone
E3. Lake sturgeon - sound coupled with air

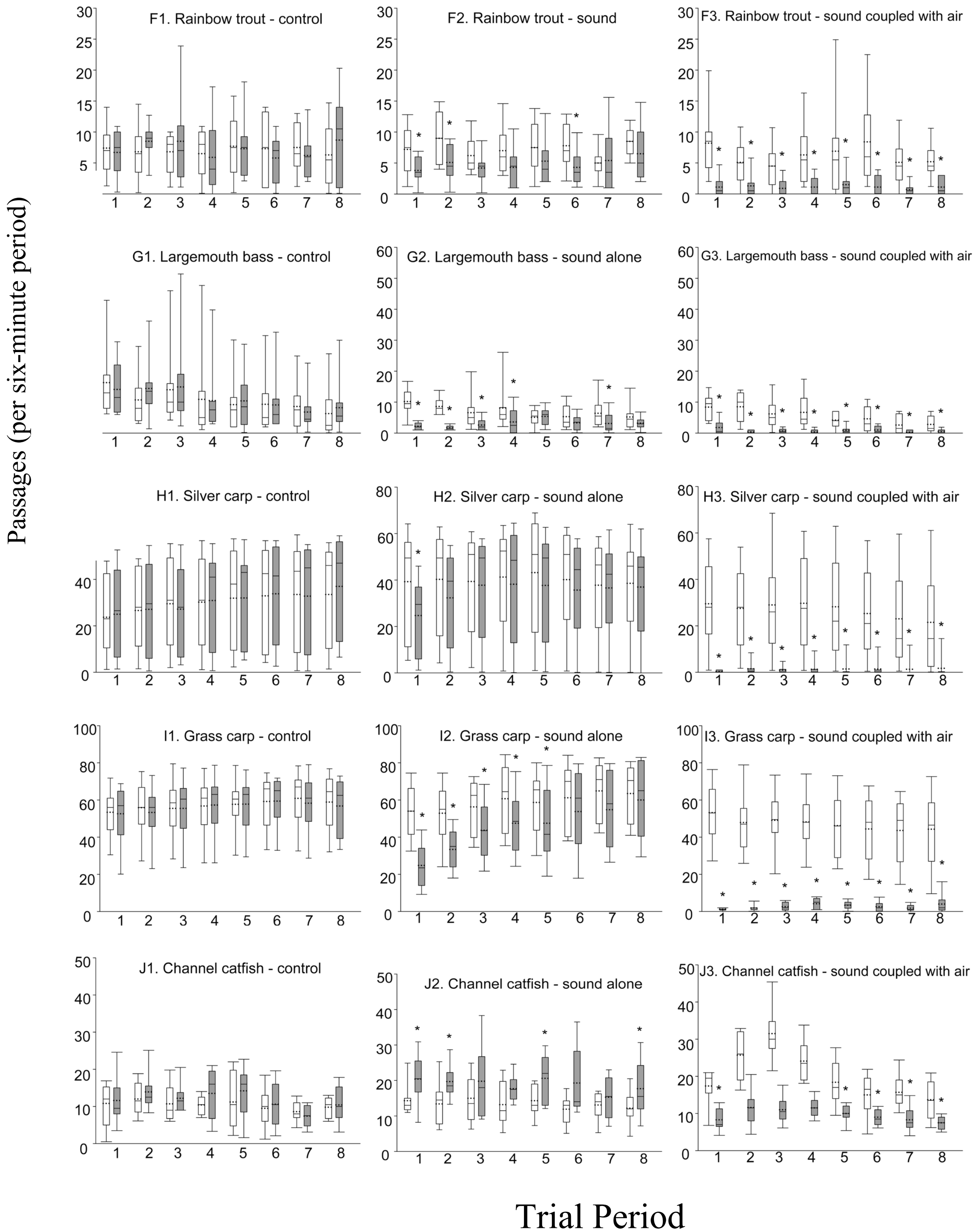
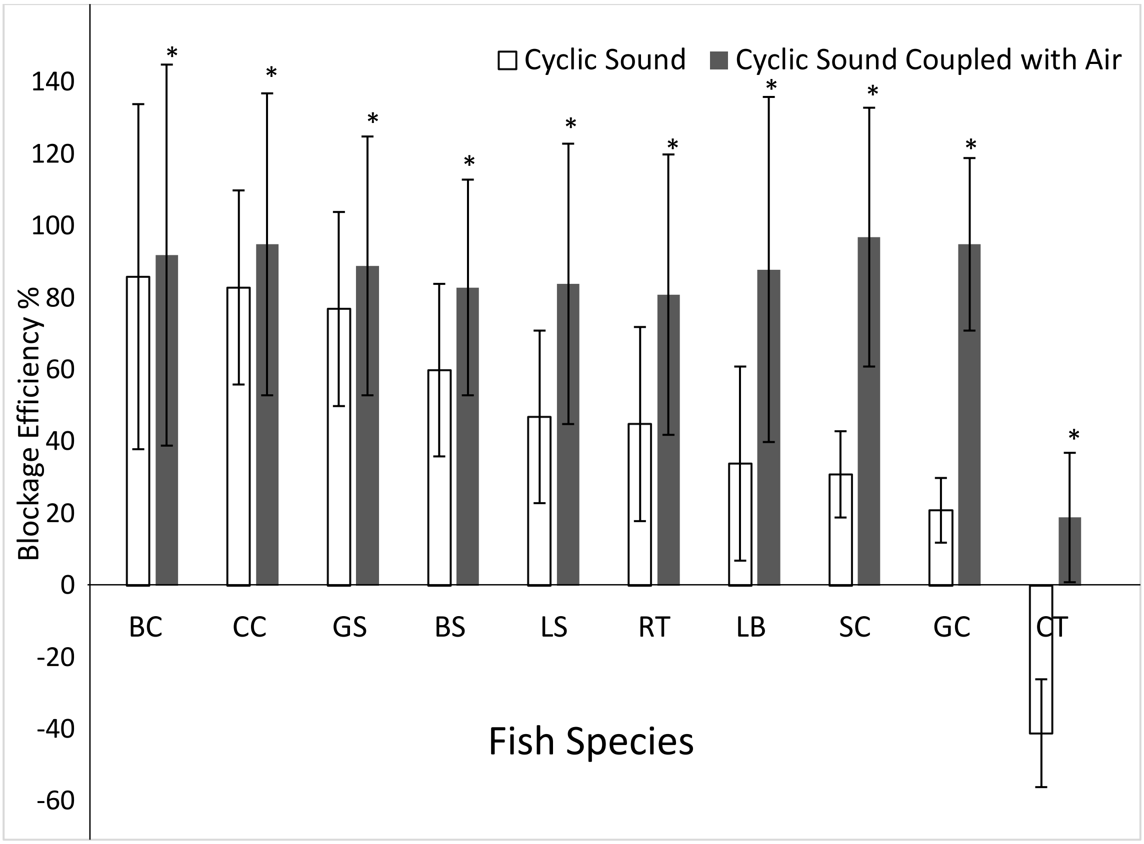

F1. Rainbow trout - control
F2. Rainbow trout - sound
F3. Rainbow trout - sound coupled with air
G1. Largemouth bass - control
G2. Largemouth bass - sound alone
G3. Largemouth bass - sound coupled with air
H1. Silver carp - control
H2. Silver carp - sound alone
H3. Silver carp - sound coupled with air
I1. Grass carp - control
I2. Grass carp - sound alone
I3. Grass carp - sound coupled with air
J1. Channel catfish - control
J2. Channel catfish - sound alone
J3. Channel catfish - sound coupled with air
Passages (per six-minute period)
Trial Period

Figure 2-2A: Box-and-whisker plots of passage rates for all experiments of five species (arranged from highest to lowest efficacy of sound alone). Y-axis shows passage rate over a six-minute period. White bars represent pre-test periods 1-8, gray bars represent test periods 1-8. Dotted lines within the bars represent passage rate mean, straight lines within the bars represent passage rate median. The x-axis represents the 8 periods. Asterisks are marked over test periods whose passage rates differ significantly from their preceding pre-test periods.

Figure 2-2B: Box-and-whisker plots of passage rates for all experiments of five species (arranged from the highest to lowest efficacy of sound alone, following the species represented in Figure 2-2A). Y-axis shows passage rate over a six-minute period. White bars represent pre-test periods 1-8, gray bars represent test periods 1-8. Dotted lines within the bars represent passage rate mean, straight lines within the bars represent passage rate median. The x-axis represents the 8 periods. Asterisks are marked over test periods whose passage rates differ significantly from their preceding pre-test periods.

Figure 2-3: Mean blockage efficiency of sound (white bars) and sound coupled with air (gray bars) with lines indicating standard deviation. Comparisons are marked with an asterisk if there is a significant difference ($p < 0.05$).



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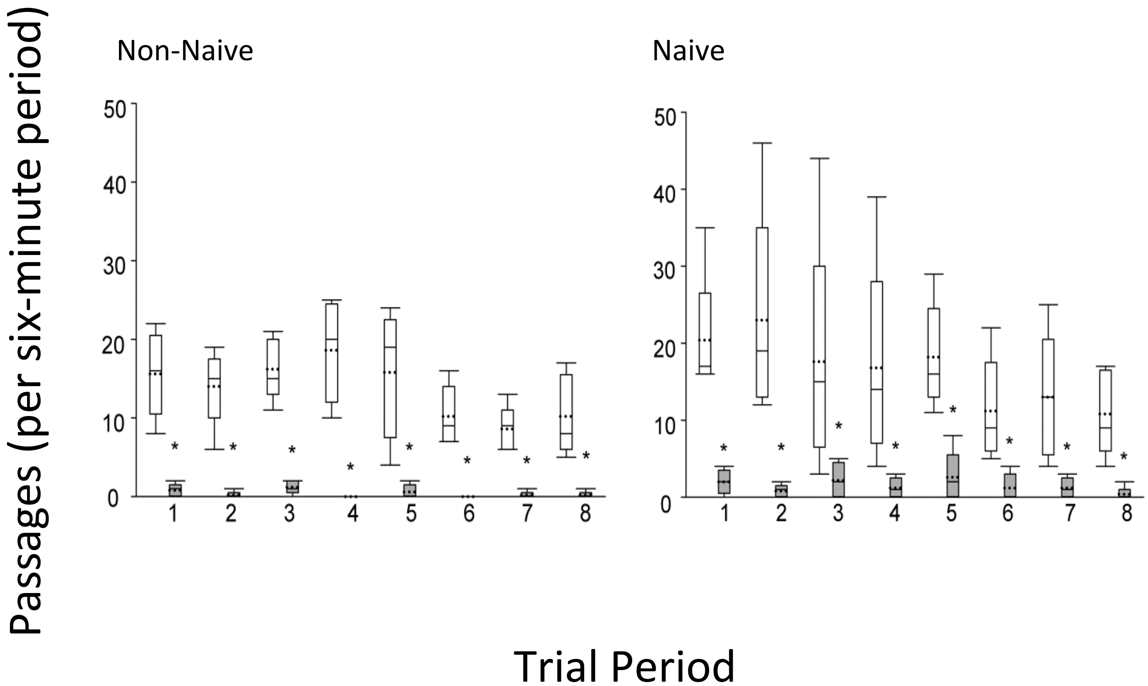
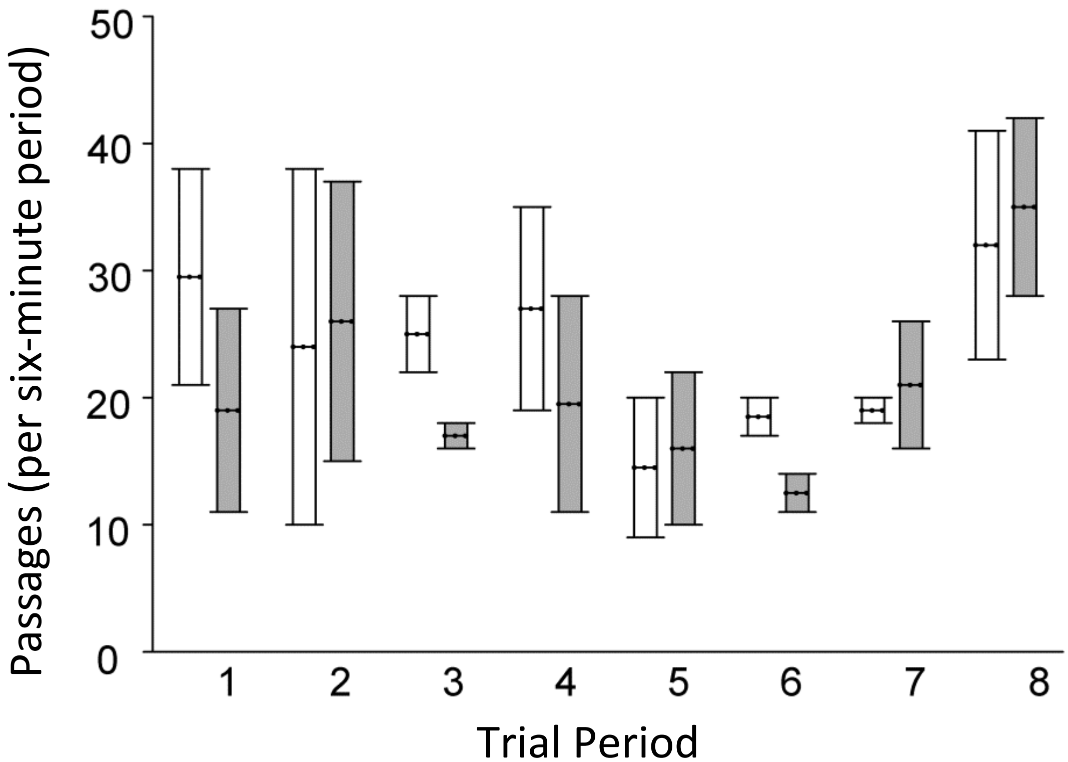
Appendix / Supplementary Data:

Figure 2-S1. Comparison between non-naïve common carp tested against sound and air (N = 5) versus naïve common carp tested against sound and air (N = 5). Non-naïve common carp were tested after five months of holding. For both sets of fish, exposure to sound and air resulted in significantly reduced passages during test periods (asterisks above the gray bars). Using a z-test to compare blockage efficiency estimates obtained from the GLMM indicate that naïve fish were not impacted to a significantly greater extent than non-naïve fish.



Species Status	Estimate	Pr (>|z|)	Z-test Comparison Estimate	Pr (>|z|)
Non-Naive	-1.966	<0.0001	1.926	0.058
Naive	-2.33	<0.0001		

Figure 2-S2. Supplementary silver carp trials against the cyclic sound signal (N = 2). When analyzed with our GLMM matrix, sound was not found to have a significant impact on their passage rates, unlike the group seen in Table 6. No test periods had significantly different passage rates than their pre-test periods. A z-test comparing the blockage efficiencies of these two groups indicated that they were significantly different ($p < 0.05$). The graph below is a box-and-whisker plot of the passage rates averaged across the two supplementary trials. White bars indicate pre-test passage rates, gray bars indicate test passage rates. The two supplementary trials used naïve silver carp procured from the same group used for the no-treatment control, sound, and sound coupled with air experiments.



Experiment	Estimate	Pr (>|z|)	Z-test Comparison Estimate	Pr (>|z|)
Silver Carp x Sound (N = 10)	-0.377	<0.0001	-9.628	<0.0001
Silver Carp x Sound (N = 2)	0.082	0.08224		

Table 2-S1: Basal passage rates for tested species. Passages were averaged over a six-minute period. Estimates of the linear change in passage rates were generated using the GLMM model. The estimate was log-transformed to determine the percent increase/decrease occurring over time. Arrows are used to indicate a significant increase (upwards arrow) or significant decrease (downwards arrow).

Fish Species	Average Passages (per 6 minutes)	Linear Estimate	% Increase/ Decrease	Pr (>|z|)
Bighead Carp	14 ± 8	-0.0265	2.62%	0.1280
Common Carp	16 ± 9	-0.0108	1.07%	0.3827
Grass Carp	57 ± 14	0.0165	1.66% ↑	0.0004
Silver Carp	30 ± 20	0.0497	5.10%↑	<0.0001
Channel Catfish	11 ± 5	-0.0416	4.07%↓	<0.0001
Golden Shiners	10 ± 5	-0.0115	1.14%	0.2809
Lake Sturgeon	11 ± 6	-0.03598	3.53%↓	0.0004
Rainbow Trout	7 ± 5	-0.0047	0.004%	0.0741
Largemouth Bass	11 ± 10	-0.1080	10.24%↓	<0.0001
Bluegill Sunfish	8 ± 3	-0.0471	4.60% ↓	<0.0001

Table 2-S2: Passage rates for tested species during pre-test periods of sound trials. Passages were averaged over a six-minute period. Estimates of the linear change in passage rates were generated using the GLMM model. The estimate was log-transformed to determine the percent increase/decrease occurring over time. Arrows are used to indicate a significant increase (upwards arrow) or significant decrease (downwards arrow).

Fish Species	Average Passages (per 6 minutes)	Linear Estimate	% Increase/ Decrease	Pr (>|z|)
Bighead Carp	11 ± 8	-0.0183	1.81%	0.1967
Common Carp	12 ± 5	-0.1022	9.72% ↑	<0.0001
Grass Carp	59 ± 16	0.0282	2.86% ↑	<0.0001
Silver Carp	40 ± 22	-0.0040	0.004%	0.4109
Channel Catfish	13 ± 5	-0.0219	2.17%	0.1027
Golden Shiners	11 ± 6	-0.1012	9.62%↓	<0.0001
Lake Sturgeon	8 ± 6	-0.1406	13.11%↓	<0.0001
Rainbow Trout	7 ± 4	-0.0121	1.20%	0.0200
Largemouth Bass	7 ± 5	-0.0871	8.34%↓	<0.0001
Bluegill Sunfish	9 ± 2	-0.0022	.002%	0.8884

Table 2-S3: Passage rates for tested species during test periods of sound trials. Passages were averaged over a six-minute period. Estimates of the linear change in passage rates were generated using the GLMM model. The estimate was log-transformed to determine the percent increase/decrease occurring over time. Arrows are used to indicate a significant increase (upwards arrow) or significant decrease (downwards arrow).

Fish Species	Average Passages (per 6 minutes)	Linear Estimate	% Increase/ Decrease	Pr (>|z|)
Bighead Carp	2 ± 2	0.0118	1.18%	0.2944
Common Carp	3 ± 4	-0.1249	11.74%	0.0117
Grass Carp	46 ± 20	0.1105	11.68% ↑	<0.0001
Silver Carp	35 ± 21	0.0389	3.97%	0.0260
Channel Catfish	19 ± 7	-0.0260	2.57%	0.0220
Golden Shiners	4 ± 3	-0.0800	7.69%↓	0.0056
Lake Sturgeon	5 ± 4	-0.1222	11.50%↓	<0.0001
Rainbow Trout	5 ± 4	0.0515	5.28%	0.0200
Largemouth Bass	3 ± 3	0.0803	8.36%	0.0090
Bluegill Sunfish	3 ± 2	0.0463	4.74%	0.1054

Table 2-S4: Passage rates for tested species during pre-test periods of sound coupled with air trials. Passages were averaged over a six-minute period. Estimates of the linear change in passage rates were generated using the GLMM model. The estimate was log-transformed to determine the percent increase/decrease occurring over time. Arrows are used to indicate a significant increase (upwards arrow) or significant decrease (downwards arrow).

Fish Species	Average Passages (per 6 minutes)	Linear Estimate	% Increase/ Decrease	Pr (>|z|)
Bighead Carp	10 ± 7	-0.0435	4.16%↓	0.0063
Common Carp	15 ± 8	-0.1249	8.95% ↓	<0.0001
Grass Carp	47 ± 16	-0.025	2.47% ↓	0.0004
Silver Carp	27 ± 20	-0.0425	4.16%	0.0292
Channel Catfish	20 ± 8	-0.0790	7.60%↓	<0.0001
Golden Shiners	10 ± 4	0.0135	1.36%	0.7757
Lake Sturgeon	10 ± 5	-0.2915	25.29%	0.0656
Rainbow Trout	6 ± 5	-0.0146	1.45%	0.4676
Largemouth Bass	6 ± 4	-0.1783	16.33%↓	<0.0001
Bluegill Sunfish	16 ± 6	.0035	.003%	0.7857

Table 2-S5: Passage rates for tested species during TEST periods of SAC trials. Passages were averaged over a six-minute period. Estimates of the linear change in passage rates were generated using the GLMM model. The estimate was log-transformed to determine the percent increase/decrease occurring over time. Arrows are used to indicate a significant increase (upwards arrow) or significant decrease (downwards arrow).

Fish Species	Average Passages (per 6 minutes)	Linear Estimate	% Increase/ Decrease	Pr (>|z|)
Bighead Carp	1 ± 0.6	-0.0505	4.92%	0.5628
Common Carp	0.9 ± 1	.1339	14.33%	0.1565
Grass Carp	3 ± 3	0.0946	0.99%	0.0072
Silver Carp	1 ± 3	0.1527	16.50%	0.1310
Channel Catfish	10 ± 3	-0.0372	3.65%	0.0256
Golden Shiners	1 ± 0.5	-0.0115	1.14%	0.2809
Lake Sturgeon	1 ± 1	-0.0140	1.39%	0.7593
Rainbow Trout	1 ± 2	-0.0260	2.57%	0.5887
Largemouth Bass	.75 ± 1	-0.1015	9.65%	0.1167
Bluegill Sunfish	2 ± 2	0.0543	5.58%	0.1692

Table 2-S6. Generalized linear mixed model (GLMM) results for Lake Sturgeon exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	**.234942**	**0.065215**	**3.58**		**0.0003**
Test Sound+AC vs No Treatment Control	**-1.84733**	**0.126411**	**-14.61**		**< 0.0001**
Test Sound vs No Treatment Control	**-0.62878**	**0.07617**	**-8.26**		**<0.0001**
Pre-Test Sound vs No Treatment Control	-0.15922	0.068695	-2.32		0.0205
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**-0.03598**	**0.010293**	**-3.50**		**0.0005**
Quadratic	0.014445	0.067968	0.21		0.8317
Cubic	-.026704	0.0670	-.40		0.6903
Quartic	-0.15247	0.65443	-2.33		0.0198
Quintic	.009120	0.065602	0.14		0.8894
Sextic	-0.75366	0.066783	-1.13		0.2591
Septic	0.013900	0.067619	0.21		0.8371
Sound + AC: Pre-Test					
Linear	-0.29153	.015836	-1.84		0.0656
Quadratic	-.002267	.104063	-.02		0.9826
Cubic	0.048993	0.104209	0.47		0.6383

Quartic	-0.12183	0.099120	-1.23	0.2190
Quintic	-0.14315	0.100707	-1.42	0.1552
Sextic	-0.20751	0.104514	-1.99	0.0471
Septic	-0.10785	0.102804	-1.05	0.2942
Sound: Pre-Test				
Linear	**-0.14056**	**0.019041**	**-7.38**	**< 0.0001**
Quadratic	0.1514	0.1243	1.22	0.0102
Cubic	-0.3182	0.1239	-2.57	0.0102
Quartic	-0.0048	0.1195	-0.04	0.9682
Quintic	-0.0810	0.1203	-0.67	0.5018
Sextic	-0.1364	0.1230	-1.11	0.2675
Septic	0.0457	0.1215	-5.26	0.7066
Sound: Test Passages				
Linear	**-0.1222**	**0.0232**	**-5.26**	**<0.0001**
Quadratic	0.2550	0.1530	1.67	0.0955
Cubic	-0.1331	0.1489	-0.08	0.9365
Quartic	-0.0119	0.1489	-0.08	0.9365
Quintic	-0.1581	0.1526	-1.04	0.3002
Sextic	-0.1983	0.1591	-1.25	0.2126
Septic	-0.0080	0.1564	-0.05	0.9595
Sound+AC Passage Rates				
Linear	-0.0140	0.0457	-0.31	0.7593
Quadratic	0.2099	0.2789	0.75	0.4516
Cubic	0.4113	0.3509	1.17	0.2411
Quartic	0.6540	0.2789	2.34	0.1903
Quintic	-0.8058	0.3339	-2.41	0.0158
Sextic	-1.0859	-0.3955	-2.75	0.0060
Septic	-0.4426	0.3059	-1.45	0.1479

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound				
Pre-Test vs Test Trial 1	**-0.5510**	**0.1376**	**-4.003**	**<0.0001**
Pre-Test vs Test Trial 2	**-0.4720**	**0.1670**	**-2.825**	**0.0047**
Pre-Test vs Test Trial 3	-0.4276	0.1914	-2.235	0.0254
Pre-Test vs Test Trial 4	-0.4754	0.1975	-2.536	0.0112
Pre-Test vs Test Trial 5	-0.5108	0.2022	-2.525	0.0116
Pre-Test vs Test Trial 6	-0.6267	0.2222	-2.820	0.0048
Pre-Test vs Test Trial 7	-0.454	0.2039	-1.989	0.0467
Pre-Test vs Test Trial 8	-0.2878	0.2412	-1.193	0.2327
Sound+AC				
Pre-Test vs Test Trial 1	**-2.1695**	**0.2758**	**-7.866**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.0797**	**0.1372**	**-7.8690**	**< 0.0001**
Pre-Test vs Test Trial 3	**-2.2432**	**0.3524**	**-6.365**	**< 0.0001**
Pre-Test vs Test Trial 4	**-1.7445**	**0.2528**	**-4.628**	**< 0.0001**
Pre-Test vs Test Trial 5	**-1.1919**	**0.2710**	**-4.399**	**< 0.0001**
Pre-Test vs Test Trial 6	**-2.8696**	**0.5934**	**-4.836**	**< 0.0001**
Pre-Test vs Test Trial 7	**-1.2296**	**0.2857**	**-4.304**	**<0.0001**
Pre-Test vs Test Trial 8	**-0.8889**	**0.3067**	**-2.898**	**0.0037**

Table 2-S7. Generalized linear mixed model (GLMM) results for Channel Catfish exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	**0.5064**	**0.0503**	**10.07**		**<0.0001**
Test Sound+AC vs No Treatment Control	**-0.2046**	**0.0565**	**-3.62**		**0.0003**
Test Sound vs No Treatment Control	**0.4654**	**0.0503**	**9.25**		**<0.0001**
Pre-Test Sound vs No Treatment Control	0.1295	0.0528	2.45		0.0143
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**-0.0416**	**0.0107**	**-3.89**		**<0.0001**
Quadratic	-0.1246	0.0672	-1.86		0.0636
Cubic	0.1506	0.0687	2.19		0.0283
Quartic	0.1538	0.0688	2.24		0.0253
Quintic	**0.1968**	**0.0689**	**2.86**		**0.0042**
Sextic	-0.5198	0.0680	-0.76		0.4448
Septic	-0.2659	0.0651	-0.41		0.6831
Sound + AC: Pre-Test					
Linear	**-0.0790**	**0.0119**	**-6.66**		**<0.0001**
Quadratic	**-0.3572**	**0.0762**	**-4.68**		**<0.0001**
Cubic	**0.4181**	**0.0744**	**5.62**		**<0.0001**
Quartic	-0.1332	0.0724	-1.84		0.6589

Quintic	-0.1498	0.0711	-2.10	0.0353
Sextic	0.0260	0.0703	0.37	0.7113
Septic	-0.0450	0.0700	-0.72	0.4726
Sound: Pre-Test				
Linear	-0.0219	0.0134	-1.63	0.1027
Quadratic	-0.0352	0.0865	-0.41	0.6841
Cubic	0.0235	0.0860	0.27	0.7865
Quartic	0.0226	0.0865	-0.82	0.4107
Quintic	-0.0712	0.0865	-0.82	0.4106
Sextic	-0.0135	0.0865	-0.16	0.8764
Septic	-0.1308	0.0857	-1.53	0.1271
Sound: Test Passages				
Linear	-0.0260	0.0114	-2.29	0.0220
Quadratic	-0.0309	0.0725	-0.43	0.6706
Cubic	-0.0004	0.0729	-0.01	0.9959
Quartic	0.0913	0.0745	1.23	0.2204
Quintic	0.1425	0.0740	1.93	0.0542
Sextic	0.0839	0.0726	1.16	0.2472
Septic	-0.0720	0.0723	-1.00	0.3191
Sound+AC Passage Rates				
Linear	-0.0372	0.0167	-2.23	0.0256
Quadratic	**-0.3105**	**0.1067**	**-2.91**	**0.0037**
Cubic	0.1716	0.1051	1.63	0.1025
Quartic	-0.0619	0.1021	-0.61	0.5442
Quintic	0.0317	0.1008	0.31	0.7531
Sextic	-0.0661	0.1001	-0.66	0.5089
Septic	0.0498	0.0978	0.51	0.6103

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound				
Pre-Test vs Test Trial 1	**0.3551**	**0.1090**	**3.26**	**0.0011**
Pre-Test vs Test Trial 2	**0.3856**	**0.1119**	**3.44**	**0.0006**
Pre-Test vs Test Trial 3	0.2777	0.1081	2.57	0.0103
Pre-Test vs Test Trial 4	0.2931	0.1150	2.55	0.0101
Pre-Test vs Test Trial 5	**0.3651**	**0.1088**	**3.36**	**0.0008**
Pre-Test vs Test Trial 6	0.4836	0.1165	4.15	**<0.0001**
Pre-Test vs Test Trial 7	0.1552	0.1190	1.30	0.1919
Pre-Test vs Test Trial 8	**0.3721**	**0.1176**	**-3.79**	**0.0002**
Sound+AC				
Pre-Test vs Test Trial 1	**-0.5387**	**0.1422**	**-3.79**	**0.0002**
Pre-Test vs Test Trial 2	-0.1387	0.1314	-1.06	0.2911
Pre-Test vs Test Trial 3	-0.3047	0.1301	-2.34	0.0192
Pre-Test vs Test Trial 4	-0.1327	0.1321	-1.00	0.3151
Pre-Test vs Test Trial 5	**-0.3521**	**0.1348**	**-2.61**	**0.0090**
Pre-Test vs Test Trial 6	**-0.2740**	**0.1438**	**-189**	**0.0018**
Pre-Test vs Test Trial 7	**-0.4510**	**0.1444**	**-3.12**	**0.0018**
Pre-Test vs Test Trial 8	**-0.4811**	**0.1501**	**-3.19**	**0.0014**

Table 2-S8. Generalized linear mixed model (GLMM) results for Grass Carp exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	**-0.1427**	**0.0325**	**-4.39**		**<0.0001**
Test Sound+AC vs No Treatment Control	**-3.0722**	**0.0787**	**-39.00**		**<0.0001**
Test Sound vs No Treatment Control	**-0.2374**	**0.0328**	**-7.24**		**<0.0001**
Pre-Test Sound vs No Treatment Control	0.0505	0.0315	1.60		0.1092
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**0.0165**	**0.0050**	**3.58**		**0.0004**
Quadratic	-0.030	0.0298	-1.02		0.3088
Cubic	-0.0126	0.0297	-0.43		0.6703
Quartic	-0.0088	0.0296	-0.30		0.7654
Quintic	0.0006	0.0296	0.02		0.9848
Sextic	-0.0002	0.0295	-0.01		0.9959
Septic	0.0078	0.0296	0.26		0.7924
Sound + AC: Pre-Test					
Linear	**-0.0250**	**0.0071**	**-3.52**		**0.0004**
Quadratic	0.0301	0.0457	0.67		0.5036
Cubic	0.0012	0.0461	0.03		0.9784
Quartic	0.0522	0.0464	1.13		0.2605

Quintic	-0.0313	0.0464	-0.67	0.4999
Sextic	0.0115	0.0463	0.25	0.8044
Septic	-0.0028	0.0461	-0.06	0.9545
Sound: Pre-Test				
Linear	**0.0282**	**0.0064**	**4.43**	**<0.0001**
Quadratic	-0.0158	0.0412	-0.38	0.7013
Cubic	-0.0229	0.0413	-0.55	0.5800
Quartic	0.0127	0.0413	0.31	0.7586
Quintic	-0.0567	0.0413	-1.37	0.1692
Sextic	-0.0095	0.0412	-0.23	0.8176
Septic	0.0283	0.0410	0.69	0.4909
Sound: Test Passages				
Linear	**0.1105**	**0.0079**	**13.98**	**<0.0001**
Quadratic	**-0.2505**	**0.0504**	**-4.97**	**<0.0001**
Cubic	**0.1387**	**0.0493**	**2.82**	**0.0005**
Quartic	-0.0128	0.0489	-0.26	0.7934
Quintic	-0.0357	0.0477	-0.75	0.4542
Sextic	0.0616	0.0463	1.33	0.1840
Septic	0.0305	0.0459	0.66	0.5069
Sound+AC Passage Rates				
Linear	0.0946	0.0352	2.69	0.0072
Quadratic	-0.5260	0.2104	-2.50	0.0124
Cubic	0.5744	0.2154	2.67	0.0077
Quartic	0.5315	0.2154	2.47	0.0136
Quintic	0.1391	0.2104	0.66	0.5084
Sextic	-0.0415	0.1982	-0.21	0.8340
Septic	0.1533	0.1745	0.88	0.3797

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound				
Pre-Test vs Test Trial 1	**-0.7781**	**0.0767**	**-10.15**	**<0.0001**
Pre-Test vs Test Trial 2	**-0.4599**	**0.0699**	**-6.58**	**<0.0001**
Pre-Test vs Test Trial 3	**-0.2533**	**0.0637**	**-3.97**	**<0.0001**
Pre-Test vs Test Trial 4	**-0.2453**	**0.0613**	**-4.00**	**<0.0001**
Pre-Test vs Test Trial 5	**-0.2117**	**0.0617**	**-3.43**	**0.0006**
Pre-Test vs Test Trial 6	-1.289	-0.0591	-2.18	0.0291
Pre-Test vs Test Trial 7	**-0.1691**	**0.0580**	**-2.92**	**0.0036**
Pre-Test vs Test Trial 8	-0.0567	0.0570	-1.00	0.3192
Sound+AC				
Pre-Test vs Test Trial 1	**-3.7750**	**0.2930**	**-12.89**	**<0.0001**
Pre-Test vs Test Trial 2	**-3.2947**	**0.2349**	**-14.03**	**<0.0001**
Pre-Test vs Test Trial 3	**-3.0433**	**0.2022**	**-15.05**	**<0.0001**
Pre-Test vs Test Trial 4	**-2.6391**	**0.1616**	**-16.33**	**<0.0001**
Pre-Test vs Test Trial 5	**-2.8169**	**0.1783**	**-15.80**	**<0.0001**
Pre-Test vs Test Trial 6	**-3.1270**	**0.2019**	**-15.49**	**<0.0001**
Pre-Test vs Test Trial 7	**-3.5531**	**0.2403**	**-14.79**	**<0.0001**
Pre-Test vs Test Trial 8	**-2.7330**	**0.1651**	**-16.56**	**<0.0001**

Table 2-S9. Generalized linear mixed model (GLMM) results for Rainbow Trout exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	0.0578	0.0887	0.652		0.5141
Test Sound+AC vs No Treatment Control	**-1.6707**	**0.1326**	**-12.596**		**<0.0001**
Test Sound vs No Treatment Control	**-0.5945**	**0.0869**	**-6.844**		**<0.0001**
Pre-Test Sound vs No Treatment Control	-0.1922	0.0817	-2.353		0.0186
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	-0.0047	0.0128	-0.367		0.7140
Quadratic	0.0613	0.0838	0.731		0.4645
Cubic	0.0924	0.0837	1.106		0.2686
Quartic	-0.0354	0.0837	-0.423		0.6726
Quintic	0.0683	0.0839	0.814		0.4157
Sextic	0.0125	0.0842	0.149		0.8817
Septic	-0.1551	0.0852	-1.821		0.0687
Sound + AC: Pre-Test					
Linear	-0.0146	0.0201	-0.726		0.4676
Quadratic	-0.0177	0.1252	-0.141		0.8877
Cubic	**-0.4819**	**0.1297**	**-3.715**		**0.0002**
Quartic	0.2801	0.1333	1.411		0.1583

Quintic	0.1880	0.1333	1.411	0.1583
Sextic	0.0794	0.1307	0.608	0.5435
Septic	0.1616	0.1254	1.289	0.1974
Sound: Pre-Test				
Linear	-0.0121	0.0181	-0.670	0.5030
Quadratic	0.0899	0.1153	0.780	0.4354
Cubic	0.1407	0.1182	1.190	0.2339
Quartic	0.1339	0.1208	1.109	0.2674
Quintic	**0.4198**	**0.1214**	**3.458**	**0.0005**
Sextic	0.0114	0.1204	0.094	0.9248
Septic	0.1141	0.1183	0.965	0.3345
Sound: Test Passages				
Linear	0.0515	0.0221	2.327	0.0200
Quadratic	0.1112	0.1453	0.770	0.4411
Cubic	0.1649	0.1465	1.126	0.2602
Quartic	-0.0483	0.1412	-0.342	0.7321
Quintic	0.1280	0.1438	0.890	0.3736
Sextic	-0.1821	0.1488	-1.225	0.2208
Septic	-0.1142	0.1469	-0.777	0.4370
Sound+AC Passage Rates				
Linear	-0.0260	0.0481	-0.541	0.5887
Quadratic	-0.0952	0.2970	-0.320	0.7486
Cubic	0.0468	0.3114	0.150	0.8806
Quartic	0.2860	0.3130	0.914	0.3609
Quintic	0.4773	0.3159	1.511	0.1309
Sextic	-0.1189	0.3129	-0.380	0.7040
Septic	-0.0394	0.2915	-0.135	0.8925

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound				
Pre-Test vs Test Trial 1	**-0.6391**	**0.2002**	**-3.192**	**0.0014**
Pre-Test vs Test Trial 2	**-0.5680**	**0.1750**	**-3.245**	**0.0012**
Pre-Test vs Test Trial 3	-0.3893	0.2000	-1.951	0.0510
Pre-Test vs Test Trial 4	-0.4875	0.1934	-2.520	0.0117
Pre-Test vs Test Trial 5	-0.3471	0.1792	-1.937	0.0527
Pre-Test vs Test Trial 6	**-0.5954**	**0.1896**	**-3.140**	**0.0017**
Pre-Test vs Test Trial 7	0.0771	0.1960	0.393	0.6942
Pre-Test vs Test Trial 8	-0.2683	0.1645	-1.631	0.1029
Sound+AC				
Pre-Test vs Test Trial 1	**-1.4605**	**0.3300**	**-4.426**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.5169**	**0.3037**	**-4.994**	**<0.0001**
Pre-Test vs Test Trial 3	**-1.5117**	**0.3623**	**-4.173**	**<0.0001**
Pre-Test vs Test Trial 4	**-1.4325**	**0.3306**	**-4.333**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.1913**	**0.2903**	**-4.104**	**<0.0001**
Pre-Test vs Test Trial 6	**-1.5405**	**0.3284**	**-4.691**	**<0.0001**
Pre-Test vs Test Trial 7	**-1.5478**	**0.4083**	**-3.791**	**0.0001**
Pre-Test vs Test Trial 8	**-1.6266**	**0.3268**	**-4.977**	**<0.0001**

Table 2-S10. Generalized linear mixed model (GLMM) results for Largemouth Bass exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	-0.0637	0.0781	-0.816		0.4145
Test Sound+AC vs No Treatment Control	**-2.1319**	**0.1607**	**-13.266**		**<0.0001**
Test Sound vs No Treatment Control	**-0.4168**	**0.0904**	**-4.611**		**<0.0001**
Pre-Test Sound vs No Treatment Control	**0.3990**	**0.0753**	**5.297**		**<0.0001**
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**-0.1080**	**0.0110**	**-9.838**		**<0.0001**
Quadratic	-0.0344	0.0740	-0.489		0.6251
Cubic	0.0421	0.0700	0.604		0.5460
Quartic	0.0281	0.0714	0.394		0.6935
Quintic	-0.0629	0.0703	-0.895		0.3709
Sextic	0.1600	0.0684	2.334		0.0200
Septic	-0.6362	0.0691	-0.920		0.3573
Sound + AC: Pre-Test					
Linear	**-0.1783**	**0.0237**	**-7.534**		**<0.0001**
Quadratic	-0.1186	0.1480	-0.802		0.4226
Cubic	0.1060	0.1478	0.717		0.4731
Quartic	0.0743	0.1520	0.489		0.6249

Quintic	0.1600	0.1488	1.075		0.2824
Sextic	0.0707	0.1419	0.499		0.6181
Septic	0.2948	0.1391	2.120		0.3399
Sound: Pre-Test					
Linear	**-0.0871**	**0.0186**	**-4.689**		**<0.0001**
Quadratic	0.1956	0.1231	1.589		0.1120
Cubic	-0.0194	0.1238	-0.157		0.8753
Quartic	-0.0597	0.1201	-0.497		0.6191
Quintic	-0.1838	0.1224	-1.501		0.1333
Sextic	-0.1651	0.1269	-1.301		0.1932
Septic	0.2216	0.1269	1.746	-	0.0809
Sound: Test Passages					
Linear	0.0803	0.0308	2.612		0.0090
Quadratic	-0.4600	0.1827	-2.518		0.0118
Cubic	-0.2010	0.1925	-1.044		0.2964
Quartic	0.4961	0.1950	2.543		0.0110
Quintic	-0.0227	0.1937	-0.117		0.9068
Sextic	-0.1139	0.1851	-0.615		0.5385
Septic	-0.2222	0.1630	-1.363		0.1730
Sound+AC Passage Rates					
Linear	-0.1015	0.0647	-1.569		0.1167
Quadratic	0.2562	0.3796	0.675		0.4997
Cubic	-0.7667	0.3992	-1.921		0.0548
Quartic	0.7534	0.4800	1.570		0.1165
Quintic	0.3400	0.4641	0.732		0.4641
Sextic	0.7628	0.4052	1.882		0.0600
Septic	-0.3440	0.4100	-0.840		0.4010

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound				
Pre-Test vs Test Trial 1	**-1.5341**	**0.2346**	**-6.538**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.6212**	**0.2649**	**-6.119**	**<0.0001**
Pre-Test vs Test Trial 3	**-0.9318**	**0.2311**	**-4.032**	**<0.0001**
Pre-Test vs Test Trial 4	**-0.8112**	**0.1999**	**-4.057**	**<0.0001**
Pre-Test vs Test Trial 5	0.0573	0.1949	0.294	0.7687
Pre-Test vs Test Trial 6	-0.4736	0.2214	-2.140	0.0324
Pre-Test vs Test Trial 7	**-0.7247**	**0.2184**	**-3.318**	**0.0009**
Pre-Test vs Test Trial 8	-0.4852	0.2243	-2.164	0.0305
Sound+AC				
Pre-Test vs Test Trial 1	**-1.9078**	**0.2626**	**-7.266**	**<0.0001**
Pre-Test vs Test Trial 2	**-3.2420**	**0.5142**	**-6.304**	**<0.0001**
Pre-Test vs Test Trial 3	**-2.4172**	**0.4015**	**-6.021**	**<0.0001**
Pre-Test vs Test Trial 4	**-3.1818**	**0.5149**	**-6.180**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.9082**	**0.3662**	**-5.211**	**<0.0001**
Pre-Test vs Test Trial 6	**-1.8401**	**0.3500**	**-5.265**	**<0.0001**
Pre-Test vs Test Trial 7	**-3.2334**	**0.5927**	**-5.455**	**<0.0001**
Pre-Test vs Test Trial 8	**-2.5147**	**0.4713**	**-5.336**	**<0.0001**

Table 2-S11. Generalized linear mixed model (GLMM) results for Bluegill Sunfish exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	**0.4754**	**0.0605**	**7.86**		**<0.0001**
Test Sound+AC vs No Treatment Control	**-1.7923**	**0.1045**	**-17.16**		**<0.0001**
Test Sound vs No Treatment Control	**-0.9151**	**0.0800**	**-11.50**		**<0.0001**
Pre-Test Sound vs No Treatment Control	0.0995	0.0624	1.59		0.1112
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**-0.0471**	**0.01190**	**-3.95**		**<0.0001**
Quadratic	0.0650	0.0770	0.84		0.4000
Cubic	0.0421	0.0780	0.54		0.5873
Quartic	0.0356	0.0781	0.45		0.6943
Quintic	0.1036	0.0783	1.32		0.1860
Sextic	-0.0028	0.0783	-0.04		0.9717
Septic	0.1252	0.0781	1.60		0.1088
Sound + AC: Pre-Test					
Linear	0.0035	0.0129	0.27		0.7857
Quadratic	**-0.2731**	**0.0844**	**-3.24**		**0.0012**
Cubic	0.0477	0.0815	0.59		0.5583
Quartic	**-0.2304**	**0.0781**	**-2.95**		**0.0032**

Quintic	0.1070	0.0769	1.39	0.1644
Sextic	-0.0766	0.0773	-0.99	0.3219
Septic	-0.0912	0.0778	-1.17	0.2411
Sound: Pre-Test				
Linear	-0.0022	0.0161	-0.14	0.8884
Quadratic	-0.0090	0.1053	-0.09	0.9321
Cubic	0.0082	0.1049	0.08	0.9373
Quartic	-0.1301	0.1007	-1.29	0.1964
Quintic	-0.0022	0.1018	-0.02	0.9825
Sextic	-0.1678	0.1048	-1.60	0.1094
Septic	-0.0597	0.1041	-0.57	0.5704
Sound: Test Passages				
Linear	0.0463	0.0286	1.62	0.1054
Quadratic	-0.4126	0.17774	-2.33	0.0200
Cubic	0.2335	0.1775	1.32	0.1882
Quartic	0.1832	0.1773	1.03	0.3013
Quintic	-0.0225	0.1735	-0.13	0.8970
Sextic	0.0305	0.1667	0.18	0.8548
Septic	0.0343	0.1580	0.22	0.8281
Sound+AC Passage Rates				
Linear	0.0543	0.0395	1.37	0.1692
Quadratic	-0.1263	0.2553	-0.49	0.6208
Cubic	-0.1159	0.2545	-0.46	0.6488
Quartic	0.0586	0.2541	0.23	0.8178
Quintic	**0.7999**	**0.2532**	**3.16**	**0.0016**
Sextic	-0.0082	0.2521	-0.03	0.9741
Septic	-0.2709	0.2516	-1.08	0.2815

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? ($\alpha = 0.05/8 = 0.0063$)

Sound				
Pre-Test vs Test Trial 1	**-1.3975**	**0.2379**	**-5.87**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.322**	**0.2125**	**-6.22**	**<0.0001**
Pre-Test vs Test Trial 3	**-0.8512**	**0.1936**	**-4.40**	**<0.0001**
Pre-Test vs Test Trial 4	**-0.7267**	**0.1935**	**-3.96**	**<0.0001**
Pre-Test vs Test Trial 5	**-0.8858**	**0.1877**	**-4.72**	**<0.0001**
Pre-Test vs Test Trial 6	**-0.8473**	**0.1990**	**-4.26**	**<0.0001**
Pre-Test vs Test Trial 7	**-1.2000**	**0.2042**	**-5.97**	**<0.0001**
Pre-Test vs Test Trial 8	**-0.8664**	**0.1958**	**-4.43**	**<0.0001**
Sound+AC				
Pre-Test vs Test Trial 1	**-2.153**	**0.3101**	**-6.93**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.859**	**0.2531**	**-7.34**	**<0.0001**
Pre-Test vs Test Trial 3	**-2.153**	**0.3105**	**-6.93**	**<0.0001**
Pre-Test vs Test Trial 4	**-2.2623**	**0.3221**	**-7.02**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.3919**	**0.2190**	**-6.36**	**<0.0001**
Pre-Test vs Test Trial 6	**-1.4448**	**0.2394**	**-6.04**	**<0.0001**
Pre-Test vs Test Trial 7	**-2.2385**	**0.2972**	**-7.53**	**<0.0001**
Pre-Test vs Test Trial 8	**-1.6311**	**0.2515**	**-6.48**	**<0.0001**

Table 2-S12. Generalized linear mixed model (GLMM) results for Golden Shiners exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	**-0.2367**	**0.0425**	**-5.57**		**<0.0001**
Test Sound+AC vs No Treatment Control	**-2.2434**	**0.1188**	**-17.08**		**<0.0001**
Test Sound vs No Treatment Control	**-1.4754**	**0.0864**	**-17.08**		**<0.0001**
Pre-Test Sound vs No Treatment Control	**-0.2442**	**0.0639**	**-3.82**		**0.0001**
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	-0.0115	0.0106	-1.08		0.2809
Quadratic	0.1473	0.0711	2.07		0.0382
Cubic	-0.1545	0.0702	-2.20		0.0278
Quartic	-0.1539	0.0694	-2.22		0.0266
Quintic	0.1301	0.0699	1.86		0.0627
Sextic	-0.0368	0.0714	-0.52		0.6061
Septic	0.0528	0.0158	0.72		0.4714
Sound + AC: Pre-Test					
Linear	-0.0396	0.0158	-2.51		0.0122
Quadratic	-0.1373	0.1027	-1.34		0.1813
Cubic	0.0953	0.1007	0.95		0.3439
Quartic	-0.1217	0.0993	-1.23		0.2202

Quintic	0.0171	0.0983	0.17	0.8616
Sextic	-0.0009	0.0979	-0.01	0.9926
Septic	0.0374	0.0988	0.38	0.7048
Sound: Pre-Test				
Linear	**-0.1012**	**0.0163**	**-6.22**	**<0.0001**
Quadratic	-0.0071	0.1061	-0.07	0.9470
Cubic	0.2116	0.1052	2.01	0.0443
Quartic	-0.0965	0.1042	-0.93	0.3543
Quintic	-0.0549	0.1040	-0.53	0.5975
Sextic	0.0050	0.1044	0.05	0.9617
Septic	-0.0778	0.1050	-0.74	0.4591
Sound: Test Passages				
Linear	**-0.0800**	**0.0289**	**-2.77**	**0.0056**
Quadratic	0.2500	0.2003	1.25	0.2119
Cubic	0.0907	0.1927	0.47	0.6379
Quartic	-0.4002	0.1877	-2.13	0.0330
Quintic	-0.2506	0.1891	-1.33	0.1850
Sextic	-0.0103	0.1969	-0.05	0.9583
Septic	-0.2686	0.2100	-1.28	0.2011
Sound+AC Passage Rates				
Linear	0.0135	0.0474	0.28	0.7757
Quadratic	-0.1464	0.3075	-0.48	0.6334
Cubic	-0.1238	0.3036	-0.41	0.6835
Quartic	-0.0765	0.2983	-0.26	0.7976
Quintic	0.0247	0.2964	0.08	0.9336
Sextic	-0.0256	0.2963	-0.09	0.9312
Septic	0.1356	0.2954	0.46	0.6460

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound				
Pre-Test vs Test Trial 1	**-1.1681**	**0.1880**	**-6.21**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.2566**	**0.1861**	**-6.75**	**<0.0001**
Pre-Test vs Test Trial 3	**-1.2067**	**0.1847**	**-6.53**	**<0.0001**
Pre-Test vs Test Trial 4	**-1.5200**	**0.2406**	**-6.32**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.3516**	**0.2389**	**-5.66**	**<0.0001**
Pre-Test vs Test Trial 6	**-1.3179**	**0.2580**	**-5.11**	**<0.0001**
Pre-Test vs Test Trial 7	**-0.8382**	**0.2113**	**-3.97**	**<0.0001**
Pre-Test vs Test Trial 8	**-1.1894**	**0.2489**	**-4.78**	**<0.0001**
Sound+AC				
Pre-Test vs Test Trial 1	**-2.3431**	**0.3319**	**-7.06**	**<0.0001**
Pre-Test vs Test Trial 2	**-2.3365**	**0.3169**	**-7.37**	**<0.0001**
Pre-Test vs Test Trial 3	**-2.4083**	**0.3311**	**-7.27**	**<0.0001**
Pre-Test vs Test Trial 4	**-1.9461**	**0.3091**	**-6.30**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.9114**	**0.3232**	**-5.91**	**<0.0001**
Pre-Test vs Test Trial 6	**-1.5639**	**0.3047**	**-5.13**	**<0.0001**
Pre-Test vs Test Trial 7	**-1.6858**	**0.3141**	**-5.37**	**<0.0001**
Pre-Test vs Test Trial 8	**-1.7982**	**0.3409**	**-5.27**	**<0.0001**

Table 2-S13. Generalized linear mixed model (GLMM) results for Common Carp exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	0.01657	0.05406	0.31	.21651	.82859
Test Sound+AC vs No Treatment Control	**-2.90613**	**0.14418**	**-20.15**	**-14.07337**	**< 0.0001**
Test Sound vs No Treatment Control	**-1.75450**	**0.08743**	**-20.07**	**-14.01750**	**<0.0001**
Pre-Test Sound vs No Treatment Control	-0.14807	0.05626	-2.63	-1.83687	0.0663
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	-0.01088	0.00871	-1.25	-0.87304	0.3827
Quadratic	0.03644	0.05672	0.64	0.44700	0.6550
Cubic	-0.04624	0.05648	-0.82	-0.57271	0.5673
Quartic	-0.01661	0.05699	-0.29	-0.20254	0.8400
Quintic	0.06508	0.05686	1.14	0.79621	0.4259
Sextic	0.04718	0.05664	0.83	0.57970	0.5622
Septic	0.04295	0.05733	0.75	0.52382	0.6004
Sound + AC: Pre-Test					
Linear	**-0.09376**	**0.01332**	**-7.04**	**-4.91695**	**<0.0001**
Quadratic	-0.16488	0.08370	-1.97	-1.37591	0.1691
Cubic	0.13778	0.08601	1.60	1.11749	0.2638
Quartic	0.14486	0.08336	1.74	1.21527	0.2243

Quintic	0.00493	0.08417	0.06	0.04191	0.9666
Sextic	-0.18468	0.08489	-2.18	-1.52258	0.1280
Septic	-0.08462	0.07924	-1.07	-0.74732	0.4551
Sound: Pre-Test					
Linear	**-0.10224**	**0.01422**	**-7.19**	**-5.02171**	**< 0.0001**
Quadratic	0.10276	0.08977	1.14	0.79621	0.4259
Cubic	0.23425	0.09401	2.49	1.73910	0.0820
Quartic	0.21338	0.09352	2.28	1.59242	0.1130
Quintic	-0.09124	0.09544	-0.96	-0.67050	0.5029
Sextic	-0.09803	0.09662	-1.01	-0.70541	0.4808
Septic	-0.08202	0.09169	-0.89	-0.62160	0.5346
Sound: Test Passages					
Linear	-0.12487	0.03464	-3.61	-2.52133	0.0117
Quadratic	-0.28519	0.21115	-1.35	-0.94288	0.3462
Cubic	-0.23016	0.21489	-1.07	-0.74732	0.4551
Quartic	0.37492	0.21829	1.72	1.20130	0.0859
Quintic	-0.17654	0.21414	-0.82	-0.57271	0.5673
Sextic	0.06763	0.20354	0.33	0.23048	0.8178
Septic	0.03971	0.18922	0.21	0.14667	0.8834
Sound+AC Passage Rates					
Linear	0.13386	0.06586	-2.03	-1.41781	0.1565
Quadratic	-0.53832	0.40737	-1.32	-0.92193	0.3571
Cubic	-0.49960	0.39617	-1.26	-0.88002	0.3789
Quartic	0.28801	0.39922	0.72	0.50287	0.6151
Quintic	-0.46032	0.38117	-1.21	-0.84510	0.3981
Sextic	0.29321	0.35305	0.83	0.57970	0.5622
Septic	-1.02580	0.33995	-3.02	-2.10926	0.0350

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound					
Pre-Test vs Test Trial 1	**-1.47010**	**0.17517**	**-8.39**	**-5.85983**	**<0.0001**
Pre-Test vs Test Trial 2	**-1.88607**	**0.22860**	**-8.25**	**-5.76205**	**<0.0001**
Pre-Test vs Test Trial 3	**-1.62308**	**0.20298**	**-8.00**	**-5.58744**	**<0.0001**
Pre-Test vs Test Trial 4	**-1.42335**	**0.19371**	**-7.35**	**-5.13346**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.47441**	**0.21301**	**-6.92**	**-4.83314**	**<0.0001**
Pre-Test vs Test Trial 6	**-1.26610**	**0.24116**	**-5.25**	**-3.66680**	**0.0003**
Pre-Test vs Test Trial 7	**-1.65822**	**0.27251**	**-6.09**	**-4.25344**	**<0.0001**
Pre-Test vs Test Trial 8	**-2.05088**	**0.29442**	**-6.97**	**-4.86810**	**<0.0001**
Sound+AC					
Pre-Test vs Test Trial 1	**-2.56067**	**0.28060**	**-9.13**	**-6.37667**	**<0.0001**
Pre-Test vs Test Trial 2	**-3.40840**	**0.45632**	**-7.47**	**-5.21727**	**< 0.0001**
Pre-Test vs Test Trial 3	**-2.19806**	**0.25927**	**-8.48**	**-5.92269**	**< 0.0001**
Pre-Test vs Test Trial 4	**-3.16950**	**0.41883**	**-7.57**	**-5.28711**	**< 0.0001**
Pre-Test vs Test Trial 5	**-2.03878**	**0.26936**	**-7.57**	**-5.28711**	**< 0.0001**
Pre-Test vs Test Trial 6	**-2.60590**	**0.42522**	**-6.13**	**-4.28137**	**<0.0001**
Pre-Test vs Test Trial 7	**-2.52587**	**0.39527**	**-6.39**	**-4.46297**	**<0.0001**
Pre-Test vs Test Trial 8	**-3.55778**	**0.58679**	**-6.06**	**-4.23250**	**<0.0001**

Table 2-S14. Generalized linear mixed model (GLMM) results for Bighead Carp exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	-0.3750	0.1619	-2.315	-1.3701	0.1707
Test Sound+AC vs No Treatment Control	**-2.5149**	**0.1956**	**-12.857**	**-7.6077**	**<0.0001**
Test Sound vs No Treatment Control	**-1.9864**	**0.1817**	**-10.929**	**-6.4669**	**<0.0001**
Pre-Test Sound vs No Treatment Control	-0.3116	0.1617	-1.927	-1.1402	0.2543
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**0.0497**	**0.0064**	**7.798**	**4.8738**	**<0.0001**
Quadratic	-0.0422	0.0647	-0.652	-0.3858	0.7002
Cubic	0.1044	0.0678	1.540	0.9112	0.3622
Quartic	0.2443	0.0663	3.687	2.1816	0.0291
Quintic	-0.0652	0.0676	-0.964	-0.5680	0.5700
Sextic	-0.2002	0.0687	-2.915	-1.7249	0.0847
Septic	0.0164	0.0639	0.2570	1.5207	0.1283
Sound + AC: Pre-Test					
Linear	**-0.0425**	**0.0097**	**-4.373**	**-2.7284**	**0.0063**
Quadratic	0.0220	0.1127	0.1960	0.1160	0.9077
Cubic	-0.0081	0.1120	-0.0720	-0.0415	0.9669

Quartic	0.0820	0.1183	0.693	0.4101	0.6817
Quintic	0.1005	0.1162	0.865	0.5118	0.6088
Sextic	0.2446	0.1112	2.200	1.3018	0.1930
Septic	0.2129	0.1139	1.870	1.1065	0.2685
Sound: Pre-Test					
Linear	-0.0183	0.0167	-2.183	-1.2917	0.1967
Quadratic	-0.1652	0.1063	-1.554	-0.9195	0.3578
Cubic	0.1116	0.1070	1.043	0.6172	0.5371
Quartic	0.0759	0.1060	0.717	0.4243	0.6713
Quintic	-0.1090	0.1057	-1.031	-0.6101	0.5418
Sextic	-0.0427	0.1050	-0.407	-0.2408	0.8097
Septic	0.0275	0.1018	0.270	0.1598	0.8730
Sound: Test Passages					
Linear	0.0118	0.0210	0.5630	0.3519	0.7248
Quadratic	-0.9249	0.2984	-3.100	-1.8343	0.0667
Cubic	0.3102	0.2717	1.142	0.6757	0.4992
Quartic	-0.3913	0.2491	-1.571	0.9296	0.3526
Quintic	0.0224	0.2297	0.098	0.0580	0.9537
Sextic	0.0541	0.2176	0.249	0.1473	0.8829
Septic	-0.0559	0.2184	-0.256	-0.1515	0.8796
Sound+AC Passage Rates					
Linear	-0.0505	0.0516	-0.978	-0.5787	0.5628
Quadratic	-0.0734	0.3373	-0.218	-0.1290	0.8974
Cubic	0.2536	0.3340	0.759	0.4491	0.6177
Quartic	-0.1325	0.3246	-0.408	-0.2414	0.8092
Quintic	0.0380	0.3252	0.117	0.0692	0.9448
Sextic	-0.1165	0.3302	-0.353	-0.2088	0.8346
Septic	0.0166	0.3293	0.505	0.2988	0.7651

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound					
Pre-Test vs Test Trial 1	**-2.7296**	**0.4204**	**-6.491**	**-3.8402**	**0.0005**
Pre-Test vs Test Trial 2	**-1.6992**	**0.2633**	**-6.454**	**-3.8189**	**0.0001**
Pre-Test vs Test Trial 3	**-1.4990**	**0.2302**	**-6.512**	**-3.8533**	**0.0001**
Pre-Test vs Test Trial 4	**-1.6957**	**0.2428**	**-6.984**	**-4.1325**	**<0.0001**
Pre-Test vs Test Trial 5	**-1.4663**	**0.2416**	**-6.058**	**-3.5846**	**0.0003**
Pre-Test vs Test Trial 6	**-1.2786**	**0.2406**	**-5.313**	**-3.1438**	**0.0016**
Pre-Test vs Test Trial 7	**-1.3373**	**0.2447**	**-5.465**	**-3.2337**	**0.0012**
Pre-Test vs Test Trial 8	**-1.6917**	**0.2903**	**-5.828**	**-3.4467**	**0.0006**
Sound+AC					
Pre-Test vs Test Trial 1	**-2.2535**	**0.3817**	**-5.904**	**-3.4935**	**0.0004**
Pre-Test vs Test Trial 2	**-1.8967**	**0.3340**	**-5.679**	**-3.3604**	**0.0008**
Pre-Test vs Test Trial 3	**-2.2610**	**0.3653**	**-6.189**	**-3.6621**	**0.0003**
Pre-Test vs Test Trial 4	**-2.2222**	**0.3519**	**-6.314**	**-3.7361**	**0.0002**
Pre-Test vs Test Trial 5	**-2.3605**	**0.3996**	**-5.908**	**-3.4959**	**0.0005**
Pre-Test vs Test Trial 6	**-2.2192**	**0.4017**	**-5.525**	**-3.2692**	**0.0011**
Pre-Test vs Test Trial 7	**-2.2312**	**0.4014**	**-5.559**	**-3.3077**	**0.0009**
Pre-Test vs Test Trial 8	**-2.1802**	**0.4022**	**-5.420**	**-3.2071**	**0.0013**

Table 2-S15. Generalized linear mixed model (GLMM) results for Silver Carp exposed to 20_2000 Hz sound and 20_2000 Hz sound coupled with Air Curtain

Fixed Effects	Estimate	Std. Error	Z value	Z value (corrected)	Pr (>|z|)
Does exposure to sound or sound+AC reduce mean passage of fish?					
Pre-Test Sound+AC vs No Treatment Control	**-0.38369**	**0.04591**	**-8.356**	**-3.61731**	**0.0002**
Test Sound+AC vs No Treatment Control	**-3.55947**	**0.12312**	**-28.910**	**-12.51515**	**<0.0001**
Test Sound vs No Treatment Control	**-0.37220**	**0.04313**	**-8.630**	**-3.61905**	**0.0002**
Pre-Test Sound vs No Treatment Control	**-0.23037**	**0.04253**	**-5.417**	**-2.34502**	**0.0095**
Do passage rates change with repeated exposure to treatments (or with time in the flume)?					
No Treatment Control					
Linear	**0.04971**	**0.006375**	**7.798**	**3.37576**	**0.0004**
Quadratic	-0.06917	0.04102	-1.686	-0.72987	0.2330
Cubic	0.01625	0.04095	0.397	0.17186	0.4318
Quartic	0.01591	0.04084	0.390	0.16883	0.4330
Quintic	0.02514	0.04058	0.618	0.26753	0.3945
Sextic	0.00283	0.04044	0.070	0.03030	0.4879
Septic	0.01259	0.04012	0.314	0.13593	0.4460
Sound + AC: Pre-Test					
Linear	-0.04249	0.00972	-4.373	-1.89307	0.0292
Quadratic	-0.14117	0.06175	-2.286	-0.98961	0.1613
Cubic	-0.01292	0.06207	-0.208	-0.09004	0.4641
Quartic	0.08466	0.06199	1.366	0.59134	0.2772

Quintic	-0.02572	0.06165	-0.417	-0.18052	0.4286
Sextic	-0.00403	0.06089	-0.066	-0.02857	0.4888
Septic	-0.00201	0.05942	-0.034	-0.01472	0.4944
Sound: Pre-Test					
Linear	-0.00406	0.00780	-0.520	-0.22511	0.4110
Quadratic	-0.07225	0.05001	-1.445	-0.62554	0.2660
Cubic	-0.00499	0.05031	-0.099	-0.04286	0.4833
Quartic	0.05142	0.05010	1.026	0.44416	0.3285
Quintic	0.05058	0.05001	1.010	0.43723	0.3310
Sextic	-0.02731	0.04994	-0.547	-0.23680	0.4067
Septic	-0.01232	0.04909	-0.251	-0.10866	0.4570
Sound: Test Passages					
Linear	0.03892	0.00867	4.488	1.94286	0.0260
Quadratic	-0.24579	0.05548	-4.430	-1.91775	0.0276
Cubic	0.16271	0.05472	2.974	1.28745	0.0990
Quartic	-0.01702	0.05392	-0.316	-0.13680	0.4459
Quintic	-0.02600	0.05315	-0.489	-0.21169	0.4164
Sextic	-0.00327	0.05240	-0.062	-0.02694	0.4896
Septic	-0.02065	0.05162	-0.400	-0.17316	0.4313
Sound+AC Passage Rates					
Linear	0.15269	0.05900	2.588	1.12035	0.1313
Quadratic	-0.59660	0.38328	-1.557	-0.50087	0.3085
Cubic	0.58729	0.35379	1.660	0.69264	0.2443
Quartic	-0.35048	0.30437	-1.151	-0.49827	0.3092
Quintic	0.51474	0.28794	1.788	0.51030	0.3049
Sextic	-0.30325	0.29160	-1.040	-0.45022	0.3264
Septic	0.16081	0.28012	0.574	0.24848	0.4019

For a specific treatment, during which trial (1-8) does exposure to light change passage rates? (α = 0.05/8 = 0.0063)

Sound					
Pre-Test vs Test Trial 1	**-0.46446**	**0.08117**	**-5.722**	**-2.47706**	**0.0066**
Pre-Test vs Test Trial 2	-0.22134	0.07466	-2.965	-1.28355	0.0998
Pre-Test vs Test Trial 3	-0.04147	0.07197	-0.576	-0.24935	0.4017
Pre-Test vs Test Trial 4	-0.07821	0.07096	-1.102	-0.47706	0.3167
Pre-Test vs Test Trial 5	-0.13626	0.07046	-1.934	-0.83723	0.2013
Pre-Test vs Test Trial 6	-0.11869	0.07270	-1.633	-0.70693	0.2401
Pre-Test vs Test Trial 7	-0.03219	0.07331	-0.439	-0.19004	0.4247
Pre-Test vs Test Trial 8	-0.04225	0.07274	-0.581	-0.25152	0.4009
Sound+AC					
Pre-Test vs Test Trial 1	**-4.61929**	**0.58026**	**-7.961**	**-3.44632**	**0.0003**
Pre-Test vs Test Trial 2	**-3.03461**	**0.26489**	**-11.456**	**-4.95931**	**<0.0001**
Pre-Test vs Test Trial 3	**-3.52313**	**0.33861**	**-10.405**	**-4.50433**	**<0.0001**
Pre-Test vs Test Trial 4	**-3.20251**	**0.28349**	**-11.297**	**-4.89048**	**<0.0001**
Pre-Test vs Test Trial 5	**-3.17326**	**0.27343**	**-11.605**	**-5.02381**	**<0.0001**
Pre-Test vs Test Trial 6	**-3.10102**	**0.27374**	**-11.328**	**-4.90390**	**<0.0001**
Pre-Test vs Test Trial 7	**-3.11356**	**0.28386**	**-10.969**	**-4.74848**	**<0.0001**
Pre-Test vs Test Trial 8	**-2.86611**	**0.24988**	**-11.470**	**-4.96537**	**<0.0001**