Acoustic and Strobe-Light Behavioural Barriers: Examining Behavioural and Movement Responses of Common Carp (*Cyprinus carpio*) at Laboratory and Mesocosm Scales

by

Paul Alexander Bzonek

A thesis submitted in conformity with the requirements for the degree of Masters of Science
Ecology and Evolutionary Biology
University of Toronto

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Acoustic and strobe-light behavioral barriers have been recognized as tools to limit the spread of Asian carps in the Great Lakes. Urgent research is needed to understand how these stimuli impact behaviour, and to evaluate barrier efficacy within realistic canal environments. In a laboratory study, Common Carp responses to stimuli were recorded with video trials (n=44). There were no differences in behavioural responses to acoustic, strobe-light, or combined stimuli. The stimulus period increased durations of carp movement, and the post-stimulus period had increases in movement duration and barrier passes. In a mesocosm study, Common Carp (n=6) and buffalo (n=3) were exposed to the same stimuli, and movement was analyzed using acoustic telemetry. Acoustic stimuli did not produce significant movement responses. Strobe lights (n=12) produced smaller utilization distributions, a decrease in relocations, and an increase in travel velocity near the stimuli (<30m). This research will help inform Asian carp management decisions.
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1 General Introduction

1.1.1 History of Asian Carps in North America

In North America, the term “Asian carps” refers to four species: Bighead (*Hypophthalmichthys nobilis*), Black (*Mylopharyngodon piceus*), Grass (*Ctenopharyngodon idella*) and Silver (*Hypophthalmichthys molitrix*) carps. These species of cyprinids have been intentionally brought over from East Asia into over 60 countries for aquaculture purposes (Kolar et al. 2005). Regarded as prolific algae and zooplankton consumers, Silver and Bighead carps were brought to the southern United States in the early 1970s for the biofiltration of lagoons in sewage facilities (Kolar et al. 2005). These species likely escaped sewage lagoons through facility flooding. In the 1990s, 5000 Bighead Carp escaped from a single farm in Missouri due to flooding (Kolar et al. 2005), however, small scale escapes had occurred since the 1970’s (Kolar et al. 2007). During this time, evidence of wild spawning was found, and Bighead and Silver caps have continued to expand their range throughout the Upper Mississippi River system (Sampson et al. 2009) (Figure 1.1).

![Figure 1.1. Silver and Bighead Carp distribution in the United States. U.S. Geological Survey (2016).](image)

Similarly, Grass Carp was brought to the United States in 1963 for biological weed control (Cudmore et al. 2004). Due to fears of escape into natural systems, triploid Grass Carp was developed in the 1980s to reduce the risk of successful invasion (Pípalová 2006). The extra set of chromosomes within triploid carp drastically reduces the likelihood of producing viable gametes.
and offspring. Grass Carp spawning has already occurred in the Sandusky River of Lake Erie since at least 2014 (Embke et al. 2016), and in the summer of 2015, eight adult Grass Carp were found along the Toronto Harbourfront (Aulakh 2015). Finally, Black Carp was stocked in the 1980s to manage snail populations and aquaculture facilities (Ledford & Kelly 2006).

1.1.2 Ecological Impacts of Asian Carps in the Mississippi

Many of the factors that make Asian carps desirable for aquaculture are the same traits that allow it to be highly invasive and destructive within new systems. The filter-feeding Silver and Bighead carps arguably produce the greatest ecological impact on novel systems due to their unique life history and ecological traits (Cudmore et al. 2004; Cudmore et al. 2012). The high fecundity, fast growth rates and broad temperature, stream velocity, and environmental tolerances permit these species to thrive in many unintentionally introduced systems (Cooke 2016) and dominate the aquatic biomass of entire regions (Vetter et al. 2015).

In the case of Bigheaded carps, the introduction of large filter feeding populations has led to complex consequences in food-web dynamics (Cudmore et al. 2012). In a simplified explanation, Bighead and Silver Carps consume zooplankton and phytoplankton respectively (COSEWIC 2004), which changes the plankton communities and can lead to a greater system primary productivity and increased turbidity (Kolar et al. 2005). Within the Mississippi water system, the majority of native fishes are affected by these changes as they consume zooplankton during their larval and juvenile stages (Vetter et al. 2015; Kolar et al. 2005). Species such as Bigmouth Buffalo that filter feed throughout the adult stages of their life history are particularly impacted by changes in zooplankton communities (Sampson et al. 2009). Finally, the changes in abiotic factors such as turbidity may affect species that rely upon visual predation, such as Walleye (Kolar et al. 2005), by reducing their capacity to capture prey.

Grass Carp consume submerged aquatic vegetation, often targeting a preferred species until it becomes scarce, and switching to another (Bain 1993). Similar to the bigheaded carps, cascading effects can then impact water quality and aquatic fauna such as benthic macroinvertebrates and fishes (Cudmore et al. 2004). Benthic invertebrates can experience loss of shelter, and greater predation and competition. Changes in fish populations are highly variable (Bailey 1978), but
Grass Carp populations have been associated with declines in diversity and gamefish size (Cudmore et al. 2004).

1.1.3 Threat of Invasion

Bigmouth and Silver carps are currently dispersing throughout the Mississippi River basin, a water system that is ultimately connected to Lake Michigan and the Great Lakes through the Chicago-Area Waterway System (Vetter et al. 2015). If no preventative measures were in place, it would be highly likely that these carps would migrate and become established within the Great Lakes (DFO 2012; Wittmann et al. 2014). These carps could utilize available food sources and suitable spawning habitat (Cooke & Hill 2010; DFO 2012), to potentially proliferate at an alarming rate and cause great damage to the ecological system. Similar ecological effects have been seen in the Mississippi River (Rasmussen et al. 2011; COSEWIC 2004). Grass Carps are already spawning in one watershed within Lake Erie (Embke et al. 2016), and models have predicted that successful recruitment could occur within multiple watersheds throughout the Great Lakes (Kocovsky et al. 2012). Preventing further invasions of all carp species would be the most effective method of avoiding all predicted negative impacts (Mack et al. 2000).

Currently, a series of electric barriers within the Chicago-Area Waterway System are the major preventative management strategy against carp invasion. However, these barriers are expensive to maintain and may partially shut down during power outages or maintenance periods (Rasmussen et al. 2011). Furthermore, the electromagnetic fields they produce are distorted when steel-hulled barges pass through (Dettmers et al. 2005), reducing the efficacy of the barrier. Due to these factors, the electric barrier may not be 100% effective, as indicated by the presence of eDNA and the capture of a Bighead Carp directly upstream of the electric barriers (Ruebush et al. 2012).

Alternative management options are available and should be researched further to help minimize the predicted risk of an Asian Carp invasion into the Great Lakes (DFO 2012). Other options include the hydrologic separation of the Mississippi and Laurentian Great Lake basins or the use of behavioural barriers that make entering novel environments undesirable for the carps. Separating the waterways would be extremely expensive with estimates ranging from $3.5-$9.5 billion in USD (Wittmann et al. 2014). Behavioural barriers are considerably cheaper.
alternatives. The potential designs range from employing visual or acoustic stimuli to emitting pheromones or producing hypoxic environments (Noatch & Suski 2012). These designs avoid the pitfalls of permanent physical barriers: such as expensive installations, impediments to water flow, and the maintenance required to remove clogging and biofouling (Flammang et al. 2014). Despite their diversity in design, one consistent trend across all behavioural barriers is that fish responses are site dependent and species specific, thereby highlighting the need for more refined research on behavioural barriers (Noatch & Suski 2012). Two strong candidates that deserve further attention are acoustic and strobe-light behavioural barriers.

1.1.4 Literature Review of Strobe-Light and Acoustic Behavioural Barriers

Since the mid-1980s, researchers have investigated the ability of sound barriers to reduce the level of fish impingement upon nearby power plants (Maes et al. 2004). The sound frequencies tested ranged from infrasound to ultrasound (Knudsen et al. 1997; Haymes & Patrick 1986; Mann et al. 1997) and produced a range of responses from not effective to highly efficient across a variety of species (Maes et al. 2004).

Recently, acoustic barrier research has shown promise when directed towards the containment of invasive Asian Carps. A study by Pegg et al. (2005) found that an acoustic barrier reduced the number of attempted passes across a barrier by 95%.

Additionally, Vetter et al. (2015) successfully chased Silver Carp away with an acoustic tone for an average of 11 times in a row. Studies have also combined multiple stimuli to produce more comprehensive barriers, such as the sound-bubble-strobe-light barrier evaluated by Ruebush et al. (2012). While these barriers appear to be quite effective at deterring carps under experimental conditions, further analysis is necessary to assess the behavioural responses to acoustic stimuli in more naturalistic settings.

Most experimental studies only analyze gross movement patterns in experimental tanks (Taylor et al. 2005; Zielinski et al. 2014; Vetter et al. 2015). Flammang et al. (2014) acknowledged that responses to barriers in studies with simplified environments may not be accurate representations of natural response behaviour. Outlining changes in general movement may not be sufficient to predict carp responses. Detailed behavioural observations should be included to adequately
interpret carp responses to stimuli. When experiments are conducted in natural settings, they often use mark-recapture methods to determine barrier success (Maes et al. 2004; Ruebush et al. 2012). This method can describe escape rates but fails to provide details regarding carp movement patterns, diurnal spatial occupation and attempted passes across a barrier. Research involving mesocosms may offer more natural responses to behavioural stimuli.

Many Asian carp behavioural barriers utilize some component of sound stimuli to take advantage of their unique anatomy. Carps and other cyprinids are hearing specialists that have a connection between their swim bladder and inner ear called a Weberian apparatus (Popper & Fay 1993) that allows them to hear a greater range of frequencies than other fish species (Figure 1.2). Sound waves create tiny compressions in the swim bladder that are transmitted to the inner ears via the Weberian ossicles. This connection through the Weberian ossicles gives carps an advantage compared to species without this anatomical feature (Lovell et al. 2006).

![Audiogram of fish hearing sensitivity comparing minimum hearing threshold against frequency. Modified from Lovell et al. (2006) and Kojima et al. (2005).](image-url)
The use of light stimuli as a behavioural barrier has received less attention, potentially due to its lack of consistency in eliciting behavioural responses under varying turbidity and background light intensity levels. Light barriers have been found to both repel and attract various fish species (Flammang et al. 2014). However, strobe-lights with a random flashing pattern may be less susceptible to habituation than other barrier strategies (Hamel et al. 2008) and could potentially be a component in species-specific integrated barriers. Strobe-light barriers have a unique advantage compared to ordinary light because fish are unable to move their iris and have a slow response to changes in light intensity (Noatch & Suski 2012). Due to these factors, intense alterations in light levels may produce an avoidance behaviour in carp species.

1.1.5 Common Carp as a Model Organism

For this thesis, Common Carp (*Cyprinus carpio*) was used as a model organism for Asian Carps. It is illegal to possess live Asian carps in Ontario. Common Carp is a strong model organism due to its biological similarity to Asian carps, its ecological importance, and its use within behavioural literature. Common Carp is in the same family Cyprinidae as the Asian carps. In addition to being hearing specialists with similar hearing capabilities (Amoser et al. 2004), Common Carp is also a social (Huntingford et al. 2010), fast-growing species capable of dramatically increasing in population size (Weber & Brown 2009). Similar to Asian carps, Common Carp is a nuisance species in North America and Australia that tend to dominate natural communities (Weber & Brown 2009). As Common Carp consumes aquatic vegetation, it re-suspends sediment and excretes high levels of nutrients that can lead to algal blooms (Chumchal et al. 2005; Weber & Brown 2009). Additionally, it reduces macrophyte abundance and habitat for benthic invertebrates (Sloan et al. 2013). These ecological impacts have led to significant scientific interest and a history of management related behavioural studies (Sisler & Sorensen 2008; Huntingford et al. 2010; Zielinski et al. 2014). Common Carp is established worldwide (Chumchal et al. 2005), and management strategies have been developed globally to control it limit its ecological damage (Weber & Brown 2009).
1.1.6 Behavioural Stimuli

The aim of this study is to investigate the behavioural and movement responses of fish to a complex acoustic signal, and stroboscopic stimuli. The visual stimulus used in this study is a 100 V random flashing underwater SeeBrite™ strobe-light which operated with a frequency of 50-60Hz (Seebrite LED, I.A.S. ltd, Vancouver, BC). Within our experimental tank, the strobe-light produced flashes of at least 120 000 lux immediately in front of the apparatus. This light per area value is slightly above the illumination of an extremely bright day. The acoustic stimulus is a three-second loop that combined a 200-1400Hz sweep, a 200-1500 Hz band sweep, and a high quality recording of a 50 hp outboard motor. The frequency sweeps cover most of the sensitive hearing frequencies for Silver and Bighead carps (Figure 1.2). Silver Carp expresses a strong jumping response to the sound of an outboard motor and can detect these tones hundreds of meters away from the source (Whitfield & Becker 2014). Asian carps exhibit a greater avoidance response, and less habituation to complex signals, than they do to single frequency tones (Vetter et al. 2015). The acoustic stimulus was produced with a 20 $V_{\text{RMS}}$ Lubell LL916H speaker within the wet-lab, and an 80 $V_{\text{RMS}}$ Lubell LL1424HP speaker within the mesocosm (Lubell Labs Inc. Columbus OH).
2 Behavioural responses of Common Carp (*Cyprinis carpio*) to acoustic and strobe-light stimuli in a laboratory setting

2.1 Abstract

The impacts of anthropogenic sound on fish fitness and behaviour are receiving a high level of interest for conservation and management purposes. Ostariophysans, including Common and Asian carps, are hearing specialists capable of hearing sounds at greater distances and lower thresholds than other hearing-generalist fishes. Acoustic behavioural barriers could be used to limit the dispersal of Asian carps into the Great Lakes while having a lesser effect on non-target fishes. Strobe-lights are another potential behavioural barrier stimuli that could be deployed in conjunction with the acoustic stimulus. This study investigated the behavioural responses of Common Carp to acoustic and strobe-light stimuli, and if the responses differed when both stimuli were combined. Common Carp (n=13) spent more time moving actively during the stimulus and post-stimulus periods, than during the control periods (30 min period duration). The number of passes across the barrier was significantly higher only during the post-stimulus period. The duration of time oriented towards the stimulus did not differ among periods. The results did not differ between acoustic, strobe-light, or combined stimuli. This research indicates that Common Carp may avoid acoustic and strobe-light stimuli; however, the combination of both stimuli may not produce an additive behavioural response.
2.2 Introduction

Anthropogenic sound produced by recreational boating, commercial shipping, power production, mining and other sources are nearly ubiquitous in many aquatic environments. Recreational boating alone has 2.5 million registered power-boaters in Canada (Whitfield & Becker 2014). This may be an issue as fishes use sound to refine their sense of the environment, understand natural and artificial structures, and conduct intraspecific communications (Popper & Carlson 1998). Similar to many anthropogenic sounds, fish communication signals, such as those used for reproduction, establishing territories and defense, are often broadband sounds (Popper & Carlson 1998). However, there is little known on how anthropogenic noise may impact fish physiology or behaviour, especially in aquatic environments (Amoser et al. 2004). As a result, there has been a high level of interest on the effects of sound on fish fitness.

Of the many factors that may influence the impacts of anthropogenic noise on fish behaviour, taxonomy plays an important role. All fishes hear through their octavolateralis system, which involves the ear and lateral line (Popper & Carlson 1998). Freshwater fishes of the superorder Ostariophysi are hearing specialists that have a connection between the inner ear and swim bladder known as the Weberian Apparatus (Popper & Carlson 1998). This connection allows fishes to perceive sound pressure waves in addition to particle movement (Popper & Fay 1993) and, by sensing both, Ostariophysian fishes can hear across a greater range of frequencies and at a lower sound intensity than hearing generalists (Lovell et al. 2006). Ostariophysian fishes may be more sensitive to anthropogenic noise than non-ostariophysian species as they can perceive a greater range of the produced sound spectra. Ostariophysian cyprinids like the Common Carp, *Cyprinus carpio*, may be able to detect powerboat noise up to 400 m away, while non-ostariophysian hearing-generalist species, such as whitefishes (Coregoninae) or perches (Percidae) can only perceive the stimulus from 30m and 200m away, respectively (Amoser et al. 2004).

The range of hearing abilities across fish taxa has important implications for how anthropogenic sound impacts fish fitness and could be exploited to help guide management and conservation efforts. Further research into the perception and behavioural responses of ostariophysians to acoustic stimuli may not only provide an opportunity to better understand mitigation issues unique to noise management, but also exploit these signals to develop behaviour-modifying
management tools. Many species in the family Cyprinidae are globally invasive. Currently, invasive Asian carps are dramatically altering the ecosystems of the Mississippi River basin and threatening to invade the Great Lakes, where they would cause similar damage (DFO 2012). The Great Lakes are not only environmentally suitable for Bighead, Black, Grass, and Silver carp populations (COSEWIC 2004; Wittmann et al. 2014), but also vulnerable to invasions unless barriers are operated to prevent their introduction and/or establishment from the Mississippi basin through the Chicago-Area Waterway System (CAWS) that hydrologically connects the two basins (Rasmussen et al. 2011). To prevent dispersal of exotic species across the CAWS, an experimental electric barrier has been deployed since 2002 (Dettmers et al. 2005). However, there are concerns regarding the reliability of the electric barrier due to shutdowns during routine maintenance and power outages (Rasmussen et al. 2011), electromagnetic and hydrological distortions during barge traffic (Dettmers et al. 2005), and the presence of carp eDNA found upstream of the barrier (Ruebush et al. 2012).

Additional barrier systems are currently being considered to supplement or replace the CAWS electric barrier (Wittmann et al. 2014). The types of barriers that could be deployed range from physical barriers, such as hydrologic separation, to physiological barriers, that use electrical or noxious stimuli, and behavioural barriers that reduce the risk of passage by altering a fish’s decision to enter a novel environment.

Physical barriers are rarely feasible due to ecological or economic implications as stream flow and native fish movement is required in most natural systems. In some situations, such as the CAWS, where artificial canals joined historically disconnected basins, hydrological separation may be feasible, but extremely expensive. Economic assessments determined that closing the CAWS would have a financial impact of approx. USD $1.3 billion annually (Schwieterman 2010), or a total cost of USD $3.5−9.5 billion (Whitfield & Becker 2014).

The collateral ecological and economic consequences of physiological barriers may be less extreme, but may not be as effective as physical barriers across all circumstances. Electrical barriers are currently in use in the CAWS, and CO₂ barriers are also receiving significant interest (Donaldson et al. 2015; Hasler et al. 2016). Both barrier technologies are still experimental in development but hold promise in mitigating potential carp invasions. Behavioural barriers may have the fewest unintended consequences but are also less secure as a preventative barrier. While
the previous physical and physiological barrier types are capable of limiting fish movement regardless of motivation and conduct, behavioural barriers are entirely dependent on altering the decision process of individuals.

Sound has many characteristics of a good behavioural barrier, such as traveling at high speeds underwater, producing a strong directional stimulus, and remaining unimpeded by turbidity or ambient light levels (Popper & Carlson 1998). By using sound as a behavioural barrier, a taxon-specific avoidance system may be developed that would have a greater impact on targeted hearing specialists over non-target hearing generalists. These acoustic behavioural barriers could be used as additional redundancies in critical dispersal corridors such as CAWS to prevent spread, or as barriers and fish guidance systems in other systems such as Great Lake tributaries to prevent establishment. However, before behaviour barriers can be deployed, their potential impacts and efficacy must be determined. Little work has been done on determining how acoustic stimuli impact carp behaviour, but this is a critical component in determining the reliability of acoustic barriers (Popper & Carlson 1998). Vetter et al. (2015) showed that complex, broadband tones can consistently produce avoidance responses in carps, but no studies have determined whether carp behaviours, such as activity or orientation, are similarly impacted.

Furthermore, little is currently known regarding the cumulative behavioural responses of carps to combinations of multiple stimuli. Ideally, acoustic stimuli could be deployed with other stimuli to provide a more comprehensive barrier system. Taylor et al. (2005) conducted a lab study to determine how effective an integrated barrier system was at deterring Bighead Carp passage. This barrier, composed of sound and bubble curtains, was 95% effective at repelling fish-passage attempts. However, the study did not examine the individual components of the barrier to determine which stimuli were driving the avoidance response, nor how the two components interacted. Further research needs to be conducted to determine how independent stimuli that impact multiple sensory systems, such as visual and acoustic components, interact with each other. No such study has determined how the combination of acoustic and strobe-light stimuli would interact and whether the behavioural responses would be additive, multiplicative, or null.

This study examines how Common Carp behaviour is impacted by acoustic, strobe-light, and combined stimuli, and how these behaviours differ during and after the stimulus is applied. The behaviours measured include the duration of active movement, duration of time oriented towards
the stimulus and the number of passes made across the stimulus region. I predict that the exposure to combined acoustic and strobe-light stimuli will elicit greater responses than each stimulus on its own. Previous studies have found the addition of multiple behavioural barrier components to have either additive or null impacts on the effectiveness of the initial barrier design (Ruebush et al. 2012; Flammang et al. 2014). Additionally, I predict that the greatest behavioural responses will be expressed during the active-stimulus period, followed by the post-stimulus period. Since Common and Asian carps have been observed to actively avoid behavioural barriers with sound components in a variety of studies and applications (Taylor et al. 2005, Ruebush et al. 2012, Flammang et al. 2014, Vetter et al. 2015), I predict both an increase in activity and a decrease in passes across stimulus during the stimulus period. Finally, since fishes are unable to control their irises (Noatch & Suski 2012), I expect to see a reduced duration of time spent oriented towards the stimulus when strobe-light or combined stimuli are active.

### 2.3 Methods

#### 2.3.1 Study Animals

Adult Common Carp were collected by boat electrofishing in Hamilton Harbour, Lake Ontario. In total, 53 Common Carp were used, with an average weight of 3.797 kg ± 1.229, and fork length of 569.48mm ± 47.40. Fish were held at the Aquatic Life Research Facility at the Canada Centre for Inland Waters (Burlington, ON, Canada). After capture, individuals were bathed in a formalin treatment to prevent parasite and disease transfer. Individuals were maintained within flow-through holding tanks for 13 days ± 9 (Mean ± SD) before being placed in experimental tanks. After either being tagged, or completing a trial, individuals were held for at least 24 hours before a new acclimation and trial session. Fish were acclimated to the experimental tank for at least 15 hours and acclimated to the stimulus for at least 45 minutes before a trial was started. Fish were held on a 10 h light – 10 h dark cycle. A total of 44 trials were conducted across the three treatment types (Table 2.1).
Table 2.1. Summary of the treatments and replicates within the wetlab experiment.

<table>
<thead>
<tr>
<th></th>
<th>Light Treatment</th>
<th>Sound Treatment</th>
<th>Combined Treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trials</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>Carp</td>
<td>45</td>
<td>45</td>
<td>42</td>
<td>53</td>
</tr>
</tbody>
</table>

2.3.2 Experiment

Wet-lab trials were conducted from July 20 - August 28, 2015 in three experimental tanks with dimensions of 2.5m*1.2m*0.4m. The behavioural responses of Common Carp to acoustic, strobe-light, and combined stimuli were recorded with a GoPro camera in the centered left end of the tank and an overhead camera aimed at the tank from the top left corner (Figure 2.1). The experimental tanks had six occupational zones with the stimulus in the middle of the tank.

Common Carp is a social species, so each trial held three carp at a time to control for potential isolation stress and to avoid a potential increase in risk perception (Huntingford et al. 2010). Before each trial, carp were anaesthetized with a Portable Electroanaesthesia System (PES, Smith-Root Inc., Vancouver, BC) and weighed, measured, photographed, and tagged with an FD-94 anchor tag (Floy Tag & Manufacturing Inc. Seattle WA) to give each carp a unique identification number. Within each video trial, carp were identified by the colour of their tag. Flagging tape of the same colour was added to the end of each tag to improve visibility and every trial was comprised of carp with three different tag colours. For all but two fish, trials were
completed in the order of strobe-light treatment, followed by the acoustic treatment, and then the combined treatment. The identity of carps within each trial was held constant across stimulus period.

Each trial lasted for 90 minutes and consisted of three components: a 30-minute control period; a 30-minute stimulus period; and, a 30-minute post-stimulus period. For each trial, three independent behavioural conditions were observed. These conditions were total duration of active movement, total duration of orientation towards stimulus, and number of passes across the stimulus. A carp is considered active if it is pumping its caudal fin to move forward, moving its dorsal fins to back up, or swimming swiftly. A carp is considered moving swiftly if it is travelling faster than ~5cm/second or travels the longitudinal length of an occupational zone in under 8 seconds. This minimum active swimming speed is approximately one third of the average active swimming speed found by Vetter et al. (2015). The main goal of the activity measurement is to differentiate between the movement of general exploratory behaviour and inactive stationary behaviour. A carp’s orientation can be categorized as either looking towards the stimulus if its head is aimed within 90° of the stimulus (Figure 2.2), or categorized as looking away from the stimulus. Passes were counted each time center of mass for the focal fish passed across the stimulus centered within the chamber.

![Figure 2.2. Illustration of orientation conditions required for fish to be considered oriented towards stimulus.](image-url)
All recorded trials were observed and scored within the observation software BORIS (Friard & Gamba 2016). Each video was viewed by one of two observers to record the times at which behavioural conditions change. Video trials were then further analyzed to produce event counts and duration summaries for each of the behavioural metrics within the statistical program R (R Core Team 2016).

### 2.3.3 Statistical Analysis

The behavioural metrics of activity, orientation, and passes across stimuli were analyzed as dependent variables within a single two-way MANOVA. The behavioural metrics were compared across the three treatment types (acoustic, strobe-light, and combined stimuli) and across the three stimulus periods (control, stimulus, and post-stimulus). To block for within-fish variation, stimulus level was nested within trial number. To account for possible observer bias between the two experienced video scorers, stimulus level and trial number were further nested within observer identity (Table 2.2). Post-hoc analyses were run on statistically relevant behavioural metrics with a general-linear-hypotheses and multiple comparisons model.

**Table 2.2. Overview of the Nested Repeated Measures Two-way MANOVA.** The duration values of each behavioural condition are compared across three treatment types at three different stimulus levels. Arrows indicate the same group of fishes observed for that trial period.
The MANOVA model was run using the ‘manova’ function within R. Post-hoc tests were run using the ‘lme’ function from the package nlme (Pinheiro et al. 2016), and the ‘glht’ function from the package multcomp (Hothorn et al. 2008).

2.4 Results

All behavioural metrics had an acceptable level of covariance for the MANOVA analysis with correlation levels of Active:Towards=0.0161; Active:Passes=0.5354; and Towards:Passes=0.1185. The three behavioural metrics were significantly different across the three stimulus periods (n=100, F=3.025, p=0.0074) and treatment types (n=100, F=2.148, p=0.0496), but no interactions were found between stimulus period and treatment type(n=100, F=0.817, p=0.6327) as seen in Figure 2.3.
Figure 2.3. Common Carp behavioural responses of activity, orientation and number of passes compared across treatment type and stimulus levels. Responses are grouped across stimulus level by trial. Letters represent significant differences between stimulus periods based on post-hoc linear mixed-effects models. Each line represents a single fish from each trial observed across the three stimulus levels.
The duration of active movement differed across stimulus level (n=102, F=5.167, p=0.0073), but there was no significant interaction between stimulus period and the treatment type of acoustic, strobe-light, and combined stimulus (n=102, F=0.016, p=0.999) (Table 2.3). The average total duration of active movement was significantly higher during stimulus (1224 s ± 384; Mean ± SD) and post-stimulus (1371 s ± 284) periods than control periods (983 s ± 428). Time spent oriented towards the barrier stimulus was not significantly different between treatment types (n=102, F=2.928, p=0.0580), or stimulus period (n=102, F=0.352, p=0.7043) (Table 2.3). The number of passes made across a barrier varied according to stimulus period (n=102, F=5.097, p=0.0078), but there was no significant interaction between stimulus period and the treatment type (n=102, F=1.884, p=0.1189) (Table 2.3). The average number of passes across the stimuli was significantly higher during post-stimulus periods (23 ± 12) than stimulus (11 ± 9) and control periods (15 ± 12).
Table 2.3. Summary statistics of Two-way-within-treatment-nested-MANOVA for behaviour metrics of activity, orientation and passes. Post-hoc ANOVAs included for comparative analysis between behaviours. Formal post-hoc Tukey’s HSD tests were conducted on Linear-mixed-effects models for each behavioural metric.

<table>
<thead>
<tr>
<th>Comprehensive MANOVA analysis</th>
<th>Df</th>
<th>F stat</th>
<th>p</th>
</tr>
</thead>
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<td>Treatment</td>
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<td>2.148</td>
<td>0.0496*</td>
</tr>
<tr>
<td>Stimulus</td>
<td>100</td>
<td>3.024</td>
<td>0.0075**</td>
</tr>
<tr>
<td>Treatment:Stimulus</td>
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<td>0.817</td>
<td>0.6327</td>
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</tbody>
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Post-Hoc Activity ANOVA

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<th></th>
<th>Df</th>
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<tr>
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<td>0.6079</td>
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<tr>
<td>Stimulus</td>
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<td>0.0073**</td>
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<tr>
<td>Treatment:Stimulus</td>
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Post-Hoc Orientation ANOVA

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Post-Hoc Passes ANOVA

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</tr>
<tr>
<td>Stimulus</td>
<td>102</td>
<td>5.097</td>
<td>0.0078**</td>
</tr>
<tr>
<td>Treatment:Stimulus</td>
<td>102</td>
<td>1.884</td>
<td>0.1189</td>
</tr>
</tbody>
</table>

2.5 Discussion

2.5.1 Behavioural Responses to Barrier Stimuli

The application of acoustic, strobe-light, or combined stimuli resulted in an increase of the average duration of active movement, but not an increase the number of passes made across the barrier. Once the stimulus ceased, activity durations remained high, but the number of passes increased to match the heightened activity levels. These changes indicate that Common Carp are likely stressed by the operating stimuli and are possibly searching for refuge without increasing their exposure to a foreign signal. Additionally, the post-stimulus results indicate that acoustic,
strobe-light, or combined stimuli may be able to produce short-term behavioural changes in Common Carp.

The application of any behavioural stimuli increased the average duration of active movement for the stimulus and post-stimulus periods. Once the stimulus began operating, Common Carp spent more time moving and maintained this higher rate of movement for the 30 min period after the stimulus had ceased. This indicates that both acoustic and strobe-light stimuli produce a short-term behavioural response in Common Carp. Individuals were likely exhibiting a fright response (Königson et al. 2002), and negative photo- and phono-taxis (Vetter et al. 2015; Ruebush et al. 2012; Taylor et al. 2005).

The number of passes made across the barrier did not differ significantly between control and stimulus periods, but were significantly higher in the post-stimulus period for all treatment types. The increase in passes made after the stimulus has ceased may be a response to high stress levels (Richards et al. 2007) and express an individual’s intent to find an escape or refuge. If this is the case, the stimulus signals would have been perceived as a foreign threat, producing an immediate avoidance response (Königson et al. 2002) and a short-term stress response (Richards et al. 2007) that remained after the stimulus ceased. When the stimulus was operating, individuals spent more time actively moving, but did not pass across the barrier more frequently as they were avoiding proximity the perceived threat. However, once the stimulus stopped, individuals began passing across the barrier more frequently, matching their heightened activity levels, and searching for an escape from the environment.

Common Carp did not express an orientation avoidance to any of the treatments when the stimulus was active. The lack of orientation is not surprising for the acoustic stimuli. Within the 3 m length of the experimental tank, Common Carp likely perceived the signal as near-field particle motion along the lateral line (Popper & Carlson 1998). While the acoustic signal’s particle motion was highly directional in its transmission from the source, an individual’s orientation towards the stimulus likely did not have a large impact on the magnitude of signal perceived. An orientation response was expected with strobe-light stimuli as an iris that was oriented away from the stimulus would receive several orders of magnitude less lux, and subsequently, less irritation produced by the bright light than if oriented towards the stimulus. It is possible that individuals perceived the strobe-light stimulus as a threat and felt more
vulnerable when not oriented towards and ‘watching’ it. Alternatively, Common Carp may not have determined that by altering their orientation, they could reduce the level of luminosity experienced. However, Königson et al. (2002) found that whitefish would turn around and swim away from an overhead a strobe-light, if the stimulus flashed light in front of the individual.

### 2.5.2 Implications for Combining Independent Stimuli

The behavioural responses did not change according to the treatment type; individuals responded similarly to acoustic, strobe-light, and combined acoustic and strobe-light stimuli. Although acoustic and strobe-light stimuli impact different sensory systems, the average behavioural response was conserved in the magnitude and direction of the response observed. It is possible that all three stimulus types lead to a common stress response elicited when Common Carp were exposed to foreign and threatening stimuli, as seen in other fish species exposed to strobe-light stimuli (Richards et al. 2007). Due to the high intensity of the acoustic and strobe-light stimuli, and the small experimental tank sizes relative to body size, Common Carp may have been expressing a strong response to each stimulus individually, leaving little room for additive responses when the stimuli were combined. Ruebush et al. (2012) conducted a mark-recapture study where they investigated the response of carps to an acoustic and a combined acoustic and strobe-light barrier. Similar to this study, Ruebush et al. did not find an additive response in the combined treatment, but had started with a high response level (95% avoidance) to the acoustic barrier on its own. Perhaps if less intense stimuli, or larger experimental tanks were used in this study, lesser behavioural responses with the individual stimuli, and an additive effect with the combined stimuli, may have been observed.

An alternative interpretation of these results may be that exposure to the combined stimuli does not increase the behavioural responses of Common Carp beyond the magnitude found by either individual stimuli. This would suggest that an acoustic barrier of sufficient magnitude would not receive any behavioural response benefits from the implementation of strobe-light components and vice versa. The addition of supplementary stimuli to exploit multiple sensory systems should then be used as a redundancy and not as a tool to modulate the magnitude of response in target fishes. Each component of the barrier would need the strength to produce the desired magnitude
of response on its own, with the addition of new components used to provide reliability across multiple environmental conditions and contexts.

2.5.3 Management Implications

Acoustic and strobe-light stimuli are both promising behavioural barrier components that produce short-term changes in Common Carp behaviour. Common Carp activity increases after exposure to either or both stimuli, and once the stimulus ceases, passes made across the barrier increase as well. These behavioural changes are likely signs of stress (Richards et al. 2007), and negative phono- or photo-taxis to acoustic and strobe-light stimuli (Vetter et al. 2015; Ruebush et al. 2012; Taylor et al. 2005). This study provides further support that acoustic and strobe-light stimuli could reduce the spread of invasive ostariophysians across geographic bottlenecks if deployed as a behavioural barrier. This research should be continued with the Bighead, Black, Grass and Silver carps of concern. Additionally, this study found that if both acoustic and strobe-light stimuli are used together in a comprehensive barrier, they may not interact to produce a greater magnitude of behavioural response. However, as acoustic stimuli are detected by the octavo-lateralis system and strobe-light stimuli are detected by the visual system, a comprehensive barrier may be more robust and capable of remaining effective across a greater range of environmental conditions.
3 Behavioural responses of Common Carp (*Cyprinis carpio*) to acoustic and strobe-light stimuli in a mesocosm setting

3.1 Abstract

The most likely pathway of Asian carp invasions into the Great Lakes is dispersal across geographic bottlenecks, such as canals or rivers. Urgent behavioural barrier research is needed to evaluate the efficacy of potential stimuli components of a barrier within realistic environments. My research examines buffalo spp. hybrid (*Ictiobus* spp.) and Common Carp (*Cyprinus carpio*) movement patterns in response to acoustic and strobe-light stimuli. An outdoor mesocosm with an environment similar to a canal was used to observe fish-utilization distributions, kernel density, proximity to stimulus, and travel velocity. Buffalo (n=3) and Common Carp (n=6) were implanted with acoustic telemetry tags and their behaviour was compared between treatments with exposure to control, acoustic, and strobe-light barriers for 60-minute periods. Trials were run during both day and night. The acoustic stimulus is a combination of a marine engine, a 200-1400Hz sweep, and a 200-1500Hz bandsweep. The acoustic stimulus was played through an underwater speaker at a depth of 4 m and a sound pressure of 175.2dB dB re 1 µPa RMS. Strobe-lights (n=12) were deployed across the mesocosm centre at water depths of 3 m and 6 m. The acoustic stimuli did not produce significant changes in utilization distributions or an association between changes in kernel density and sound pressure. The strobe-light stimuli did produce a significantly smaller utilization distribution, a decrease in telemetry relocations near the stimuli (<30m), and faster average travel velocity near the stimuli. No meaningful differences were found between the responses of buffalo and Common Carp to either stimulus.
3.2 Introduction

3.2.1 Asian Carp Management

In an effort to limit further Asian carp invasions and mitigate ecological impacts where populations have established, a bi-national, multi-agency integrated pest-management system for Asian carps has been implemented. In 2007, the Asian Carp Working Group and Aquatic Nuisance Species Task Force outlined seven goals for Asian carp management and control (Conover et al. 2007). These goals include: preventing the accidental or deliberate introduction of Asian carps into novel systems; containing and controlling carp expansions; extirpate or minimize effects of feral populations; provide information to the public, commercial entities, and government agencies; conduct research relevant to effective Asian carp management; and plan, implement, and evaluate Asian carp management and control efforts.

Canada does not have established Asian carp populations within its waters; therefore, Canadian contributions to the integrated pest-management system are constrained to supporting goals related to preventing accidental introduction, containing expansion, providing public education, and conducting research. Currently, Canada has taken actions in support of all of these goals (Invasive Species Centre 2016). As of 2005, Ontario enacted legislation to make the possession of live Asian carps illegal. In 2015, Canada made it illegal to import live Asian carps and required all imports to be eviscerated. Fisheries and Oceans Canada operates an Early Detection Asian Carp monitoring program that samples throughout Lake Ontario and Lake Erie to monitor current fish communities, search for rogue Asian carps, and respond to reported Asian carp sightings (Marson et al. 2016). Further monitoring efforts include a partnership with the Ontario Ministry of Natural Resources and Forestry to conduct eDNA sampling throughout both lakes. In partnership with the Ontario Invasive Species Centre, Fisheries and Oceans Canada also maintains an active outreach program that produces news, public surveys, and advertisements to increase awareness and engage the public (www.asiancarp.ca). Fisheries and Oceans Canada is also funding behavioural barrier research, such as this study, to help prevent the invasion of Asian carps into the Great Lakes.
3.2.2 Field Studies
Considerable efforts are underway to advance our understanding of acoustic barriers and to progress from lab to mesocosm and field studies. In May 2016, a multi-agency meeting was held to determine the current state of science and future priorities for acoustic Asian carp deterrence (USGS 2016). Scientists and managers from federal, state, provincial, and academic organizations agreed that urgent validation of the barrier technology in realistic settings is a necessary next step for the deterrence strategy (USGS 2016). Continued research would help to determine the efficacy and limitations of acoustic barriers, its potential in augmenting other dispersal deterrents, and its suitability for future deployment across a range of environments. Barrier concerns include the effects of site-specific structures and substrate on sound propagation, habituation, and impacts to non-target fishes (USGS 2016). Acoustic barrier technologies could also be integrated with other deterrent stimuli and harvest strategies to produce a comprehensive integrated pest management system (USGS 2016). Deployment sites at lock-and-dam structures are currently being identified along the Upper Mississippi, Ohio, and Illinois rivers for future installation (USGS 2016).

Asian carps continue to disperse throughout the Mississippi, Illinois, Missouri, and Maumee rivers with the potential to invade the Great Lakes (Cuddington et al. 2014). The benefits of refining barrier research must be weighed against the potential costs of delaying site deployment. Furthermore, there is significant bi-national public pressure to act and protect the Great Lakes from an invasion of Asian carps (Rasmussen et al. 2011; Hinterthuer 2012; Donaldson et al. 2015). Applied research involving the effectiveness of barrier technology needs to be urgently conducted and with direct applications towards future barrier deployment to avoid site-deployment delays. This research should address the outlined concerns of environmental influences on sound propagation, habituation, impacts on non-native fishes, and interactions with other barrier technologies.

3.2.3 Common Carp and Buffalo Hybrids
Conducting barrier studies on Asian carps at field sites ahead of the invasion front could be disastrous to the integrated management plan if individuals escaped their experimental boundary and into the wild. Since Canada does not have any reproducing Asian carp populations, Canadian
barrier studies are being conducted with the use of Common Carp as a proxy for Asian carps. Common Carp is a strong model organism for Asian carps due to their biological similarity as a hearing specialist, its ecological importance (Weber & Brown 2009), and its use in behavioural literature (Sisler & Sorensen 2008; Huntingford et al. 2010; Zielinski et al. 2014).

As outlined in the Acoustical Deterrent Workshop (USGS 2016), the potential impacts of acoustic stimuli on native species are also a major concern. Native species, such as Gizzard Shad (*Dorosoma cepedianum*) and Bigmouth Buffalo (*Ictiobus cyprinellus*), have close diet overlap with Bighead and Silver carps and are very likely to be negatively impacted by a Great Lakes invasion (Sampson et al. 2009). Bigmouth Buffalo population size and area of occupancy within the Great Lakes has increased over the last 20 years (COSEWIC 2009), which allowed it to be downlisted from Special Concern to Not at Risk in the Canadian Great Lakes basin (COSEWIC 2009). However, if Asian carps invade the Great Lakes, buffalo populations could begin to decline (Sampson et al. 2009).

Special consideration should be made to determine how potential barriers placed within the Great Lakes may impact Bigmouth Buffalo, especially in ecosystems where Bigmouth Buffalo competing with Asian carps could compound fitness concerns. Within the Great Lakes basin, Bigmouth Buffalo readily hybridize with two other *Ictiobus* species, the Smallmouth and Black buffaloes (Bart et al. 2010), making the three species difficult to distinguish (Holm et al. 2009).

### 3.3 Methods

To determine how acoustic and strobe-light stimuli influence buffalo and Common Carp movement, an acoustic telemetry study was conducted in a mesocosm. A ship slip was used to form an experimental arena with acoustic and strobe-light stimuli, and block nets to contain fishes. Wild buffalo and Common Carp were electro-fished from Hamilton Harbour and outfitted with acoustic tags to be detected by an acoustic telemetry array installed in the mesocosm. The sound-pressure profile of the mesocosm under ambient and acoustic treatments was mapped. To investigate fish-movement responses, kernel-density and utilization-distribution analyses were conducted for both stimuli types. For acoustic stimuli trials, change in kernel densities was also regressed against stimulus sound-pressure level. For strobe-light trials, the number of relocations
near the stimulus (<20 m) was analysed with a Chi-squared test, and travel velocity near the strobe-light stimulus was investigated with a two-way nested ANOVA.

3.3.1 Study Site
The mesocosm experiment was conducted in a ship slip immediately adjacent to the Burlington Bay Canal under the Burlington Skyway Bridge (Figure 3.1). It is enclosed with concrete and corrugated metal sheathing on three sides and opens to Hamilton Harbour on the fourth side with a double net preventing fish from escaping or debris from entering. The mesocosm substrate was predominantly silt sediment. The block nets were 12 x 42 m nylon nets with a 2.5 cm mesh spacing that were secured from top to bottom along the ship-slip walls, and above the waterline with aircraft cable secured beyond the mesocosm walls (Rainbow Net and Rigging Limited, Dartmouth NS). Due to its original purpose and construction alongside the Burlington Bay Canal, the mesocosm accurately simulates the environmental conditions of shipping canals within the Great Lakes basin. The mesocosm is 107.5 meters long, 34.5 meters wide, and 8 meters deep, holding a water volume greater than eight Olympic swimming pools. The acoustic stimulus was placed in the centre of the mesocosm and suspended at a depth of 4 m with aircraft cable. The strobe-light stimuli were produced with 12 underwater strobe-lights that bisected the mesocosm and were placed equidistant to each other. The strobe-lights were suspended at alternating depths of 3 and 6 m with aircraft cable. Experimental trials occurred over six days and nights, June 22-28, 2015. Within the mesocosm, fishes were fed with pellets (Profishent Trout Chow, Martin Mills Inc. Elmira ON) ad libitum to 1% fish weight through two automatic fish feeders (AX275 12-V, Aquatic Xtreme Fish Feeders, San Antonio TX). Fish feeders were placed near the northeast and southwest corners of the mesocosm.
Figure 3.1. Location of the experimental mesocosm in Burlington, ON, at the Canadian Centre for Inland Waters.

3.3.2 Study Animals
Buffalo (n=13) and Common Carp (n=13) were tagged for experimental use within the mesocosm. Wild fishes were electrofished from Hamilton Harbour and held within the Aquatic Life Research Facility as described in Chapter 2.3.1. Individuals were anaesthetized with a Portable Electroanaesthesia System (PES, Smith-Root Inc., Vancouver, BC) and then weighed, measured, photographed, and tagged with a 19 mm long HTI™ acoustic tag (Model 900LV, Hydroacoustic Technology Inc., Seattle, WA) via surgical incision. The acoustic tags were inserted into the peritoneal cavity through a 10 mm ventral incision posterior to the pelvic fins. The wound was closed with 2-3 independent sutures. Fish were manually irrigated with oxygenated water throughout the surgery. After a monitored recovery, individuals were transferred to holding tanks for 24 hours to monitor health post-surgery before they were transferred to the mesocosm. Once in the mesocosm, buffalo and Common Carp were allowed to acclimate for 60 hours before the first experimental trial. Of the 13 tagged buffalo released into the mesocosm, 10 individuals were detected by the hydrophone array, seven individuals continued to be detected throughout the duration of the study, and three individuals showed signs of movement by the end of the study. Individuals that did not move for durations greater than one day were considered to have died or lost their tag. Every individual that stopped moving for the duration of a day, remained stationary throughout the remainder of the experiment. Of the 13 tagged Common Carp released into the mesocosm, six individuals were detected by the
hydrophone array. All six Common Carp were used in the study, but two individuals were no longer detected by the hydrophone array after the first day of the experiment. Hydrophone detection may have failed if individuals escaped beyond the block nets, or if tags were improperly programmed, malfunctioned, or lost battery power. No holes or obvious escape routes were apparent within the block-net installation. Acoustically tagged fish (15 Common Carp, five Gizzard Shad, three Channel Catfish (*Ictalurus punctatus*) one Smallmouth Bass (*Micropterus dolomieu*), and one Bowfin (*Amia calva*)) from a previous study were still enclosed within the mesocosm for the duration of this study. Only the movement patterns of the naïve fishes were investigated for this study.

### 3.3.3 Telemetry Tracking

The mesocosm array was designed such that a subject could always be triangulated between at least three hydrophone locations and two depth levels. This allowed for the accurate estimation of latitude, longitude, and depth for each tagged individual. Each hydrophone is an underwater acoustic receiver that listens to the pings produced by the acoustic tags. A hydrophone records when it receives a ping and an acoustic telemetry receiver (ATR, Hydroacoustic Technology Inc., Seattle, WA) interprets the difference in detection times from multiple hydrophones (Figure 3.2) to produce an estimate of fish location during that ping. Individuals were identified by the frequency of their ping with periods ranging from 2146 ms to 3448 ms. An estimated relocation was produced for every ping, which means that the temporal resolution of fish movement was 2.1 – 3.4 s, depending on the individual.

Processing and positioning of the acoustic telemetry data was completed in consultation with experts at HTI, the company which produced the acoustic array, and Adrienne McLean, an aquatic technician at Fisheries and Oceans Canada (McLean et al. 2016). Relocation estimation errors can occur due to background noise, signal reflections, and other factors. A 95% error probable was constructed to determine the accuracy of the telemetry array. The error probable determines the radius in which 95% of the relocations of a stationary tag fall within. Three acoustic tags were used to produce a 95% error probable of 2.01 m ± 0.8979 (Mean ± SD) (McLean et al. 2016). A post-processing data filter was used to further remove erroneous relocation data inherent to acoustic telemetry data, and maximize accuracy of location estimates.
(Donaldson et al. 2015; Romine et al. 2015). Any relocations that did not occur within 2000-3000 ms of the previous relocation were removed. Next, any relocation that was >12 m away from the previous relocation was removed, as carps would not likely travel that distance within the 2-3 s period. Finally, if the number of relocations for an individual within an hour-long trial period did not fall within 0.75-1.1x the expected number of relocations for that individual and trial period, the trial data for that fish were removed. The number of relocation estimations for all individuals throughout the duration of the study was filtered from $3.68 \times 10^6$ data points to $1.04 \times 10^6$ data points. This was a relatively aggressive filtering process compared to other behavioural barrier studies (McLean et al. 2016; Hasler et al. 2016; Donaldson et al. 2015; Romine et al. 2015), but this was necessary to maximize accuracy for sensitize metrics such as travel velocity, and sufficient data remained for a robust statistical analysis.

Trials ran for one hour with a control trial run immediately after each stimulus trial. During the day trials, the first stimulus period started at 0900, and the last period started at 1500. During the night trials, the first stimulus period started at 2100, and the last period started at 0300. Trials were run for 1 h periods to minimize within-trial habituation and maximize replicates.

![Figure 3.2. Schematic diagram of the important experimental features of the mesocosm. The mesocosm is a ship slip enclosed with concrete on three sides. The entrance is blocked with a net to contain acoustically tagged subjects. Twelve hydrophones attuned to the acoustic tags were installed within the mesocosm to constantly record the location of every tagged fish. Six hydrophones were installed near the surface aiming downwards and six other hydrophones were installed at 7.5 m deep aiming upwards.](image-url)
3.3.4 Acoustic Profiles

Sound pressure was recorded within the mesocosm with a JASCO Ocean Sound Meter at a depth of 4 m. Ambient sound pressure was measured at seven points along the centered length of the mesocosm, and acoustic stimulus sound pressure was measured with 35 points along seven transects spanning the width of the mesocosm. Sound pressure was measured over a 60 s period, with the peak and root-mean square (RMS) values recorded under ambient- and acoustic-stimulus periods. Acoustic plots were then produced with the spline function in ArcGIS (ESRI 2014). Sound pressure is measured in decibels and, in underwater applications, is usually referenced to a pressure of 1 microPascal, which is lower than the reference used in standard air applications (Popper & Fay 1993). Peak sound pressure ranged from 137.8 to 156.6 dB re 1 µPa under ambient conditions and 170.5 to 191.6 dB re 1 µPa during stimulus periods (Figure 3.3). RMS sound pressure ranged from 128.0 to 129.7 dB re 1 µPa under acoustic conditions, and 152.7 to 175.2 dB re 1 µPa during stimulus periods. In both control and treatment periods, the open end of the mesocosm had the quietest acoustic conditions.

![Acoustic profiles of mesocosm sound pressure during ambient and acoustic-stimulus periods. Peak sound pressure over a 60 s period was plotted.](image)

3.3.5 Statistical Analysis

To plot the density of fish relocations estimated with the acoustic-telemetry array, kernel-density plots were calculated for the mesocosm. Kernel-density plots apply a smoothing function to discrete points to model the density of points across 2-D space (Calenge 2011). These plots were produced using ArcGIS (ESRI 2014). Conceptually, kernel densities are produced by building a 3D surface over the space of interest and adding a rounded cone centered on each
point to the surface. As cones overlap, they positively interact to produce peaks in areas of high point density (Figure 3.4). The conceptual elevation of the surface is used to produce the density plots. Kernel-density plots allow for the visualization of the relative density of fish relocations within the mesocosm. Since only relocations are being analyzed, kernel-density plots do not differentiate between a few individuals spending a long time in an area, or many individuals spending a short time in an area.

![Conceptual representation of a kernel-density smoothing function in 1D space. Red crosses represent discrete point features. Blue waves represent individual kernel ‘cones’ centred on each discrete point. Black line represents the surface elevation produced by the positive interference of interacting kernels. Image modified from http://fedc.wiwi.hu-berlin.de/xplore/tutorials/](image)

**Figure 3.4.** Conceptual representation of a kernel-density smoothing function in 1D space. Red crosses represent discrete point features. Blue waves represent individual kernel ‘cones’ centred on each discrete point. Black line represents the surface elevation produced by the positive interference of interacting kernels. Image modified from [http://fedc.wiwi.hu-berlin.de/xplore/tutorials/](http://fedc.wiwi.hu-berlin.de/xplore/tutorials/).

To estimate the space that individuals occupied across control, acoustic and strobe-light treatments, utilization-distribution plots were constructed. Utilization distributions are bivariate models used to estimate the ‘home range’ of individual with the use of x-, y-coordinate relocation data (Calenge 2011). A home range is an area where an individual performs its core activities and excludes any excursive activities (Hodder et al. 1998). The utilization distribution is modeled with a kernel function and coordinate data similar in concept to kernel-density estimations. Utilization-distribution plots were estimated using the ‘kernelUD’ function and habitat-area estimations were completed with the ‘mcp.area’ function in the R package adehabitatHR (Calenge 2006). The utilization distributions were not bound by mesocosm borders, so estimations were allowed to inflate beyond the real mesocosm structure. The estimated full habitat ranges of both buffalo and Common Carp were compared across treatment type (Control, Light, Sound) using a one-way ANOVA. Buffalo and Common Carp were
combined within the analysis to increase sample size. A Tukey’s HSD test was conducted post-hoc to determine where groups statistically differed.

Utilization distributions can use various proportions of relocation data to construct a home range and exclude extreme relocations considered to be excursive or exploratory in nature (Calenge 2011). Although, it is common to produce 95% home ranges and exclude the most extreme 5% of relocations (Hasler et al. 2016), even rare exploratory events could have dispersal consequences in the context of behavioural barrier; therefore, in this study, a 100% home range is used that includes all data points that passed the reliability filter.

To determine the association between fish relocations and sound-pressure levels, linear regression was used. To produce the regression, the mesocosm was divided into 422 hexagonal ‘bins’, each with an attributed kernel density value for each species and a peak sound-pressure value for the ambient- and acoustic-stimulus trials (Figure 3.5). Changes in kernel-density values were taken from the kernel-density plots and averaged for each bin. These values were then normalized as a percentage of the maximum bin-density value for that treatment type. The normalized density values from the ambient trials were subtracted from the values recorded during acoustic trials to determine the average change in kernel density for each bin between ambient and acoustic trials. To determine if there was a correlation between sound pressure and a decrease in fish density, the average change in bin kernel density was regressed against peak sound pressure during acoustic trials for each species. The analysis was conducted within the statistical program R (R Core Team 2016).
To determine if buffalo and Common Carp had fewer relocations near the strobe-light stimuli during operation, a Chi-squared test was conducted. The proximity between an individual and the strobe-light stimulus was determined for each relocation during control and strobe-light treatments. The profile of the strobe-light stimulus, which bisects the mesocosm, was mapped with 35 points spaced approximately 1 m apart and arranged linearly (Figure 3.6). Similar to Donaldson et al. (2015), the Euclidean distance between each fish relocation and all of the strobe-light profile points was calculated, and the shortest distance between each fish relocation and a strobe-light point was recorded.
Relocations were binomially categorized as either near the stimulus when Euclidean distances were <20 m, or far from the stimulus when Euclidean distances were >20 m. A distance of 20 m was used as the stimulus intensity beyond this distance was likely not behaviourally significant (Bzonek, pers. obs.). A study conducted on Rainbow Smelt (*Osmerus mordax*) determined that the application of strobe-light stimuli could produce an avoidance response to strobe-light stimuli up to a distance of 15-21 meters (Hamel et al. 2008). To determine whether the relative frequency of relocations near the strobe-light stimulus was impacted by the activation of the stimulus, a Chi-squared test was calculated using the statistical program R (R Core Team 2016).

To determine if buffalo and Common Carp had fewer and faster average travel velocity near the strobe-light stimuli during operation, a nested two-way ANOVA was conducted. Only travel velocity near (< 20 m) the stimulus was analysed. Travel velocity was compared across treatment type (control, strobe-light) and species (buffalo, Common Carp), with time of day (day, night) nested within treatment type. As with the near-far classification, a distance of 20 m was used as individuals beyond this distance from the stimulus would likely not be behaviourally impacted by the activation of the strobe light. A Tukey’s HSD test was conducted post-hoc to determine differences between groups. The analysis was conducted within the statistical program R (R Core Team 2016).
3.4 Results

3.4.1 Kernel Density

High buffalo relocation densities appeared throughout the mesocosm during control trials, with some apparent preference towards the open end of the mesocosm (Figure 3.7). During the acoustic-stimulus periods, there appeared to be some avoidance of the mesocosm centre (Figure 3.8), but no changes were apparent between the control and strobe-light stimuli (Figure 3.9). Across all treatment types, Common Carp appeared to avoid the centre and strongly preferred the open end of the mesocosm (Figure 3.10; Figure 3.11; Figure 3.12), which makes interpreting avoidance during the stimulus trials difficult.

When investigating the change in kernel density of the acoustic trials compared to control trials, it appears that both buffalo and Common Carp are avoiding the mesocosm centre where the acoustic stimulus is placed (Figure 3.13; Figure 3.15). For both buffalo and Common Carp, the application of strobe-light stimuli did not produce a bisecting decrease in kernel density (Figure 3.14; Figure 3.16), as would be expected if individuals were avoiding the strobe-light stimulus.
Figure 3.7. Kernel density of buffalo relocation densities across all control trials.

Figure 3.8. Kernel density of buffalo relocation densities across all acoustic trials.

Figure 3.9. Kernel density of buffalo relocation densities across all strobe-light trials.
Figure 3.10. Kernel density of Common Carp relocation densities across all control trials.

Figure 3.11. Kernel density of Common Carp relocation densities across all acoustic trials.

Figure 3.12. Kernel density of Common Carp relocation densities across all strobe-light trials.
3.4.1.1 Relative Change in Kernel Density

Figure 3.13. Change in relative kernel density of buffalo relocations between control and acoustic trials.

Figure 3.14. Change in relative kernel density of buffalo relocations between control and strobe-light trials.
Figure 3.15. Change in relative kernel density of Common Carp relocations between control and acoustic trials.

Figure 3.16. Change in relative kernel density of Common Carp relocations between control and strobe-light trials.
Utilization Distribution

The estimated utilization-distribution surface areas for tagged fishes were determined to differ across the control, acoustic, and strobe-light treatment types ($F_{\text{stat}} = 4.922$, $p=0.0177$, DF=21) (Figure 3.17). Individuals occupied a smaller area during the strobe-light treatments ($0.3466 \text{ ha} \pm 0.0249$) than they did for the control ($0.3796 \text{ ha} \pm 0.0184$) and acoustic treatments ($0.3564 \text{ ha} \pm 0.0206$). Variance in utilization-distribution surface area between individuals was smaller in control treatments ($0.00035 \text{ ha}$) than in strobe-light ($0.00062 \text{ ha}$) or acoustic treatments ($0.00042 \text{ ha}$).

![Figure 3.17. 100% kernel-density habitat-area estimations for buffalo and Common Carp individuals across control, strobe-light, and acoustic stimuli. Letters denote statistical differences in mean area estimations across treatments. Home-range plots above the letters show individual utilization distributions for all fishes across treatment types. Buffalo or Common Carp with smaller utilization distributions are stacked above individuals with larger distributions.](image)

3.4.2 Acoustic-Stimulus Regression

Peak sound pressure explained very little of the variation in the change in bin kernel density for buffalo ($r^2=0.029$, df=420, tstat=3.691, $p=0.0003$) and Common Carp ($R^2=0.06$, df=420, tstat=1.931, $p=0.0541$) (Figure 3.18). In both species, the greatest changes in kernel density were found in bins with relatively low sound pressure (<175).
Figure 3.18. Association between mesocosm sound pressure and change in normalized bin kernel density for buffalo and Common Carp. Change in normalized bin kernel density was calculated by subtracting normalized ambient density values from acoustic density values.

3.4.3 Strobe-Light Chi-squared for Proximity

Buffalo and Common Carp spent most of their time >30m away from the strobe-light stimuli regardless of whether the stimulus was operating (Figure 3.19). As had been seen with the kernel-density plots (Figure 3.7; Figure 3.10), individuals preferred the open-ended edge of the mesocosm over all other areas.

Figure 3.19. Violin plots displaying the relative proportion of relocations found at varying distances from the strobe-light stimulus. Relocations are compared across species, treatments, and time of day.
In both buffalo (DF=1, Chi²=422, p=8.5e⁻⁹⁴) and Common Carp (df=1, Chi²=716, p=8.9e⁻¹⁵⁸), an association was found between relocation proximity and treatment type. When the strobe-light stimulus was operating, there were significantly fewer relocations near the stimulus than when the strobe light was off. For buffalo, the proportion of relocations near the stimulus decreased from about 8.7% during control trials to 5.6% during strobe-light trials (Figure 3.20). For Common Carp, the proportion decreased from 2.8% during control trials to 1.1% during strobe-light trials (Figure 3.20).

![Figure 3.20. Total count of relocations near (<20m) or far (>20m) from the stimulus during all control or strobe-light treatments. Both buffalo and Common Carp expressed an association between treatment type and proportion of relocations near the strobe-light stimuli.](image)

### 3.4.4 Strobe-Light ANOVA for Travel Velocity

Travel velocity differed across treatment type, time of day, and species as determined by nested two-way ANOVA (Table 3.1). During the day, the application of strobe-light stimuli did not increase travel velocity of buffalo or Common Carp; however, during the night, the application of strobe-light stimuli did increase travel velocity for both buffalo and Common Carp (Figure 3.21). Across treatment types and time of day, Common Carp consistently had faster travel
velocities than buffalo. During the day trials, buffalo mean travel velocity was 0.185 m/s under control treatments and 0.232 m/s during strobe-light treatments. Common Carp velocity during the day was 0.523 m/s under control treatments and 0.556 m/s under strobe-light treatments. During the night trials, buffalo mean travel velocity increased from 0.140 m/s during control treatments to 0.257 m/s during strobe-light treatments. Common Carp mean travel velocity increased from 0.283 m/s during night control trials to 0.581 m/s during night strobe-light treatments.

Table 3.1. Statistical results from movement velocity nested two-way ANOVA. Travel velocity was compared across treatment type and species with time of day nested within treatment type. Stars beside factors indicate significant differences between groups.

<table>
<thead>
<tr>
<th>Type</th>
<th>Df</th>
<th>Sum Sq</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residuals</td>
<td>16809</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>*Species</td>
<td>1</td>
<td>198</td>
<td>879</td>
<td>2e-16</td>
</tr>
<tr>
<td>*Type</td>
<td>1</td>
<td>43</td>
<td>190</td>
<td>2e-16</td>
</tr>
<tr>
<td>*Species:Type</td>
<td>1</td>
<td>7</td>
<td>29</td>
<td>7e-8</td>
</tr>
<tr>
<td>* Species:Type:AmPm</td>
<td>4</td>
<td>69</td>
<td>77</td>
<td>2e-16</td>
</tr>
</tbody>
</table>
Figure 3.21. Influence of treatment type, species type and time of day on estimated travel speed. Letters indicated statistical differences between groups as determined by nested two-way ANOVA. Box represents 25th, 50th and 75th percentiles, and whiskers represent 1.5x interquartile range.

3.5 Discussion

Acoustic stimuli failed to produce a significant difference in home-range size, or a significant correlation between change in kernel density and stimulus sound pressure. This is likely due, in part, to buffalo and Common Carp preferences towards the open end of the mesocosm, and the unusually loud sound profile. The strobe-light stimuli produced a significantly smaller home-range size and fewer relocations, and a faster night-time travel velocity near an operating strobe light. Travel velocity was not significantly different between control and strobe-light trials during the day. There were no meaningful differences between buffalo and Common Carp for any of the metrics analyzed.
3.5.1 Acoustic Stimulus

This study did not find a significant response in fishes exposed to the acoustic stimuli, regardless of the metric used. Kernel-density plots investigated the relative density of fish relocations for all tagged individuals within a species, and all trials within a treatment. Density values were highest near the open end of the mesocosm for both the control (Figure 3.7; Figure 3.10) and the acoustic treatment (Figure 3.8; Figure 3.11). Additionally, changes in kernel density between the control and acoustic treatment are smaller in magnitude near the stimuli than near the open end of the mesocosm (Figure 3.13; Figure 3.15). This bias was seen in both species, but was more apparent in Common Carp. Any changes between stimulus and control kernel densities in the open end of the mesocosm may not be necessarily driven by stimulus-induced behavioural changes, but rather stochastic movement, or responses to environmental changes such as water flow. The kernel-density bias towards the open end would increase the relative impact of stochastic movement on the change in kernel-density plots (Figure 3.13; Figure 3.15).

The utilization-distribution home-range area did not appear to decline with the application of acoustic stimuli. It was expected that fishes would avoid regions of high sound pressure (Maes et al. 2004) and, as a consequence, occupy less space within the closed mesocosm. The lack of a significant decline in home range may be due to an insufficient avoidance response to the acoustic stimuli, or a result of individuals being held within an enclosure smaller than their natural home range. Hasler et al. (2016) conducted a home-range analysis on Bluegill (Lepomis macrochirus) and found that, while fish expressed other behavioural changes, home range did not change in response to CO$_2$ concentration. Their study proposed that the truncation of potential home-range size may have confounded the ability to resolve any changes in home range. Similar to Hasler et al. (2016), fishes enclosed in my mesocosm were unable to realize their full home-range size potential.

Finally, the application of acoustic stimuli did not produce the expected reduction in kernel densities in regions of high sound pressure. Stimulus sound-pressure level was expected to be negatively correlated with changes in kernel density, but sound pressure did not explain a meaningful level of variation ($r^2=0.029$).
3.5.2 Strobe-light Stimulus

This study found significant movement responses to strobe-light stimuli for some, but not all, metrics used. Kernel densities during the strobe-light stimulus were highest near the open end of the mesocosm (Figure 3.9; Figure 3.12), similar to the densities of the acoustic and control treatments. This also led to the changes in kernel density between the control and strobe-light treatments expressing a greater magnitude near the stimuli than near the open end of the mesocosm (Figure 3.14; Figure 3.16). This bias was seen in both species, but was more apparent in Common Carp.

Fishes occupied a significantly smaller home range under strobe-light stimuli than they did during the control or acoustic treatment. This may be because fishes were less likely to conduct exploratory behaviour when exposed to the foreign stimuli or that they were avoiding areas near the strobe-light stimuli. A field study investigating the response of Rainbow Smelt to strobe lights determined that the operation of the stimulus led to a startle response and an abrupt decline in fish density near the stimuli (Hamel et al. 2008). In a similar response, buffalo and Common Carp may be avoiding the stimulus, leading to individuals occupying a smaller home-range within the enclosed mesocosm.

Further evidence of buffalo and Common Carp avoiding the strobe lights can be seen with the reduction in relocations found near the stimulus when active compared to inactive. This is consistent with a study conducted on Sockeye Salmon (*Oncorhynchus nerka*) that found an 80% reduction in salmon density within 30 m of a strobe-light stimulus (Maiolie et al. 2001). Across both treatments, Common Carp spent less time near the stimulus than buffalo. This is likely because Common Carp were more heavily biased towards the open end of the mesocosm than the buffalo.

The strobe-light stimulus was also capable of increasing the travel velocity of buffalo and Common Carp during the night, but not during the day. At night, mean travel velocity within 20 m of the strobe light was 1.81 times faster when the stimulus was active for buffalo, and 2.05 times faster when the stimulus was active for Common Carp. These results are consistent with Konigson et al. (2002), who studied European Whitefish (*Coregonus lavaretus*) at night within a net-pen enclosure and found that individuals turned away and swam faster after being exposed to strobe-light stimuli. In both studies, movement is likely an avoidance response (Richards et al.
to an aversive, non-natural stroboscopic stimulus (Mesquita et al. 2008).

3.5.3 Comparison of Acoustic and Strobe-light Stimuli

The acoustic stimulus did not perform as a behavioural barrier, but the strobe-light stimulus did, for most metrics tested (Table 3.2). The change in kernel-density plots did not express a significant avoidance near the acoustic stimulus, home range did not decrease relative to the control, and sound pressure did not explain variation in changes in kernel-density plots. The home range may not have decreased during the acoustic treatment due to the mesocosm truncating the potential home range size of individuals. However, home ranges exhibited during the strobe-light trials would have been similarly affected, but they exhibited significantly smaller home ranges than the control or acoustic treatment. The strobe-light treatment also did not produce predicted changes in kernel density. However, application of the strobe-light stimulus produced significantly smaller home ranges, fewer relocations near the stimulus, and a faster travel velocity at night (Table 3.2).

Table 3.2. Summary of the statistical tests conducted to evaluate whether acoustic and strobe-light stimuli acted as behavioural barriers based on movement responses of buffalo and Common Carp.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Analysis</th>
<th>Barrier?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acoustic</td>
<td>Kernel Density</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Home Range</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Acoustic Regression</td>
<td>No</td>
</tr>
<tr>
<td>Strobe-light</td>
<td>Kernel Density</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Home Range</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Proximity Chi-squared</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Travel Velocity ANOVA: Day</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Travel Velocity ANOVA: Night</td>
<td>Yes</td>
</tr>
</tbody>
</table>
No differences between buffalo and Common Carp were found in their responses to acoustic or strobe-light treatments. Common Carp did have a significantly faster travel velocity than buffalo (Figure 3.21), but both species responded to the stimulus in the same way. This indicates that the behavioural barriers tested may not express the species specificity required to inhibit targeted invasive species, but not non-target native species.

3.5.4 Comparison to Other Studies

The lack of significant movement responses to the acoustic stimulus is inconsistent with recent findings regarding the use of acoustic stimuli as a deterrent against Asian carp passage (Vetter et al. 2015; Flammang et al. 2014; Sloan et al. 2013; Ruebush et al. 2012). This may be due to the acoustic stimulus and resulting acoustic environment being much louder in this study than other studies. The mesocosm acoustic stimulus had a RMS sound pressure of 175.2 dB re 1 µPa, which was 45.5 dB above ambient conditions, while Vetter et al. (2015) had a stimulus pressure of 150 dB re 1 µPa, 30 dB above ambient, and Zielinski et al. (2014) had a stimulus pressure of 130 dB, 50 dB above ambient conditions (N.B. sound pressure values from literature are assumed to be RMS values). The quietest region of the mesocosm during the acoustic treatment was 152.7 re 1 µPa, which is louder than the loudest regions of the other two studies. If fishes did not deem the quietest regions of the mesocosm to be suitable acoustic refuge, they may have continued to search for an escape, or further refuge, and shown no preference for these areas. This has important implications for the maximum stimulus and ambient sound pressure that could be used for an effective acoustic barrier.

The avoidance response to the strobe-light stimuli was less significant in the mesocosm than found in previous literature (Maiolie et al. 2001). Maiolie et al. (2001) found an 80% reduction in salmon density within 30 m of a strobe-light stimulus while my study found a 35.7% reduction in buffalo, and a 60.7% reduction in Common Carp within 20 m of the stimulus. This may be due to differences in strobe-light design (Sullivan et al. 2016) or study design. Maiolie et al. (2001) conducted their study in an unenclosed pelagic region of natural lakes, as opposed to an enclosed mesocosm. Barrier stimuli may be less effective in mesocosms than in natural environments due to a simpler, smaller environment, and the consequent re-encountering of the stimulus (Flammang et al. 2014). Additionally, there are likely taxon-specific responses to strobe
Richards et al. (2007) investigated the stress responses of Fathead Minnow (*Pimephales promelas*), Chinook Salmon (*O. tshawytscha*), and other species to stroboscopic stimuli, and determined that cyprinids may be less sensitive than salmonids to strobe lights. Thus, the open pelagic environment, and greater taxon-specific sensitivity to the strobe-light stimulus may have led to a greater avoidance response in the Maiolie et al. (2001) study than found in my study.

Time of day also has important implications for strobe-light efficacy. Many strobe-light studies have investigated fish responses at night or in darkness (Sullivan et al. 2016; Flammang et al. 2014; Mesquita et al. 2008; Richards et al. 2007; Königson et al. 2002). Fewer studies have investigated movement or behavioral responses of fishes to strobe-light stimuli during the day (Rooper et al. 2015; Maiolie et al. 2001). Within the mesocosm, buffalo and Common Carp did not increase travel velocity in response to strobe-light stimuli during the day. This indicates that potential strobe-light behavioural barriers may be less effective during the day than at night. Previous studies have determined that acclimation to ambient lighting and the intensity of strobe-light stimuli can impact the magnitude of fish response (Mesquita et al. 2008). The lack of a travel-velocity response in the mesocosm at day may be due to the ambient luminosity of the mesocosm reducing the relative impact of the strobe-light stimuli.

### 3.5.5 Mesocosm Shortcomings and Future Studies

Across all species (buffalo, Common Carp) and treatments (control, acoustic, strobe-light), fishes appeared to strongly prefer the open end of the mesocosm. Individuals may have been attracted there by the presence of algae along the block nets or the passive movement of water into the mesocosm. Future mesocosm studies should include positioning the barrier stimuli near the open end of the mesocosm. Successful behavioural barrier stimuli would be capable of driving fishes away from the open end of the mesocosm. Vetter et al. (2015) successfully drove Silver Carp from end to end in a small-scale laboratory setting, but there has been no published studies where driving fishes end to end was attempted with acoustic stimuli at a larger scale.

This study did not find any significant movement responses in fishes exposed to the acoustic stimuli. This may be due, in part, to the extremely high sound pressure throughout the mesocosm during the acoustic treatment and the lack of sufficient acoustic refuge. If there is a limit to the
effective sound pressure that can be used in an acoustic barrier, this has important implications for the deployment options of an acoustic barrier. The ambient acoustic profile of the mesocosm was unusually high with an RMS sound pressure of 128.0 dB re 1 µPa, compared to ambient sound pressures in the lab of 120 dB re 1 µPa (Vetter et al. 2015) and 80 dB re 1 µPa (Zielinski et al. 2014). The mesocosm was unusually loud because it was within 100 meters of a major highway at the Burlington Bay Skyway. Additionally, the skyway was under maintenance construction during the operation of trials. Ambient sound pressure is an important factor because a sufficient pressure gradient is necessary to produce an avoidance response (Noatch & Suski 2012). If there is a maximum stimulus sound pressure, beyond which the avoidance behaviour breaks down, then acoustic barriers are limited to environments where the ambient acoustic profile is low enough to allow for a sufficient sound pressure gradient that does not surpass the behavioural sound pressure maximum near the stimulus. As a consequence, acoustic barriers may not be suitable near major highways and existing barriers may be negatively impacted by nearby construction. Future work should measure fish responses to varying sound pressure levels within the same site to determine if avoidance behaviour breaks down after at extremely high sound pressure levels. Additionally, acoustic studies could investigate the movement responses of fishes to the same acoustic stimulus sound pressure, and varying magnitudes of ambient acoustic profiles.

3.5.6 Conclusion

In conclusion, the strobe-light stimulus displayed some characteristics of a successful behavioural barrier, but the acoustic stimulus did not. Fishes were consistently attracted to the open end of the mesocosm, and the ambient and acoustic stimulus sound profiles were louder than seen in other barrier studies. These factors likely led to the lack of significant responses to the acoustic stimulus. Fishes exposed to strobe lights occupied less space, avoided the stimulus, and traveled faster near the stimulus at night. There were no meaningful differences between buffalo and Common Carp for any of the metrics analyzed.
4 General Conclusions

To my knowledge, no previous study has used both laboratory and mesocosm experiments to investigate the responses of fishes to the same aversive stimuli within the context of potential Asian carp behavioural barriers. This study did so with both a stroboscopic stimulus and a complex acoustic signal as potential behavioural-barrier stimuli. Close comparisons can be made between the two experimental scales to help develop a more comprehensive understanding of the responses likely to be seen with a strobe-light or acoustic behavioural-barrier deployment.

This study determined that behavioural responses within a laboratory setting are consistent with movement responses in a mesocosm setting. This was seen in the strobe-light stimulus with the increase in time spent moving actively within the laboratory (Figure 2.3) and the increase in average travel velocity within the mesocosm (Figure 3.21). An indirect consequence of active movement is a faster travel velocity. Consequently, Common Carp within the mesocosm, which had a faster travel velocity near the operating strobe-light stimulus, would also have spent a greater proportion of time moving actively. This finding is important as it provides validation for laboratory studies (Vetter et al. 2015; Zielinski et al. 2014; Flammang et al. 2014) and indicates that stimulus-induced behavioural changes can lead to the larger-scale movement responses, which would be required for behavioural barriers to effectively prevent Asian carps from traveling across dispersal corridors.

The behavioural findings from the laboratory study may have illuminated methodological issues within the mesocosm study. The laboratory video trials indicated that exposure to acoustic and strobe-light stimuli induce short-term behavioural changes in Common Carp, even after the stimulus has stopped operating. The duration of time spent moving actively and the number of passes made across the stimulus by Common Carp were both higher in the post-stimulus period than in the control period (Figure 2.3). This indicates that the trial schedule within the mesocosm may not have sufficiently separated the movement responses between the control and stimuli trials. Control trials occurred directly after acoustic or strobe-light trials. If the fish responses during control trials were still influenced by the stimulus trials, then any true responses to the strobe-light and acoustic stimuli may have been confounded.

The combination of both experimental scales may also yield deeper understanding of the utility of strobe lights within behavioural-barrier technologies. Strobe lights did show movement
responses in both buffalo and Common Carp, but efficacy was impacted by daylight and ambient lux. Additionally, strobe lights failed to produce a significant orientation response within the laboratory study. This is a concern as a leaky barrier, less effective during the day, could dramatically increase the likelihood of Asian carp establishment in the Great Lakes, even if only a few fish pass the barrier each year (Cuddington et al. 2014). Finally, previous studies have determined that, in addition to luminosity, strobe-light barriers are also susceptible to changes in turbidity, water velocity, and biological factors such as acclimation, prey attraction, and fish life stage (Richards et al. 2007). Due to these vulnerabilities found at both levels of investigation, strobe lights may not be suitable as a stand-alone behavioural barrier.

Finally, findings from both study scales can help inform management efforts on the potential strengths and weaknesses of a comprehensive integrated-barrier system. As determined through the video-recorded laboratory trials, the combination of strobe-light and acoustic stimuli did not increase the magnitude of response over either individual response (Figure 2.3). This has implications for how comprehensive barrier systems could be implemented. Comprehensive barriers should not be designed with additional stimuli used to modulate the magnitude of response, but rather, stimuli from multiple sensory systems should be used to produce a more robust barrier that remains effective across a greater range of environmental conditions and is less susceptible to habituation (Noatch & Suski 2012). For example, acoustic stimuli may be less effective during periods of high background noise, such as construction or barge traffic; during these times, strobe-light stimuli could be used to produce the appropriate behavioural response. Alternatively, strobe-light stimuli are most effective at night and in clear water (Noatch & Suski 2012), so acoustic components could be used when these conditions are not met.

In conclusion, acoustic and strobe-light stimuli are both capable of producing responses in ostariophysian fishes. The application of acoustic stimuli in the laboratory produced behavioural responses of increased activity and increased post-stimulus passes beyond the stimulus. However, in the mesocosm study, an acoustic stimuli that produced extremely high sound-pressure levels could not produce significant avoidance responses. The strobe-light stimulus produced the same behavioural responses in the laboratory study and produced avoidance responses in the mesocosm study. Finally, the combination of both acoustic and strobe-light stimuli did not increase the magnitude of any behavioural response in the laboratory study. In the mesocosm study, buffalo and Common Carp did not differ in their movement responses to either
stimulus. Based on these results, acoustic and strobe-light stimuli both elicit dispersal-modifying behaviour that may be worthwhile contributions to an integrated barrier. However, both stimuli are vulnerable to changes in environmental conditions and failed to produce an absolute avoidance response. Thus, neither stimulus may be suitable as a stand-alone barrier technology within critical Asian carp dispersal corridors. Future work should continue to use detailed, behavioural laboratory studies to investigate the responses of Asian carps and other ostariophysians to acoustic and strobe-light stimuli. Additionally, proof-of-concept studies in natural systems are necessary before any behavioural stimuli is deployed within a dispersal corridor. This is needed to ensure behavioural barrier reliability, and maximize efficacy. Finally, other physiological and behavioural stimuli, such as CO₂, should be investigated on their own and in combination with acoustic and strobe-light stimuli to further define the strengths and weaknesses of potential integrated barriers.
5 References


Anon, 2016. asiancarp.ca. Invasive Species Centre. Available at: http://asiancarp.ca.


