Effects of broadband sound and alarm cue on the behaviour of Common Carp

Jaewoo Kim, Kyle D. Crans and Nicholas E. Mandrak

Fisheries and Oceans Canada
Asian Carp Program
867 Lakeshore Road
Burlington, ON
L7S 1A1

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EFFECTS OF BROADBAND SOUND AND ALARM CUE ON THE BEHAVIOUR OF COMMON CARP

by

Jaewoo Kim, Kyle D. Crans and Nicholas E. Mandrak

Fisheries and Oceans Canada
Asian Carp Program
867 Lakeshore Road, Burlington, ON, L7S 1A1
Email: jkim1017@hotmail.com

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ABSTRACT

Kim, J., Crans, K.D. and Mandrak, N.E. 2021. Effects of broadband sound and alarm cue on the behaviour of Common Carp. Can. Manuscr. Rep. Fish. Aquat. Sci. 3209: iii + 13 p.

Permanent barriers are considered most effective in preventing the movement of invasive fishes, but may not be feasible due to costs and/or logistical constraints. Alternative non-permanent barriers using electricity, light, sound, pressure, and bubbles are currently being developed and deployed for the management of aquatic invasive species. However, the effectiveness of these barriers is not well understood. We conducted a series of laboratory trials to examine the effects of broadband sound and alarm cue on the behaviour of Common Carp, *Cyprinus carpio* (Linnaeus 1758). In response to either the broadband sound or alarm cue, Common Carp did not change their positions relative to the origin of each stimulus within an experimental tank. In addition, their behavioural responses did not significantly differ among two, five, 15, and 30 minute exposures. Our results indicate that broadband sound or alarm cue may not be an effective control tool for Common Carp and highlight the importance of testing non-permanent fish-barrier technologies in a laboratory setting when considering management options for dealing with invasive species.

RÉSUMÉ

Kim, J., Crans, K.D. and Mandrak, N.E. 2021. Effects of broadband sound and alarm cue on the behaviour of Common Carp. Can. Manuscr. Rep. Fish. Aquat. Sci. 3209: iii + 13 p.

Les barrières permanentes sont considérées comme le moyen le plus efficace pour empêcher la circulation des poissons envahissants, mais celles-ci peuvent ne pas être réalisables en raison de contraintes de coût ou de logistique. Des barrières de rechange non permanentes utilisant l’électricité, la lumière, des sons, la pression ou des bulles sont en cours de conception et de déploiement aux fins de la gestion des espèces aquatiques envahissantes. L’efficacité de ces barrières demeure cependant mal comprise. Nous avons réalisé une série d’essais en laboratoire afin d’examiner les effets de signaux d’alarme à large bande et sur le comportement de la carpe commune (*Cyprinus carpio*) [Linnaeus 1758]. En réponse aux signaux d’alarme à large bande, les carpes communes n’ont pas changé de position relativement à l’origine de chaque stimulus à l’intérieur d’un bassin expérimental. De plus, leurs réponses comportementales n’ont pas varié de manière considérable lorsqu’elles ont été soumises à des expositions de deux, de cinq, de quinze et de trente minutes. Nos résultats indiquent que les signaux d’alarme à large bande ne semblent pas constituer un outil de contrôle efficace pour la carpe commune et font ressortir l’importance de mettre à l’essai en laboratoire des technologies de barrières à poissons non permanentes afin de déterminer les meilleures options pour la gestion des espèces envahissantes.

INTRODUCTION

Common Carp, *Cyprinus carpio* (Linnaeus 1758) is native to Eurasia and has been introduced to fresh waters globally (Scott and Crossman 1973; Sorensen and Bajer 2011). Common Carp is considered detrimental to native fish populations as it increases turbidity by uprooting submerged vegetation (Scott and Crossman 1973; Weber and Brown 2009). Extensive research and management efforts have been conducted in an attempt to limit the spread of Common Carp because of their significant environmental impacts through vegetation removal, such as loss or degradation of wetlands and nursery habitats for fishes, birds, and other species (Sisler and Sorenson 2008; Weber and Brown 2009; Huntingford et al. 2010; Zielinski et al. 2014; Kim and Mandrak 2017).

To manage invasive species, such as Common Carp, there has been a long history of barriers being used to stop, slow, or direct fish movement, with mixed success. Barriers fall into one of two main categories, physical and non-physical. Physical barriers, while effective, are often expensive to install and maintain, inhibit navigation, prevent native fish movements, and modify the natural physical processes of the waterway (e.g., flow regimes). Non-physical barriers, which can include stimuli such as sound, light, bubbles, electricity, and chemicals, either alone or in concert, have been the recent focus of many studies (Noatch and Suski 2012; Ruebush et al. 2012; Gross et al. 2013; Johnson et al. 2014; Zielinski et al. 2014; Vetter et al. 2015, 2017; Sullivan et al. 2016; Kim and Mandrak 2017; Zielinski and Sorensen 2017). These barriers can offer an attractive alternative to physical barriers as they are often less costly than physical barriers, can be deployed quickly, can be taxon-specific, and may not inhibit natural processes or navigation (Noatch and Suski 2012; Murchy et al. 2016). However, the effectiveness of non-physical barriers at preventing the movement of fishes is still being explored.

To date, there have been many studies examining the efficacy of non-physical barriers. Bubble barriers have been shown to reduce the passage of juvenile Common Carp by up to 80% in the lab, and 60% in the field, when the barrier was deployed across the entire width of the channel (Zielinski et al. 2014, Zielinski and Sorensen 2016). Often, bubble barriers are used as a sound-generation tool, rather than a visual barrier (Zielinski et al. 2014). Juvenile Silver Carp, *Hypopthalmichthys molitrix* (Valenciennes 1844) showed negative phototaxis to the origin of broadband sound (defined as a recording of an outboard motor; 0 – 10 000 Hz) in a semi-natural experimental pond (Vetter et al. 2015). Similar results were found with juvenile Bighead Carp, *Hypophthalmichthys nobilis* (Richardson 1845), juvenile Silver Carp, and adult Common Carp (Vetter et al. 2017; Zielinski and Sorensen 2017). In addition, the effects of strobe lights have been tested on Common Carp in a laboratory setting. Adult Common Carp were found to avoid the area around a lit strobe light in experimental tanks during light periods, perhaps pointing towards the effectiveness of this technology as a potential deterrent (Kim and Mandrak 2017). The use of chemicals as potential non-physical barriers is a growing area of research. One such naturally occurring chemical is the alarm cue (Smith 1992; Brown et al. 2011; Lucon-Xiccato et al. 2016). This is a substance that fishes release in response to injuries received from predators (Kim et al. 2004; Kim et al. 2011; Di Rocco et al. 2014; Perrault et al. 2014). The release of an alarm cue can prompt a variety of reactions by other fishes in the area of the release. In a study using juvenile Rainbow Trout, *Oncorhynchus mykiss* (Walbaum 1792), fish exposed to a conspecific skin homogenate containing alarm cue prior to interaction with a completely novel predator showed a stronger anti-predator response (e.g., reduced activity and foraging behaviour) than fish that had not been exposed (Mirza and Chivers 2003). The use of a necromone (Wagner et al. 2011), a pheromone released from decaying flesh, has been shown to be effective in eliciting avoidance behaviour in the field by Sea Lamprey, *Petromyzon marinus* (Linnaeus 1758). Such a result indicates that alarm cues are candidates for incorporation into non-physical barriers (Imre et al. 2010; Wagner et al. 2011). Integration of pest control using pheromones including alarm cues have been recommended as viable and practical management options for nuisance fish control (Sorensen and Stacey 2004; Sorensen and Johnson 2016). The body of research on this topic has been growing, but more insights are still needed into the efficiency of these barriers in different environments including lab, mesocosm, and field scales, and combinations of, and their effects on different fish species and age classes.

Common Carp is well suited for this study, as this species continues to be one of the most widespread invasive species in the world (Weber and Brown 2009). Common Carp belongs to Ostariophysii, a superorder of fishes that possess Weberian apparatus (Bird and Hernandez 2007). The primary function of the Weberian apparatus is to transfer mechanical energy caused by oscillations in the swim bladder, due to interactions with the sound pressure waves, to the inner ear (Bird and Hernandez 2007; Vetter et al. 2017). Common Carp is most sensitive to frequencies between 800 and 1000 Hz (Kojima et al. 2005). In contrast, the Paddlefish, *Polyodon spathula* (Walbaum in Artedi 1792) and Lake Sturgeon, *Acipenser fluvescens* (Rafinesque 1817) display frequency sensitivity between 100 – 500 Hz (Lovell et al. 2005; Lovell et al. 2006). This disparity between auditory capacities is important, as it shows potential for sound to be developed into a species-specific barrier in the future. Laboratory tests were conducted on Common Carp to evaluate the effectiveness of both sound and an alarm-cue stimulus as a non-physical barrier. This was a proof of concept test to determine if these specific deterrents would have potential to block or influence the movement of Common Carp.

METHODS

ANIMAL HUSBANDRY

Adult Common Carp were captured from Hamilton Harbour, at the western end of Lake Ontario, via boat electrofishing. The individuals collected averaged 3.71 kg (± SE = 0.11, N = 54) in weight and 510 mm (± SE = 5.8, N = 54) in fork length. Prior to trials, fish were examined for external injuries and only those with no obvious external injuries were used in the trials. Fish were housed in a series of recirculating tanks (689 and 1,647 l) with a stocking density of 50 g/l at the Aquatic Life Research Facility at the Canada Centre for Inland Waters in Burlington, Ontario, Canada. All fishes were housed in several large aerated tanks with dechlorinated water (12 h:12 h light:dark photoperiod; water temperatures 12–15 °C). Fish were given a two-week quarantine after being brought into the laboratory to prevent the spread of disease by keeping the holding tanks on a flow-through system (0.5-1 l/g biomass/day with no water turnover per day) and performing formalin treatments when necessary. After the quarantine period, tanks were switched to a recirculation schedule of at least 10% water turnover per day. Common Carp were fed a commercial sinking pellet (Proficient Trout Chow, Martin Mills, Inc.) at 0.5-1% of fish biomass in each tank three times a week.

ALARM CUE PREPARATION

To prepare the alarm-cue solution, a skin sample was taken from a euthanized Common Carp (Kim et al. 2011) immediately after euthanization. The alarm cue was filtered and diluted to meet a concentration of 0.1 cm^2/ml of skin extracts, which have been shown to elicit anti-predator responses in freshwater fishes including ostariophysians (Brown et al. 2011; Kim et al. 2011). The skin was cut from the trunk of the fish (posterior of the gills to the caudal peduncle) with a fileting knife. The scales were then carefully removed from the skin sample. The skin was cut into smaller pieces (<15 cm^2) and placed in 500 ml of water. The skin sample was homogenized using a blender to release as much alarm cue as possible. A cotton filter was used to separate the homogenate (skin tissue) from the alarm cue released in the water. The filter was then tied off to prevent skin pieces from escaping and the rest of the water was poured over it to maximize the extraction of the alarm cue. The alarm-cue solution was divided into smaller portions and frozen at or below -20 °C until use. During the entire procedure, as well as during the behavioural trials, skin and water samples were kept on ice to minimize degradation of the alarm cue (Kim et al. 2004; Kim et al. 2011).

EXPERIMENTAL TANKS

Behavioural experiments were conducted in a series of three rectangular experimental tanks, each made of ceramic and plastic materials (3.6 X 1.1 X 0.39 m) with water filled to the top. All experimental tanks were set up with a grid system (Figure 1), where it was divided into six identical sections (0.55 X 1.2 m), with red duct tape. The tank was surrounded by opaque curtains to prevent fish from being disturbed during trials. Prior to each trial after the acclimation period, flow and aeration was turned off to maximize the visibility of camcorders, and minimize any interference including sound with flow and bubbles. The tanks were drained and cleaned between each trials.

BEHAVIOURAL EXPERIMENTS

Prior to each trial, three individual fish were introduced to an experimental tank at least 16 hours before the trial for acclimation. Each trial consisted of three consecutive phases of 30 minutes. Each phase was recorded using an underwater camcorder (GoPro) that was positioned at one end of the tank to allow for filming down the length of the tank and an overhead camcorder (XA25, Canon) that was placed at the same end of the tank (Figure 1). All individuals were used only once for either alarm-cue or sound trials. For alarm-cue trials, there were pre-stimulus, control stimulus, and alarm-cue stimulus phases. For the sound trials, there were pre-stimulus, stimulus, and post-stimulus phases.

For the alarm-cue trials, during the pre-stimulus phase, nothing was added to the tank. At the beginning of the control-stimulus phase, 50 ml of deionized water (with additional 30 ml of deionized water to flush the airline tubing) was added to the tank using a syringe and airline tubing (Figure 1). At the beginning of the alarm-cue stimulus phase, 50 ml of prepared alarm cue (0.1 cm^2/ml of skin extracts) was added to the tank and then 30 ml of deionized water was added to flush the airline tubing.

For the sound trials, the pre-stimulus phase consisted of no stimulus. During the stimulus phase, sound (a tone rising from 2 – 2 000 Hz on a three second loop) was played for 30 minutes at maximum volume on a single Oceaners DRS-8 speaker (Oceaners, OH, USA), which was placed in the tank throughout the three phases. For the post-stimulus phase, the speaker was turned off for 30 minutes. While we did not have capabilities to measure the sound levels at the time of the study, a subsequent study (Bzonek 2016) measured the ambient sound intensity as 129 - 139 dB re 1 µPa root-mean-square (RMS) in the same experimental tanks with a JASCO Ocean Sound Meter (JASCO, Halifax, NS).

After each trial, all fish were weighed (g) and measured for fork length (mm) before being placed in a recovery tank. To facilitate the measuring process, all fish were anaesthetized to reach stage IV (i.e., induction stage, Summerfelt and Smith 1990; Kim et al. 2017) using a portable electroanaesthesia system (PES, Smith-Roots, Inc.) using a setting of 100 V, 30 Hz cycle frequency, 3 pulses, 500 Hz burst frequency, and duty cycle of 62% (Kim et al. 2017).

DATA ANALYSIS

The video analysis was conducted similar to Kim and Mandrak (2017). During the analysis, each individual fish position per minute were scored by watching both the overhead and underwater videos for all three phases (i.e., 3 X 30 minutes). A positional score (1-6) was recorded at each one-minute interval for each fish, depending on the location that the fish occupied on the pre-marked grid. If fish occupied two or more grids, the grid occupied the longest by the fish was scored. Positional scores were tallied and analyzed for four time-intervals (two, five, 15, and 30 minutes).

To examine the effects of sound or alarm cue on the positioning behaviour of Common Carp, repeated measures Analysis of Variance (RM-ANOVAs) were used to test the effects of treatment on the mean positional scores among the three phases as within subjects: pre-stimulus, control stimulus, alarm-cue stimulus for alarm-cue trials; and, pre-stimulus, stimulus, post-stimulus for sound trials. All statistical analyses were conducted with SPSS V 12.0.1 with $\alpha = 0.05$ (Zar 1996).

RESULTS

SOUND TRIAL

For all intervals, Common Carp did not significantly change their positions within the tank in response to broadband-sound stimulus.

At two-minute intervals, Common Carp did not significantly change their positions among the three phases (RM-ANOVA: within subjects: $F_{1, 8} = 3.42$, $p = 0.10$; Figure 2A). On average, Common Carp were positioned in the middle part of the tank away from the sound stimulus throughout the three phases. At five-minute intervals, Common Carp did not significantly differ in their positions among the three phases (RM-ANOVA, within subjects: $F_{1, 8} = 4.04$, $p = 0.07$; Figure 2A). Although not significant, it appears that Common Carp may have moved closer to the underwater speaker during the stimulus phase when the underwater speaker was turned on (Figure 2A). In general, Common Carp spent most time in the middle part of the tank. At 15-minute intervals, Common Carp did not significantly change their positions within the tank among the three phases (RM-ANOVA, within subjects: $F_{1,8} = 0.43$, $p = 0.53$). Overall, at 30-minute intervals, there was no significant difference in the position of the fish among the three phases (RM-ANOVA, within subjects: $F_{1,8} = 0.03$, $p = 0.87$).

ALARM CUE TRIAL

There was no significant difference in the positions of fish among the three phases at two-minute intervals (RM-ANOVA, within subjects: $F_{1,8} = 0.77$, $p = 0.41$; Figure 2B), five-minute intervals (RM-ANOVA, within subjects: $F_{1,8} = 0.25$, $p = 0.63$; Figure 2B), 15-minute intervals (RM-ANOVA, within subjects: $F_{1,8} = 0.03$, $p = 0.86$; Figure 2B), and 30-minute intervals (RM-ANOVA, within subjects: $F_{1,8} = 0.18$, $p = 0.68$; Figure 2B). For all intervals, on average, Common Carp spent most time in the middle part of the tank across the three phases.

DISCUSSION

Common Carp did not significantly respond to either broadband sound or alarm cue in our study. In response to either broadband sound or alarm cue, Common Carp spent most time in the middle part of the experimental tank regardless of the experimental phases.

The testing area may have been too small to observe any significant effect, as many experiments testing the effect of non-physical deterrents are either conducted in the field or in larger experimental tanks. However, Zielinski and Sorensen (2017) observed a negative phonotaxis response in Common Carp, which were 40% smaller than those used in our study, in an enclosure 18% smaller than the enclosure used in our experiment, though that experiment was conducted in near to total darkness. Additionally, we used these same experimental tanks to test the effect of strobe lights on Common Carp and other species and did find an avoidance effect (Kim and Mandrak 2017), making it unlikely that the size of the tanks was a contributing factor to the null results observed. However, sound travels much more efficiently and creates complex fields in small tanks compared to strobe lights, which may have influenced reactions (Akamatsu et al. 2002; Rogers et al. 2016; Gray et al. 2016; Zielinski and Sorensen 2017). Unfortunately, we did not have the equipment to measure and examine the sound intensity observed within our experimental tank. Another possibility is that Common Carp do not have the same magnitude of response to sound stimuli as other Asian carps. In Zielinski and Sorensen (2017), although a negative phonotaxis response to a complex sound was noted initially in Common Carp, Silver Carp, and Bighead Carp, there was a quick habituation to the sound in all three species and Common Carp seemed to be less sensitive to the sound than the other two species both initially and through prolonged trials. In a series of experiments on the effect of broadband sound on swimming behaviour, Common Carp was found to be less sensitive and to habituate to the sound, whereas Silver and Bighead Carp did not (Vetter et al. 2015; Murchy et al. 2016). It is also possible that Common Carp used in our study were different from those used in other studies (Vetter et al. 2015; Murchy et al. 2016; Zielinski and Sorensen 2017) in terms of fish size, location, prior history and experience, and health status when captured prior to the study which may have contributed to different results.

Some studies have shown that sound elicited response in fishes to avoid an area and perhaps can act as a barrier or guidance technology. For example, Silver Carp elicited both escape responses and area avoidance to a broadband sound source but not to a monotone source in outdoor experimental ponds (Vetter et al. 2015). Vetter et al. (2015) used an outdoor pond setting, larger experimental area, and different species. The size of experimental area could influence the degree to which fish can exhibit a detectable avoidance response. The experimental area in our study was considerably smaller than that used by Vetter et al. (2015), which may have limited the ability of fish to escape the sound field. The species tested could influence whether fish can detect the sound and whether the sound acts as a deterrent. The species used in our study has a Weberian apparatus, similar to Vetter et al. (2015), but recent literature suggests Common Carp may be less sensitive to sound changes than Silver Carp due to their habituation to the sound stimulus and difference in visual acuity (Murchy et al. 2016; Zielinski and Sorensen 2017). There have also been successes in using sound for area avoidance in other fish species, usually to prevent entrainment in hydroelectric facilities and around the cooling vents of power plants. These successes have been shown in Alewife, *Alosa pseudoharengus* (Wilson 1811) (71 – 99% effectiveness), cyprinids (up to 80%), and European Eel, *Anguilla anguilla* (Linnaeus 1758) (57%), with researchers concentrating on frequencies that were biologically relevant to the target species (Haymes and Patrick 1986; Sand et al. 2000; Sonny et al. 2006).

In addition to broadband sound, studies using a combination of bubble curtains and broadband sound and/or strobe light have shown that Common Carp, Silver Carp, and Bighead Carp have responded to bubble curtains by reducing their swimming activities and/or diverting to unblocked channels (Ruebush et al. 2012; Zielinski and Sorenson 2016). In one case, a bubble barrier was found to produce sounds between 100 – 1000 Hz at a volume of 145 dB, which is in the lower half of the frequency range used in this study (Taylor et al. 2005; Zielinski et al. 2014; Zielinski and Sorenson 2016) and within the hearing range of the Asian carps (Popper and Fay 1993; Lovell et al. 2006). Bubble curtains may be more effective than pure sound sources at eliciting responses as they form a visual barrier, which is likely to be more effective at causing area avoidance than a localized point source deterrent (Zielinski et al. 2014; Zielinski and Sorensen 2016). It has also been shown that the bubbles trap and amplify noise, perhaps multiplying the deterrent effect of the sound (Taylor et al. 2005). However, Zielinski et al. (2014) argued that bubble curtains do not act as visual barriers. Their tests with speaker arrays and lighting indicated that carp avoidance of the bubble curtain involved responses to sound and fluid motion rather than visual cues. Varying results highlight the importance of identifying the underlying mechanisms related to the broadband sound, bubble curtains, and strobe lights.

The alarm-cue failed to elicit detectable changes in relative position for Common Carp in our experimental tanks. There are at least three reasons why this might be. First, the alarm cue may not have been present in high enough concentrations or amount. In our experiment, we used 1 cm^2 of skin for every 10 ml of water during preparation of the alarm cue, which equated to 5 cm^2 of skin being used in each experiment (50 ml added to the tank). In an experiment conducted by Lawrence and Smith (1989) on Fathead Minnow, *Pimephales promelas* (Rafinesque 1820), 1 cm^2 of skin was sufficient to elicit an avoidance response. Thus, we anticipate enough alarm cue was present to elicit an avoidance response if adult Common Carp respond to conspecific alarm cues. Second, adult Common Carp may not respond to conspecific alarm cues. Predation on adult Common Carp in Lake Ontario is thought to be low due to their large size (Scott and Crossman 1973). Third, adult Common Carp may require the presence of a predator in addition to alarm cues to elicit an avoidance response, similar to what was found in Slimy Sculpin, *Cottus cognatus* (Richardson 1836) (Chivers et al. 2001). Therefore, the larger Common Carp used in this experiment, despite the presence of damage-released alarm cue, may have visually assessed the threat and, as a result, did not exhibit an avoidance response.

In conclusion, in our study, broadband sound (2-2000 Hz) or the presence of alarm cue did not affect the usage of area in an experimental tank by adult Common Carp. Careful consideration should be exercised when interpreting our results as another laboratory study showed that smaller Common Carp do avoid broadband sound in dark environments (Zielinski and Sorensen 2017). This highlights the importance of understanding the mechanisms triggering avoidance behaviours in fish to each stimulus. In the future, studies could investigate the effect of different types of sound with varying volumes/intensities of sound on adult and juvenile Common Carp. For alarm cue, it would be important to test the effects of alarm cue on juvenile Common Carp. Testing and comparisons across types and intensity of stimuli, target life stage and/or species of target would help identify and provide more robust solutions for management and conservation when dealing with invasive species.

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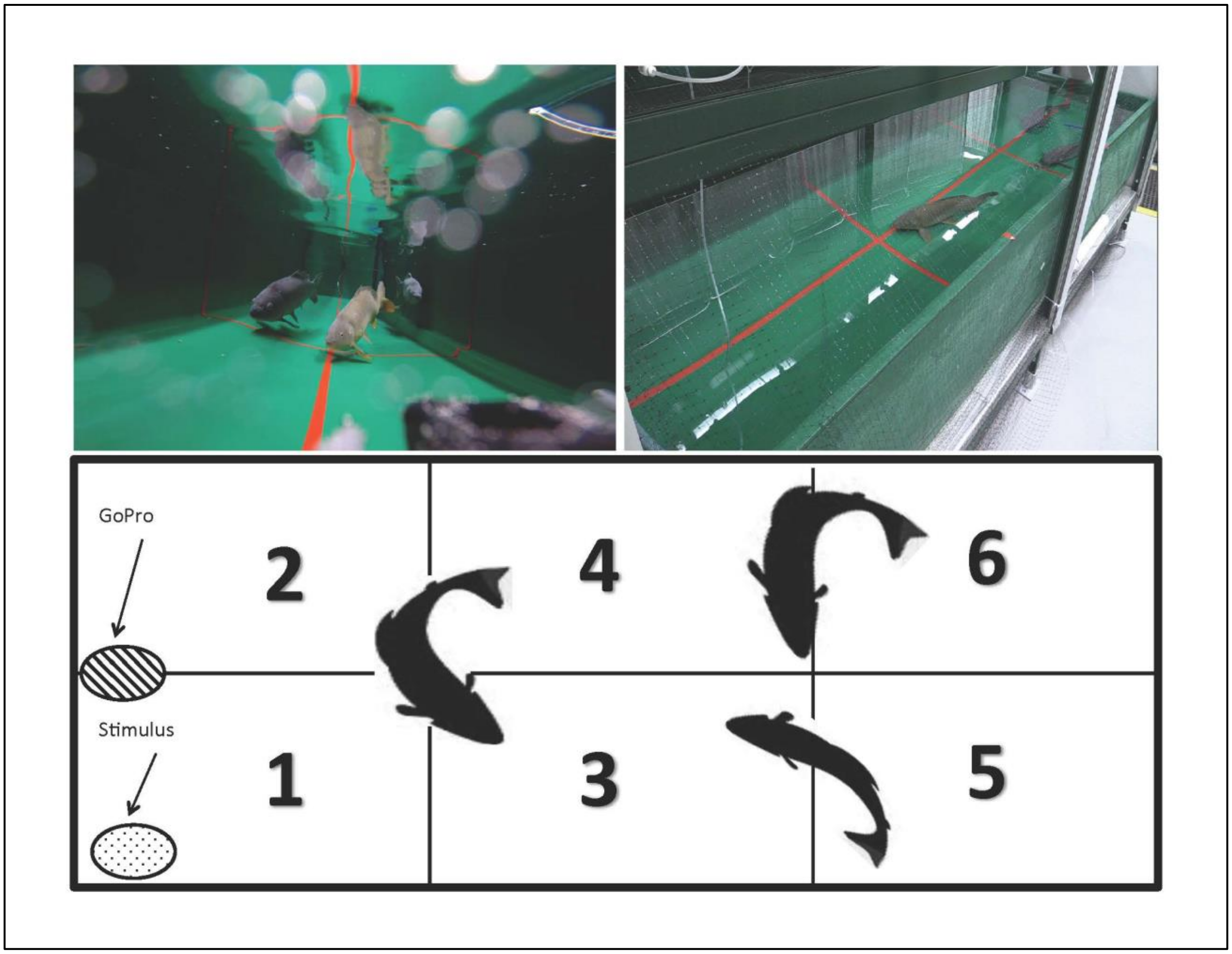

Figure 1. Experimental tank set up. Underwater view from GoPro Camera of three Common Carp in the experimental tank (top left). Overview picture of experimental tank with three Common Carp (top right). Layout of experimental tanks with scoring grid (bottom).

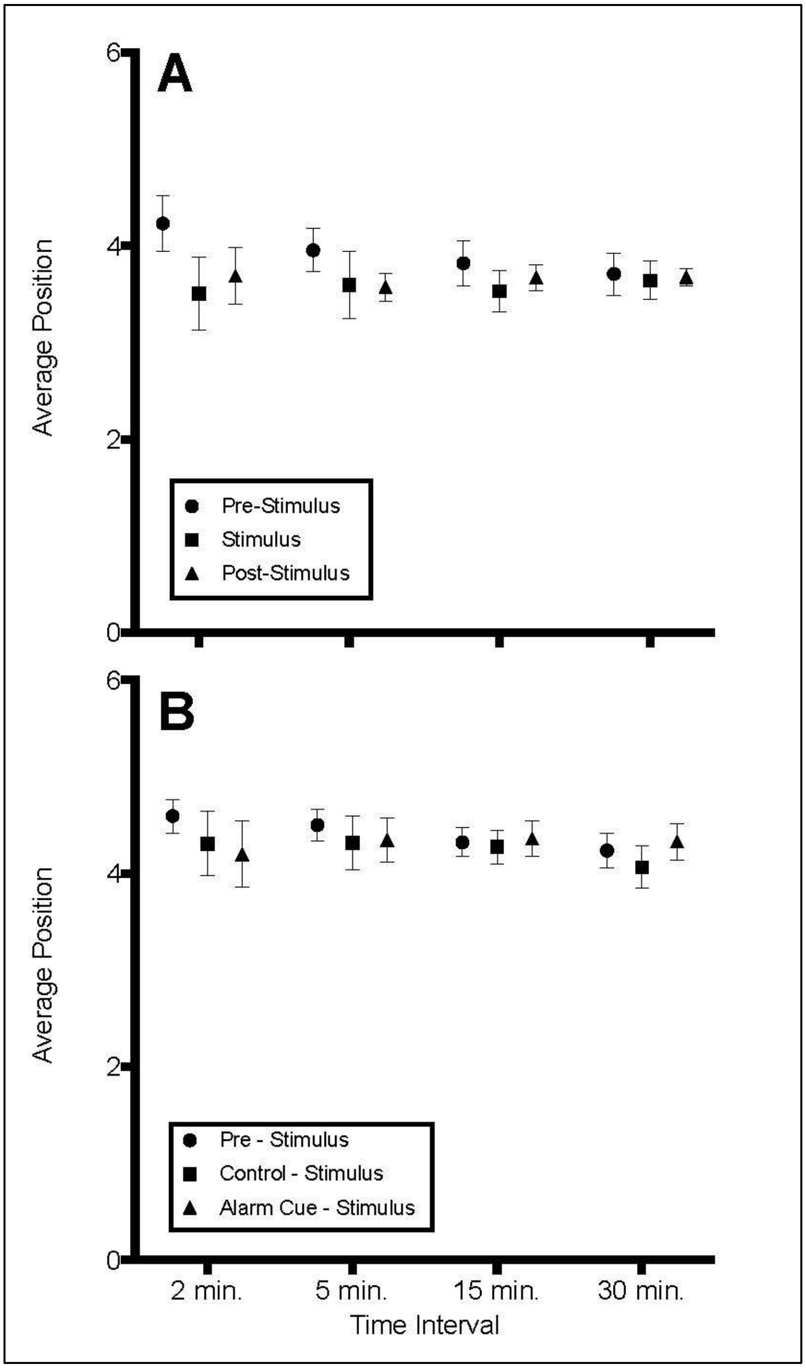

Figure 2. Mean (± standard error) position within experimental tank of common carp in response to: (A) Broadband sound (2 – 2000 Hz) stimulus, (B) alarm cue stimulus.