PREDATOR RECOGNITION AND ANTI-PREDATOR BEHAVIOR IN JUVENILE ASIAN CARP

BY

JARED CLAYTON WILSON

THESIS

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Master's Committee:

Adjunct Professor David H. Wahl, Director of Research
Assistant Professor Eric R. Larson
Adjunct Assistant Professor Andrew F. Casper

ABSTRACT

Predation is a determining factor influencing the strength and success of a year class in many prey fishes. Chemical, social, and similar predator cues may all inform an individual of a possible impending predatory threat. Bighead (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*) widespread invasive fishes, and can be vulnerable to predation during their first year. Because they now overlap the niches of many native prey species, they may shoal with these species and learn about predation while doing so. Additionally, generalizing predators that have similar characteristics (e.g. odors or body morphometry) may increase the survival of an individual. The objective of this study was to evaluate how juvenile bighead and silver carp respond to threatening situations, as well as how adaptive and plastic are these responses. To achieve these objectives, I performed three separate studies that aimed to define the alarm response of these fishes, assess how group composition may influence the alarm response, and determine if the response could be generalized when exposed to taxonomically similar predators. Bighead and silver carp responded to conspecific alarm cues and both species reduced their activity, however bighead carp did so in much tighter schools than silver carp. In same-species groups of bighead carp, largemouth bass (*Micropterus salmoides*) odor induced similar behavioral changes, provided at least one experienced individual was present. However, when naïve bighead carp were paired with experienced golden shiners (*Notemigonus crysoleucas*), they matched their behavior to that of the golden shiner. Groups of silver carp conditioned to recognize largemouth bass odor only showed behavioral changes when exposed to the same predator odor, and were unable to generalize the odor of largemouth bass to recognize smallmouth (*M. dolomieu*) odor. These three studies indicate that there is some degree of flexibility in the response of bighead and silver carp exposed to different threatening situations, however more tests are needed to identify how the different responses may impact the relative success of these invaders as they approach novel environments.

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Dedicated to my pupperinos, Millie and Mosby. Also, the cat, Chester.

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Chapter 1: Behavioral Response of Juvenile Silver and Bighead Carp to Conspecific and Heterospecific Alarm Cues

Abstract

Predation is an important factor influencing the strength and success of a year class in many prey fishes. Many adaptations, including chemical cues, benefit shoaling groups by informing members of a possible impending predatory threat. Bighead (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*) are widespread invasive fishes that spends much of the first year at a size vulnerable to predation. The objective of this study was to evaluate the fright response of juvenile bighead and silver carp when exposed to alarm cues from conspecific and heterospecific fishes. Groups were exposed to 5 mL of alarm cue from either bighead carp, silver carp, golden shiner (*Notemigonus chrysoleucas*), or fathead minnow (*Pimphales promelas*). Behavior was recorded before and after the application of the treatment. Our results indicate that both species were unable to recognize cues from native cyprinids, yet both were able to recognize conspecific alarm cues. However, the two species of Asian carp respond to conspecific chemicals differently. Silver carp increase distance among individuals, whereas bighead carp reduce distance between individuals. Both show a reduction in activity after chemical application. Both species recognize conspecific alarm cues, but bighead carp exhibit similar fright patterns when exposed to alarm chemicals from either Asian carp species. For future management application, information is needed to determine if fright response is sustained following the continuous application of alarm chemicals.

Introduction

Predation is an important factor when accounting for larval and juvenile fish survival (Rice et al. 1997). Individuals often recognize potential threats by processing chemical, visual, and social cues that occur before, during, and after predatory encounters (Lima and Dill 1990). Adapting to highly diverse and rapidly changing aquatic habitats, many fish have evolved highly sensitive chemosensory organs and utilize many different chemical cues to detect and assess the risk of predation (Hara 1994). One chemical cue utilized by some prey fish is an alarm substance (Schreckstoff) present in the skin tissue of many cyprinids that, when damaged, informs conspecifics to the threat of danger (Krause 1993, Brown 2003). In some cases, the close proximity of schools of heterospecific fishes has also been shown to benefit some prey fishes, suggesting that the capacity for recognizing more than one alarm cue allows a faster response when danger is imminent (Chivers et al. 1995). Thus, threat recognition through chemical alarm cues of conspecifics and sympatric heterospecifics informs an individual when assessing predation risks.

Genetic and environmental factors are thought to influence selection of effective anti-predator strategies in prey fishes (Pitcher 1986). Because of the innate variability of these factors, the functionality of alarm responses can vary widely across taxa in order to maximize the efficiency of fitness related activities under the threat of danger (Lima and Dill 1990, Chivers and Mirza 2001). Over long periods of evolutionary time, it is possible that one strategy for predator avoidance may be selected for over other strategies. Therefore, the functional importance of an appropriate alarm response to predation threats can be dependent on several community, morphological, ontogenetic, or environmental factors (Pitcher 1986). Inappropriate or inefficient anti-predator behaviors may increase vulnerability to various predator feeding strategies (Magurran 1990, Šmejkal et al. 2018). Such mismatches may be one reason so many introduced species either fail to establish a population altogether (Williamson and Fitter 1996), or serve as a resource subsidy to native predators (Crane et al. 2015). Despite the critical role behavior has on the success of animal invasions (Holway and Suarez 1999, Deacon and Magurran 2016), behaviors of the rapidly expanding populations of Asian carp are still largely understudied. Although several studies in North America have investigated adult feeding behaviors (Kolar et al. 2005) and larval swimming behaviors (George and Chapman 2013), almost none have investigated alarm response behaviors of these fishes. Therefore, little is known about the mechanisms used by Asian carp to recognize threats and make fitness related decisions in the presence of different alarm chemicals.

Bighead (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*), hereafter referred to as Asian carp, are prolific non-native cyprinids distributed across much of the Midwestern United States (Kolar et al. 2005, Baerwaldt et al. 2013) and are presumed to pose devastating economic and ecosystem-wide threats to the Laurentian Great Lakes (Mandrak and Cudmore 2004, Tsehaye et al. 2013). Asian carp spend much of their first year of life at a size vulnerable to predation (<250 mm), and visually resemble common native prey items like golden shiners (*Notemigonus crysoleucas*) and shad (*Dorosoma spp*) (Kolar et al. 2005, Williamson and Garvey 2005). Previous laboratory studies indicate a high selection for juvenile Asian carp over native forage species (Sanft 2014), and field studies suggest that juvenile Asian carp are consumed by native predators (Anderson 2015). Previous electro-olfactogram tests (EOG) suggest that Asian carp respond to hormonal pheromones (Stacey and Cardwell 1995, Little 2014), and presumably the dermal club cells that produce alarm cues in Ostariophysians are present in Asian carp, supporting the idea that Asian carp may respond to conspecific alarm cues if a predator attacks a shoal mate. Additionally, EOG data suggest that closely related species of teleost fish use similar hormonal pheromones (Sorensen and Stacey 1999), and laboratory studies by Chivers et al. (1995) suggest that diverse communities of prey fish benefit the individuals that are capable of recognizing sympatric heterospecific alarm cues. Although Asian carp are nonnative to North America, their relatedness to species that are native may allow an innate recognition of heterospecific alarm substances.

Exploitation of alarm behaviors has been shown to be a viable management tactic, especially when paired with other removal techniques, thus alarm responses of Asian carp should be of interest to managers. Other nuisance fish species, such as sea lamprey (*Petromyzon marinus*) (Wagner et al. 2011, Hume et al. 2015) and Eurasian ruffe (*Gymnocephalus cernua*) (Maniak et al. 2000), have been the subjects of successful field tests using putrefaction cues to divert movements. Similarly, managers may be able to exploit the behavioral response to alarm cues shown by Asian carp as a way to increase the efficiency of various removal techniques (Sorensen and Johnson 2016). Successfully using alarm cues to mitigate the movement and spread of Asian carp is contingent on understanding how specific chemical cues inform behavioral strategies.

Herein, we assess the chemical recognition capabilities and behavioral strategies used by invasive Asian carp to help develop management strategies seeking to prevent their impending invasion of the Great Lakes. Specifically, we aimed to characterize the behavioral response (if any) of bighead and silver carp to alarm chemicals from a) conspecific fishes, b) closely related heterospecific fishes, and c) distantly related native heterospecific fishes. To do this, we quantified a suite of behavioral responses before and after the fish were exposed to the respective chemical treatment. We predicted that the Asian carps would show some form of behavioral response, though this response may be limited to only conspecific or closely related heterospecific alarm chemicals.

Methods

Test Species

Juvenile silver carp were obtained from the U.S. Geological Survey's Columbia Environmental Research Center in Columbia, Missouri. Juvenile bighead carp were obtained from Osage Catfisheries, Inc. in Osage Beach, Missouri. Fathead minnows (*P. promelas*) and golden shiners (*N. crysoleucas*) were obtained from a local bait shop near Sullivan, IL. All fish used were hatchery reared, and thus provided naïve test subjects with no prior experience to predation. All fish were obtained during the summer of 2016 and housed at the Kaskaskia Biological Station in Sullivan, IL in separate indoor raceways, using dechlorinated, filtered, and aerated water, with ambient temperatures at 22 ± 1 °C. Bighead and silver carp were fed a daily crushed commercial pellet diet, whereas golden shiners and fathead minnows were fed a commercial flake food. Waste removal and partial water changes occurred biweekly to promote health and prevent water quality induced stress. Fish were allowed to acclimate to ambient laboratory conditions for at least 7 days prior to experimental testing. Mean fork length (FL) of all fish used was held uniform at 50.6 ± 7.3 mm. Prior to testing, single species groups of 3 fishes were transferred to 37 L testing aquaria filled with 35 L of aerated, dechlorinated water and allowed to acclimate for 24 hours.

Alarm Cue Preparation

Stimulus preparation followed similar methods as Ferrari et al. (2007) and Brown et al. (2006). Skin tissue was removed from 15 bighead carp (mean FL 50.1 ± 4.2 mm), 15 silver carp (mean FL 50.6 ± 4.7 mm), 30 fathead minnows (mean FL 51.3 ± 5.2 mm) and 30 golden shiners (mean FL 50.7 ± 3.6 mm). All fish used in this study were euthanized in accordance with the Illinois Institutional Animal Care and Use Committee (IACUC) by removing their heads at the base with a scalpel. Skin fillets from both sides of the fish were removed. Length and depth of the filets were measured to the nearest hundredth of a centimeter and placed in chilled deionized water. The total area of skin tissue collected was approximately 45.09 cm^2 for bighead carp, 45.54 cm^2 for silver carp, 92.34 cm^2 for fathead minnows, and 91.26 cm^2 for golden shiners. Skin fillets were then homogenized with a blender and filtered through 20 µm mesh to remove larger particles. Samples were diluted to ~1 cm^2 tissue/5ml of deionized water and stored at −20 °C in 45 ml aliquots until use. During experimentation, 5 ml of the resulting solution (~1 cm^2 of tissue) was applied to the tanks containing 35 L of water. To elicit overt antipredator response in cyprinids, these are considered high concentrations (Ferrari et al. 2005), thus alarm responses, if they exist, were expected to be observed.

Experimental Design

To verify that our design appropriately described fright response, we first investigated the alarm responses of fathead minnows and golden shiners, two native cyprinids with established alarm behaviors (Chivers and Smith 1994, Godard et al. 1998). Treatment groups for these species consisted of their respective conspecific alarm chemical and a control (*n* = 15 each). To understand generic fright response of both species of Asian carp, treatment groups for both species consisted of a control and conspecific cues. Next, we wanted to understand their response when presented with alarm cues of potential shoal mates in their invaded range, therefore treatments included cues from a close taxonomic relative (e.g., bighead carp treated with silver carp cues and vice-versa), and cues from both previously tested native cyprinids (fathead minnows and golden shiners), resulting in a total of five treatment groups for each species of Asian carp (*n* = 15 each).

Groups of 3 individuals from each species were transferred from the holding raceways to each of the treatment tanks. Fish were fed to satiation, and any excess food was removed. Groups of three were used to avoid introducing any unnecessary isolation stress, since Asian carp are a schooling fish (Brown et al. 2006, Ferrari et al. 2007, Ghosal et al. 2016). To prevent external stimuli from influencing behavioral responses, experimental tanks were painted white on the external side of the glass and held inside a curtained area during testing. Alarm chemicals were added to experimental tanks via an injection through air tubing placed over a submersible aquarium pump that recirculated water through the tank. Preliminary tests showed that the system could disperse food coloring throughout the tank within 15 seconds. A 7.6 cm x 15.2 cm x 10.2 cm mesh shelter was constructed and placed in the middle of each tank to provide cover. GoPro (model Hero 6) cameras mounted above each tank were used to record each trial.

Each experimental session lasted for ten minutes, where fish activity and behavior was recorded for five minutes pre-stimulus to develop a neutral baseline for behavior, followed by a five minute post-stimulus period. Prior to testing, 100 ml of ambient tank water was removed from each tank through the check valve system and discarded, while an additional 100 ml was removed and used later as a flush. Treatment applications were administered by injecting 5 ml of a randomly assigned alarm cue or control (ambient tank water) into the tubing, and flushed with the 100 ml of ambient tank water that was previously removed. After flushing the treatment through the tubing, cues were allowed to disperse for 30 seconds before the post-stimulus period began recording activity. Following the testing period for each trial, the length of each fish was measured, and then the fish was euthanized using the same methods described in the stimulus preparation section. At the completion of each trial, tanks and tubing were thoroughly cleaned with a mild detergent and dried before being reused for further trials.

Video recordings of each trial were reduced to 5 frames per second using the open-source software VirtualDub. Measurements were then extracted on a frame-by-frame basis for each individual in the tank using the behavioral tracking software Lolitrack (Loligo Systems, Viborg, Denmark). Recorded behaviors included four common alarm metrics: shelter use (Mirza et al. 2001), activity (Wisenden et al. 2009), group cohesion (Brown et al. 1970), and distance from the cue (Ferrari et al. 2005). Shelter use was measured using a count of total shelter visits and the total time (s) spent within the shelter per minute. Individuals were considered active when the software registered that the position of the fish had changed >10 pixels (10 pixels ≈ 0.5 cm). When the software registered a fish as active, variables representing activity included the total time (s) spent active per minute, distance moved per minute, and the average velocity (cm s^{-1}) per minute. Group cohesion was measured by two values, which were determined by calculating the distance of the nearest neighbor (cm) when a fish was considered active and when it was considered idle. To adjust for size differences among fish, post-injection nearest neighbor values were converted to a ratio against the baseline average value for each fish. Values >1 indicate increased nearest neighbor distance and values <1 indicate decreased nearest neighbor distance. Distance from the source of the cue was based on the average distance of the fish from the source of the cue as it entered each tank. Behavioral values were aggregated by minute for each individual within the experimental tank, then averaged together to produce a tank-wide average at each minute. A pre-stimulus baseline value for each of the variables consisted of averaging the values for minutes 1-5 prior to applying the appropriate treatment.

Statistical Analysis

A repeated measures, non-parametric, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was conducted using the PERMANOVA+ add-on of PRIMER v.7 (Anderson et al. 2008, Clarke and Gorley 2015) to test how behaviors for each species changed when a prescribed treatment was added to the aquarium. Within the framework, we used a conservative approach when analyzing the data. Before performing the analyses, data for each species were first square-root transformed to reduce the influence of outliers, then converted into triangular normalized Euclidean distance matrices to reduce the influence of metrics with larger values (e.g. distance traveled may average 300cm for a minute, but the average active time that minute was only 5s) (Anderson et al. 2008, Shirkhorshidi et al. 2015). Factors in the analysis included time, treatment, and replicate ID. Time was held as a fixed repeated factor, which included the pre-stimulus baseline average (time = 0), and each of the five minutes following the treatment application (6 levels). Treatment was also considered a fixed factor (fathead minnows: 2 levels, golden shiners: 2 levels, silver carp: 5 levels, and bighead carp: 5 levels). Replicate ID was considered a random factor nested within treatment. For each model, inference was drawn by comparing measured values to a simulated distribution generated by 9999 permutations of the observed data, with fixed effects summed to zero and significance judged at a 5% significance level with a type III sum of squares (Anderson et al. 2008). Significant model terms, most importantly interaction (Treatment by Time) effects, were investigated with pair-wise post-hoc comparisons to see how post-stimulus minutes differed from the pre-stimulus baseline. Due to multiple comparisons, post-hoc *p*-values were based on adjusted alpha-levels using the Holm-Bonferroni adjustment method (Holm 1979).

Temporal patterns for each treatment group were visualized using non-metric multi-dimensional scaling (nMDS; Kruskal 1964) based on the normalized Euclidean distance matrices. To reduce the number of data points and allow for a clear demonstration of behavioral change across treatment groups, ordination plots consisted of treatment by time centroids. Personalities, such as boldness, can largely impact behavioral differences amongst groups (Leblond and Reebs 2006). However, general patterns of behavioral change amongst localized groups can be representative of the overall behavioral response (Schaerf et al. 2017). As such, the relative positions of each centroid in multivariate space can illustrate how the different treatment groupings influence behavior (Anderson et al. 2008). As a final step, hierarchical agglomerative clustering was used to group centroids into clusters, with statistically significant clusters identified by similarity profiles using the SIMPROF routine (Clarke and Warwick 1994, Clarke and Gorley 2015). Behavioral variables contributing the most to cluster separation were identified through similarity percentage analyses (Clarke 1993, Cabecinha et al. 2009, Boys and Williams 2012).

In this framework, conducting *post-hoc* pairwise tests and using a single point to represent each treatment by time centroid requires the data be similarly dispersed across the treatment by time groups (Anderson et al. 2008). These assumptions were tested using a permutational analysis of multivariate dispersion using the PERMPDISP routine (Clarke and Gorley 2015) and indicated no significant difference in levels of dispersion of the treatment by time points within any of the species groups (fathead minnow: $F_{11,\ 168}$=0.8613, P= 0.83; golden shiner: $F_{11,\ 168}$=0.1676, P=0.17; bighead carp: $F_{29,\ 420}$=1.7242, P=0.13; silver carp: $F_{29,}$

$_{420}$=1.4963, P=0.34). Therefore, *post-hoc* comparisons could be made and each treatment by time centroid could be represented by a single point within the ordination plots.

Results

PERMANOVA/PERMDISP

All four species showed a significant ($P<0.05$) or marginally significant ($P<0.10$) treatment by time interaction effect, indicating that behavioral change was occurring over time (Table 1.1). *Post-hoc* pairwise comparisons of each minute post-injection relative to the pre-injection baseline revealed no departure from the baseline behavior (P>0.05; Table 1.2) when any of the four species were present with an ambient tank water control. In contrast, all species showed a significant departure from their baseline when presented with a conspecific alarm cue (P<0.05; Table 1.2), indicating that there were detectable alarm responses for all four species. Neither bighead carp nor silver carp showed significant departures from their baseline behaviors when presented with alarm chemicals from either of the two native species (P>0.05; Table 1.2). When silver carp were exposed to alarm chemicals from bighead carp, there was no detectable difference from the baseline; however, bighead carp expressed an alarm response when presented with alarm chemicals from silver carp and showed a significant response three out of five minutes (Table 1.2).

nMDS/Cluster Analysis

In each nMDS for the four species tested, there was an identifiable “baseline” cluster that included the baseline average of each treatment tested as well as all the points from the control treatment (Figures 1.1 and 1.2). Additionally, centroids representing the conspecific alarm cue for each species formed one or more “response” clusters. SIMPER analysis showed that groups of fathead minnows exposed to conspecific chemicals increased distance from the source of the cue and increased shelter use (Table 1.3). The nMDS and cluster analysis for fathead minnows revealed an “initial” response, which included minutes 1, 3, and 4 from the conspecific alarm cue treatment, as well as a “secondary” response consisting of just minute 5 from that treatment (Figure 1.1). The “initial” response cluster showed increased distance from the source and increased time spent in shelter accounted for much of the separation of the clusters (Table 1.3). Although increases in velocity and distance moved were also a factor, it is possible this could be an artifact of the groups of fish moving into shelter, as they were idle for much of the baseline period. As the responses proceeded through time, the “secondary” response cluster is characterized by more shelter visits, activity, and reduced school cohesion than the “baseline” cluster, indicating that individuals may be assessing threats while moving in and out of shelter (Table 1.3). The cluster analysis of the centroids for golden shiners revealed only one “response” cluster that could be separated from the “baseline” cluster (Figure 1.1). The “response” cluster included minutes 2, 4, and 5, from the conspecific alarm cue treatment (Figure 1.1). SIMPER analysis showed increased active time and distance moved were among the strongest contributors to cluster separation (Table 1.4).

The “baseline” cluster for bighead carp included all centroids for each treatment’s baseline average (time = 0). In addition, all five time centroids for the control and native alarm cue treatments were included in this “baseline” cluster, indicating a lack of ability to recognize alarm cues from native cyprinids (Figure 1.2). Cluster analysis revealed a brief “initial” response consisting of the first minute post-injection for both Asian carp alarm cues tested and a longer “secondary” response cluster that includes the remaining 4 minutes from each Asian carp alarm cue (Figure 1.2). The “initial” response cluster is typified by increased shelter usage and increased school cohesion compared to the “baseline” cluster while the “secondary” response cluster is characterized by reduced active time and distance moved and increased velocity and school cohesion (Table 1.5).

Cluster analysis revealed silver carp had a similar pattern to that of bighead carp. The “baseline” cluster was comprised of all time centroids from the control treatment, baseline averages from all other treatments, and most time centroids from the two native species (Figure 1.2). Minutes two and four from the golden shiner cue treatment were not included in this baseline cluster, though they were not significantly different from their baseline average. Unlike bighead carp, the “initial” response of silver carp contained only the first minute from the conspecific cue. The “secondary” response included the remaining four minutes from the conspecific alarm cue, all five minutes of the closely related bighead carp alarm cue, and minutes two and four from the golden shiner alarm cue treatment (Figure 1.2). The “initial” response cluster was driven by increased distance from the source, reduced active time, and reduced distance moved. The “secondary” response cluster showed reduced school cohesion, increased swim velocity, and a reduction in distance moved compared to the “baseline” cluster (Table 1.6).

Discussion

Defining specific alarm response behaviors of Asian carp increases the possibility of successful management efforts by exploiting the behavioral response of these invaders. We confirmed that bighead and silver carp respond to alarm chemicals. It is believed that most Ostariophysians possess an innate ability to identify threats when a nearby injured conspecific releases subdermal alarm cues, and our results indicate that juvenile bighead and silver carp are no exception. Both bighead and silver carp responded to conspecific alarm chemicals, however the species differed in their ability to recognize heterospecific chemicals. Although bighead carp could recognize cues from silver carp, there was no evidence that silver carp could recognize the cues from bighead carp. For both Asian carp species, neither changed behaviors when exposed to alarm chemicals from native fishes.

We defined the behavioral response of bighead and silver carp to alarm chemicals. To do this, we used a unique approach. Commonly used to study community structures, PERMANOVA can be a useful tool to study multiple behavioral responses simultaneously, which is particularly useful when the behavioral response of a species is previously uncharacterized (Kuehne et al. 2012, Parker et al. 2012, Carthey and Banks 2016), such as the case with Asian carp. We first verified this approach by using species of fish with documented alarm responses. We found that conspecific alarm chemicals caused fathead minnows to increase their shelter utilization and golden shiners to increase their activity, and similar behaviors have been described previously in the literature (Mathis et al. 1996, Godard et al. 1998). With these results, we were able to corroborate the usefulness of this approach, and used it to describe species specific alarm response behaviors for the two species of Asian carp.

Bighead carp exposed to conspecific alarm chemicals reduced their activity, moved away from the source of the cue and into shelter, and increased overall school cohesion. These behavioral changes closely resemble the “selfish herd” theory in which individuals reduce predation threat by moving to the center of a group, putting other conspecifics between themselves and potential predators (Hamilton 1971). Alarm responses such as these promote decreased vulnerability to predation for members at the center of the aggregate, however there are drawbacks. Maintaining school cohesion can be largely dependent on the less perceptive and slower members (Beauchamp 2007, Eshel et al. 2011), thereby reducing the speed at which a group identifies and escapes from threats. To that end, slow-moving, tightly schooled prey fish may be at a disadvantage when encountering a predator capable of ingesting multiple individuals at once, such as a largemouth bass (*M. salmoides*)(Wainwright and Lauder 1986).

Similar to bighead carp, silver carp exposed to conspecific alarm chemicals reduced their activity and avoided the source of the cue. However, unlike bighead carp, they showed a decrease in school cohesion. Silver carp commonly leap out of the water as a response to boat motors and other auditory stimuli (Kolar et al. 2005). Despite these previous observations, there were no occurrences of individuals leaping out of tanks following any of the chemical stimuli. The relative increases in intra-school distances that we found are associated more with the "look-around" or "defensive" schooling behaviors (Pavlov and Kasumyan 2000), in which individuals of the group rely heavily on visual inputs after the school is alerted to a threat. Additionally, these "look-around" schools maximize school mobility at the cost of the integrity of the school, often fracturing and rejoining.

Previous studies suggest that relatedness can influence chemical structures of alarm cues and pheromones (Sorensen and Stacey 1999), and our study supports this hypothesis. When presented with the alarm cue from the closely related silver carp, bighead carp showed similar behavioral changes as if they were presented with a conspecific alarm chemical. Conversely, when silver carp were presented with cues from the closely related bighead carp, behavioral changes did not significantly change from the baseline. Behavioral values for silver carp exposed to bighead carp alarm cues did cluster in the same vicinity as the groups exposed to silver carp cue. Although not as equally strong of a response, similar trends indicate that silver carp may still respond, albeit not as intensely, to bighead carp cue. Both species of Asian carp showed no significant alteration in behavior when exposed to alarm cues from either fathead minnows or golden shiners. All four species belong to the same family (Cyprinidae); however, the Asian carps evolved on a separate continent and are therefore separated by a much greater phylogenetic distance (Cunha et al. 2002). Even though taxonomic relatedness may influence the *innate* ability for Asian carp to recognize a native heterospecific alarm chemical, current sympatry with native cyprinids may ultimately lead to a *learned* behavioral response, particularly when mixed species groups are exposed to threatening situations (Larson and McCormick 2005).

Alarm responses of fishes *in situ* are complex, and can be influenced by any number of social, sensory, or environmental variables. Furthermore, exposure to alarm chemicals may not always elicit immediately detectable responses, and prolonged exposure may lead to less obvious physiological and morphological responses. For example Crucian carp (*Carassius carassius*) can develop deeper body shapes, allowing them to reach size refuge and outgrow gape limitations of potential predators earlier in life (Bronmark and Pettersson 1994). Because we focused on the immediately detectable behavioral responses, it is unknown if Asian carp express any such lagged morphological response to alarm chemicals. If any of the Asian carp were to undergo morphological changes as a response to alarm chemicals like Crucian carp, predation mortality may decrease earlier in life, which could lead to more individuals recruited into the reproductive population. Under these scenarios, continuous applications of alarm chemicals would not be recommended.

Understanding behavioral changes may provide managers a tool to increase the efficiency of any number of removal tools. Similar differences in school cohesion of silver carp and bighead carp have been shown (Ghosal et al. 2016), and these differences of the fracturing/regrouping “look-around” schools of silver carp and the “selfish herd” schools of bighead carp may explain the stark differences in capture rate reported by Green and Smitherman (1984). They found only 38% capture rate of silver carp compared to almost 99% capture rate of bighead carp in ponds following a single seine haul. Therefore, the schooling behaviors of these fish may help guide how managers approach removing these fish from a given area. Pairing alarm chemicals with active removal gears, such as seines and trawls, may be more successful in removing tightly schooled bighead carp. Whereas, pairing alarm chemicals with passive removal gears such as gill and fyke nets may be more successful at removing silver carp, as it may increase the number of times individuals encounter these gears as their schools fracture and rejoin. Future inquiries into how alarm chemicals may be used with different removal techniques and their effects on capture rates of these fish may help managers when targeting removal of either species of Asian carp.

Figures

Figure 1.1: nMDS of Treatment by Time centroids for each treatment group that (a) fathead minnows and (b) golden shiners were exposed to in tank experiments. Numbers represent time, with time = 0 indicating baseline average and 1-5 indicating minutes following treatment application. Grey circles represent significant clusters identified by similarity profiles.

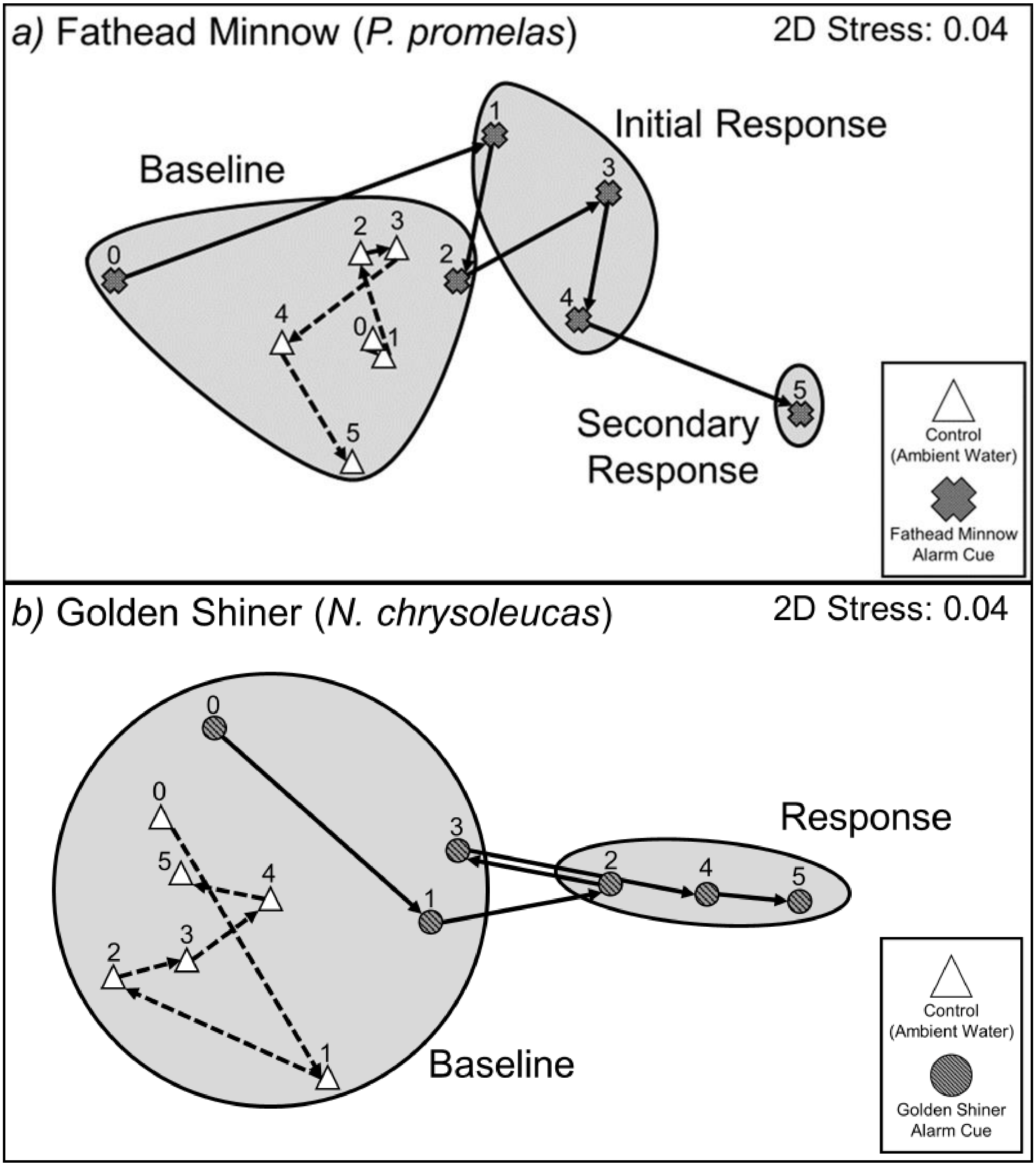
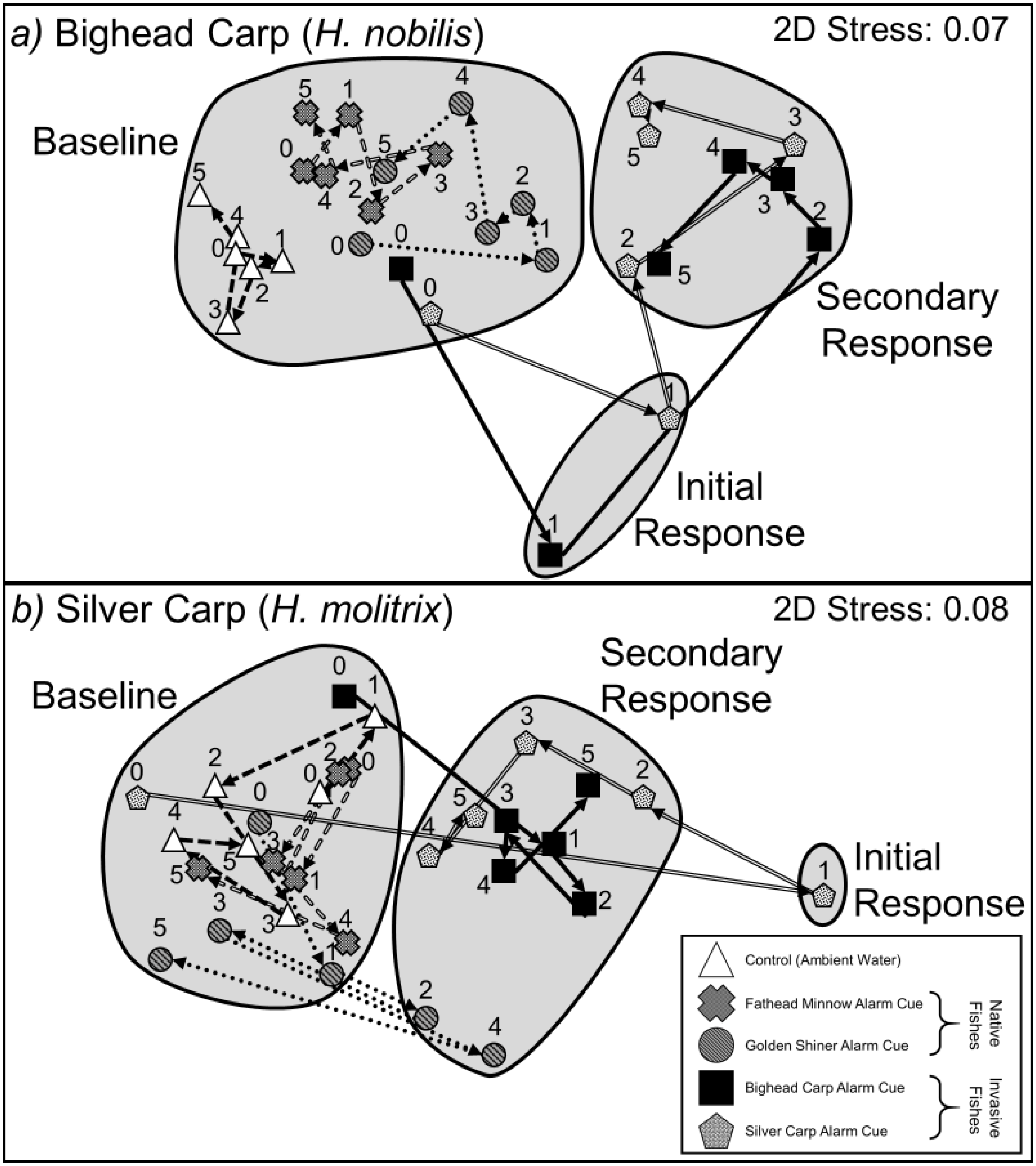


Figure 1.2: nMDS of Treatment by Time centroids for each treatment group that (a) bighead carp and (b) silver carp were exposed to in tank experiments. Numbers represent time, with time = 0 indicating baseline average and 1-5 indicating minutes following treatment application. Grey circles represent significant clusters identified by similarity profiles.



Tables

Table 1.1: Repeated-measures, non-parametric, PERMANOVA, conducted on each species group (fathead minnow, golden shiner, bighead carp, silver carp). Each native species was tested with conspecific alarm chemicals against a control. For the two species of Asian carp, treatment groups consisted of bighead carp alarm cue, silver carp alarm cue, fathead minnow alarm cue, golden shiner alarm cue, and a control. In all tests, replicate ID was a random factor nested within treatment.

	df	SS	MS	Pseudo-F	P(Perm)	Unique Perms
Fathead Minnow						
Treatment	1	10.2	10.2	0.3440	0.87	9935
Minute	5	37.1	7.4	1.9687	0.006	9913
ID(Treatment)	28	829.5	29.6	7.8702	0.0001	9794
Treatment x Minute	5	28.3	5.7	1.5041	0.08	9905
Residual	140	527.0	3.8			
Total	179	1432.0				
Golden Shiner						
Treatment	1	2874.7	2874.7	0.9162	0.37	9952
Minute	5	1715.3	343.1	2.3265	0.005	9929
ID(Treatment)	28	87855.0	3137.7	21.2790	0.0001	9848
Treatment x Minute	5	1504.8	301.0	2.0411	0.02	9903
Residual	140	20644.0	147.5			
Total	179	114590.0				
Bighead Carp						
Treatment	4	6520.5	1630.1	1.3685	0.20	9930
Minute	5	2759.5	551.9	5.2276	0.0001	9934
ID(Treatment)	70	83380.0	1191.1	11.2830	0.0001	9761
Treatment x Minute	20	3353.4	167.7	1.5882	0.01	9858
Residual	350	36951.0	105.6			
Total	449	132960.0				
Silver Carp						
Treatment	4	4437.7	1109.4	1.1603	0.33	9936
Minute	5	1581.9	316.4	2.3971	0.01	9927
ID(Treatment)	70	66930.0	956.1	7.2442	0.0001	9815
Treatment x Minute	20	3887.5	194.4	1.4727	0.03	9891
Residual	350	46196.0	132.0			
Total	449	123030.0				

Table 1.2: Post-hoc comparisons of the Treatment by Time interaction term for treatment groups of each species (fathead minnow, golden shiner, bighead carp, silver carp) against the respective baseline value (Time = 0). For both species of Asian carp, groups were treated with an ambient tank water control (CTRL), bighead carp alarm cues (BHC), silver carp alarm cues (SVC), fathead minnow alarm cues (FHM), and golden shiner alarm cues (GS). Native species (fathead minnows and golden shiners) tested their respective conspecific cues against an ambient control.

	Minute 1		**Minute 2**		**Minute 3**		**Minute 4**		**Minute 5**	
	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms
Bighead Carp										
CTRL	0.24	9976	0.20	9976	0.30	9961	0.21	9957	0.26	9959
BHC	0.004	9954	0.03	9967	0.03	9957	0.06	9965	0.08	9969
SVC	0.06	9960	0.07	9966	0.05	9960	0.02	9951	0.02	9976
GS	0.06	9959	0.26	9952	0.32	9963	0.38	9966	0.24	9962
FHM	0.10	9974	0.10	9959	0.07	9978	0.06	9965	0.07	9977
Silver Carp										
CTRL	1.74	9947	0.67	9956	1.69	9942	1.38	9975	1.03	9945
SVC	0.02	9952	0.02	9935	0.02	9950	0.06	9950	0.03	9957
BHC	0.33	9936	0.19	9947	0.20	9952	0.27	9940	0.19	9953
GS	0.50	9939	0.42	9951	0.96	9944	0.35	9957	1.42	9943
FHM	1.96	9955	1.65	9946	1.16	9943	0.76	9941	2.10	9948
Fathead Minnow										
CTRL	0.32	9960	0.37	9958	0.22	9952	0.38	9963	0.39	9959
FHM	0.03	9952	0.006	9956	0.003	9943	0.01	9951	0.005	9960
Golden Shiner										
CTRL	0.80	9947	0.86	9953	0.98	9943	0.64	9952	0.53	9949
GS	0.03	9944	0.02	9948	0.18	9949	0.004	9952	0.002	9944

Table 1.3: SIMPER analysis of the relevant clusters within the nMDS for fathead minnows (Figure 1.1), comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Initial Resp. Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Initial Response		***Average Squared Distance = 16.47***			
Distance From Source	-0.580	1.160	3.880	1.28	23.6
Shelter Time	-0.583	1.170	3.480	1.73	21.1
Velocity	-0.145	0.947	2.110	0.71	12.8
Distance Moved	-0.165	-0.153	1.650	0.90	10.0
Nearest Neighbor when Active	-0.370	0.296	1.500	0.76	9.1
Active Time	-0.392	0.402	1.480	0.82	9.0
Shelter Visits	-0.400	0.427	1.450	0.57	8.8
Nearest Neighbor when Idle	-0.331	0.072	0.910	0.65	5.5
Baseline vs. Secondary Response		***Average Squared Distance = 39.99***			
Nearest Neighbor when Idle	-0.331	2.430	8.040	1.98	20.1
Nearest Neighbor when Active	-0.370	2.070	6.400	1.86	16.0
Shelter Visits	-0.400	1.920	6.020	1.20	15.1
Active Time	-0.392	1.930	5.990	1.54	15.0
Distance Moved	-0.165	1.780	4.280	1.30	10.7
Shelter Time	-0.583	1.150	3.320	1.84	8.3
Distance From Source	-0.580	1.150	3.060	2.95	7.7
Velocity	-0.145	-1.680	2.870	1.62	7.2

Table 1.4: SIMPER analysis of the relevant clusters within the nMDS for golden shiners (Figure 1.2), comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Response Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Response		***Average Squared Distance = 23.06***			
Active Time	-0.493	1.480	4.160	2.21	18.1
Distance Moved	-0.485	1.460	4.130	1.79	17.9
Shelter Time	0.410	-1.230	3.280	1.23	14.2
Distance From Source	0.349	-1.050	2.850	1.14	12.4
Nearest Neighbor when Idle	0.321	-0.963	2.490	0.93	10.8
Shelter Visits	0.312	-0.935	2.410	0.81	10.4
Velocity	0.276	-0.828	2.180	0.69	9.5
Nearest Neighbor when Active	0.126	-0.379	1.550	1.10	6.7

Table 1.5: SIMPER analysis of the relevant clusters within the nMDS for bighead carp (Figure 1.3), comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Initial Resp. Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Initial Response		***Average Squared Distance = 31.66***			
Shelter Time	-0.452	2.130	7.650	1.47	24.2
Nearest Neighbor when Active	0.490	-1.530	4.720	1.64	14.9
Distance From Source	-0.260	1.730	4.560	1.48	14.4
Nearest Neighbor when Idle	0.473	-1.380	4.000	1.25	12.6
Shelter Visits	0.197	1.830	3.850	1.05	12.2
Distance Moved	0.564	-1.050	3.380	1.18	10.7
Active Time	0.603	-1.040	3.140	1.37	9.9
Velocity	-0.488	-0.394	0.366	0.82	1.2
Baseline vs. Secondary Response		***Average Squared Distance = 23.01***			
Velocity	-0.488	1.320	4.150	1.22	18.03
Active Time	0.603	-1.250	3.800	1.66	16.5
Distance Moved	0.564	-1.150	3.430	1.49	14.9
Nearest Neighbor when Idle	0.473	-0.837	2.790	0.89	12.1
Nearest Neighbor when Active	0.490	-0.842	2.600	1.12	11.3
Shelter Time	-0.452	0.597	2.230	0.91	9.7
Shelter Visits	0.197	-0.950	2.030	0.86	8.8
Distance From Source	-0.260	0.219	1.980	0.89	8.6

Table 1.6: SIMPER analysis of the relevant clusters within the nMDS for silver carp (Figure 1.4), comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Initial Resp. Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Initial Response		***Average Squared Distance = 41.36***			
Distance From Source	-0.550	3.060	13.40	3.17	32.4
Distance Moved	0.683	-2.370	9.560	3.04	23.1
Active Time	0.196	-2.690	8.780	1.96	21.2
Velocity	-0.362	1.480	3.790	1.40	9.2
Nearest Neighbor when Idle	-0.548	0.765	2.130	1.37	5.2
Nearest Neighbor when Active	-0.372	-1.160	1.430	0.81	3.5
Shelter Time	-0.223	0.378	1.260	0.77	3.0
Shelter Visits	0.374	0.658	1.010	0.68	2.5
Baseline vs. Secondary Response		***Average Squared Distance = 19.12***			
Nearest Neighbor when Idle	-0.548	0.828	3.040	0.97	15.9
Distance Moved	0.683	-0.902	2.910	1.40	15.2
Nearest Neighbor when Active	-0.372	0.714	2.440	0.84	12.8
Velocity	-0.362	0.458	2.430	0.63	12.7
Shelter Visits	0.374	-0.671	2.420	0.73	12.6
Shelter Time	-0.223	0.331	2.170	0.79	11.4
Distance From Source	-0.550	0.622	2.030	0.99	10.6
Active Time	0.196	-0.077	1.680	0.84	8.8

Chapter 2: The Effect of Social Influence on the Behavior of Juvenile Bighead Carp in Same and Mixed Species Groups

Abstract

Learning about threats through social cues expressed by shoal mates is one strategy used by prey species to gather information about their environment. By mimicking the response of an experienced individual, naïve individuals are more likely to survive. Overlapping the niches of many native prey species, the widely distributed invasive Asian carp (*Hypophthalmichthys* spp.) spend much of their early lives vulnerable to predation. By shoaling in single species or mixed species groups, the survivability of these fishes may be increased. We evaluated the response of naïve juvenile bighead carp (*Hypophthalmichthys nobilis*) when grouped with either experienced conspecific or heterospecific golden shiner (*Notemigonus crysoleucas*) shoal mates and exposed to a predator kairomone. Groups were exposed to 20 mL of largemouth bass (*Micropterus salmoides*) odor, and behavior was recorded before and after application. Our results indicate a decrease in activity and nearest neighbor distance for naïve carp paired with conspecifics, however individuals paired with experienced golden shiners imitated the response of the golden shiners, indicating the alarm response of the bighead carp may be plastic. Future research is needed to determine if naïve individuals express similar responses to subsequent applications of predator kairomones after the experienced individual is removed. These results suggest that adaptive behaviors may contribute to Asian carp's success in North America.

Introduction

Non-native bighead (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*), hereafter referred to as Asian carp, are invasive fishes that have moved throughout the Mississippi watershed and are distributed across much of the Midwestern United States (Kolar et al. 2005). Juvenile Asian carp use highly productive shallow backwater areas as nursery habitat before moving into the deeper main channel habitats (Calkins et al. 2011). As adults, Asian carp are voracious planktivores capable of profoundly influencing the zooplankton and phytoplankton communities that serve as forage for many native fishes (Cremer and Smitherman 1980, Sampson et al. 2009, Sass et al. 2014, Collins et al. 2018). As juveniles, Asian carp are vulnerable to predation (Lampo et al. 2017), and are even selected for by largemouth bass (*Micropterus salmoides*) over other native prey items (Sanft et al. 2018; *in review*). Juvenile Asian carp have similar morphology and swimming behavior as commonly consumed native prey (e.g., gizzard shad) (Kolar et al. 2005). Although Asian carp are non-native to North America, their prolific expansion has proven they are well equipped to mitigate predation pressure.

Co-evolved prey species and predators can develop unique adaptations to successfully coexist. For example, naïve hatchery-reared juvenile chinook salmon (*Oncorhynchus tshawytscha*) show an innate response to predator odors from northern pikeminnow (*Ptychocheilis oregonensis*), even if the salmon came from a population that was allopatric to that predator (Berejikian et al. 2003). Non-native fishes, like Asian carp, lack this co-evolutionary "arms-race" period with the myriad of predators found within their new range, and therefore, non-native juvenile fishes may be inefficient or have inappropriate behaviors as a response to new forms of predation risk (Pitcher 1986, Magurran 1990). Along with other inherent differences with native prey species, non-native fishes may enhance prey resources available for native predators (Crane et al. 2015, Crane and Einhouse 2016). Conversely, species capable of rapidly responding to novel predation hazards likely have a higher probability of successfully establishing populations in the face of biotic resistance from native predators (Reader and Laland 2003, Rehage et al. 2005, Alofs and Jackson 2014, Deacon and Magurran 2016).

Aggregation can be an important strategy used by fishes to increase the likelihood of survival (Pitcher 1986). Asian carp are known aggregators, and this behavior may greatly contribute to their success (Kolar et al. 2005, Ghosal et al. 2016). As fish coalesce into social groups (shoaling), they transmit an array of social cues to nearby members (Magurran and Higham 1988). This in turn allows the collective shoal to process much more information than a lone individual, and abrupt changes in the behavior of one individual can inform other members of the shoal about food availability and potential predation threats (Hamilton 1971, Krause 1993, Parrish and Edelstein-Keshet 1999). Within these groups, knowledge of predators can be indirectly shared. For instance, one individual may have prior experience with a predator (e.g. learned through first-hand encounter) and recognize an impending threat, while others within the group do not. Behavioral differences by members of the group are likely to draw more attention from predators, therefore social processes (e.g. conformity) within the group exert considerable influence over the behaviors of the naïve individuals (Webster and Ward 2011, Krause et al. 2017). For example, groups of European minnows (*Phoxinus phoxinus*) were able to transfer information about a potential threat and induce behavioral change in naïve individuals unable to observe a predator (Magurran and Higham 1988). Thus, by imitating the behavioral response shown by other members of the group to threatening cues, naïve individuals increase their survivability (Pavlov and Kasumyan 2000).

Aggregates of fish are not always homogenous. Mixed species groups are reportedly common between juvenile cyprinids and similarly sized adult cyprinids (Mainardi 1980, Krause et al. 1996, Pavlov and Kasumyan 2000), even more so when their food and habitat preferences overlap or when predation pressure is high (Morse 1977, Ward et al. 2003). Like same-species groups, imitation behavior and learned predator recognition through social cues can occur in mixed-species groups (Mathis et al. 1996). As juveniles, Asian carp occupy similar trophic niches, have similar behavioral and morphological characteristics, and are similar in size to many native cyprinids (Lee et al. 1983, Kolar et al. 2005, Boros et al. 2014, Johnson et al. 2017). Although many fish inherently select same-species groups, the adaptive flexibility hypothesis suggests that successful invaders can adopt the beneficial behavioral strategies of native species (Wright et al. 2010). Therefore, there is a possibility mixed-species groups of Asian carp and native cyprinids occur, and that these mixed-species groups facilitate the success of Asian carp. Although resource competition may occur in larger groups (Clark and Mangel 1986), same-species and mixed-species groups offer many opportunities for juvenile Asian carp to learn about predation threats without ever having direct experience with a predator.

Herein, we assess how plastic the behavior of bighead carp can be when experience level and group composition change. Our first objective was to assess the role experience plays in same-species group behavior of bighead carp and identify if the strength of the alarm response degrades as fewer experienced individuals occupy the group. Second, we assessed the influence mixed-species groups had on the behavior of naïve bighead carp, and evaluated if this effect degraded with decreasing numbers of experienced, heterospecific group mates. We used golden shiners (*Notemigonus crysoleucas*) as a model native heterospecific species in mixed-species groups because their morphology, trophic niche, and habitat use overlaps with juvenile bighead carp, and evidence suggests that they shoal with juvenile fishes from other species (Gascon and Leggett 1977, Cremer and Smitherman 1980, Krause et al. 1996, Kolar et al. 2005, Johnson et al. 2017). In the same species groups, we predicted that changes in group behavior would occur, but weaken in groups with fewer experienced individuals. In mixed species groups, we expected that if bighead carp and golden shiners form mixed-species shoals, the experienced heterospecifc fishes could inform naïve bighead carp about threats in a similar fashion, though this effect may be dampened if bighead carp outnumber the golden shiners.

Methods

Test Species

Juvenile bighead carp were obtained from Osage Catfisheries, Inc. in Osage Beach, Missouri. Golden shiners were obtained from a local bait shop near Sullivan, IL. Adult largemouth bass were collected from Lake Shelbyville in Sullivan, IL using boat electrofishing. All prey fish used were hatchery reared, and thus provided naïve test subjects with no prior experience to predation. All fish were obtained during the summer of 2016 and housed at the Kaskaskia Biological Station in Sullivan, IL in separate indoor raceways, using dechlorinated, filtered, and aerated water, with ambient temperatures at 22 ± 1 °C. Bighead carp were fed a daily crushed commercial pellet diet, whereas golden shiners were fed a commercial flake food, and largemouth bass were fed juvenile (<50 mm) bluegill (*Lepomis macrochirus*) as a non-cyprinid diet to avoid any diet-based recognition during experiments. Waste removal and partial water changes occurred biweekly to promote health and prevent water quality induced stress.

Fish were acclimated to laboratory conditions for at least 7 days prior to experimental testing. Mean fork length (FL) of all prey fish used was 51.0 ± 7.3 mm. Mean FL of largemouth bass ranged from 250 mm to 500 mm.

Experienced Fish and Stimulus Preparation

Alarm chemicals and predator odors were generated using methods by Ferrari et al. (2007) and Brown et al. (2006). To generate alarm chemicals, skin tissue was removed from 10 bighead carp (mean FL 50.3 ± 6.8 mm) and 10 golden shiners (mean FL 51.0 ± 4.4 mm). All fish used in this study were euthanized in accordance with the Illinois Institutional Animal Care and Use Committee (IACUC) by removing their heads at the base with a scalpel. Skin fillets from both sides of the fish were removed. Length and depth of the filets were measured to the nearest hundredth of a centimeter and placed in chilled deionized water. The total area of skin tissue collected was approximately 30.06 cm^2 for bighead carp and 31.42 cm^2 for golden shiners. Skin fillets were then homogenized with a blender and filtered through 20-µm mesh to remove larger particles. Samples were diluted to ~1 cm^2 tissue/5ml of deionized water and stored at −20 °C in 45 ml aliquots until use.

To generate predator odors, largemouth bass were transferred into individual 94-L aquaria filled with dechlorinated tap water and held for 24 hours to allow for waste elimination from their last meal. Largemouth bass were then transferred to clean, 94-L aquaria filled with dechlorinated tap water and left for 24 hours to ensure adequate odorization of the water. Following this 24-hour period, fish were placed back in the holding raceways. The odorized water was stirred and stored frozen in 60 ml aliquots until needed during the conditioning and testing phases.

We used methods similar to Mathis et al. (1996) to generate groups of experienced bighead carp and golden shiners. Representative fish from each species (bighead carp and golden shiners) were added in groups of three to separate, clean 37-L aquaria filled with 35 L of aerated, dechlorinated water and acclimated for 24 hours. Following acclimation, we applied 20 ml of generated predator odor and 5 ml of the generated alarm cue to each tank, pairing the two stimuli to simulate a predatory encounter. Fish were exposed to predator and alarm odors for approximately 3 hours before individuals were removed from the tanks and transferred into the appropriate experimental tank and acclimated for 24 hours. Pairing predator odors with alarm cues at this concentration has been shown to be an effective strategy when conditioning individuals to associate threat with a novel stimulus (Ferrari et al. 2005).

Experimental design

Same-species groups were randomly assigned one of the following treatment groupings: (a) three experienced individuals, (b) two experienced individuals and one naïve individual, (c) one experienced individual and two naïve individuals, or (d) three naïve individuals (n = 15 each). Similarly, mixed-species groups were randomly assigned one of the following treatment groups: (e) two “experienced” golden shiners and one “naïve” bighead carp, and (f) one “experienced” golden shiner and two “naïve” bighead carp (n = 15 each). Fish were fed to satiation, and any excess food was removed. Groups of three were used to avoid introducing any unnecessary isolation stress, since bighead carp are a schooling fish (Brown et al. 2006, Ferrari et al. 2007, Ghosal et al. 2016). To prevent external stimuli from influencing behavioral responses, experimental tanks were painted white on the external side of the glass and held inside a curtained area during testing. Odors were added to experimental tanks via an injection through air tubing placed over a submersible aquarium pump that recirculated water through the tank.

Preliminary tests showed that this system could disperse food coloring throughout the tank within 15 seconds. A 7.6 cm x 15.2 cm x 10.2 cm mesh shelter was constructed and placed in the middle of each tank to provide cover. GoPro (model Hero 6) cameras mounted above each tank were used to record each trial.

Each experimental trial lasted for ten minutes. Trials consisted of recording fish activity and behavior for five minutes pre-stimulus to develop a neutral baseline for behavior, followed by a five-minute post-stimulus period. Prior to testing, 100 ml of ambient tank water was removed from each tank and discarded, while an additional 100 ml was removed and used later as a flush. Treatment applications were administered by injecting 20 ml of the largemouth bass predator odor, and flushed with the 100 ml of ambient tank water that was previously removed. Predator odors were allowed to disperse for 30 seconds before recording activity during the post-stimulus period. Following the testing period for each trial, each fish was measured to total length and euthanized using the same methods described in the stimulus preparation section. At the completion of each trial, tanks and tubing were thoroughly cleaned with a mild detergent and dried before being reused for further trials.

Video recordings of each trial were reduced to 5 frames per second using the open-source software VirtualDub. Measurements were then extracted on a frame-by-frame basis for each individual in the tank using the behavioral tracking software Lolitrack (Loligo Systems, Viborg, Denmark). Recorded behaviors included four common alarm metrics: shelter use (Mirza et al. 2001), activity (Wisenden et al. 2009), group cohesion (Brown et al. 1970), and distance from the cue (Ferrari et al. 2005). Shelter use was measured using a count of total shelter visits and the total time (s) spent within the shelter per minute. Individuals were considered active when the software registered that the position of the fish had changed >10 pixels (10 pixels ≈ 0.5 cm).

When the software registered a fish as active, variables representing activity included the total time (s) spent active per minute, distance moved per minute, and the average velocity (cm s^{-1}) per minute. Group cohesion was measured by two values, which were determined by calculating the distance of the nearest neighbor (cm) when a fish was considered active and when it was considered idle. To adjust for size differences among fish, post-injection nearest neighbor values were converted to a ratio against the baseline average value for each fish. Values >1 indicate increased nearest neighbor distance and values <1 indicate decreased nearest neighbor distance. Distance from the source of the cue was based on the average distance of each fish from the source of the cue as it entered each tank.

Behavioral values were aggregated by minute for each individual within the experimental tank. Due to difficulties identifying which individuals in the same-species treatment groups were naïve, values were averaged across individuals to produce a tank-wide, per-minute average. In the mixed-species groups, the two species were easily distinguishable, and values of behavioral variables were averaged by species for each trial. In both same- and mixed-species groups, a pre-stimulus baseline value for each of the variables consisted of averaging the values for minutes 1-5 prior to injection of the predator odor.

Statistical Analysis

A two factor, repeated-measures, non-parametric, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was conducted for same-species treatment groups to test how behaviors for each of the groups changed when largemouth bass odor was added to the aquarium. Factors in the same-species analysis included time, treatment, and replicate ID. Time was held as a fixed repeated factor, which included the pre-stimulus baseline average (time = 0) and each of the five minutes following the treatment application for a total of 6 levels. Treatment was also considered a fixed factor (4 levels), and replicate ID was considered a random factor nested within treatment (60 levels). Within this model, a significant Treatment x Time interaction would indicate that the experience level of the group effected behavioral change of the group through time.

To test how mixed-species groups influenced the behavior of predator-naïve juvenile bighead carp, we used a three-way repeated measures PERMANOVA. As with the same-species analysis, time was held as a fixed repeated factor (6 levels). Because each species could be easily distinguished, species was included as a fixed factor (2 levels). Treatment was also considered a fixed factor (2 levels), and replicate ID was considered a random factor nested within both species and treatment. Within this model, a significant Treatment x Time interaction would signal differing effects of group composition on the behavioral change through time. A significant three-way interaction (Treatment x Species x Time) would indicate that the magnitude of response varied between the two species within each treatment group.

Statistical analysis for both tests was performed using the PERMANOVA+ add-on of PRIMER v.7 (Anderson et al. 2008, Clarke and Gorley 2015). Within this framework, we used a conservative approach when analyzing the data. Before performing each analysis, data were first square-root transformed to reduce the influence of outliers, then converted into triangular normalized Euclidean distance matrices to reduce the influence of metrics with larger values (e.g. distance traveled may average 300 cm for a minute, but the average active time that minute was only 5 s) (Anderson et al. 2008, Shirkhorshidi et al. 2015). For each model, inference was drawn by comparing measured values to a simulated distribution generated by 9999 permutations of the observed data, with fixed effects summed to zero and significance judged at a 5%

significance level with a type III sum of squares (Anderson et al. 2008). Significant model terms, most importantly interaction effects, were investigated with pair-wise post-hoc comparisons to examine how post-stimulus minutes differed from the pre-stimulus baseline. Due to multiple comparisons, post-hoc *P*-values were based on adjusted alpha-levels using the Holm-Bonferroni adjustment method (Holm 1979).

Temporal patterns for each treatment group were visualized using non-metric multi-dimensional scaling (nMDS; Kruskal 1964) based on the normalized Euclidean distance matrices of same- and mixed-species groups. To reduce the number of data points and allow for a clear demonstration of behavioral change across treatment groups, ordination plots consisted of treatment by time centroids. Personalities, such as boldness, can largely impact behavioral differences amongst groups (Leblond and Reebs 2006). However, general patterns of behavioral change amongst localized groups can be representative of the overall behavioral response (Schaerf et al. 2017). As such, the relative positions of each centroid in multivariate space can illustrate how the different treatment groupings influence behavior (Anderson et al. 2008). As a final step, hierarchical agglomerative clustering was used to group centroids into clusters, with statistically significant clusters identified with similarity profiles using the SIMPROF routine (Clarke and Warwick 1994, Clarke and Gorley 2015). Behavioral variables contributing the most to cluster separation were identified through similarity percentage analyses (Clarke 1993, Cabecinha et al. 2009, Boys and Williams 2012).

In this framework, conducting post-hoc pairwise tests and using a single point to represent each treatment by time centroid requires the data be similarly dispersed across the treatment by time groups (Anderson et al. 2008). These assumptions were tested using a permutational analysis of multivariate dispersion using the PERMDISP routine (Clarke and

Gorley 2015) and indicated no significant difference in levels of dispersion of the centroids within both same- and mixed-species groups (Same-Species groups: $F_{23, 336}$=1.0451, P= 0.59; Mixed-Species groups: $F_{23, 336}$=2.1470, P=0.07). Therefore, post-hoc comparisons could be made and each treatment by time centroid could be represented by a single point within the ordination plots.

Results

Same-Species Groups

Within the homogenous groups of bighead carp, behavioral change over time was detected, indicating that there was a response to the predator odor ($P<0.05$, Table 2.1). For each of the treatment groups containing experienced individuals, application of the largemouth bass odor initiated detectable behavioral differences compared to each group's behavioral baseline average (Table 2.2). Groups of three experienced bighead carp showed the strongest response, with significant differences from the behavioral baseline average detected at all five minutes ($P<0.05$; Table 2.2). Groups with two experienced individuals showed a somewhat weaker response. Differences were detected for two of the five minutes whereas a marginally significant response ($P<0.10$) was detected for the remaining three minutes (Table 2.2). The group with only one experienced individual showed similar results. Differences were detected for the first three minutes and marginally significant differences for the fourth minute (Table 2.2). Although not significant, minute five seemed to follow the same pattern, indicating a possible degraded response is occurring with time. The group without an experienced individual did not show any innate response to the largemouth bass odor, as differences in behaviors were not detected for any of the post-stimulus minutes (Table 2.2).

Groups containing at least one experienced individual expressed similar trajectories of behavioral change from their baseline average, whereas the group with three naïve fish did not significantly differ from their baseline behavior (Figure 2.1). Within each treatment group containing at least one experienced individual, groups expressed similar behavioral trajectories from their baseline. Among these groups, the trajectory of minute 1 consistently moved away from the baseline, whereas subsequent minutes began to trend back towards the baseline (Figure 2.1). Cluster analysis of centroids from all groups identified three separate clusters. Among these clusters, there was a discernable “baseline” cluster that included each treatment’s baseline average, as well as all of the five time centroids for the group of three naïve bighead carp. Additionally, two response clusters were identified. Despite similar trends in behavior among the groups with at least 1 experienced individual, the response cluster with the largest difference from the baseline cluster consisted solely of minute 1 from the group of three experienced bighead carp. The response cluster is characterized primarily by increased group cohesion when the fish are active and increased velocity, though their active time and distance moved was greatly reduced compared to the baseline cluster (Table 2.3). The second response cluster consisted of the remaining minutes from the group of three experienced bighead carp, as well as all post-injection minutes for the other two treatment groups with at least one experienced individual (Figure 2.1). Similar to the previous response cluster, separation from the baseline cluster is defined by less time spent active and less distance moved. However, unlike the first cluster, group cohesion was increased primarily when the fish were idle and fish visited shelter quite a bit less (Table 2.3). Among the two response clusters, differences in behavior were observed when at least one member of a group of bighead carp have experience with a predator. The trajectory of these behavioral changes matched the alarm response of bighead carp when exposed only to alarm cues, and the behavioral change was dominated by increased group cohesion and reduced activity (Chapter 1), indicating that bighead carp may associate the predator odor with potential threats and act in a similar fashion.

Mixed-Species Groups

Within the two treatment groups, there was no significant effect of species detected, and interaction effects that included this term were also not significantly different, indicating that the two species within the treatments were behaving similarly (Table 2.1). There was, however, a significant Treatment by Time effect, indicating that there was a difference between the two treatment groups at some point in time (Table 2.1). Pairwise *post-hoc* tests conducted on the interaction effect indicated that, although the baseline average (Time=0) for the two treatments were significantly different (*Pairwise Treatment by Time PERMANOVA*: at Time=0, adjusted P=0.04), both treatments differed significantly from their own respective baselines at each of the five minutes post-stimulus (Table 2.2). Additionally, minutes following the baseline were not significantly different for the two groups (*Pairwise Treatment by Time PERMANOVA*: at Time=1, 2, 3, 4, and 5, adjusted P>0.13). Baseline behaviors for mixed species groups may be influenced by group composition. However, once an experienced group mate alters its behavior in the presence of a novel cue, the behavioral patterns of the two groups ultimately converge. Further, no effect of species was detected and behavior during the minutes following the injection was not significantly different between the two groups. A uniform behavioral response by both species occurred in the two treatment groups.

In both treatment groups for the mixed species groups, behavior changed throughout time in a similar fashion. Within each of the two treatment groups, the ordination plot revealed that the bighead carp and golden shiners in these groups changed their behavior similarly through time, as centroids for the species within the two groups stayed close together at each time interval (Figure 2.2). Cluster analysis of the centroids identified three separate clusters. Although there was a significant difference between the baseline behavioral average in the PERMANOVA model, nMDS ordination and cluster analysis still identified the baseline values for both species and treatments as a singular baseline cluster (Figure 2.2). Additionally, cluster analysis also revealed two separate response clusters: one for each treatment group. Despite the differences in the response clusters, the trajectory of behavioral change for each group from the baseline followed similar patterns. Simper analysis on the two response clusters revealed that, compared to the baseline cluster, the response cluster representing the group with 2 experienced golden shiners increased their active time and distance moved while decreasing their velocity and time spent in shelter (Table 2.4). Similarly, these same variables loaded with the same trajectory for the group with only one experienced golden shiner, except this group saw an increase in shelter visits while time spent in shelter did not contribute as much to differences from the baseline.

Discussion

Imitation behavior appears to be a strong factor in determining the behavioral response of naïve bighead carp exposed to novel predator cues. We show group behavior of same-species groups of bighead carp containing at least one experienced individual changed significantly following exposure to largemouth bass odor. Further, our results indicate that such imitation behavior may not be limited to same-species groups. Mixed-species groups of experienced golden shiners and naïve bighead carp also displayed changes in group behavior when exposed to the predator odor. In both same- and mixed-species groups, the resulting behavioral changes appear to be largely dependent on specific alarm behaviors of the experienced individual(s) in the group. Alarm behaviors displayed by the same-species groups of bighead carp matched findings from a previous study (Chapter 1), and were defined by increased group cohesion and reduced activity. In the mixed-species groups, the naïve bighead carp mimicked the golden shiners by increasing activity and distance moved, a response commonly shown by golden shiners exposed to threatening stimuli (Godard et al. 1998, Rosenthal 2015, Chapter 1) . Although group composition may be much more adaptive *in situ*, our results indicate a clear example of naïve bighead carp imitating behavioral changes of group mates, regardless of species, when exposed to a novel stimulus.

We sought to determine if same-species groups of bighead carp showed degraded or diluted behavioral responses when there were fewer experienced individuals to inform naïve group mates about potential threats. Our results indicate similar behavioral changes occurred even when only one individual was considered “experienced.” Additionally, behavioral changes by groups with an experienced individual matched previously described alarm behaviors of bighead carp exposed to only chemical cues (Chapter 1), indicating that the experienced individuals could associate the predator odor as a potential threat. Through continuous exposure to experienced individuals, naïve fish can develop conditioned responses to different predator odors (Leshcheva and Zhuikov 1989). Rapidly conditioned responses are likely how naïve invaders learn about predators in their new environments.

There was no detectable behavioral change for the group of three naïve Bighead carp. Prey naïveté often serves to facilitate top predators (Sih et al. 2010) and this failure to display an innate recognition of largemouth bass odor puts juvenile bighead carp at a disadvantage when encountering predators in North America. To successfully avoid predation, a naïve individual likely requires social input from experienced group mates. However, the context for a fast growing species like bighead carp to learn from older experienced conspecific group mates is likely limited, given that the size discrepancy of juveniles and adults precludes groups of juveniles and adults from forming (Pitcher et al. 1985, Bonabeau and Dagorn 1995, Kolar et al. 2005). It is possible bighead carp mitigate this disadvantage by forming mixed-species groups. In fact, it is often the case that juveniles from one species form groups with phenotypically similar heterospecific fish (Pavlov and Kasumyan 2000). Adult golden shiners have been shown to readily form mixed-species groups, and often with the young-of-year of a much larger fish, like the White sucker (*Catostomus commersoni*) (Krause et al. 1996). Therefore, it may be advantageous for juvenile bighead carp to group with a native species and learn about dangers from more experienced group members (Mainardi 1980).

We also assessed if bighead carp in mixed-species groups would respond to subtle changes in behaviors of heterospecific group mate(s), and what that response would entail. Behaviors responsible for the separation of the baseline cluster and the two response clusters indicated that bighead carp did respond to the changes of their experienced partners. Golden shiners, who utilize fast-starts to flash-expand and observe/assess threat based on the response of other school mates (Strandburg-Peshkin et al. 2013) seemed to influence the behavior and separation between the baseline and response clusters in both mixed-species treatment groups. Both treatment groups increased active time and distance moved, that matches the expected behavioral response for golden shiners. These results indicate that bighead carp mimic the alarm response of heterospecific group mates when exposed to a novel cue. If groups form like this *in situ*, the delayed response by bighead carp to follow the dashing/darting response of the golden shiners may work simultaneously to create a delayed response. As the golden shiner dashes away to assess the situation, the bighead carp follows suit, keeping close and imitating the behavior of the experienced group mate. Delayed responses of prey movements can be efficient defense strategies employed by schooling fish, introducing confusion and reducing the number of prey caught (Demšar et al. 2015). Mixed-species aggregates are not without drawbacks. Contrasting patterns of movement may be increasingly more evident to predators in smaller groups. As morphometric and physiological differences of the species cause differences in swimming behavior, predators have more success capturing individuals, exposing the entire group to increased levels of predation (Pitcher and Wyche 1983, Landeau and Terborgh 1986). However, at larger group sizes these differences become significantly less noticeable, and the most vulnerable members of the mixed-species group are the stragglers singled out by the predators (Alevizon 1976, Herbert-Read et al. 2013).

We demonstrate the propensity for Asian carp to school; even in mixed-species groups, they are inveterate aggregators. More importantly, our results show that bighead carp can rapidly recognize subtle behavioral changes made by group mates in both same- and mixed-species groups. Within these groups, the response to external stimuli can be quite flexible, as even one experienced individual can influence the group's behavior. By picking up on these social contexts and conforming to the group response, bighead carp are displaying a high degree of behavioral flexibility. It is unknown the extent that bighead carp select same- or mixed-species groups in natural systems. However, the flexibility of their response shown in our experimental system does provide an opportunity to speculate how they can benefit. In dense habitat patches, ambush predators often experience more success as schools of fish inadvertently move close by (Savino and Stein 1989). Hampered by the lack of visual cues, and the lack of an innate response to predator odors, same-species groups of juvenile bighead carp are likely more vulnerable than juveniles operating within mixed-species groups containing native prey fish. In fact, the flexibility to utilize social contexts from experienced conspecific and heterospecific group mates is one factor that facilitates the success of invasive species (Wright et al. 2010). Studies focusing on how bighead carp in same- and mixed-species groups exposed to different predator feeding strategies could provide further insight into how bighead carp utilize the available social cues and the relative success of the fish in each group.

Figures

Figure 2.1: nMDS of Treatment by Time centroids for each same-species group. Treatment groups a) 3 experienced members, b) 2 experienced members/1 naïve member, c) 1 experienced member/2 naïve members, d) 3 naïve members of bighead carp were exposed to largemouth bass predator odors. Numbers represent time, with time = 0 indicating baseline average and 1-5 indicating minutes following treatment application. Grey circles represent significant clusters identified by similarity profiles.

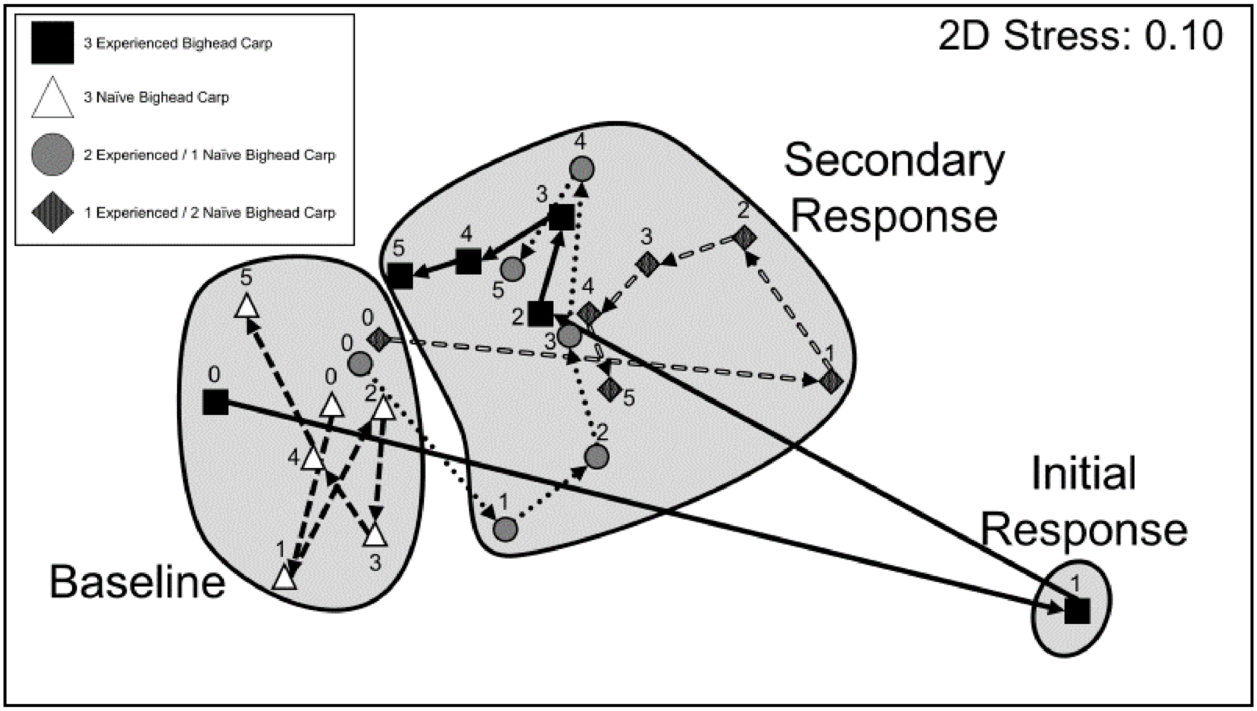
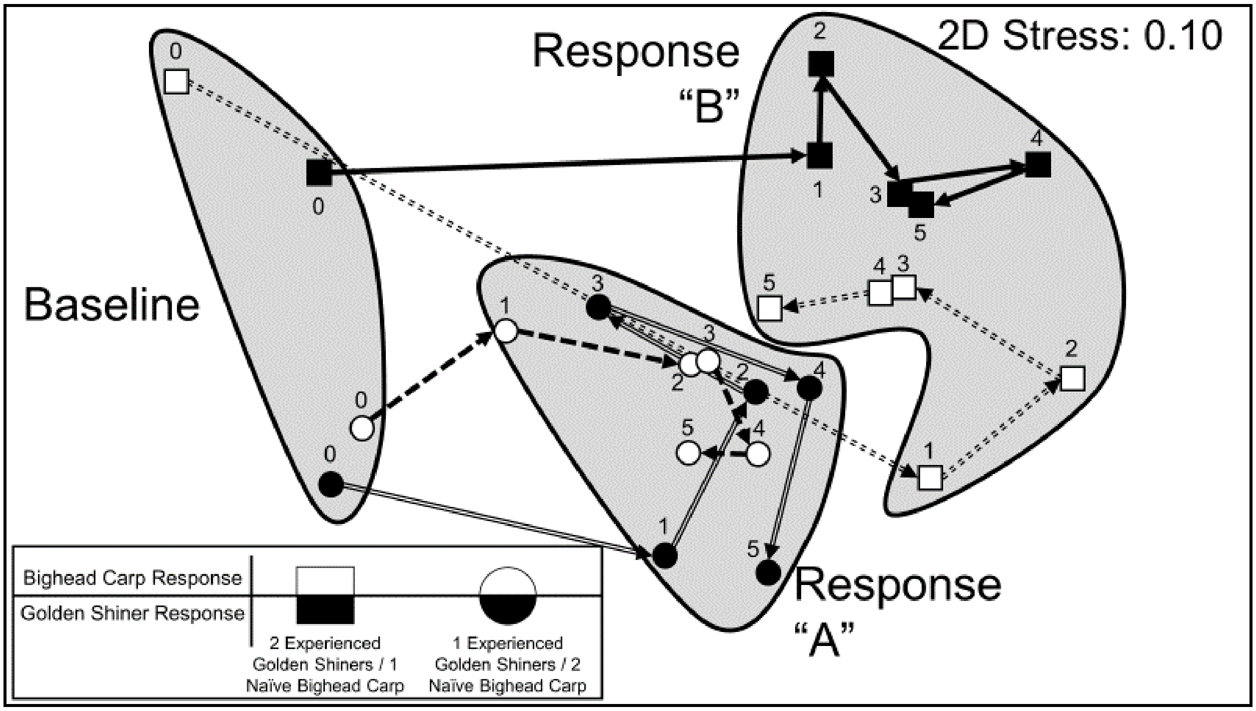


Figure 2.2: nMDS of Treatment by Time centroids for each mixed-species treatment group of bighead carp and golden shiners. Treatment groups a) 2 experienced golden shiners/1 naïve bighead carp and b) 1 experienced golden shiner/2 naïve bighead carp were exposed to Largemouth bass predator odors. Numbers represent time, with time = 0 indicating baseline average and 1-5 indicating minutes following treatment application. Grey circles represent significant clusters identified by similarity profiles.



Tables

Table 2.1: Repeated-measures, non-parametric, PERMANOVA, testing how treatment group a) 3 experienced members, b) 2 experienced members/1 naïve member, c) 1 experienced member/2 naïve members, d) 3 naïve members effected behavioral change through time for same species groups of juvenile bighead carp. Also included is the three-factor, repeated-measures, non-parametric, PERMANOVA, testing how species composition (golden shiner and bighead carp) and treatment group a) 2 experienced golden shiners/1 naïve bighead carp, b) 1 experienced golden shiner/2 naïve bighead carp effected behavioral change through time for naïve juvenile bighead carp and experienced golden shiners. Replicate ID was a random factor nested within treatment for same-species groups and nested within the treatment by species interaction for mixed-species groups.

	df	SS	MS	Pseudo-F	P(Perm)	Unique Perms
Same-Species Groups						
Treatment	3	3022.6	1007.5	1.4047	0.19	9947
Minute	5	2864.2	572.9	7.6123	0.0001	9929
ID(Treatment)	56	40165.0	717.2	9.5309	0.0001	9785
Treatment x Minute	15	1853.0	123.5	1.6416	0.01	9879
Residual	280	21071.0	75.3			
Total	359	68975.0				
Mixed-Species Groups						
Species	1	5.1	5.1	0.2210	0.96	9943
Treatment	1	67.4	67.36	2.9370	0.02	9945
Minute	5	145.3	29.1	6.3490	0.0001	9907
Species x Treatment	1	18.6	18.6	0.8110	0.54	9936
Species x Minute	5	16.3	3.3	0.7120	0.85	9899
Treatment x Minute	5	36.6	7.3	1.5990	0.03	9903
ID(Treatment x Species)	56	1284.2	22.9	5.0100	0.0001	9684
Species x Treatment x Minute	5	20.5	4.1	0.8950	0.61	9876
Residual	280	1281.7	4.6			
Total	359	2875.6				

Table 2.2: Post-hoc comparisons of the Treatment by Time interaction term (Table 2.1) for Same-Species treatment groups of bighead carp (BHC) and the significant Treatment by Minute term (Table 2.1) by minute for the Mixed-Species groups of bighead carp and golden shiners (GS) against their respective baseline values (Time = 0).

	Minute 1		**Minute 2**		**Minute 3**		**Minute 4**		**Minute 5**	
	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms	Adjusted *P*	Unique Perms
Same-Species Groups										
3 Exp. BHC	0.002	9957	0.002	9960	0.001	9972	0.01	9935	0.04	9947
3 Naïve BHC	0.10	9950	0.51	9963	0.11	9964	0.37	9953	0.24	9960
2 Exp. BHC/ 1 Naïve BHC	0.06	9953	0.02	9946	0.05	9963	0.01	9951	0.05	9970
1 Exp. BHC/2 Naïve BHC	0.01	9961	0.02	9955	0.02	9967	0.08	9958	0.11	9953
Mixed-Species Groups										
2 Exp. GS/ 1 Naïve BHC	0.001	9947	0.0003	9945	0.0004	9948	0.001	9945	0.001	9942
1 Exp. GS/ 2 Naïve BHC	0.03	9945	0.001	9953	0.001	9944	0.001	9948	0.001	9957

Table 2.3: SIMPER analysis of the relevant clusters within the nMDS for Same-Species groups (Figure 2.1), comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Initial Resp. Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Initial Response		***Average Squared Distance = 56.78***			
Nearest Neighbor when Active	0.572	-3.640	17.800	6.24	31.4
Velocity	-0.651	3.360	16.200	5.93	28.5
Active Time	1.030	-1.790	8.130	3.20	14.3
Distance Moved	1.020	-1.640	7.220	2.98	12.7
Shelter Time	-0.068	1.770	3.790	1.55	6.7
Nearest Neighbor when Idle	0.725	-0.495	2.020	0.83	3.6
Distance From Source	0.485	0.302	1.340	0.53	2.4
Shelter Visits	0.850	0.684	0.291	1.10	0.5
Baseline vs. Secondary Response		***Average Squared Distance = 17.84***			
Distance Moved	1.020	-0.537	2.980	1.10	16.7
Shelter Visits	0.850	-0.595	2.970	1.23	16.7
Active Time	1.030	-0.534	2.950	1.18	16.6
Nearest Neighbor when Idle	0.725	-0.431	2.630	0.84	14.7
Distance From Source	0.485	-0.333	2.510	0.61	14.1
Shelter Time	-0.068	-0.083	1.560	0.74	8.7
Velocity	-0.651	0.179	1.260	0.76	7.1
Nearest Neighbor when Active	0.572	-0.108	0.977	0.97	5.5

Table 2.4: SIMPER analysis of the relevant clusters within the nMDS for Mixed-Species groups (Figure 2.2), comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Response Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Response "A"		***Average Squared Distance = 4.20***			
Active Time	-0.787	0.357	1.350	2.90	32.1
Velocity	0.744	-0.160	0.918	1.56	21.8
Distance Moved	-0.607	0.335	0.912	3.05	21.7
Shelter Time	0.221	-0.264	0.422	0.99	10.1
Distance From Source	-0.096	0.091	0.212	0.75	5.1
Shelter Visits	-0.194	-0.146	0.173	1.00	4.1
Nearest Neighbor when Idle	0.003	0.090	0.126	1.02	3.0
Nearest Neighbor when Active	-0.116	-0.073	0.091	0.92	2.2
Baseline vs. Response "B"		***Average Squared Distance = 2.63***			
Velocity	0.744	-0.138	0.880	1.55	33.4
Active Time	-0.787	-0.042	0.606	1.95	23.0
Shelter Visits	-0.194	0.224	0.413	0.77	15.7
Distance Moved	-0.607	-0.092	0.307	1.47	11.7
Shelter Time	0.221	0.175	0.198	1.48	7.5
Nearest Neighbor when Active	-0.116	0.125	0.104	0.84	3.9
Distance from Source	-0.096	-0.053	0.085	0.79	3.2
Nearest Neighbor when Idle	0.003	-0.091	0.042	0.73	1.6

Chapter 3: Non-Native Silver Carp Fail to Generalize Behaviors When Exposed to Odors from Three North American Predators

Abstract

Habitats within the invaded range of Asian carp (*Hypophthalmichthys* spp.) may vary considerably, and as a result, so may the predator assemblages. Predator-prey interactions exert significant influence over the survival of juvenile cohorts. Closely related predators often share similar characteristics (e.g. odors or body morphometry), and prey species capable of expressing generalized behavioral responses to predators with similar characteristics may increase their chances for survival. Here, we examined how naïve juvenile silver carp (*Hypophthalmichthys molitrix*) respond to three predator odors from predators commonly found in Midwestern lakes and rivers: two congeneric species of bass (largemouth *Micropterus salmoides* and smallmouth *M. dolomieu* bass) and longnose gar (*Lepisosteus osseus*). Additionally, we tested how silver carp conditioned to recognize largemouth bass odor responded to the same three predator odors. We found that juvenile silver carp showed no innate response to any of the three predator odors. Additionally, although they could be conditioned to recognize predator odors from largemouth bass, they were unable to generalize the predator odors of the bass and groups conditioned to recognize largemouth bass odors did not respond when exposed to smallmouth bass or longnose gar odor. Though we speculate as to why the lack of generalized behavior as a response to predator odors may be the case, future studies are needed to discern the effects these results may have on the mortality of juvenile silver carp *in situ*.

Introduction

The dynamic nature of predator and prey interactions is largely influenced by the experiences they have with one another (Lima and Dill 1990). In aquatic environments, both predator and prey are exposed to numerous stimuli from many sources (e.g. chemical, visual, auditory, cultural, etc.). Although the result of predatory encounters is generally a life or death binary for the prey, how they arrive at one result or the other is contingent on how they interpret the cues they are given (Dall et al. 2005). Rapidly detecting predators before being detected by the predator is often a requisite for the survival of the prey (Reader and Laland 2003). In some instances, it may even be beneficial for prey species to develop an innate response to a sympatric predator, particularly if the predator and prey coexist for long periods of evolutionary time (Berejikian et al. 2003). More often than not, however, prey species are faced with threats from a variety of predators.

It is well understood that predation is an important factor when accounting for larval and juvenile survival of fishes (Rice et al. 1997). Predatory fish assemblages are diverse and prey fishes may face changing predator assemblages throughout their lives. However, closely related piscivores tend to possess several similar morphological and physiological adaptations (Bower and Piller 2015), and selection favors prey species capable of adaptively responding to predators as the individual progresses through its specific life history (Wright et al. 2010). Therefore, prey species capable of expressing generalized behavioral responses to predators with similar characteristics would be at a selective advantage. The basis of these concepts was demonstrated by Ferrari et al. (2007), finding that fathead minnows (*Pimephales promelas*) trained using lake trout (*Salvelinus namaycush*) odor as a predatory reference generalized their anti-predator behavioral responses to the odors of closely related brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*), yet failed to exhibit similar responses when exposed to northern pike (*Esox lucius*) or white suckers (*Catostomus commersoni*). In the context of non-native prey species, generalizing behavioral responses to similar native predators is likely useful when predation pressure would otherwise prevent successful invasion (Elton 1958, Deacon and Magurran 2016). Consequently, aquatic systems can be much more resistant to invasions if propagules are exposed to high predation pressure from species-rich predator assemblages (Carey and Wahl 2010, Bajer et al. 2012).

Since the 1970's, the fast-growing Asian carp have established themselves in rivers across much of the Midwestern United States (Kolar et al. 2005, Williamson and Garvey 2005). These highly successful invaders have been the cause of much concern, predominately due to the economic and ecological threats they pose to the Great Lakes fishery (Mandrak and Cudmore 2010, Tsehaye et al. 2013, Collins et al. 2018). As a result, studies have been conducted to better understand the biology and life-histories of these fishes. Throughout their lives, these fish are voracious planktivores, and exploit these resources to grow quickly, however they are likely vulnerable to predation during their first year (Williamson and Garvey 2005, Sass et al. 2014, Lampo et al. 2017). During early ontogeny, juvenile Asian carp use highly productive shallow backwater areas as nursery habitat before moving into deeper main channel riverine habitats as adults (Calkins et al. 2011), and this ontogenetically influenced habitat use exposes juvenile Asian carp to a myriad of predators. However, if Asian carp can generalize novel predation risks based on a previous predatory experience, predation pressure may be reduced as they move between habitats.

Understanding and characterizing factors that influence the vulnerability of a habitat to invasion is critical for effective management (Degrandchamp et al. 2008). Despite the influence behavior can have on the success of an invasive fish (Deacon and Magurran 2016), very few studies have investigated how these fish apply experience to behavioral decisions. For Asian carp, the ability to generalize novel predation risks may be a factor in predicting the likelihood of establishment in new areas. The objective of our study was to assess how juvenile silver carp (*Hypophthalmichthys molitrix*) could generalize their experience with one predator odor and apply it to a close relative of that predator. To do this, we created two groups of silver carp: one conditioned to recognize largemouth bass odor (*Micropterus salmoides*) and one that was not. We compared how behaviors of these groups changed when exposed to predator odors from largemouth bass, smallmouth bass (*Micropterus dolomieu*), and longnose gar (*Lepisosteus osseus*). Since Asian carp are invasive and have cohabitated with North American predators for a relatively short period of time, we hypothesized that they could not innately recognize North American predator odors. Because of this, we predicted the group not conditioned to recognize largemouth bass odor would show no significant changes in behavior when exposed to any of the three predator odors. If silver carp were capable of generalizing predator odors, we predicted that prior conditioning with the largemouth bass odor would result in a similar behavioral change when exposed to either of the congeneric bass odors, yet there would be little to no behavioral change when exposed to the longnose gar odor.

Methods

Test Species

Juvenile silver carp were obtained from the U.S. Geological Survey’s Columbia Environmental Research Center in Columbia, Missouri. Adult largemouth bass and adult longnose gar were collected using boat electrofishing from Lake Shelbyville in Sullivan, IL.

Adult smallmouth bass were collected using boat electrofishing from the Middle Fork of the Kaskaskia River near Penfield, IL. All fish were obtained during the summer of 2016 and housed at the Kaskaskia Biological Station in Sullivan, IL in separate indoor raceways, using dechlorinated, filtered, and aerated water, with ambient temperatures at 22 ± 1 °C. All silver carp were hatchery reared from wild broodstock, and thus provided naïve test subjects with no prior experience to predation. Silver carp were fed a crushed commercial pellet diet daily, whereas all predators were fed juvenile (<50 mm) bluegill (*Lepomis macrochirus*) as a non-cyprinid diet to avoid any diet-based recognition during experiments. Waste removal and partial water changes occurred biweekly to promote health and prevent water quality induced stress. Fish were acclimated to laboratory conditions for at least 7 days prior to experimental testing. Mean fork length (FL) of all juvenile silver carp was held uniform at 50.2 ± 7.1 mm. Mean FL of all predators ranged from 250 mm to 500 mm.

Stimulus Preparation

Alarm chemicals and predator odors were generated using methods by Ferrari et al. (2007) and Brown et al. (2006). To generate alarm chemicals, skin tissue was removed from 10 silver carp (mean FL 51.1 ± 7.9 mm). All fish used in this study were euthanized in accordance with the Illinois Institutional Animal Care and Use Committee (IACUC) by removing their heads at the base with a scalpel. Skin fillets from both sides of the fish were removed and length and depth of the filets were measured to the nearest hundredth of a centimeter, and placed in chilled deionized water. The total area of skin tissue collected was approximately 30.66 cm^2. Skin fillets were then homogenized with a blender and filtered through 20 μm mesh to remove larger particles. Samples were diluted to ~1 cm^2 tissue/5 ml of deionized water and stored at −20 °C in 45 ml aliquots until use.

To generate predator odors, each predator was transferred into individual 94-L aquaria filled with dechlorinated tap water and held for 24 hours to allow for adequate waste elimination. Predators were then transferred to clean, 94-L aquaria filled with dechlorinated tap water and left for 24 hours to ensure adequate odorization of the water. After this 24-hour period, fish were placed back in the holding raceways. The odorized water was stirred and stored at −20 °C in 60 ml aliquots until needed during the conditioning and testing phases.

Experimental Design

All tanks used during the conditioning and experimental periods were 37-L aquaria filled with 35 L of aerated, dechlorinated water. Each tank was affixed with a single line of tubing that was run to a check valve system outside of a curtained area to prevent any external stimuli from influencing the behavioral responses of the test fish while the researchers applied the various stimuli. This tubing was placed directly over a submersible aquarium pump that recirculated water throughout the tank. Preliminary tests showed that this system dispersed food coloring throughout the tank within 15 seconds. Additionally, all tanks had a 7.6 cm x 15.2 cm x 10.2 cm mesh shelter at the center to provide cover, as well as an air stone, which was used for the duration of the acclimation and conditioning periods, but removed during the testing period. Silver carp are believed to be a schooling fish (Ghosal et al. 2016), therefore to prevent isolation stress, groups of three silver carp were added to each tank and acclimated for 24 hours before conditioning occurred. After the fish were added to each tank, and before the conditioning and testing periods, fish were fed to satiation, and any excess food was removed.

Two conditioning groups were created using methods similar to that of Ferrari et al. (2007) and each tank was randomly assigned to one of the conditioning groups. After the first acclimation period, 100 ml of water was removed from the tubing connected to the check valve system and discarded. An additional 100 ml was removed and saved to later flush the stimuli back through the tubing into the tank. After the ambient water was removed, 20 ml of largemouth bass odor was flushed through the check valve system into the tank and followed by either 5 ml of the generated alarm cue (Alarm Group) or 5 ml of aerated, dechlorinated tap water (Null Group). Fish from each conditioning group were exposed to the paired stimuli for approximately 3 hours before being removed from the tanks and transferred into the experimental tanks. Once again, fish were fed to satiation, excess food was removed, and fish were allowed to acclimate again for another 24-hour period.

Once in the experimental tanks, treatment groups were then randomly assigned one of the three predator odors: largemouth bass, smallmouth bass, or longnose gar. Ten replicates of each treatment (predator odor) were performed within the two conditioning groups (Alarm and Null groups). One hour prior to the testing period, GoPro (model Hero 6) cameras were mounted above each tank and used to record each trial. As we did during the conditioning period, 100 ml of water was removed and discarded from each tank, while an additional 100 ml was removed and set aside to flush the treatment stimuli back into the tank. Treatment applications were administered by injecting 20 ml of the assigned predator odor into each tank, followed by the ambient tank water flush. Each experimental session lasted for ten minutes. Each session consisted of recording fish activity and behavior for five minutes pre-stimulus to develop a neutral baseline for behavior, followed by a five-minute post-stimulus period. After flushing the assigned treatment into each tank, predator odors dispersed for 30 seconds before recording activity for the post-stimulus period. Following the testing period, all tanks and fixtures were thoroughly drained and cleaned, and each fish was measured to total length and euthanized using the same methods described in the stimulus preparation section.

Video recordings of each trial were reduced to 5 frames per second using the open-source software VirtualDub. Measurements were then extracted on a frame-by-frame basis for each individual in the tank using the behavioral tracking software, Lolitrack (Loligo Systems, Viborg, Denmark). Recorded behaviors included four common alarm metrics: shelter use (Mirza et al. 2001), activity (Wisenden et al. 2009), group cohesion (Brown et al. 1970), and distance from the cue (Ferrari et al. 2005). Shelter use was measured using a count of total shelter visits and the total time (s) spent within the shelter per minute. Individuals were considered active when the software registered that the position of the fish had changed >10 pixels (10 pixels ≈ 0.5 cm). When the software registered a fish as active, variables representing activity included the total time (s) spent active per minute, distance moved per minute, and the average velocity (cm s^{-1}) per minute. Group cohesion was measured by two values, which were determined by calculating the distance of the nearest neighbor (cm) when a fish was considered active and when it was considered idle. To adjust for size differences among fish, post-injection nearest neighbor values were converted to a ratio against the baseline average value for each fish. Values >1 indicate increased nearest neighbor distance and values <1 indicate decreased nearest neighbor distance. Distance from the source of the cue was based on the average distance of each fish from the source of the cue as it entered each tank. Behavioral values were aggregated by minute for each individual within the experimental tank, then averaged together to form a tank-wide average at each minute. Within each tank, a pre-stimulus baseline value for each of the variables consisted of averaging the values for minutes 1-5 prior to applying the appropriate treatment. This baseline average is referred to in the analysis portion as time = 0.

Statistical Analysis

A three-factor, repeated-measures, non-parametric, permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was conducted to test how the different conditioning groups of silver carp may influence how they respond to one of three different predator odors. Within this model, factors included conditioning group, treatment, time, and replicate ID. Time was held as a fixed, repeated factor, which included the pre-stimulus baseline average (time = 0) and each of the five minutes following the treatment application (time = minutes 1-5) for a total of 6 levels. Conditioning group (2 levels) and treatment (3 levels) were also considered fixed effects. Replicate ID was considered a random factor nested within treatment and conditioning group. A significant two-way interaction effect of Treatment x Time would tell of the effects the different treatments have on the behavior of silver carp, but would not tell how conditioning would affect behavior. Similarly, a significant interaction of Condition x Time would only tell us the effect conditioning group has on the behavior through time without assessing the effects of the different treatments. Because of this, we are most interested in a significant three-way interaction effect (Condition x Treatment x Time), as this would indicate that the magnitude of response through time varied between conditioning groups exposed to the different treatments.

Statistical analysis was performed using the PERMANOVA+ add-on of PRIMER v.7 (Anderson et al. 2008, Clarke and Gorley 2015). Within this framework, we used a conservative approach when analyzing the data. Before performing this analysis, data were first square-root transformed to reduce the influence of outliers, then converted into triangular normalized Euclidean distance matrices to reduce the influence of metrics with larger values (e.g. distance traveled may average 300cm for a minute, but the average active time that minute was only 5s) (Anderson et al. 2008, Shirkhorshidi et al. 2015). Inference was drawn by comparing measured values to a simulated distribution generated by 9999 permutations of the observed data, with fixed effects summed to zero and significance judged at a 10% significance level with a type III sum of squares (Anderson et al. 2008). Significant model terms, most importantly interaction effects, were investigated with pair-wise post-hoc comparisons to examine how post-stimulus minutes differed from the pre-stimulus baseline. Due to multiple comparisons, post-hoc *P*-values were based on adjusted alpha-levels using the Holm-Bonferroni adjustment method and significance was judged at a 10% significance level (Holm 1979).

Temporal patterns were visualized using non-metric multi-dimensional scaling (nMDS; Kruskal 1964) based on the normalized Euclidean distance matrices. To reduce the number of data points and allow for a clear demonstration of behavioral change across conditioning and treatment groups, ordination plots consisted of condition by treatment by time centroids. Personalities, such as boldness, can largely impact behavioral differences amongst groups (Leblond and Reebs 2006). However, general patterns of behavioral change amongst localized groups can be representative of the overall behavioral response (Schaerf et al. 2017). As such, the relative positions of each centroid in multivariate space can illustrate how the different treatment groupings influence behavior (Anderson et al. 2008). As a final step, hierarchical agglomerative clustering was used to group centroids into clusters, with statistically significant clusters identified with similarity profiles using the SIMPROF routine (Clarke and Warwick 1994, Clarke and Gorley 2015). Behavioral variables contributing the most to cluster separation were identified through similarity percentage analyses (Clarke 1993, Cabecinha et al. 2009, Boys and Williams 2012).

In this framework, conducting *post-hoc* pairwise tests and using a single point to represent each centroid requires the data be similarly dispersed across the condition by treatment by time groups (Anderson et al. 2008). The assumption was tested using a permutational analysis of multivariate dispersion judged at a 5% significance level using the PERMDISP routine (Clarke and Gorley 2015) and indicated no significant difference in levels of dispersion of the condition by treatment by time points ($F_{35, 324}$=1.636, P= 0.06). Therefore, *post-hoc* comparisons could be made and each condition by treatment by time centroid could be represented by a single point within the ordination plots.

Results

As expected, none of the two-way interactions within our model were significant ($P >$ 0.10, Table 3.1), indicating that neither the conditioning group nor the treatment applied sufficiently explained behavioral differences through time. However, a significant three-way interaction (Condition x Treatment x Time) was detected ($P <$ 0.10, Table 3.1), and was accompanied by a significant Time effect ($P <$ 0.10, Table 3.1), indicating that combinations of condition and treatment affected the behaviors of the silver carp through time. Pairwise *post-hoc* tests conducted on this interaction effect held Condition and Treatment constant while comparing the minutes within each Condition and Treatment group. Silver carp conditioned with largemouth bass odor and conspecific alarm chemicals and later exposed to largemouth bass odor significantly altered their behavior when comparing the baseline average (Time=0) against each of the post-stimulus minutes ($P \leq$ 0.10, Table 3.2), indicating that silver carp can recognize a predator once exposed to a simulated threatening experience. Within this same conditioning group, silver carp did not significantly change their behavior when exposed to the smallmouth bass odor, nor the longnose gar odor ($P >$ 0.10, Table 3.2). Additionally, treatment groups of silver carp conditioned with largemouth bass odor and the dechlorinated tap water did not alter their behaviors when exposed to any of the three predator odors ($P >$ 0.10, Table 3.2).

Ordination plots revealed similar baseline behaviors (Time = 0) for all treatment and conditioning groups (Figure 3.1), and cluster analysis of the centroids within this ordination mirrored the results from the pairwise PERMANOVA in Table 3.2. Silver carp that were conditioned with largemouth bass odor and a conspecific alarm chemical were the only group to deviate significantly from their baseline (Figure 3.1). Minutes 1-5 for this group were the only centroids not to be included within the baseline cluster, and instead formed a separate cluster (Figure 3.1). SIMPER analysis indicated that the trajectory of behavioral change between the baseline and response clusters were best described by a reduction in group cohesion (when fish were either active or idle; Table 3.3).

Discussion

Our results demonstrate silver carp can learn to recognize odors from a North American predator, though they may not do so innately or by generalizing predators with similar odors. Among the groups of silver carp that were not conditioned with the paired largemouth bass odor and alarm cue, we did not find any differences in behavior when exposed to the three North American predator odors. Innate recognition of predators is generally thought to be dependent on the coevolutionary history of the predator and prey (Ferrari et al. 2010). The prey naïveté hypothesis developed by Sih et al. (2010) suggests that the lack of evolutionary history between North American predators and silver carp should disproportionately favor the predators, provided anti-predator responses of the prey are inappropriate. In a similar study, tadpoles experienced higher predation mortality when exposed to an invasive predator (Gomez-Mestre and Díaz-Paniagua 2011). Failure to show any response to predator cues may dramatically increase mortality, but learned recognition of predators may be more practical than recognizing a predator based on some innate intuition, as individuals may be more situationally adaptive (Brown and

Chivers 2005, Wright et al. 2010). We show that silver carp can learn to recognize predator odors, as groups conditioned with the paired odor/alarm cue were found to display significant behavioral differences after being exposed to largemouth bass odor. The increased distance among individuals within these groups reflects similar alarm behaviors of juvenile silver carp exposed to conspecific alarm chemicals (Chapter 1). We did not test how the mortality rates of experienced and naïve juvenile silver carp varied when exposed to actual predators. However, predator naïve silver carp have been shown to be more vulnerable to predation by largemouth bass than other native prey (Sanft et al. 2018, *in review*), and this may indicate that their alarm responses may not be appropriate.

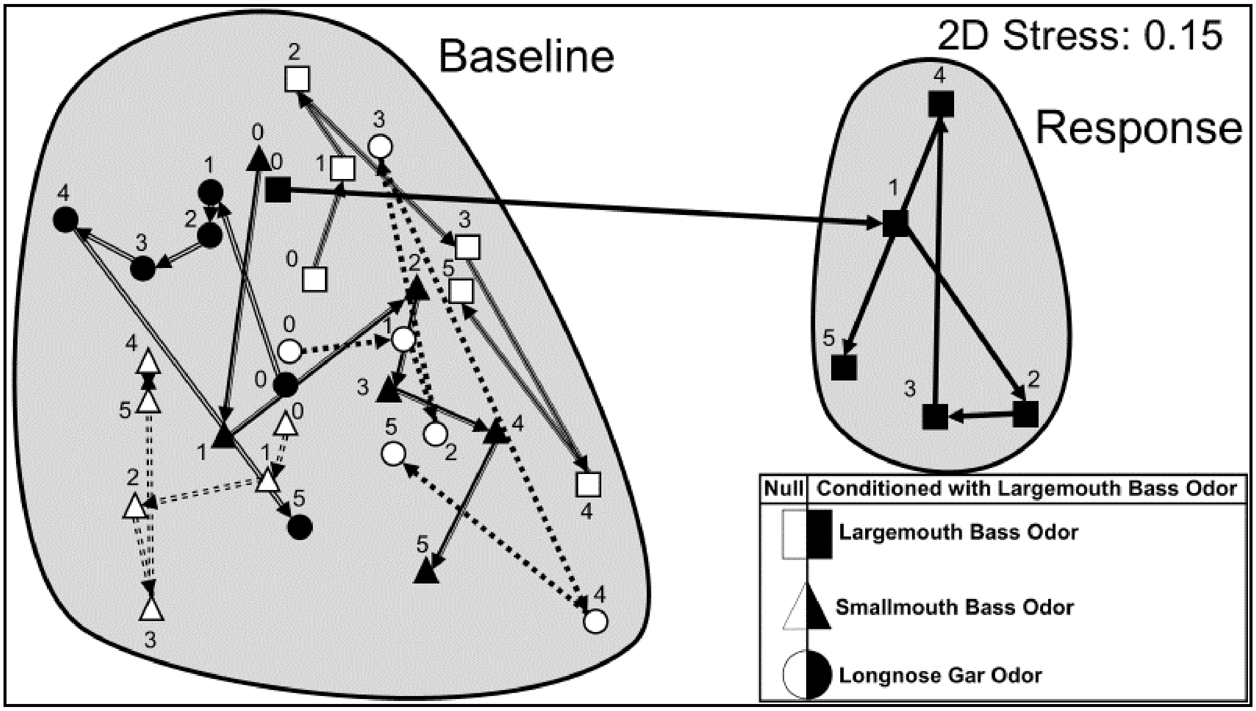
Generalizing predators is an adaptive way prey species apply previous experience to novel predators by co-opting similar odors into a singular “predator” category, ultimately increasing predator detection probability in environments with multiple predators (Ferrari et al. 2016). In our system, we did not find any evidence that silver carp could generalize predator odors, as groups conditioned to recognize largemouth bass odor did not change their behavior when exposed to predator odors of the similar smallmouth bass or the more distant longnose gar. Several reasons could explain why their behavior did not change in this context. For example, the predator odors of largemouth and smallmouth bass may not be as similar as we had predicted. Odor signatures allow prey fish to generalize odors between predators, and is presumed to be influenced by the taxonomic relatedness of the predators (Ferrari et al. 2007). Largemouth and smallmouth bass are closely related and express many ecological similarities, such as diet overlap and morphometry (Philipp et al. 1979, Olson and Young 2003). However, they show preference for different habitats: largemouth bass are commonly found in vegetated lakes, whereas smallmouth bass prefer cooler mid-order streams (Edwards et al. 1983, Raibley et al. 1997). Environmental conditions of each species' habitat may influence their respective body chemistries in a way that could obfuscate the similarities of their odors.

Failing to associate predator odors for congeneric predators may actually benefit silver carp. Simply put, they may not need to generalize predators. When organisms perceive less threat, they are more likely to spend more time foraging (Brown et al. 2006). For example, the invasive mosquitofish (*Gambusia affinis*) has been shown to experience higher levels of foraging success, even in the presence of predators, than its native counterparts (Rehage et al. 2005). Although this could lead to increased juvenile mortality with many serving as forage for native predators (Carlsson et al. 2009), increased resource consumption, rapid growth, and high fecundity (Williamson and Garvey 2005) likely ensures some level of survivorship as individuals outgrow the gape limitations of many potential predators across their invaded range (Hambright et al. 1991).

Detecting predators through olfaction has been shown to be widespread across fish taxa (Hara 1994, Ferrari et al. 2010), and juvenile silver carp appear to be no exception. Although generalization of predators by silver carp may occur through other vectors, like visual cues (McCormick and Manassa 2007), our results indicate that this does not occur as a result of predator odors. However, we did not test how visual detection may influence juvenile Asian carp survival. Additionally, how Asian carp respond to predator cues and whether or not the behavioral response increases the likelihood of survival when faced with North American predators is also unclear. Without additional tests, discussion about the role predator generalization has on the survival of silver carp in novel environments remains speculative. Future studies using different types of cues or different predator combinations would provide a more definitive idea of how behavior and predator recognition affects survival of silver carp.

Figure

Figure 3.1: nMDS of Treatment by Time centroids for silver carp conditioned and unconditioned with largemouth bass odor exposed to various treatment groups. Numbers represent time, with time = 0 indicating baseline average and 1-5 indicating minutes following treatment application. Grey circles represent significant clusters identified by similarity profiles.



Tables

Table 3.1: A three-factor, repeated-measures, non-parametric, PERMANOVA testing how conditioning groups a) largemouth bass odor/distilled water and b) largemouth bass odor/silver carp alarm cue and treatment groups a) largemouth bass odor, b) smallmouth bass odor, and c) longnose gar odor effected behavioral change through time for juvenile silver carp. Replicate ID was a random factor nested within the treatment by conditioning interaction.

	df	SS	MS	Pseudo-F	P(perm)	Unique Perms
Treatment	2	75.255	37.628	1.336	0.22	9938
Condition	1	20.699	20.699	0.735	0.56	9937
Minute	5	30.809	6.162	1.558	0.05	9896
Treatment x Condition	2	47.046	23.523	0.835	0.57	9922
Treatment x Minute	10	32.523	3.252	0.822	0.80	9865
Condition x Minute	5	21.901	4.380	1.107	0.32	9892
ID(Treatment x Condition)	54	1521.300	28.172	7.123	0.0001	9704
Treatment x Condition x Minute	10	54.512	5.451	1.378	0.05	9847
Residual	270	1067.900	3.955			
Total	359	2872.000				

Table 3.2: Pairwise *post-hoc* comparisons for largemouth bass (LMB), smallmouth bass (SMB) and longnose gar (GAR) odors tested within each conditioning group (Alarm or Null) comparing each of the minutes post stimulus against the respective baseline value (Time = 0).

		Minute 1		**Minute 2**		**Minute 3**		**Minute 4**		**Minute 5**	
Conditioning Group	Treatment Group	Adj. *p*-val	Unique Perms	Adj. *p*-val	Unique Perms	Adj. *p*-val	Unique Perms	Adj. *p*-val	Unique Perms	Adj. *p*-val	Unique Perms
Alarm	*LMB*	0.08	9922	0.10	9943	0.04	9934	0.05	9941	0.10	9924
	SMB	0.25	9929	0.26	9935	0.33	9942	0.32	9946	0.24	9942
	GAR	0.36	9942	0.79	9931	0.51	9934	0.36	9938	0.57	9944
Null	*LMB*	0.73	9941	0.99	9942	1.16	9943	0.83	9938	1.32	9934
	SMB	0.91	9955	1.78	9942	0.74	9940	2.11	9938	1.51	9940
	GAR	0.67	9940	3.10	9940	0.11	9926	0.03	9936	0.49	9946

Table 3.3: SIMPER analysis of 2 clusters within the nMDS, comparing which variables accounted for separation of the baseline cluster and each significantly different cluster identified by the similarity profiles of the centroids.

	Baseline Avg. Value	Response Avg. Value	Avg. Sq. Distance	Sq. Distance / SD	Contributing %
Baseline vs. Response		***Average Squared Distance = 3.29***			
Nearest Neighbor when Idle	-0.158	0.978	1.360	2.30	41.23
Nearest Neighbor when Active	-0.144	0.894	1.160	1.92	35.32
Shelter Visits	-0.015	0.095	0.205	0.81	6.25
Distance from Source	0.007	-0.044	0.167	0.74	5.07
Velocity	0.011	-0.067	0.148	0.74	4.49
Shelter Time	0.021	-0.130	0.125	0.75	3.80
Distance Moved	0.007	-0.041	0.072	0.73	2.19
Active Time	-0.011	0.071	0.054	0.73	1.65

Chapter 4: Conclusions

The adaptive response of bighead and silver carp to potentially threatening scenarios is undoubtedly a contributing factor to their success in North America. Though neither species showed a detectable response to alarm cues from native cyprinids, they were able to respond to conspecific alarm chemicals. The difference in response between the two species of Asian carp was reflected primarily in how close-knit their respective schools were, with bighead carp showing higher levels of school cohesion than silver carp. Nevertheless, the ability for bighead and silver carp to recognize conspecific alarm chemicals indicates that they are able to learn about threats from their environments, particularly when a conspecific is injured.

The extent to which this ability to learn about threats may not be limited only to the learned individual. Following exposure to predator odors from largemouth bass, groups of bighead carp containing as few as one experienced conspecific altered their behavior as if they were being exposed directly to alarm chemicals from their own species. Additionally, though they were unable to respond to alarm chemicals from native cyprinids, naïve bighead carp paired with experienced golden shiners also altered their behavior once exposed to the predator odor. However, the bighead carp’s response was not similar to the previously determined alarm behavior. Instead, the response shown matched the response of their shoal mates, indicating that, at least on some level, there is a degree of behavioral plasticity that may be influenced by social mechanisms. *In situ*, if mixed-species groups are occurring, bighead carp may benefit by schooling with native fishes with prior predator experience.

Though we were able to demonstrate silver carp possessed the ability to learn about a particular predator following a conditioning period that paired predator odor with alarm chemicals from other silver carp, they do not appear capable of generalizing components of that odor and applying it to a predator with similar characteristics. However, predator generalization can be based on many types of cues, and therefore may not be limited to only chemical cues. Instead of chemical cues, they may in fact rely on some other form of predator cues to inform them of danger, such as movement or body shape.

Our study highlights a few key components that managers may want to consider when managing for these invasive fish, particularly if they are seeking to improve the efficiency of trapping and removal efforts. However, the intensity of the response and age of the fish is something that should be considered if they wish to remove adults. We used juvenile individuals from both species, and the behavior of juvenile fish may not necessarily be representative of adult behavior. Future studies should aim to understand if similar behaviors are displayed by an adult in a controlled environment before undertaking a large removal effort.

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