THE BEHAVIORAL RESPONSE OF *Notropus artherinoides* TO A SIMULATED AVIAN

PEDATORY ATTACK AND THE EFFECT OF STIMULUS PROFILE ON SCHOOL DENSITY

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Abstract

This study was conducted to determine how schooling characteristics of the emerald shiner, *Notropis artherinoides,* change in response to the introduction of an avian predatory stimulus. An additional objective was to determine if minnows differentiate between potential predatory threats based on predator profile. Three silhouettes representing a circle, a square, and a cross were flown over a school of 37 shiners. This was repeated three times with varying orders of presentation. Schooling was video-recorded and measured in terms of distance to nearest fish. It was observed that school density was lowest during the introduction of a stimulus, and that there was no significant difference in density between schools observed before and after a stimulus. Furthermore, it was found that distance to nearest fish did not vary with respect to stimulus profile. These findings suggest that emerald shiners do not distinguish potential avian predators based on profile and that passing shadows stimulate a cooperative defensive response.

Introduction

Organisms utilize a variety of tactics, both independently and cooperatively, to reduce risk of predation. Schooling, the practice of congregating with similar individuals and swimming in a synchronized manner, is a common example of a cooperative defense mechanism (Partridge, 1982) and is observed almost ubiquitously among fishes (Lund, 2011). The practice of schooling possesses both advantages and disadvantages for an individual. Disadvantages include exposure to high volumes of waste and increased competition during feeding. These disadvantages, however, are often outweighed by factors such as the increased ease of finding a mate, and decreased risk of predation by avian and aquatic predators alike. As a result, the structural characteristics of a school of fish often changes over time and may fluctuate greatly in response to stimuli such as the introduction of a predator (Partridge, 1982).

Multiple hypotheses have been proposed to explain the defensive advantage of schooling in response to predators. One concept, the confusion hypothesis, states that a high density of prey moving within a given area reduce the ability of predators to focus on, and therefore target, individual prey (Neill, 1974). A second concept, the 1/N Hypothesis, states that a fish's chance of becoming prey is negatively related to the number of fish in a school (Partridge, 1980).

Notropis artherinoides, commonly referred to as the emerald shiner, is a species of minnow common in the littoral zone of inland lakes of Northern Michigan. Feeding primarily on cladoceran zooplankton, the emerald shiner is generally observed in large aggregations or schools (Savitz, 1997), a tactic it utilizes for defense against aquatic predators such as the walleye, *Sander vitrius,* (Pothoven 2009) and avian predators such as the Belted Kingfisher, *Megaceryle alcyon* (Ryser, 1985).

The purpose of this study is to investigate changes in schooling patterns in *N. artherinoides* in response to a simulated predatory attack. Furthermore, this research examines the role of profile as a stimulus for changes in schooling behavior. It is expected that schooling characteristics of the emerald shiner will change in response to the introduction of a predatory stimulus. Additionally, it is expected that emerald shiners distinguish avian predators based on profile. If this is the case, we predict that schooling density will be greatest in response to a stimulus based on the profile of a kingfisher. An increase in school density in response to stimulus would support the notion that the schooling structure of *N. artherinoides* varies in response to changes in the level of perceived predatory threat.

Methods

Two hundred emerald shiners were acquired from Young's Bait and Party store in Alanson, Michigan during August of 2011. The minnows were retained in five 37.9 L holding tanks throughout the experiment.

The study consisted of three series of simulated predatory attacks. During each series of simulations, 37 emerald shiners were placed in a 241L tank and allowed to acclimate for 60 minutes. Following acclimation, three different wood cutouts were pulled lengthwise over the tank at five-minute intervals at a height of 0.4 m above the surface of the water. A five -pound weight was utilized to propel the cutouts over the tanks at a constant rate of 2 m/s.

The three cutouts were created in the shape of a square, circle, and a cross. The cross was designed to reflect the area and proportions of a Belted Kingfisher silhouette at 8:19 scale. This resulted in a cross-shaped silhouette with an area of 134.56 cm^2 and this area was held constant among all three silhouettes.

Four spotlights were placed facing downward at a height of 2.15 m above the surface over the water in order to cast a shadow on the aquarium floor during simulation. For each set of experiments, a new group of minnows was used to avoid desensitization. In addition, during each set of experiments, order of presentation was randomized.

During each series of simulations, minnows were video recorded from above the tank. Following each series, footage was reviewed during, and at 10 seconds before and after each “fly-over”. At each of these points, ImageJ was utilized to measure distance to nearest fish for each individual fish. Fish were measured from the center of their body mass. These data were used to calculate the mean distance to the nearest shiner for each group of fish both 10 seconds before, and after, each simulated attack.

An ANOVA was utilized to determine variance in distance to nearest shiner among all simulated predatory attacks. The three time intervals were compared in terms of mean distance to nearest fish using a Tukey HSD test. Differences in mean distance to nearest fish among different stimulus profiles were compared using a Post-Hoc test. Statistical analyses were performed using SPSS.

Results

Mean distance to nearest fish was analyzed against silhouette shape, order of presentation, and time relative to introduction of a stimulus (Table 1). Mean distance to nearest fish varied significantly with respect to time at which measurements were taken relative to the introduction of a stimulus (p=0.006). A post hoc test showed that mean distance to nearest fish was significantly greater during the introduction of the stimulus than either before or after the stimulus was introduced (Table2). There was no significant difference in mean distance to nearest fish between before and after the stimulus (p=0.932).

Stimulus shape had no significant effect on distance to nearest fish (p=0.069) (Table 3). Post-hoc tests showed that there was a significant difference in distance to nearest fish between schools exposed to the square silhouette and schools exposed to the round silhouette (p=0.05). There was no significant difference in distance to nearest fish between schools exposed to the cross and schools exposed to the square or circle. Order of presentation of the different stimuli was found to have no significant effect on distance to nearest fish (p=0.852).

Discussion

The purpose of this experiment was to determine the influence of an avian predatory threat on the schooling behavior of shiner minnows. In addition, this study sought to determine whether the shape of a predatory stimulus evokes a greater response with respect to schooling in emerald shiners.

The presence of a moving shadow caused a direct response in the form of dispersal, followed by congregation. Based on the measurement of distance to nearest fish, however, it was determined that the density of shiners after a stimulus was similar to the density of the school prior to the stimulus. The reason for this is not clear, although the presence of a dense school prior to each test suggests that the shiners may have been exposed to some disturbance prior to experimental trials. A possible source of disturbance was the mounting of the video camera above the tank prior to the introduction of each stimulus. Such activity in close proximity to the minnows may have been viewed as a predatory threat and therefore may have promoted a defensive response in the form of schooling.

In addition to schooling, it was our general impression that, upon introduction of a stimulus, emerald shiners tended to shift position toward the direction from which the stimulus originated. Although this observation was not measured quantitatively, it is believed that the shiners swam in opposite direction of the perceived threat in order to minimize the length of exposure to an apparent predator (Litvak, 1993).

Testing also showed that shiners did not distinguish between potential predators based on shape, although the corresponding p-value (p=0.069) suggests variance would be significant given a larger sample size. There was significant difference in schooling density between fish exposed to the circle and fish exposed to the square. The reasons for this variance is unknown, although the absence of straight lines in the circle may increase the likelihood that such a stimulus be mistaken for other environmental factors such as a passing cloud, or a leafy branch swaying in the wind.

Throughout the experiment, certain limitations were noted which may ultimately have impacted results to varying degrees. A high rate of fish mortality caused us to limit the extent of time for which minnows were allowed to acclimate before and between simulations. This may have been responsible for the schooling behavior that was observed prior to the introduction of each stimulus. In addition, fluctuations in lighting conditions may have impacted the minnow’s ability to distinguish between stimuli.

Despite these limitations, the results of this experiment identify the presence of a moving shadow as the stimulatory factor responsible for eliciting a defensive response to a perceived avian predatory threat in emerald shiners. In addition, results demonstrate that emerald shiners do not distinguish between potential predators based on profile. Future studies of this nature may seek to determine how schooling response varies between fish exposed to avian predators and fish exposed to aquatic and terrestrial threats.

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Table 1: Mean distance to nearest fish versus shape of stimulus, time relative to introduction of stimulus, and order of presentation.

Source	Type III Sum of Squares	Degrees of Freedom	Mean Square	F	Significance
Corrected Model	6.146[a]	6	1.024	3.319	0.020
Intercept	60.932	1	60.932	197.431	0.000
Stimulus Shape	1.889	2	0.945	3.061	0.069
Time of Stimulus	4.156	2	2.078	6.733	0.006
Order of Stimulus	0.100	2	0.050	0.162	0.852
Error	6.172	20	0.309		
Total	73.250	27			
Corrected Total	12.318	26			

Table 2: Mean distance to nearest fish versus time relative to introduction of stimulus.

Time of Stimulus		Mean Distance to Nearest Fish Difference	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
After	Before	-0.090268	0.2517110	0.932	-0.722581	0.542046
	During	-0.873731	0.2517110	0.006	-1.506045	-0.241418
Before	After	0.090268	0.2517110	0.932	-0.542046	0.722581
	During	-0.078346	0.2517110	0.014	-1.415778	-0.151150
During	After	0.873731	0.2517110	0.006	0.241418	1.506045
	Before	0.783464	0.2517110	0.014	0.151150	1.415778

Table 3: Mean distance to nearest fish versus silhouette shape

Shape of Stimulus		Mean Distance to Nearest Fish Difference	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
Circle	Cross	0.230703	0.251711	0.636	-0.401611	0.863017
	Square	0.639740	0.251711	0.05	0.007426	1.272053
Cross	Circle	-0.230703	0.251711	0.64	-0.863017	0.401611
	Square	0.409037	0.251711	0.26	-0.223277	1.041351
Square	Circle	-0.639740	0.251711	0.05	-1.272053	-0.007426
	Cross	-0.409037	0.251711	0.26	-1.041351	0.223277