Rock Bass Learn to Associate Food with a Visual Cue and
Remember the Association when Food is Absent

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ABSTRACT

We explored the foraging ability of rock bass (*Ambloplites rupestris*) by testing three hypotheses consistent with the predictions of optimal foraging theory: 1) fish can learn to associate food with a visual cue; 2) trained fish will go to a visual cue faster than untrained fish; and 3) over time, without the reinforcement of food, trained fish will exhibit a diminished response to a visual cue. Our results supported each hypothesis. During the first 96 h of testing, 88 to 100% of trained fish went to the visual cue first; 50% of the trained fish went to the visual cue first after 312 h. None of the untrained fish went to the visual cue first. Trained fish went to the visual cue significantly faster (11.0 cm sec^{-1}) than untrained (1.6 cm sec^{-1}) fish. There were no significant differences in velocity to the visual cue among the times tested for control fish (0.8 to 2.6 cm sec^{-1}). However, velocities of experimental fish were significantly higher from 0 to 24 h (16.7 cm sec^{-1}) than from 48 to 312 h (6.7 cm sec^{-1}), suggesting that they began extinguishing their responses as the time since the last food reward associated with the cue increased. If rock bass use these abilities in their natural habitats, they likely improve their foraging efficiency and, thus, their overall fitness.

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INTRODUCTION

In the absence of overriding evolutionary pressures (e.g., high predation rates, sexual selection for body size), animals should maximize energy ingested (E) and minimize the time spent (T) searching for, pursuing, and handling prey (Schoener 1971). In an environment with heterogeneous prey distributions, one way to maximize E/T is to locate and spend more time feeding in patches with greater densities of high E/T food and to search for better areas if food supplies are depleted. However, for many freshwater fishes, feeding areas (e.g., often riffles in streams) may not be suitable for other daily or seasonal activities such as resting (i.e., conserving energy), hiding (e.g., pools or cover), or spawning (e.g., moving to another location, such as from lake to tributary). Therefore, the ability to learn and remember features of the physical environment associated with high E/T values should be advantageous for individual organisms and populations.

Temperate streams with diverse substrates have patchy distributions of benthic macroinvertebrate food sources for fish, with varying rates of renewal and productivity (Merritt et al. 1996). Infrequent or seasonal renewal of food sources or patchy spatial distributions can be overcome if a forager can remember the best locations for food and when it is available. Feeding in a spatially and temporally heterogeneous environment requires that information from the immediate past be combined with information from the more distant past in order to track environmental change (Hirvonen 1999). Therefore, efficient foragers should learn the positions of food sources and visit them according to rates of renewal (Hughes and Blight 2000). Shape, pattern, size, and spatial relationships are visual characteristics that foragers may use as cues for both food and its surroundings. Therefore, greater success as a forager or predator should be related to an ability to learn and remember. Previous studies have shown that some fishes can learn and remember associations between visual cues and food (Salas et al. 1996, Ohnishi 1997, Hughes and Blight 1999 and 2000, Lopez et al. 2000a and 2000b).

We tested the following hypotheses with rock bass (*Ambloplites rupestris*): 1) fish can associate food with a visual cue, 2) trained fish will go to a visual cue faster than untrained fish, and 3) over time, without the reinforcement of food, trained fish will exhibit a diminished response to the visual cue and look elsewhere for food. We chose the rock bass because it has not been tested for learning and memory and it is hardy and resilient in the laboratory. The rock bass is native to the Great Lakes-St. Lawrence River, Mississippi River, and Hudson Bay watersheds, but it has been transplanted throughout eastern North America. It prefers vegetated and rocky substrate in water that is clear and free of silt. It is mostly a benthic feeder, and its diet consists of small fish and benthic macroinvertebrates (Scott and Crossman 1973).

METHODS AND MATERIALS

Subjects and Feeding

Forty juvenile (70 to 110 mm; Scott and Crossman 1973) rock bass were netted in a local stream in the fall of 2002 and transported to the laboratory. For several months, fish were held at 12 to 19 ºC with a 12/12 light-dark cycle in two, 400 L stainless steel

tanks with filtered and aerated water. Fish were then held in optimal, 20-22 ºC water (Scott and Crossman 1973) for several weeks before testing. Fish were initially fed live minnows and chopped earthworms mixed with frozen chironomids. Feeding with live food decreased until fish were fed only chironomids. Chironomids were pipetted onto four randomly placed terracotta plates in each tank so that fish might begin to associate plates and food. At the end of each feeding period, the plates were removed.

Establishing Experimental and Control Subjects

A four-arm maze in a large cylindrical tank was used to select suitable subjects (i.e., fish that would eat after handling). For 2 d before testing in the maze, food was withheld. For the trials, frozen brine shrimp or chironomids were placed on a terracotta plate at the end of each arm of the maze. At the beginning of a test, one fish was put in a cylinder placed in the center of the maze. After 15 sec, the cylinder was lifted. Fish that actively fed in the maze after handling were considered suitable subjects for further testing. From a group of 20 suitable fish, 10 were randomly chosen as experimental animals, and 10 were the controls.

Training and Experimentation

Group Training and Acclimation. Each of the control and experimental tanks was divided into three chambers (Fig. 1). The two end sections were pre- and post-test holding areas. The middle portion of each tank was the holding/feeding area and testing arena. This larger portion of each tank was divided into 5 x 5 cm quadrats visible only to the observers. For one month before testing, fish lived and were fed in the combined holding/feeding area and testing arena. The visual cue was an L-shaped, white ceramic tile (10 cm x 10 cm) that stood on the bottom of a tank. Five terracotta plates and one visual cue with a plate on it were placed in randomly chosen quadrats at the same locations in the tanks with experimental and control fish.

Previous studies have shown that social foraging and schooling assist learning behavior and increase willingness to take risks (Lachlan et al. 1998, Grand and Dill 1999, Reebs 2000, Galef and Giraldeau 2001, Swaney et al. 2001). After 2 d without food, four groups of five fish were netted and placed in the holding areas, two each in the control and experimental tanks (Fig. 1). Those areas were covered with black plastic to prevent the subjects from observing the experimental protocols before group training began. In the tank with experimental fish, food was always pipetted onto the plate on the white tile (visual cue); in the tank with control fish, food was pipetted onto a randomly chosen plate that may or may not have been on an identical visual cue. Then a group of five fish was released to feed. After 5 min, the feeding fish were recaptured and placed back into their holding area. Group training was repeated three times over a 3-d period. The procedure allowed the fish to associate food with the terracotta plates and acclimated them to the handling they would receive during individual tests.

Individual Training. Individual training sessions were run in the same way as group training, except that only one fish was placed in the testing arena at a time. After 2 d without food, an individual was netted and placed in a cylinder (Fig. 1) for 15 sec, released, and allowed to forage. After 5 min, the fish was recaptured and placed in a post-test holding area. After testing all 20 individuals, the visual cue, terracotta plates

and black plastic dividers were removed, allowing fish full access to their tanks but with no spatial cues other than the unchanging surroundings of the laboratory and tank.

To correct for the differing distance of the randomly placed visual cue during each test, a velocity for each fish was calculated as (distance from starting point to visual cue)/(time to visual cue from starting point). The fish were trained until the average velocity to the visual cue of the 10 fish in the experimental group reached a plateau.

Experimentation. Fish were moved to one holding area at the end of each tank and covered with black plastic. The terracotta plates and visual cue were placed at the same randomly chosen locations in each tank, but no food was used. The distance from visual cue to the release point of the fish was measured. A stopwatch was used to record the time from release of each fish into the test arena until it investigated the terracotta plate on the visual cue. We hypothesized that the experimental fish would reach the visual cue more quickly on average because, unlike the control fish, they had been trained to associate the visual cue with food. To assess memory of learned associations between the visual cue and food, control and experimental fish were tested without food 24, 48 and 96 h after the initial test and, after *ad libidum* feeding, 312 h after the initial test.

Data Analysis

To evaluate the effect of training on the experimental group, two specific measures of performance were recorded and analyzed—percentage of fish going to the visual cue first during a trial and velocity to the visual cue. Percentage data were appraised with a chi-square test, and velocity data with a two-way ANOVA general linear model. Kruskal-Wallis ANOVA and was used to distinguish mean velocities at different testing times within the control and experimental treatments (Statistix 1999).

RESULTS

The two groups of 20 fish, from which 10 control and 10 experimental fish were drawn, ranged in size from 73 to 101 (91.0 ± 3.2) mm and 70 to 110 (89.2 ± 3.1) mm, respectively, and the groups did not differ in length (df = 38, F = 0.38, P = 0.543). Fish that fed in preliminary testing were not measured again. Three of the 20 fish died during the 10-week training and testing period. The control fish was replaced, resulting in a constant sample size of 10, but the two experimental fish that died were not replaced.

Training enhanced the ability of rock bass to associate food with the visual cue (df = 9, chi-square = 249.53, P < 0.0001). From 0 to 96 h, 88 to 100% of experimental fish went to the terracotta plate on the visual cue first at an average velocity of 16.9 ± 2.7 to 5.7 ± 2.9 cm sec^{-1} (Table 1). After 312 h, 50% of the experimental fish went to the visual cue first at an average velocity of 6.2 ± 2.9 cm sec^{-1}. No control fish went to the visual cue first, and their maximum average velocity from 0 to 312 h was 2.6 ± 0.6 cm sec^{-1}.

Trained fish swam faster to the visual cue than controls (df = 1, F = 64.68, P < 0.0001), especially before 48 h (Fig. 2). No difference in the velocities of control fish was detected with respect to time (df = 4, F = 1.20, P = 0.325). However, there was a significant difference among velocities of trained fish at times $\leq$ 24 h and $\geq$ 48 h (df = 4, F = 5.14, P = 0.001). Thus, there was a significant interaction (df = 4, F = 4.61, P = 0.002) between experimental and control treatments and test times (0, 24, 48, 96, 312 h).

DISCUSSION

Our data support the hypothesis that rock bass can associate food with a visual cue. Except for the last trial (312 h, 50%), 88-100% of the trained fish went to the visual cue first after their release, whereas none of the control fish went to the visual cue first at any time. These results are consistent with studies of other fishes (e.g., goldfish, *Carassius auratus*, Warburton 1990; pumpkinseed, *Lepomis gibbosus,* Kieffer and Colgan 1991; and bluegill, *Lepomis macrochirus,* Ehlinger 1990, Wildhaber et al. 1994) and establish learning and memory ability for a third member of the centrarchid family. Our data also support the hypothesis that trained fish go to a visual cue faster than untrained fish. Rock bass in the experimental group went to the visual cue faster (4.8-20.5 times) than those in the control group, but the differences in velocity between the two groups decreased after 24 h. Finally, our data support the hypothesis that the response of rock bass to the visual cue declines over time in the absence of reinforcement of the association with food.

Several factors may account for the observed decline in fidelity and velocity to the visual cue by experimental fish with respect to time since training. Because they were not receiving a food reward at the visual cue during the trials, the experimental fish may have begun to disassociate the cue with food—extinguishing the trained behavioral response. Consistent with predictions for optimal foraging (Schoener 1971, Wildhaber et al. 1994, Milinski 1994), the rock bass may have begun to look in other places for food, or they may have given up on finding food. Another possible factor contributing to the decline of memory and velocity over time is that individuals have varying abilities to perceive, respond, learn and remember (cf. Odling-Smee and Braithwaite 2003). Alternatively, in a study on sticklebacks (*Gasterosteus* spp.), Milinski (1994) noted that memory windows may change in response to environmental changes. Another study of sticklebacks found that memory windows were 8, 10, and >25 days in fish from marine, anadromous, and freshwater populations (Mackney and Hughes 1995).

Fish may rely on one or more of five mechanisms to find food—spatial cues, temporal changes in behavior, olfaction, vision, or learning and memory. In our study, spatial cues can be dismissed as the way rock bass found food because the visual cue and plates were randomly distributed during each training session in identical tanks. Thus, no association could be made using other objects or memory of plate locations. Because the feeding trials were run during the same 3-h period each day, any behavioral changes that could have resulted from differences in testing time were eliminated as well. Because food was used during training, it is possible that the fish relied on odor or seeing the food to associate the visual cue with food, but this was not possible during the experimental trials because no food was in the tank. Therefore, learning to associate the visual cue with food and memory of that association best explain our results.

Our results support the idea that rock bass recognize changes in their feeding environment and that they may be capable of responding to temporal and spatial depletions and renewals of food in their natural environment. For a predator to be successful, it must know where its food is located. In our experiments, rock bass were

able to learn where their food was located and to remember that location based on a visual cue. Therefore, it can be hypothesized that rock bass can recognize landmarks in their natural environment associated with the food they eat.

ACKNOWLEDGMENTS

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Table 1. Percentage of experimental and control fish going to the visual cue first, and their mean velocity $\pm$ standard error to the visual cue.

Trial Time (h)	Experimental Fish		Control Fish	
	% First	Velocity (cm sec^{-1})	% First	Velocity (cm sec^{-1})
0	100	16.4 ± 2.7	0	0.8 ± 0.2
24	100	16.9 ± 2.7	0	2.6 ± 0.6
48	88	8.2 ± 2.9	0	1.7 ± 0.9
96	88	5.7 ± 2.9	0	1.2 ± 0.4
312	50	6.2 ± 2.9	0	1.7 ± 0.7

LIST OF FIGURES

| Holding area | Testing   arena   C | Holding area |