Effects of Environmental Enrichment on Learning a Discrimination Task by Captive White-spotted Bamboo Sharks (*Chiloscyllium plagiosum*)

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Abstract

It has been demonstrated experimentally that several species of sharks are capable of learning by association but no studies have investigated the effects of environmental enrichment on sharks' learning abilities. The main objective of this study was to test the hypothesis that environmental enrichment would improve learning performance in captivity of white-spotted bamboo sharks (*Chiloscyllium plagiosum*). During training and testing with a food reward before environmental enrichment, the sharks appeared to associate a discriminative stimulus (black vs. white tile) with food, but they did not discriminate between the tiles without a food reward. After a 68-d non-testing period followed by 10 d of exposure to enrichment objects (two plastic hula hoops) in the sharks' tank, the experiment was repeated with the same result. There were no statistically significant differences in learning and memory before and after environmental enrichment. This negative result may have resulted from 1) small sample size (N=2 sharks), 2) ineffective enrichment objects, 3) insufficient time for enrichment effects to occur, or 4) inability of this species to learn a discrimination task. Future studies on enrichment should include larger sample sizes within species, multiple species of sharks, and testing with different enrichment objects.

Dedication

I dedicate this thesis to my supportive parents, my loving husband, and to my twin sons Cameron and Adrian; may you always follow your dreams.

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I thank Dr. Haynes for statistical and writing support; the other members of my thesis committee, Drs. Jacques Rinchard and Lori Forzano, for their advice, support, and patience; Melissa O'Meara for coding videos and assisting with data collection; and my family for support and encouragement. I am also appreciative of the IACUC at Brockport and also at the Aquarium of Niagara for supporting this study, particularly Dan Arcara for providing background information and the other aquarists who helped deliver reinforcement. Finally, I thank the sharks themselves for inspiring me to pursue this study.

Biographical Sketch

- I obtained my Bachelor of Science degree in Animal Behavior Research from the University at Buffalo in 2009 (courses concentrated in psychology, biology and anthropology)
- I traveled to Puerto Rico in 2009 to assist with Humpback whale research for the Bioacoustics Lab, Psychology Department, University at Buffalo. As a Research Assistant, I assisted with data entry and analysis of sound recordings of Humpback whale song; tested sound equipment, and applied for research grants to obtain funds for lab equipment and travel expenses
- I worked for the Niagara Aquarium Foundation, a non-profit organization, for seven years as a Customer Service Representative while also gaining experience in the Education Department and as a volunteer in the Marine Mammal Care Department
- I traveled to Fiji in 2011 to study shark behavior and conservation while completing a research project.
- I will obtain my Master of Science degree in Environmental Science from the State University of New York College at Brockport in 2012 (relevant courses included wildlife ecology, univariate and multivariate statistics, animal behavior, and grant writing)

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Introduction

Sharks have been misrepresented by the media for much of the twentieth century. Many popular movies, books, and paraphernalia have portrayed these species as blood thirsty human-devouring monsters (Maniguet 2007). The mere mention of the name ”shark” invokes fear and brings to mind images of mindless eating machines with intimidating jaws (Benchley 2003) and little brain function, intent only on terrorizing any human foolish enough to enter its domain.

These widely held beliefs are changing as more accurate representations of sharks emerge. New appreciation is due in part to keeping sharks in captivity. There has been much debate regarding the ethics of animals in captivity (Mason et al. 2007) but there is much to be gained by exposing humans to sharks in a simulating yet beneficial environment. Just as exposure to animals in zoos has led to calls by the public to stop the destruction of terrestrial megafauna, exposure to sharks in aquariums might lead to similar calls to protect increasingly threatened shark populations around the world. Also, much needed research can be conducted and benefit from observing/ monitoring sharks in captivity for an extended period of time, a feat that is difficult to accomplish when studying sharks in the wild.

There are approximately 400 species of sharks in eight orders (Carwardine and Watterson 2002). Sharks belong to the class Chondrichthyes, fish that have skeletons made of cartilage instead of bone, which is further broken down into two subclasses, one of which, Elasmobranchii, includes sharks, skates, and rays (Parker 2008).

To date there have been no studies that investigate the effects of environmental enrichment on shark learning. There are several reasons why such studies may be important. (1) There are still gaps in basic knowledge about many aspects of shark behavior. (2) In terms of learning capabilities, it is evident that sharks possess the necessary neurological equipment to perform discrimination and conditioning tasks; their brains are comparable in size to those of mammals and birds. Maniguet (2007) noted that sharks have well developed eyesight and are capable of discriminating between contrasting colors. In a review by Snelson et al. (1984) it was noted that bull sharks (*Carcharhinus leucas*) in captivity were capable of visually discriminating between different colored nets. Klimely (2003) reported that sharks learn more quickly than goldfish and comparably to mice. (3) More information about shark behavior can help close the gap between misrepresentation and fear and reality, while at the same time increase public awareness about the plight of sharks and, ideally, improve the public's appreciation of sharks' abilities.

Overharvesting of sharks is a serious threat to their continued existence, as it is estimated that approximately 50-100 million sharks are killed each year (Parker 2008). In addition, pollution, destruction of habitat, by-catch and depletion of food sources contribute to the problem of decline in shark populations. Basic knowledge of the life history of sharks indicates the need for conservation measures. Sharks are 'K' selected species which mature late in life, reproduce slowly, and bear few offspring (Parker 2008). It is important in terms of conservation and marine ecosystem balance (many sharks are apex predators) for the public to be aware of and actively support shark conservation and management.

Learning by Sharks

There are two forms of associative learning: classical and operant conditioning (Guttridge et al. 2009). During classical or Pavlovian conditioning, an animal learns to give the same response to an unconditioned stimulus (e.g., food) after repeated pairings with a conditioned stimulus (e.g., a bell) (Powell et al. 2009). During operant or instrumental conditioning, an association is made between a behavior and a consequence (e.g., a rat learns that pressing a lever results in the presentation of a food reward). An animal can manipulate its environment to receive a reward. Operant behaviors are voluntary and are either reinforced or punished (Powell et al. 2009). Positive reinforcement involves presenting a rewarding stimulus to increase the likelihood that a behavior will be repeated in the future. Negative reinforcement involves removing an aversive stimulus to increase the likelihood that a behavior will be repeated in the future. A response to a discriminative stimulus will be reinforced when the stimulus is present and not reinforced when absent (Powell et al. 2009). A discrimination task involves rewarding the selection of one object over another and is achieved by using a training or shaping procedure using a step-by-step process of reinforcing successive approximations to the desired end behavior (Powell et al. 2009).

In a review, Parker (2008) reported that sharks are capable of learning and have high levels of intelligence, based in part on instinct but also on memory. Parker stated that nurse (*Ginglymostoma cirratum*) and lemon (*Negaprion brevirostris*) sharks can readily learn to associate food with auditory, visual and temporal cues. They are also able to discriminate between different shapes and orient in a maze task.

The majority of operant conditioning tasks with sharks were performed in the 1960s, revealing a substantial lapse of time and knowledge about shark learning (Guttridge et al. 2009). Wright and Jackson (1964) taught adult and juvenile lemon and bull sharks to press a target which would ring a bell. The target was a square piece of plywood, each side measuring 5.75 in, painted white with a single, central black dot. Food items were used to attract the sharks to the target, and eventually the sharks started bumping the target. Upon target contact, a bell, which was powered by a battery, would ring at the surface to indicate to the researchers that a correct response was made by the shark. During the testing period, the sharks also made target contact in the absence of a food reward.

Tester and Kato (1966) performed a visual discrimination study with juvenile blacktip sharks (*Carcharhinus melanopterus*) to investigate their visual capabilities regarding several properties, such as color, brightness, orientation and form. Sharks were trained using negative reinforcement to associate targets with an electrical shock. The targets were square pieces of vinyl floor tile (9 in^2) covered with different colors of high gloss paper. One task involved pairing each color with a standard grey, and another task investigated different shapes (triangle, circle, rectangle) oriented in different positions (vertical vs. horizontal). Some sharks showed signs of discrimination, but the authors suggested that further work was needed to draw more precise conclusions.

Clark (1959) trained adult lemon sharks to push a target, causing a bell to ring, in order to obtain a food reward. The target was a square piece of plywood, 41 cm^2, painted white. A shaping procedure was used to train the sharks to bump the target with their snouts by throwing pieces of fish into the water, progressively getting closer to the target. Eventually, fish pieces were tied to the target, and the sharks learned to press the target to obtain a food reward. Interestingly, these sharks retained the response without receiving the stimulus (target) or the reward (food) for a 10 week period.

Klimely (2003) used operant conditioning to train a nurse shark to bump a white Plexiglass square (3 in x 3 in) which was mounted on a piece of wood that could be lowered to the bottom of the tank. By using a shaping procedure, or rewarding step-by-step behaviors to achieve a novel behavior, the shark was rewarded for swimming toward the target, eventually leading to physical contact with the target. Even after the food reward was withdrawn, the shark continued the behavior of bumping the target, indicating that learning had taken place.

Gruber and Myrberg (1977) performed a series of operant conditioning experiments on lemon sharks, in which the sharks entered a Y-shaped maze through a brightly illuminated entrance in order to obtain a food reward. The sharks were subjected to another discrimination task in which they had to choose between a black and a white target. The sharks were rewarded for selecting the white target only and learning was defined by a reduced number of errors; after training only two errors occurred in twenty trials.

In summary, successful learning of a discrimination task has been demonstrated for lemon, bull and nurse sharks but results were equivocal for blacktip sharks. In one study (Clark 1959), lemon sharks remembered the discriminative stimulus for ten weeks in the absence of a food reward.

Enrichment Studies

Enrichment has been used to stimulate animals' senses, break the perceived monotonous routine of captivity, offer the animals choices and some control in their environments, and, ultimately, improve animal welfare (Disney's Animal Programs 2009). Specifically, in a laboratory or captive setting, the goal of enrichment is to reduce stereotypic or repetitive behaviors such as pacing or pattern swimming which are thought to indicate an animal is experiencing boredom or anxiety (Mason et al. 2007). Disney's Animal Enrichment Philosophy (2009) provides a 6-step process for developing novel enrichment items, using the acronym S.P.I.D.E.R. (setting goals, planning, implementing, documenting/evaluating, and readjusting). With a species-specific behavioral goal in mind, a plan is made to offer enrichment which allows the animal to have choices and control in its environment. All enrichment plans that are implemented are documented, evaluated for their effectiveness and readjusted as needed. Due to criteria for novel enrichment items put forth by The American Zoo and Aquarium Association (1999), items must be assessed for risk and hazard to the animal before implementation. New enrichment items given to the Buffalo Zoological Society's collection of education animals must first be approved by the Zoo's Veterinarian (personal communication, Kenny Coogan, former Animal Care Supervisor). New enrichment items given to the penguins at the Aquarium of Niagara are chosen to be 'toddler safe,' void of small pieces that could break off and sharp edges that could injure the animal (personal communication, Dan Arcara, Exhibits Department Supervisor).

An enriched environment is complex and variable, including different forms of enrichment, such as changes to the exhibit structure, dietary items, novel objects (e.g., toys or tunnels), or social interaction (van Praag et al. 2000). Environmental enrichment improves learning by heightening sensory feedback and increasing neuron growth. The same brain areas (nucleus accumbens and prefrontal cortex) that are responsible for responding to enriched environments are also related to learning (reviewed in Wood et al. 2006). Enrichment studies have been performed with rats (Woodcock and Richardson 2000; Wood et al. 2006), olive hybrid baboons (Bourgeois and Brent 2005); pigs (de Jong et al. 2000), three-spine sticklebacks (Brydges and Braithwaite 2009), and cichlids (Kotrschal and Taborsky 2010); however, no studies have investigated the effects of enrichment on sharks.

Woodcock and Richardson (2000) discovered that rats (*Rattus norvegicus*) improved memory capabilities involving contextual information more rapidly when housed in an enriched environment. Similarly, Wood et al. (2006) examined the effects of environmental enrichment on an operant conditioning task with rats. The enriched rats were housed in cages containing toys and interactive objects, such as tunnels, which were repositioned several times a week. A shaping procedure was used to train the rats to poke their noses through a single illuminated hole to receive a food reward. During the first phase of the shaping procedure, a feeder cue (tone and light) was paired with the food reward. Rats received a reward for poking whichever hole was illuminated during the second phase, but were rewarded for poking the only illuminated hole during phase three. The enriched-housed rats reached phase three of the shaping procedure faster than the standardly-housed rats. Surprisingly, the standardly housed rats, which received no enrichment, performed just as well as the enriched rats during phases one and two, but not on phase three of the study. This finding may have resulted because the incentive stimuli were more rewarding to the standardly housed rats, or because these rats learn just as well in simple learning tasks.

Bourgeois and Brent (2005) examined four enrichment techniques (positive reinforcement training, food enrichment, non-food enrichment, and social enrichment) for olive hybrid baboons (*Papio anubis*) in order to reduce abnormal behaviors. They found that social enrichment (caged with conspecifics) had the most profound effect on improving the animals' overall well-being.

The effects of enrichment on exploration of novel items, learning and memory tasks were investigated in pigs (de Jong et al. 2000). At an early age, randomly chosen piglets (*Sus scrofa domesticus*) were offered the opportunity to explore a novel passageway (enrichment) outside their pen. Next, the piglets had to run three different variations of a maze to obtain a food reward. When tested at a later age, the enriched pigs outperformed the barren-housed pigs in terms of long-term memory on variation one of the maze task. However, there was no difference in learning ability reported for the two groups of pigs. The authors concluded that barren- housing conditions negatively affected the welfare of the pigs, measured by physiological factors such as salivary cortisol concentrations which indicate stress.

In a study investigating the effect of enrichment on the behavior of three-spine sticklebacks (*Gasterosteus aculeatus*), the 'enriched-condition' fish were housed in a tank containing gravel, plants, objects for the fish to swim through (upside down flower pots), and a variable feeding schedule (Brydges and Braithwaite 2009). The fish were then subjected to a learning task during which they had to learn the association between a visual cue and a foraging patch that contained a food reward. Enriched fish had no advantage in the learning task over the non-enriched fish. The authors explained this may have been due to the fact that the enrichment they provided was not stimulating to the fish or that learning may have been more strongly influenced by genetics and thus not affected by environmental enrichment.

Another study with cichlids (*Simochromis pleurospilus*) (Kotrschal & Taborsky 2010) provided more encouraging results for enrichment. Enriched fish were given a varied diet at an early age and tested as adults to associate a food reward with a visual cue. A variable food diet was used to reflect unpredictable environmental changes likely to be encountered in the wild. The results showed that the enriched fish performed better than the non-enriched fish, supporting the authors' hypothesis that environmental variation enhances learning. The authors argued that enriched environments provide novel, complex situations which promote neural growth.

In summary, three mammals (rats, olive hybrid baboons, pigs) generally, but not always, demonstrated better learning performance after environmental enrichment. Among fishes, three-spine sticklebacks showed no improved learning after environmental enrichment, a cichlid species showed improved learning after enrichment, and no enrichment studies have been conducted with sharks.

Study Goal and Objectives

By nature sharks are curious and investigate novel objects in their environment such as boats, fishing gear, dive equipment, and even divers (Parker 2008). If sharks explore in the wild, I hypothesized that, using similar methods of enrichment for fish and mammal studies, they would also explore novel items in a captive setting at the Aquarium of Niagara. The sharks at the Aquarium have never received enrichment items.

The goal of this study was to examine the effects of environmental enrichment on learning by sharks. I predicted that the sharks would learn a discrimination task faster (as measured by number of approaches to a target) after exposure to an enriched condition than in a non-enriched condition. The novelty of this project was investigating the discriminative abilities of species of sharks which have not previously been studied in terms of learning or conditioning tasks. The discrimination task of this study tested the sharks' ability to differentiate between two objects of contrasting colors (black and white). It has been shown that sharks have well-developed eyesight, with retinas containing both rods and cones which provides evidence for color vision and the ability to distinguish contrasts (Tester and Kato 1966; Maniguet 2007).

Methods

Subjects

The subjects were naive sharks, two each of three species, housed in two separate exhibits at the Aquarium of Niagara in Niagara Falls, NY. Four of the sharks, two white-spotted bamboo sharks (*Chiloscyllium plagiosum*) and two blacktip sharks, were housed together in one exhibit, and two catsharks (*Scyliorhinus rotifer*) were housed in a separate exhibit.

The first exhibit was a 37,900 L tank, (216 cm long x 104 cm wide x ~259 cm deep), of which approximately 30,320 L was available for the sharks to swim in. The exhibit was hexagonal with a wooden plank (366 cm long) above the surface of the water which ran the length of the tank; it was stood on when the staff fed the sharks. The water was kept at 25.5°C and the pH was 8.1-8.2. This exhibit contained two adult white-spotted bamboo sharks obtained through private donations from different individuals. The male was donated in 2004 and was approximately 7-8 years-old, the female has been at the Aquarium for at least 12 years. Both sharks were ~76 cm long and ~1.3-1.8 kg. Also in this exhibit were two juvenile blacktip sharks estimated at 1 year of age. They came from a wholesaler in Los Angeles (captured in Eastern Asia). Sexing of these juvenile blacktips (~61 cm long and 0.9 kg) was difficult, as it was hard to see the claspers (indicating a male) without handling the sharks.

The second exhibit was a 758 L tank (152 cm long x 51 cm wide x ~51 cm deep). It was refrigerated to 10° C and pH was 7.9-8.0. It contained two adult catsharks, one male and one female, which came from the Virginia Marine Science Museum in 1998. Both sharks were ~61 cm long, approximately ~0.22-0.45 kg and at least 13 years old.

Procedures

The study was conducted from August – November 2011. All phases were conducted during the working hours of the Aquarium's Exhibits' staff (7 am-3:30 pm) and at regular feeding times, so as not to interfere with the Aquarium's regular schedule. The bamboo sharks and blacktips were fed every other day at 11:30 am, and the catsharks were fed on Mondays, Wednesdays, and Fridays at varying times of day (see Appendix A for a feeding/training/testing schedule). The sharks were fed with pieces of capelin (*Mallotus villosus)* or rainbow smelt (*Osmerus mordax*) on a wire attached to a long metal pole. Due to Aquarium regulations and IACUC (Institutional Animal Care and Use Committee) specifications, the animals were never removed from their exhibits for testing.

The pre-enrichment procedure was a four-phase process, including familiarization, training, and two testing phases. During the familiarization phase I observed the sharks' normal behaviors and noted identifying characteristics of individuals before any training took place. During the training phase the sharks learned to associate a novel stimulus with a food reward. Phase 1 of testing paired the discriminative stimulus with a food reward, and Phase 2 of testing presented the discriminative stimulus without a food reward. Testing phases 1-2 were repeated 78 d after pre-enrichment testing ended (68 d of no research activity and 10 d of exposure to enrichment objects).

Like the discrimination objects used by Clark (1959) and Tester and Kato (1966), the objects in this study were pieces of ceramic floor tile (i.e. non-porous) with areas of 103 cm^2 in the large exhibit and 58 cm^2 in the small exhibit. One object was two black tiles glued (using GE 100% Silicone, clear, no latex) back to back so the object would look the same on both sides. The other object consisted of white tiles of the same size, composition and construction. These objects tested each shark's ability to discriminate between two objects of contrasting colors.

Both discrimination objects were fastened approximately 30 cm apart with twine on a 1.9 cm diameter PVC pipe (see Wright and Jackson 1964 for a similar procedure using weighted plywood), held by me. I stood midway (183 cm mark) on the wooden plank above the large exhibit, and placed the objects ~15 cm below the surface of the water for the blacktip sharks. For the bamboo sharks the discrimination objects were placed 15 cm above the bottom of the tank. For the catsharks in the small exhibit, the objects were placed ~25 cm below the surface, midway between the surface and bottom.

<u>Familiarization Phase</u>: During this phase, I spent time observing the sharks' routine behavior, before any training or testing took place, in order to become familiar with each individual and to habituate the sharks to my presence in front of and above the exhibit. Sexing and identification of unique characteristics (scars, markings or dorsal/caudal fin sizes) and swimming patterns (e.g., fast vs. slow) were completed during this phase so that I could reliably identify each individual shark. Observations were made for 1 h on each of 2 d (40 min devoted to the larger tank containing four sharks and 20 minutes observing the two sharks in the smaller exhibit each day). Each shark was observed individually for 10 min.

<u>Training Phase</u>: The training phase lasted for 5 d (every other day so that delivery of reinforcement corresponded with the feeding schedule), enabling each shark to establish an association between a novel stimulus and a response through repeated pairings. The training target was a single black target (described above), chosen by flipping a coin, which was lowered into the water by twine. Initially, the

training target was placed in the tank for 10 min to allow the sharks to acclimate. Then, a shaping procedure (see Appendix B) was used to train the sharks to approach the training target within ~30 cm (large exhibit) or ~15 cm (small exhibit), using a step-by-step process of reinforcing with food successive approximations to the desired end behavior (see Powell et. al. 2009 for similar procedure).

Upon insertion of the training target, the first step was to reward a shark for a turn or orientation toward the direction of the target. Next, the shark was rewarded for an approach to the target (initially the shark had to come half way from its starting point to the target). The next reward followed an approach to the target within one quarter of the distance from the shark's starting point to the target. Finally, the shark was only rewarded for coming within ~30 or ~15 cm (depending on the species) of the target. If the shark did not attenuate to the object after 2 min the object was removed. The shark then received a rest interval for 9 min before being trained again.

The sharks were rewarded with pieces of fish that were part of their regular diet using their regular feeding pole. The aquarist was always responsible for delivering the reinforcer. The blacktips were fed 0.11-0.23 kg of fish daily, the bamboo sharks were fed 0.22-0.34 kg every other day, and the catsharks were fed 0.056 kg three times per week. To avoid overfeeding, during the experiments food items were cut into small pieces to extend the length of feeding/training time. The sharks received the regular portion of their daily diet whether reinforced or not. If the shark did not attenuate to the target or participate in the training procedure, the target was removed and after one minute the shark was fed the rest of its diet using the regular feeding procedure.

Testing Phase 1 (non-enriched; with a food reward): During this phase (4 trials, 1 trial/day), which began 2 d after the Training Phase ended, each shark's learning capabilities on a discrimination task was assessed by counting the number of approaches to the discrimination objects (white and black) from the time they were lowered into the water. To remain consistent, the discriminative stimulus was again black (Blanco et al. 2006; Ibsen et al. 2007; Morisaka and Okanoya 2009; Hothersall et al. 2010). Each testing session lasted as long as the sharks' regular feeding time (approximately 20-30 mins.) The orientation of the black and white objects was randomly alternated during and between sessions (i.e., presentation to the subjects was switched from left to right using an odd-even random number series).

A shark was rewarded with food for approaching the black discriminative stimulus within ~30 or ~15 cm. When a shark did not attenuate to the objects or participate in the testing procedure, the objects were removed and after one minute the shark was fed the rest of its diet using the regular feeding procedure. Number of approaches to either target was recorded during testing (see Appendix C for raw and tabulated data).

Testing Phase 2 (non-enriched; without a food reward): Testing phase 2 (3 trials, 1 trial/day) began 2 d after testing phase 1 ended. The sharks were tested before their regular feeding time and were not rewarded with food for selecting the black discriminative stimulus to determine if they remembered the correct response for this discrimination task. Testing continued until the sharks no longer preferentially selected the discriminative stimulus.

Testing Phases 1-2 (after enrichment): After 68 days of no testing, the enrichment objects were introduced to the larger exhibit of sharks on November 4th, and remained in the exhibit for ten days to allow the sharks to become familiar with and explore the objects. The enrichment object was one the sharks could swim through. This technique has been used for rats (van Praag et al. 2000) and three-spine sticklebacks (Brydges and Braithwaite 2009). This enrichment item did not interfere with the sharks' normal feeding schedule or the use of food items as reinforcers. The enrichment objects were two brightly colored rings (hula-hoops) weighted down by a dive weight so that they rested on the bottom of the exhibit. The sharks were able to explore the rings while being viewed by Aquarium guests.

Initially, the sharks were observed with the novel enrichment items for 30 min, looking for erratic swimming patterns or behaviors, to ensure they were not causing them harm or stress. If the enrichment objects were attacked or appeared to be causing the sharks distress, they would have been removed and an alternative enrichment item would have been substituted. Post-enrichment testing phases 1-2 resumed on November 17th, after the enrichment objects were removed from the exhibit. Again, the black discriminative stimulus was used.

Experimental Design

This study was a within subjects design. For each trial, the total number of approaches to either discrimination object was recorded for each individual of the species during its testing period. For each phase a percentage was calculated for how many correct choices were made out of the total number of approaches to the two discrimination objects. If the sharks learned to associate the discriminative object with food, it was expected that the number of correct choices would improve during Phase 1 of testing and attenuate with time during Phase 2 when no food reward was offered.

Count data were log10-transformed and percentage data were arcsine-transformed before analysis. A two-tailed, two-sample t-test was used to compare the responses of male and female sharks during the pre-enrichment training phase. One-tailed, paired t-tests were used to compare the percentages of correct vs. random (50: 50 correct: incorrect) responses to the discriminative stimulus in pre- and post-enrichment testing phases 1 and 2. One tailed, two-sample t-tests were used to compare testing phase 1 and 2 responses before and after enrichment. Shapiro-Wilk Normality tests confirmed that use of t-tests was appropriate in all cases (see Appendix D for all statistical analyses).

Results

Behavioral Observations during the Familiarization Phase

The white-spotted bamboo sharks did not move at all during the observation period. They were both resting on the bottom of the exhibit, one in front of and one behind a ceramic pot. One blacktip was very active, swimming constantly near the surface. He usually swam the entire circumference of the tank (pattern swimming) but did swim to the bottom of the exhibit once and stayed there for ~45 sec. The catsharks did not move at all during observation. I noticed the only difference between the two individuals was the banding just behind the first dorsal fin.

Ultimately, tests were conducted on only the two white-spotted bamboo sharks. One blacktip shark became ill and died before the testing phases; because the other blacktip did not show interest in the training object after its conspecific died, testing never commenced. The catsharks never responded to a visual stimulus with food, so testing was ended for this species.

Pre-Enrichment Training and Testing

Training Phase (non-enriched; with food reinforcer): During 5 d of training, there was no significant difference ($p = 0.0936$) in the total number of approaches to or contacts with food near the black training tile by the female (8.6 ± 2.2) and male (6.4 ± 1.5) white-spotted bamboo sharks.

Testing Phase 1 (non-enriched; with food reinforcer): During 4 d of testing, the two white-spotted bamboo sharks correctly approached the black target within ~30 cm (frequently bumping it) 70.0% $\pm$ 12.8% of the time. This result was significantly greater ($p = 0.0025$) than a 50: 50 random response (Table 1; Figure 1).

Testing Phase 2 (non-enriched; without food reinforcer): During 3 d of testing, starting 2 d after phase 1 testing concluded, the two white-spotted bamboo sharks correctly approached the black target within ~30 cm 54.5% $\pm$ 16.4% of the time. This result was not significantly different from a 50: 50 random response ($p = 0.2410$); hence learning and memory of the discriminative stimulus were not demonstrated during pre-enriched conditions (Table 1; Figure 1).

Post-Enrichment Testing

The female shark responded immediately to the enrichment objects when they were placed in the exhibit. She stopped in front of the hula hoop where it was fastened to the dive weight, then circled around it. The female was still exploring the hula hoops when observed 2 d later. The male was not observed responding to the enrichment objects.

Testing Phase 1 (enriched; with food reinforcer): During 3 d of testing, the two white-spotted bamboo sharks correctly approached the black target within ~30 cm (frequently bumping it) 64.2% $\pm$ 10.7% of the time. This result was significantly greater ($p = 0.0139$) than a 50: 50 random response (Table 1; Figure 1).

Testing Phase 2 (enriched; without food reinforcer): During 3 d of testing, the two white-spotted bamboo sharks correctly approached the black target within ~30 cm 54.0% ± 16.3% of the time. This result was not significantly different from a 50: 50 random response ($p = 0.2563$); hence learning and memory of the discriminative stimulus was not demonstrated during post-enriched conditions (Table 1; Figure 1).

Post-enrichment vs. Pre-enrichment

After 68 d of inactivity and 10 d of exposure to the enrichment objects, testing phases 1 (with food reward) and 2 (without food reward) were repeated. There was no significant difference in approaching the discriminative stimulus between post- (64.2% ± 10.7%) and pre- (70.0% ± 12.8%) enrichment for testing phase 1 ($p = 0.8179$), nor was there a significant difference in approaching the discriminative stimulus between post- (54.0% ± 16.3%) and pre- (54.5% ± 16.4%) enrichment for testing phase 2 ($p = 0.5181$).

Discussion

Although lemon (Clark 1959; Wright and Jackson 1964; Gruber and Myrberg 1977), bull (Wright and Jackson 1964) and nurse (Klimely 2003) sharks have been shown to respond (learning and memory) to discriminative stimuli similar to those I used, the sharks in my study ignored (catsharks) or appeared to associate the discriminative stimulus with food but did not remember the response after 2 d (white-spotted bamboo sharks). In a previous study some blacktip sharks showed signs of discrimination, but the authors (Tester and Kato 1966) suggested that further work was needed to draw more precise conclusions, a result similar to mine. After the death of its conspecific, the other juvenile blacktip shark in my study became very "shy and skittish" and experienced a loss of appetite. Although he showed interest in the target (by circling about two feet underneath it, near the bottom of the tank where he normally did not swim), he did not respond to training. According to Dan Arcara, (Exhibits Supervisor, Aquarium of Niagara Falls), because the remaining blacktip was the only shark swimming in that part of the water column in the exhibit, it was "exposed, vulnerable and unwilling to approach the targets."

The visual discrimination task turned out to be inappropriate for the adult catsharks. I noticed right away that it took an unusually long time for these two sharks to attenuate to the target/ discriminative stimulus, even after repeated pairings with a food reward. These sharks are a deepwater species with very poor eyesight (Parker 2008). I then chose another learning task for these two sharks that I thought might better suit their capabilities. I developed an electroreception task that honed in on the ability of many sharks to detect food by receiving electrical impulses through small pores on their snout, the ampullae of Lorenzini (Parker 2008). One metal tile (stainless steel) and one ceramic tile were each paired with food and buried under gravel at the bottom of the exhibit. Even this task proved to be unsuccessful, perhaps because this species does not feed by electroreception.

Alternatively, these two sharks are old, having lived at the Aquarium for at least 13 years. They may have lost their ability to actively search for food which has always been brought to them on the end of a feeding stick.

In the end, only the results obtained from the two adult white-spotted bamboo sharks were retained; the other subjects' results had to be omitted for the reasons described above. In pre- and post-enrichment testing phase 1, the adult white-spotted bamboo sharks appeared to learn to associate the discriminative stimulus quickly with a food reward but without food they failed to distinguish the discriminative target from the alternative target 2-8 days later. After a 68-d period of no research activities, followed by 10 d of exposure to enrichment objects, the white-spotted bamboo sharks again failed to remember the correct target without a food reward, and their performances in post-enrichment testing phases 1 and 2 did not improve in relation to pre-enrichment testing phases 1 and 2. It appears that during testing phase 1 of the pre- and post-enrichment studies the white-spotted bamboo sharks approached the discriminative stimulus because food was nearby but, as demonstrated in testing phase 2, failed to associate the correct target without food and remember the association, unlike the results obtained for lemon and bull sharks (Wright and Jackson 1964) and nurse sharks (Klimely 2003). Although I found no previous enrichment studies on sharks during my literature review, among bony fishes (class Osteichthyes) environmental enrichment improved learning and memory in a cichlid (Kotrschal and Taborsky 2010) but not in three-spine sticklebacks (Brydges and Braithwaite 2009).

Study Limitations

Any of shark species available to study, small sample size, uncontrolled testing environments, enrichment object chosen, insufficient time to explore enrichment objects, not enough training and testing trials, or the discrimination task chosen for this study may have produced the key result: two white-spotted bamboo sharks did not learn or remember the association of food with a discriminative stimulus before and after 10 d of environmental enrichment with hula-hoops in the exhibit.

The hula-hoops may not have been stimulating to the white-spotted bamboo sharks, similar to the results found with three-spine sticklebacks (Brydges and Braithwaite 2009). In that study, the fish living in an enriched environment (one that included upside down flower pots for the fish to swim through) performed equally well with the non-enriched fish. The authors suggested that these enrichment objects may not have been stimulating to the fish or ineffective at improving their learning capacity.

Due to Aquarium and IACUC regulations, my options for enrichment items were limited. Enrichment in the form of live prey was recommended against, so that it would not interfere with the sharks' normal diet and the use of food as a reinforcer. I was also advised not to use any form of tubing or crates that the sharks could swim into but then get stuck, or be hidden from public viewing.

The sharks may have needed more than 10 days to explore and benefit from the enrichment objects in their exhibit. In the study with three-spine sticklebacks (Brydges & Braithwaite 2009), the subjects were exposed to enriched conditions for 10 months. Due to regulations, the sharks could not be removed from their exhibits, so they were housed with another shark of the same species, other species of sharks, and other fish, all of which may have had an effect on exploration of the enrichment object, the training target, and the discriminative stimulus.

I was not allowed to deliver the food reinforcer myself; the aquarists (five helped with my study) fed the sharks and each had his or her own feeding style. Occasionally, a mistake was made and the reinforcer was delivered to the wrong shark which could have inadvertently strengthened an incorrect response. At other times, the food item fell off the feeding pole before it could be delivered to the appropriate shark. Also, there was often a lapse in time between a correct response by the shark and the delivery of the reinforcer; it takes time for an aquarist to 'reload' the feeding pole at the surface and quickly get it back into the water.

Recommendations and Summary

Additional studies are needed that investigate sharks' learning and memory. Which species can learn a discriminative stimulus? How long can they retain the memory of an association without receiving practice or training? Which species benefit from environmental enrichment? For future work, I would suggest a before and after enrichment study with two different groups of sharks, so that not all sharks have prior experience with the learning task. This way, it would be easier to determine whether a change in performance is caused by the introduction of the enrichment or prior experience with the learning task. I would also suggest extending the testing period to determine the sharks' memory and extinction time, and also using juvenile sharks that may respond more favorably to the enrichment objects than older sharks.

My study built upon knowledge gained from previous conditioning studies involving sharks and other species. An unexpected benefit of my study came from raising public awareness about sharks (in the Aquarium setting) and their learning capabilities and getting people interested in their behavior. Many Aquarium visitors talked with me about the project, and the project was publicized in the Aquarium's Sea Star Newsletter (Kay 2011) and Niagara Falls Gazette (Deluca 2011).

The more we know about the way sharks learn and their physiology, behavior and sensory capabilities, the more we can advocate for their protection and conservation and help to eradicate the public's misperceptions about sharks. In a small way, the project advanced my career goal, to dispel the public's view that sharks are mindless, vicious predators whose destruction worldwide should not concern us.

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Table 1. Percent success of white-spotted bamboo sharks at approaching the discriminative stimulus before (pre) and after (post) enrichment (correct vs. random [50:50] responses), and p-values associated with comparisons shown. TP1 = with food reward; TP2 = without food reward.

Percent Correct	Random	TP1pre	TP2pre
TP1pre (70.0%)	0.0015	----	----
TP2pre (54.5%)	0.2682	----	----
TP1post (64.2%)	0.0015	0.8074	----
TP2post (54.0%)	0.2863	----	0.5176

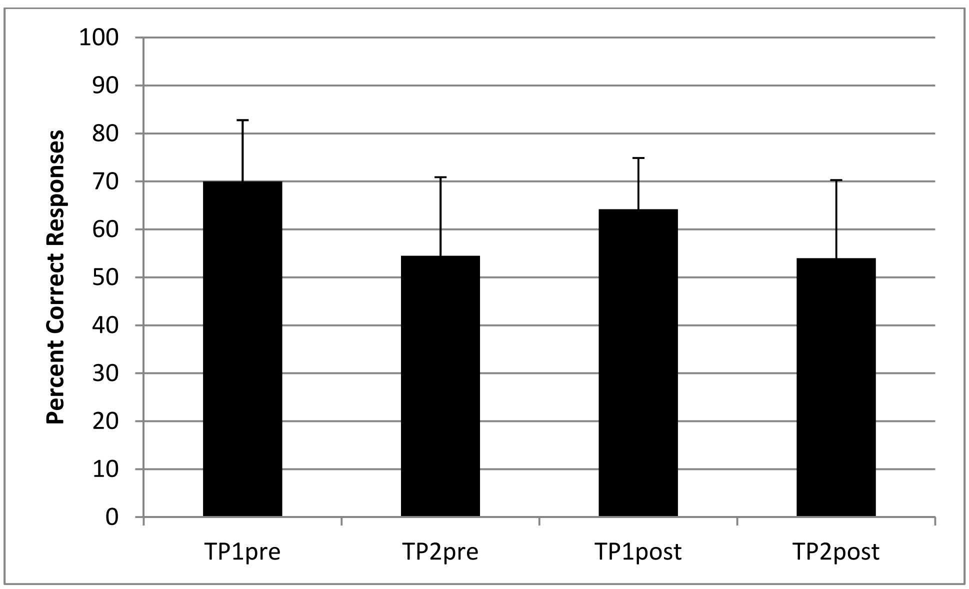

Figure 1. Percent success of white-spotted bamboo sharks at approaching the discriminative stimulus. TP1 = with food reward; TP2 = without food reward. Pre = pre-enrichment; Post = post-enrichment

Appendix A

Shark Feeding/Training/Testing Schedule

(All feedings began at 11:30 am)

Date (Day)	Phase	Which Sharks Fed
8/3 (Wed.)	Familiarization (day 1)	All
8/4 (Thurs.)	Familiarization (day 2)	Blacktip
8/5 (Fri.)	Training (day 1)	All
8/8 (Mon.)	Training (day 2)	Catsharks, Blacktip
8/9 (Tues.)	Training (day 2)	Bamboo sharks, Blacktip
8/10 (Wed.)	Training (day 3)	Catsharks, Blacktip
8/11 (Thurs.)	Training (day 3)	Bamboo sharks, Blacktip
8/12 (Fri.)	Training (day 4)	Catsharks, Blacktip
8/13 (Sat.)	Training (day 4)	Bamboo sharks, Blacktip
8/15 (Mon.)	Training (day 5)	All
8/17 (Wed.)	Testing- phase 1- day 1	Bamboo sharks
8/19 (Fri.)	Testing- phase 1- day 2	Bamboo sharks
8/21 (Sun.)	Testing- phase 1- day 3	Bamboo sharks
8/23 (Tues.)	Testing- phase 1- day 4	Bamboo sharks
8/25 (Thurs.)	Testing -phase 2- day1	Bamboo sharks
8/29 (Mon.)	Testing- phase 2- day 2	Bamboo sharks
8/31 (Wed.)	Testing- phase 2- day 3	Bamboo sharks
9/1-11/3	No testing	
11/4- 11/14	Enrichment Period; no testing	
11/17 (Thurs.)	Testing- phase 1-day 1	Bamboo sharks
11/21 (Mon.)	Testing- phase 1-day 2	Bamboo sharks

11/23 (Wed.)	Testing- phase 1-day 3	Bamboo sharks
11/25 (Fri.)	Testing- phase 2-day 1	Bamboo sharks
11/27 (Sun.)	Testing- phase 2-day 2	Bamboo sharks
11/29 (Tues.)	Testing- phase 2-day 3	Bamboo sharks

Appendix B

Shaping Procedure*

1.) The shark was rewarded for a turn or orientation toward the direction of the target (upon entry of target)

2.) The shark was rewarded for an approach to the target (the shark had have to come half way from its starting point to the target).

3.) The next reward came after an approach to the target within one quarter of the distance from the shark's starting point to the target.

4.) Finally, the shark was only rewarded for coming within ~30 cm. (or ~15 cm. depending on the species) of the target.

* If the shark did not attenuate to the object after 2 minutes, the object was removed. The shark then got a rest interval for 9 min before being trained again.

Appendix C

<u>Raw and Tabulated Data</u>

Training

Number of responses with food reward

DaySex	**Approaches**	**Contacts**
D1f	5	3
D1m	5	1
D2f	5	3
D2m	6	2
D3f	5	4
D3m	4	1
D4f	4	2
D4m	4	1
D5f	7	5
D5m	6	2

black=randomly chosen training target

Day	**Female**	**Log10F**	**Male**	**Log10M**
D1	8	0.90309	6	0.77815
D2	8	0.90309	8	0.90309
D3	9	0.954243	5	0.69897
D4	6	0.778151	5	0.69897
D5	12	1.079181	8	0.90309
Mean	8.6		6.4	
SD	2.19089		1.516575	

Pre-Enrichment Testing

Testing Phase 1: Pre-Enrichment

Number of responses WITH food reward

DaySex	Correct	Incorrect	DaySex	Correct	ArcsinC	Random	ArcsinR
D1f	8	1	D1f	0.888889	1.094914	0.5	0.5235988
D1m	5	1	D1m	0.833333	0.985111	0.5	0.5235988
D2f	3	3	D2f	0.5	0.523599	0.5	0.5235988
D2m	3	1	D2m	0.75	0.848062	0.5	0.5235988
D3f	8	3	D3f	0.727273	0.81434	0.5	0.5235988
D3m	4	2	D3m	0.666667	0.729728	0.5	0.5235988
D4f	4	2	D4f	0.666667	0.729728	0.5	0.5235988
D4m	4	3	D4m	0.571429	0.608246	0.5	0.5235988
black=randomly chosen testing target			Mean	0.700532		0.5	
			SD	0.128425		0	

Pre-Enrichment Testing

Testing Phase 2: Pre-Enrichment

Number of responses WITHOUT

food reward

DaySex	Correct	Incorrect	DaySex	Correct	ArcsinC	Random	ArcsinR
D1f	2	4	D1f	0.333333	0.339837	0.5	0.5235988
D1m	4	5	D1m	0.444444	0.460554	0.5	0.5235988
D2f	4	3	D2f	0.571429	0.608246	0.5	0.5235988
D2m	4	4	D2m	0.5	0.523599	0.5	0.5235988
D3f	9	2	D3f	0.818182	0.958242	0.5	0.5235988
D3m	3	2	D3m	0.6	0.643501	0.5	0.5235988
black=continuing testing target			Mean	0.544565		0.5	
			SD	0.164493		0	

Post-Enrichment Testing (11 weeks later)

Testing Phase 1: Post-Enrichment

Number of responses WITH food reward

DaySex	Correct	Incorrect	DaySex	Correct	ArcsinC	Random	ArcsinR
D1f	4	3	D1f	0.571429	0.608246	0.5	0.5235988
D1m	2	1	D1m	0.666667	0.729728	0.5	0.5235988
D2f	4	4	D2f	0.5	0.523599	0.5	0.5235988
D2m	3	2	D2m	0.6	0.643501	0.5	0.5235988
D3f	5	2	D3f	0.714286	0.795603	0.5	0.5235988
D3m	4	1	D3m	0.8	0.927295	0.5	0.5235988
black=randomly chosen testing target			Mean	0.642063		0.5	
			SD	0.10743		0	

Post-Enrichment Testing

(11 weeks later)

Testing Phase 2: Post-

Enrichment

Number of responses WITHOUT

food reward

DaySex	Correct	Incorrect	DaySex	Correct	ArcsinC	Random	ArcsinR
D1f	2	2	D1f	0.5	0.523599	0.5	0.5235988
D1m	2	1	D1m	0.666667	0.729728	0.5	0.5235988
D2f	4	1	D2f	0.8	0.927295	0.5	0.5235988
D2m	2	3	D2m	0.4	0.411517	0.5	0.5235988
D3f	3	5	D3f	0.375	0.384397	0.5	0.5235988
D3m	1	1	D3m	0.5	0.523599	0.5	0.5235988
black=continuing testing target			Mean	0.540278		0.5	
			SD	0.163505		0	

Appendix D

Statistical Analyses, Including Tests for Normality and Equal Variance

Training

Two-Sample, Two-Tailed T Test for Log10 Female - Log10 Male

Variable	Mean	N	SD	SE
Log10F	0.9236	5	0.1086	0.0485
Log10M	0.7965	5	0.1026	0.0459
Difference	0.1271			

Null Hypothesis: difference = 0

Alternative Hyp: difference <> 0

				95% CI for Difference	
Assumption	T	DF	P	Lower	Upper
Equal Variances	1.90	8	**0.0936**	-0.0269	0.2811
Unequal Variances	1.90	8.0	0.0937	-0.0270	0.2812

Test for Equality	F	DF	P
of Variances	1.12	4,4	**0.4575**

Shapiro-Wilk Normality Test

Variable	N	W	P
Female	5	0.9316	0.6071
Male	5	0.8030	0.0857
Log10F	**5**	**0.9572**	**0.7883**
Log10M	**5**	**0.8127**	**0.1024**

Number of Approaches/Contacts: Male = Female

Tendency toward Female > Male

Pre-Enrichment Testing

Testing Phase 1: Pre-Enrichment WITH Food Reward

Paired, One-Tailed T Test for Arcsin Correct - Arcsin Random

Null Hypothesis: difference = 0

Alternative Hyp: difference > 0

Mean	0.2681
Std Error	0.0663
Mean - H0	0.2681
Lower 95% CI	0.1113
Upper 95% CI	0.4249
T	4.04
DF	7
P	**0.0025**

Shapiro-Wilk Normality Test

Variable	N	W	P
Correct	8	0.9766	**0.9439**
Random	na		

Correct > Random WITH food reward

Pre-Enrichment Testing

Testing Phase 2: Pre-Enrichment WITHOUT Food Reward

Paired, One-Tailed T Test for Arcsin Correct - Arcsin Random

Null Hypothesis: difference = 0

Alternative Hyp: difference > 0

Mean	0.0654
Std Error	0.0861
Mean - H0	0.0654
Lower 95% CI	-0.1560
Upper 95% CI	0.2868
T	0.76
DF	5
P	**0.2410**

Shapiro-Wilk Normality Test

Variable	N	W	P
Correct	6	0.9362	**0.6287**
Random	n		

Correct = Random WITHOUT food reward

Post-Enrichment Testing (11 weeks later)

Testing Phase 1: Post-Enrichment WITH Food Reward

Paired, One-Tailed T Test for Arcsin Correct - Arcsin Random

Null Hypothesis: difference = 0

Alternative Hyp: difference > 0

Mean	0.1811
Std Error	0.0590
Mean - H0	0.1811
Lower 95% CI	0.0295
Upper 95% CI	0.3326
T	3.07
DF	5
P	**0.0139**

Shapiro-Wilk Normality Test

Variable	N	W	P
Correct	6	0.9805	**0.9540**
Random	na		

Correct > Random WITH food reward

Post-Enrichment Testing (11 weeks later)

Testing Phase 2: Post-Enrichment WITHOUT Food Reward

Paired, One-Tailed T Test for Arcsin Correct - Arcsin Random

Null Hypothesis: difference = 0

Alternative Hyp: difference > 0

Mean	0.0598
Std Error	0.0848
Mean - H0	0.0598
Lower 95% CI	-0.1584
Upper 95% CI	0.2779
T	0.70
DF	5
P	**0.2563**

Shapiro-Wilk Normality Test

Variable	N	W	P
Correct	6	0.8899	**0.3175**
Random	na		

Correct = Random WITHOUT food reward

No basis for going on to Post-Enrichment Testing Phase 3: Extinction time without reward

Pre- vs. Post-Enrichment Results

Two-Sample, One-tailed T Test for Correct Responses:

Arcsin Post- vs. Arcsin Pre-Enrichment WITH food reward

Variable	Mean	N	SD	SE
ArcsinPos	0.7047	6	0.1444	0.0590
ArcsinPre	0.7917	8	0.1876	0.0663
Difference	-0.0871			

Null Hypothesis: difference = 0

Alternative Hyp: difference > 0

				95% CI for Difference	
Assumption	T	DF	P	Lower	Upper
Equal Variances	-0.94	12	**0.8179**	-0.2882	0.1141
Unequal Variances	-0.98	12.0	0.8270	-0.2804	0.1063

Test for Equality	F	DF	P
of Variances	1.69	7,5	**0.2922**

Shapiro-Wilk Normality Test

Variable	N	W	P
ArcsinPos	6	0.9805	**0.9540**
ArcsinPre	8	0.9766	**0.9439**

Post- = Pre-Enrichment responses WITH food reward

Pre- vs. Post-Enrichment Results

Two-Sample, One-tailed T Test for Correct Responses:

Arcsin Post- vs. Arcsin Pre-Enrichment WITHOUT food reward

Variable	Mean	N	SD	SE
ArcsinPost	0.5834	6	0.2078	0.0848
ArcsinPre	0.5890	6	0.2110	0.0861

Difference -5.64E-03

Null Hypothesis: difference = 0

Alternative Hyp: difference > 0

				95% CI for Difference	
Assumption	T	DF	P	Lower	Upper
Equal Variances	-0.05	10	**0.5181**	-0.2750	0.2637
Unequal Variances	-0.05	10.0	0.5181	-0.2750	0.2638

Test for Equality	F	DF	P
of Variances	1.03	5,5	**0.4873**

Shapiro-Wilk Normality Test

Variable	N	W	P
ArcsinPost	6	0.8899	**0.3175**
ArcsinPre	6	0.9362	**0.6287**

Post- = Pre-Enrichment responses WITHOUT food reward