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Creating a Discrete-Trial Laboratory Experiment for Goldfish

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ABSTRACT

The project was designed to create an alternative to teaching basic operant conditioning techniques with rodents by developing a means to illustrate discrete-trial water maze training with goldfish. It first utilized two comet goldfish trained to eat from a food wand. The subjects were placed in a start area within the maze and timed, with each trial concluding once they reached the designated finish area and consumed a food reinforcer. Three smaller comet goldfish were later run in the same maze using floating food as the reinforcer (versus delivery via the food wand). Data was recorded on sheets created specifically for use with this procedure; response time from start to finish decreased over subsequent trials, as did errors (wrong turns) within the maze. Overall, four of five fish showed marked improvement throughout the study. The average initial completion time was 9.21 minutes, while the average final trial time was 1.88 minutes. This computes an average improvement from the first trial to the last of 7.33 minutes. These results show that goldfish can indeed be used to demonstrate behavioral principles such as the law of effect and discrete-trial training, foundational concepts in the field of behavior analysis. This study was part of a larger project that aims to create a complete operant lab manual for use with goldfish, which would allow students the opportunity to participate in a lab that is less costly and more manageable than traditional rat lab.

Keywords: animal learning; experimental analysis of behavior; law of effect;

Students learning the foundational principles of behavioral science stand to benefit from direct experiences with the material. However, many laboratory activities used in universities are prohibitively expensive. The conventional rat lab utilizes operant chambers that range from hundreds to thousands of dollars (Devarakonda et al., 2016; Gurley, 2019). Educators should have more affordable means of demonstrating behavioral principles to students firsthand. As such, there is room for learning tools that minimize cost without sacrificing engagement.

Cost-effective experiments that are accessible to colleges and high schools, as an alternative to the traditional rat lab, are worth investigating (Devarakonda et al., 2016). Low-maintenance animals, like cockroaches and goldfish, can learn novel tasks comparable to those completed by mammals (Rodriguez et al., 1994; Zajonc et al., 1969). Affordable homemade equipment can be used in place of an operant chamber to run laboratory activities with these inexpensive subjects. For example, prior research has shown that the comet goldfish can learn to navigate a variety of different mazes (Churchill, 1916; Kleerekoper et al., 1974; Muntz & Cronly-Dillon, 1966). The goldfish in these studies were able to successfully navigate mazes of varying complexity over repeated trials.

The ability to navigate a novel learning apparatus is indicative of the law of effect. Thorndike's (1927) law of effect states that behavior is a result of its consequences. An organism's responses are more strongly connected with the situation when they are met with satisfaction, while any responses met with dissatisfaction are weakened. Essentially, when the subject gets something desirable by behaving in a certain way, it is more likely to behave that way in the future. Likewise, behavior that led a subject to an outcome marked by "annoyingness" is less likely to be repeated; an animal will make fewer ineffectual movements with each successive trial to reach "states of affairs" with greater "satisfyingness" (Thorndike, 1927, p. 212).

The homemade apparatus devised in the present study also demonstrates discrete trial training (DTT). Discrete trial training is one of two dominant learning paradigms in behavioral science, with the other being free-operant learning (Chance, 2014). DTT entails placing the subject in a situation with a device that allows for a single display of desirable behavior per trial (Hachiya & Ito, 1991). The reinforced behavior is the subject's interaction with the device. Once the desired response is emitted, the trial ends because the device must be reset for the subject. In this way, the subject has one opportunity in each trial to exhibit the desired behavior and have it reinforced. Free-operant learning is distinguished from DTT because free-operant allows the subject to emit the desired response multiple times during a single session. The device being interacted with does not need to reset once the desired behavior occurs (Hachiya & Ito, 1991). Rather, the subject can freely interact with the device as its behavior is reinforced each time.

An overview of costs accrued by lab activities involving rodents compared to costs associated with goldfish highlights the savings presented by this alternative maze lab procedure. Rodents ordered from a biological supply company vary in cost depending on their age/size but cost no less than $25 per subject (vs. under 50 cents for goldfish). In addition, the shipping costs for live animals are very high ($250 per crate) vs. picking up some fish at the local pet store. Rats can be obtained at a local pet store, but even small feeder rats typically cost between $6 and $10 apiece. Rats also require bedding, which runs $25 per bale, and weekly cage cleaning is necessary, resulting in a much greater reoccurring cost and response effort than that for fish (adding tap water and monthly filter changes). Mazuri rat chow is $25 per 25-lb. bag, whereas the Tetra fish flakes in the present research were $7 for a container that lasted an average of 6 months.

As for the in-house setup, the costs for a rodent colony are much more significant (laboratory-quality caging, cage racks, water bottles) vs. a $50 10-gallon tank set up from Amazon. Polycarbonate double cages used for rodents cost $1,050 for five, and the wire cage tops are another $550 (actual price quote from Ancare, Inc., 2022). In addition, the dedicated space needed for a rodent colony is significant, and due to the potential for allergies in humans, must be kept separate from human spaces and/or have highly effective ventilation. A fish tank can be placed just about anywhere and takes up relatively little space. Today, a basic operant chamber costs $4,700 (actual price quote from Lafayette Instruments, Inc., 2022). Fish can be trained in the home tank, or smaller isolated plastic tanks for a fraction of that price.

Taken altogether, the present study sought to (a) demonstrate the utility of an inexpensive, homemade aquatic maze to study operant conditioning in goldfish and (b) characterize the learning process by measuring maze completion time across successive trials. It was hypothesized that the goldfish would solve the maze faster with each successive trial. This research was meant as a proof of concept supporting the utilization of cost-effective learning apparatuses in behavioral psychology courses. The maze was intentionally built using material readily available to anyone interested in building their own experimental apparatus.

METHODS

Subjects

There were two groups of fish trained in the aquatic maze. The first group was trained in the fall of 2022 and consisted of two goldfish named Comet and Galaxy. They each measured approximately 3.5 inches in length. Comet and Galaxy were kept together in a 10-gallon tank in a separate room from the maze. The second group was trained in the spring of 2023 and consisted of three goldfish: Orange, Blanca, and Midnight. Each fish measured approximately 2 inches in length. Given their smaller size, Orange, Blanca, and Midnight were housed in another 10-gallon tank right next to the maze. The fish were typically fed once a day in the afternoon with commercially available Tetra goldfish flakes. This amounted to roughly a teaspoon of food per day.

The water temperature was maintained near 22°C in both 10-gallon tanks as well as the aquatic maze. The home tanks and the maze were kept at the same temperature to avoid stress related to a sudden temperature change. The two 10-gallon tanks were set up and sufficiently cycled before the subjects were kept in them. The maze and the home tanks were filled with treated tap water. The water was treated using Tetra AquaSafe Aquarium Water Conditioner. Two teaspoons of the solution conditioned an entire 10-gallon tank. This same conditioner was also used in the maze. The treatment was reapplied following monthly cleanings of the tanks and the maze. The maze water was reused twice each week before being cleaned. Tap water was also added to the tanks as needed. Filter changes were completed monthly ($2 per filter). The kit with the tank, filter, heat, etc. was $50 from Amazon. However, there was no filtration system in the maze, which is a limitation addressed in the discussion section.

Materials

Table 1 provides an itemized list of the materials, their cost, and the retailer they were purchased from. The bulk of the materials were purchased from Amazon and Walmart to maximize ease of accessibility and cost-effectiveness. Any item that would be submerged in the maze or have frequent contact with the water was verified to be aquarium safe

Table 1. Comet Goldfish Discrete Trial Training Maze Supplies

Item	Cost	Retailer
Akfix 100AQ Aquarium Safe Silicone Sealant - Waterproof Bond to Glass, Clear 100% Silicone Sealer for Freshwater & Saltwater Aquarium, Solvent-Free, Non-Toxic | 2 - Pack, 9.5 Oz. Transparent.	$18.99	Amazon
Aluminum Wire, Anezus 9 Gauge 12 Gauge 18 Gauge Bendable Metal Wire Armature Aluminum Craft Wire for Wreath Making Beading Floral (Silver, 3 mm Thickness).	$10.00	Amazon
Aqua Culture Aquarium Gravel, Caribbean, 25 lb.	$20.38	Walmart
Aqua Culture Glass Thermometer.	$1.63	Walmart
AQUANEAT Aquarium Bio Sponge Filter Media Pad 36" x 12" x 1" / 72" x 12" x 1" Open Cell Foam Sheet Cut-to-Fit for Fish Tank Sump (36" x 12" x 1").	$17.99	Amazon
Duck Brand 1.88 in. x 20 yd. Black Colored Duct Tape.	$3.94	Walmart
Sicce Jolly Heater, 20 Watt.	$24.99	Petco
Sterilite 66 Quart Stadium Blue Ultra Storage Box.	$24.98	Walmart
Total	$122.90	

Procedure

First, the sides of an under-bed storage unit were taped off to obstruct all distracting views of the outside. Then aquarium-safe silicone was used to seal the holes in that storage unit. To form the maze walls, wires were run through water-safe foam sheets, which were then bent to the desired shape. These walls were placed inside the storage unit and attached to the rim with the segments of exposed wire. Silicone was used to secure the foam to the sides of the maze. Once the walls were secure, 25 pounds of gravel was added to cover the floor, and the storage unit was filled with water just below the rim of the maze. A water heater and thermometer were later added for temperature regulation.

Comet and Galaxy were each transported from their home tank to the maze in a bucket. The maze was kept in a room separate from the home tank. Researchers would fill the bucket with water from the home tank, net the fish, and place them in it. This bucket was then walked to the other room, where a researcher would net the fish and place them into the maze at the start of the experiment. When it was time to move the fish, they were netted and placed into the bucket of water. If that same fish was doing another trial that same day, they waited in the bucket while the maze was reset for the next trial. If the fish was not doing another trial that day, then the researcher walked the bucket back to the room with the home tank. The fish was then netted and placed back into the home tank. The water in the bucket from the tank was poured back in.

To make proceedings easier for the second group, Orange, Blanca, and Midnight were kept in a tank right next to the maze. The researcher would net the fish and move them directly to the start of the maze. This change was made to reduce the time it took to begin each trial. After each trial, the researcher would net the fish and move them back into the home tank.

Sessions were conducted on weekday afternoons during the fall and weekday mornings during the spring. A camera was mounted above the maze to film the fish while observers manually recorded other data. Data was recorded on sheets created specifically for use with this procedure.

The comet goldfish were deprived of food for twenty-four hours before each session. With the first group of fish, a wall was placed at the start which would be lifted once the timer commenced. With the second group, the timer simply commenced once the fish exited the submerged netting. Also, with the first group, a food reinforcer was administered to the fish upon successful completion of the maze via a food wand. However, due to the limited availability of the food wand, the second group had to forgo the wand, so the food instead floated on the surface of the water at the end of the maze. Each trial was completed when the fish obtained the food reinforcer; simply swimming near the endpoint was not sufficient. Upon completion, the fish were netted and transported back to their home tank. However, the immediacy of that netting proved important to the experiment.

Fish Group 1, Task A

The first group of comet goldfish was made up of two: Comet and Galaxy. In trials where fish had to reach the endpoint labeled “Task A”, the timer stopped once the subject obtained the food reinforcer. The fish were netted immediately following consumption of the food reinforcer in this earliest version of the experiment. However, this proved problematic as both Comet and Galaxy were subsequently observed avoiding the end of the maze. It was speculated that the net was an aversive stimulus that the fish were avoiding. In consideration of this, the endpoint of the maze was reassigned in “Task B” (see Figure 1).

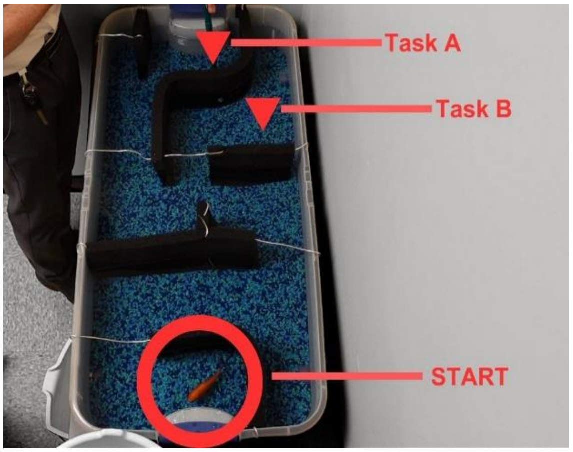

Figure 1. Discrete Trial Training Maze Layout (view from the mounted camera)

Task B was a rendition of the DTT maze meant to solve the problem that had been observed with Task A. The key difference in Task B was that the goldfish were allowed to swim freely for 30 seconds to a minute after obtaining the food reinforcer. This length of time was allotted for the fish because it was roughly how long it took researchers to record data from the trials, stop the video recording, and collect the camera. Allowing the subjects to linger in the maze was to break any association that may have formed between the end of the maze and the aversive stimulus that was the net.

Fish Group 2, Task B

The second group of comet goldfish consisted of Orange, Blanca, and Midnight. These three were tested in reaching the same Task B endpoint that the previous group was. The floating food reinforcer was originally meant to float at the surface within a small ring corral, but the fish were observed avoiding the ring at the end of the maze in several trials. Suspecting that the corral may be obstructing them from reaching the food, the ring was removed, and the food was left to float freely.

Data Analysis

The single-subject design in the present study did not warrant the use of inferential statistical techniques to analyze the data. The single-subject design allowed for an examination of the cause-effect relations at the level of the individual subject. To demonstrate that goldfish can learn to navigate the homemade maze, completion times across trials were compared. Trends toward decreased navigation times were expected to support the stated hypothesis.

RESULTS

Cross-Group Results for Task B

The date and length of time for each trial can be seen in Table 2. Galaxy's performance improved following the change from Task A to Task B. The first successful trial lasted 6.98 minutes, the second for 4.67 minutes, the third for 4.43 minutes, the fourth for 2.23 minutes, the fifth for 0.63 minutes, the sixth for 0.45 minutes, the seventh for 0.62 minutes, and the final for 0.35 minutes, decreasing 6.63 minutes from the first to last trial. Following the switch to Task B, Comet's first trial ran for 8.70 minutes and the second for 4.37 minutes. The third and final trial was incomplete; however, the improvement from the first to second trial was still 4.33 minutes.

Table 2. Comet Goldfish Discrete Trial Training

Comet		Galaxy			
Date	**Time**	**Date**	**Time**		
11/28/2022	8.7	11/28/2022	6.98		
11/29/2022	4.37	11/28/2022	4.67		
11/29/2022	--	11/28/2022	4.43		
		11/29/2022	2.23		
		11/29/2022	0.63		
		11/29/2022	0.45		
		11/30/2022	0.62		
		11/30/2022	0.35		
Blanca		**Midnight**		**Orange**	
Date	**Time**	**Date**	**Time**	**Date**	**Time**
2/24/2023	--	2/24/2023	7.98	2/25/2023	--
2/26/2023	--	2/25/2023	--	2/28/2023	13.17
3/3/2023	--	2/26/2023	13.45	3/1/2023	22.6
3/9/2023	--	2/28/2023	4.23	3/3/2023	9.5
3/10/2023	--	3/1/2023	5.13	3/9/2023	3.12
		3/3/2023	3.7	3/10/2023	2.05
		3/9/2023	3.03		
		3/10/2023	0.73		

In the second group, Blanca did not complete any of the five trials that were run. Orange completed five out of the six trials. The first successful trial ran for 13.17 minutes, the second for 22.60 minutes, the third for 9.50 minutes, the fourth for 3.12 minutes, and the final for 2.05 minutes, for an improvement from the first to last trial of 11.12 minutes. Midnight completed seven out of the eight trials. The first successful trial ran for 7.98 minutes. Following a single incomplete run, the second successful trial lasted 13.45 minutes. The third lasted 4.23 minutes, the fourth, 5.13 minutes, the fifth, 3.70 minutes, the sixth, 3.03 minutes, and the final, 0.73 minutes, decreasing 7.25

minutes from the first to last trial. Both Orange and Midnight exhibited improved performance following the switch to free-floating fish flakes.

Overall, four out of five fish demonstrated marked improvement throughout training, finishing the maze faster with almost all subsequent trials. The average improvement across all four successful fish from the first trial to the last was 7.33 minutes.

DISCUSSION

The present study built an affordable experimental apparatus and demonstrated that goldfish can learn to navigate it. Four out of the five fish showed marked improvement in navigating the maze, supporting the case for this experiment as a learning activity. Results from the first goldfish group indicate that the net is an aversive stimulus. The goldfish exhibited noticeable avoidance of the endpoint when training on Task A. Switching to Task B and increasing the time between the goldfish consuming the food reinforcer and then being netted seemed to improve Galaxy's performance from 6.98 minutes to 0.35 minutes. Comet improved over only two successful trials, from 8.70 minutes to 4.37 minutes. It is therefore important that the fish not be netted immediately following delivery of the reinforcer, otherwise, an association may form between the food and the net.

Having learned from the fall experiments and modifying the procedure accordingly, the goldfish in the spring were not allowed to form any association between the endpoint of the maze and an aversive stimulus. Two out of the three fish seemed to benefit from this change: Orange improved from 13.17 minutes to 2.05 minutes and Midnight improved from 7.98 minutes to 0.73 minutes. However, this procedure did not affect Blanca. While Blanca would sometimes approach the endpoint, they did not consume the food reinforcer. This may be due to the 24-hour food deprivation period not sufficiently motivating Blanca as it did the other fish. See Figure 2 for a graphical summary of these results.

The present study has limitations to address. First, inter-observer reliability was not accounted for. The reason was that two observers were not always available to oversee trials. In the trials conducted in the spring, only one observer carried out the experiments. Video footage of the training was used to verify the data collected by that researcher. Future studies should include measures of inter-observer reliability to ensure that trials are recorded accurately.

Second, future studies should give the subjects a consistent length of time to linger in the maze following consumption of the food reinforcer. The goldfish in the present study were allowed to remain in the maze after completing each trial for 30-60 seconds. Allowing all subjects to have a regular 60-second intermission would improve the replicability of the study, as well as lend more validity to comparisons between subjects.

Third, the aquatic maze was not aerated. This may have led to differences in pH levels between the home tanks and the maze. Future studies using the maze for an extended period should aerate the water using a standard air pump and aeration stone. Adding a filter to the maze would also improve water quality and potentially reduce the stress placed on the fish. Sudden changes in pH can influence behavior, so these levels should be controlled. In addition to testing the pH levels of both the home tank and the maze, it may be worthwhile to test ammonia, nitrite, and nitrate levels in the home tank because they can also influence behavior.

Fourth, future studies should also investigate the practicality of a more careful technique for transporting the fish. Goldfish have a mucus layer that covers their body and helps to protect them from disease and parasites (Dash, Das, Samal, &

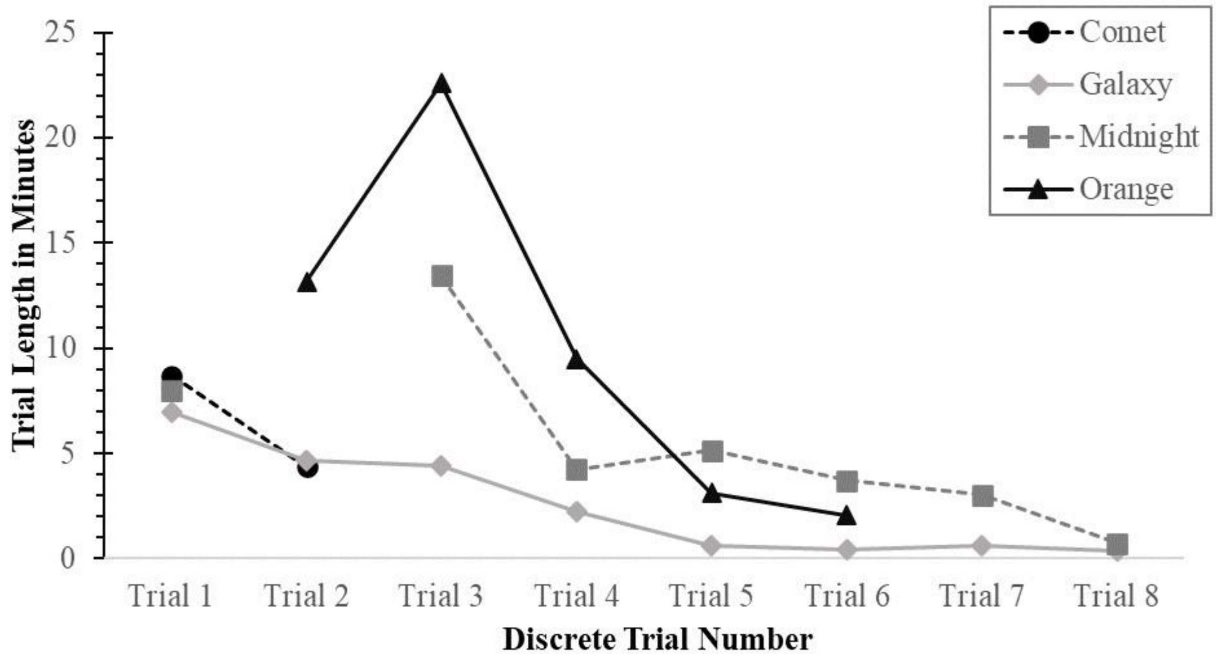

Figure 2. Changes in Maze Completion Times Across Trials

Thatoi, 2018). Netting the fish can damage their slime coat. Treating both the home tank and arena water with API Stress Coat (available through Amazon & Walmart) can be very helpful in reducing the damage. Also rinsing the net in between fish and soaking it in water treated with Stress Coat can be helpful. If the fish's epidermis is damaged, it may cause them to produce a chemical substance known as "Schreckstoff", which is a German word roughly meaning "fright" or "fear stuff" (Ajay, 2012). This alarm acts as a chemical message to other fish who may then demonstrate an alarm reaction, e.g., escape behavior and other fear-related behaviors. In the present study, reusing the water in the aquatic maze for multiple fish may have affected their behavior due to the Schreckstoff lingering in the water.

To avoid damage to the subjects, especially the epidermis, a technique incorporating more handling may be worthwhile. To reduce the stress posed to the fish upon first entering the maze, fish may be netted out of the home tank and placed in a bucket for a few minutes. This intermission – maybe three minutes of wait time – would be meant to decrease fish stress in much the same way as the intermission following the consumption of the food reinforcer. Once it is time to move the fish from the bucket to the maze, carefully pouring them in would pose much less risk to their epidermis than would a net (Dash et al., 2018). Therefore, an integrated technique for transporting the fish that combines netting, brief intermission, and gentle handling may go a long way to making the fish more comfortable in the maze.

Lastly, two out of the five comet goldfish underperformed for reasons not immediately clear. It is possible that Comet never dissociated the end of the maze with the net and so avoided the food reinforcer in Task B. As for Blanca, their food deprivation period may not have been long enough, thus rendering the reinforcer ineffectual. Future studies should further optimize the maze to ensure consistent success across all subjects. One way might be to shape how the fish navigate the maze by first presenting the food reinforcer close to the starting point, then gradually moving the food further towards the endpoint with each successive trial (Skinner, 1953). In this way, changing the criteria for how far the fish must swim and in what direction to obtain food may lead to more rapid and consistent success. This adjustment would come with the added benefit of demonstrating the concept of shaping that the rat lab does.

The law of effect demonstrated in the present experiment is similar to the concept of shaping in that both are foundational in behavioral science (Chance, 2014). Shaping is the gradual teaching of a new behavior by successive approximations, positive reinforcement, and extinction (Skinner, 1953). An individual who shapes behavior may begin by reinforcing rough approximations of the target behavior. Once the subject reliably makes these rough approximations, the behavior is gradually refined as greater and greater criteria must be met before the behavior exhibited by the subject is reinforced (Chance, 2014). With these successive approximations, behavior that more closely resembles the target behavior is positively reinforced. Shaping is what lab activities with rodents typically demonstrate.

The shaping demonstrated by a conventional rat lab is not identical to the law of effect demonstrated in the present study. Shaping would require students to gradually increase what they demand from the subject (Skinner, 1953). In contrast, the training in this study did not involve changing the criteria for reinforcement. Since the criteria for reinforcement in the aquatic maze did not change, it provides a stronger demonstration of the law of effect. However, the two principles are essential to introductory behavioral science courses and can be taught with hands-on demonstrations (Chance, 2014). Therefore, having an affordable means of demonstrating one – Thorndike's (1927) law of effect – would be worthwhile to both educators and students.

In conclusion, a DTT maze for goldfish can serve as a learning tool for behavioral studies. The present study's findings are consistent with previous research on goldfish's ability to learn how to effectively navigate mazes (Churchill, 1916; Rodriguez et al., 1994). Devising more cost-effective ways of demonstrating foundational behavioral principles is worthwhile for educators and students. The cost of constructing the homemade apparatus used in this research was a fraction of conventional operant chambers (Devarakonda et al., 2016; Gurley, 2019). The water maze is a discrete trial paradigm that lets students study operant conditioning using live subjects. Future researchers should continue investigating learning tools that are affordable and accessible. This study was part of a larger project that aims to create a complete operant lab manual for use with goldfish, which would allow students the opportunity to participate in a lab that is less costly and more manageable than a traditional rat lab.

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