

Visual perception of planar-rotated 2D objects in goldfish (*Carassius auratus*)

Caroline M. DeLong^{a,*}, Irene Fobe^a, Taylor O'Leary^a, Kenneth Tyler Wilcox^b

^a Department of Psychology, College of Liberal Arts, Rochester Institute of Technology, 18 Lomb Memorial Dr., Rochester, NY 14623, USA

^b Department of Psychology, College of Arts and Letters, University of Notre Dame, 390 Corbett Family Hall, Notre Dame, IN 46556, USA



ARTICLE INFO

Keywords:

Goldfish
Object constancy
Object discrimination
Visual perception

ABSTRACT

This study examined the ability of goldfish to visually identify 2D objects rotated in the picture plane. This ability would be adaptive for fish since they move in three dimensions and frequently view objects from different orientations. Goldfish performed a two-alternative forced choice task in which they were trained to discriminate between two objects at 0°, then tested with novel aspect angles (+/− 45°, +/− 90°, +/− 135°, 180°). Stimuli consisted of an arrow and half circle (Experiment 1) and line drawings of a turtle and frog (Experiments 2 and 3). In the first two experiments, the S+ and S− were presented at the same aspect angle. Performance in these experiments exceeded chance on four of seven novel aspect angles. Overall accuracy was not significantly different with complex stimuli (animal drawings) vs. simple stimuli (geometric shapes). In Experiment 3, when fish were tested with the S+ at varying aspect angles and the S− always presented at 0°, the fish failed to discriminate among the stimuli at all but one aspect angle. These goldfish viewing planar-rotated 2D objects did not display viewpoint-invariant performance, nor did they show a systematic decrement in performance as a function of aspect angle.

1. Introduction

The ability to recognize an object from any viewpoint is called object constancy. It is considered an essential property of human visual perception (Jolicoeur and Humphrey, 1998). Many studies have addressed the ability of non-human animals to visually recognize rotated objects (for a review see Peissig and Goode, 2012), but few fish subjects have been tested (e.g., Bowman and Sutherland, 1969; Schluessel et al., 2014; Wang and Takeuchi, 2017). It would be advantageous for fish to possess visual object constancy. Swimming fish make complex three-dimensional motions in which they can view objects and animals from many different orientations (Kleerekoper et al., 1974). Unlike most terrestrial organisms, fish move frequently up and down along the y-axis. They are often exposed to views of objects from the side and above and below as they swim. Delius and Hollard (1995) suggested that organisms that experience their environment from a variety of rapidly changing orientations should recognize objects regardless of orientation. Fish use visual object recognition for navigation, foraging, and identifying social partners, prey and predators (Brown et al., 2011). Further, fish are capable of complex discrimination tasks using vision (e.g., Agrillo et al., 2013; Brown et al., 2011; DeLong et al., 2017a; Newport et al., 2016; Truppa et al., 2010). The purpose of this study was to investigate whether goldfish visually recognize 2D objects

rotated in the picture plane.

Studies on visual object constancy in humans have produced varying results that led to competing theories of how individuals represent objects (Jolicoeur and Humphrey, 1998; Lawson, 1999). In object-centered theories, a single underlying 3D representation is constructed of the object specifying structural relations between geons (e.g., Biederman and Gerhardstein, 1993; Marr, 1982). If a new view of a familiar object is encountered, then the structural representation of the object will be activated as long as the relationships between the geons are preserved. This means recognition performance on familiar and novel views should be equivalent and the results are classified as viewpoint-independent. According to viewer-centered theories, the visual system stores several views of an object, a novel view is matched to the nearest stored representation and different 2D object representations are formed with each different view (e.g., Tarr, 1995; Tarr and Pinker, 1989). These theories predict that novel views will require some processing, which results in differences in performance between familiar and novel views (i.e., viewpoint-dependent performance). Manipulating variables such as experience with the task, familiarity with the stimuli, object type, object set size, the similarity of the objects within the sets, rotation plane, and rotation scheme can cause performance to shift between viewpoint-independence and viewpoint-dependence (Jolicoeur and Humphrey, 1998).

* Corresponding author.

E-mail address: cmdgsh@rit.edu (C.M. DeLong).

<https://doi.org/10.1016/j.beproc.2018.10.009>

Received 25 May 2018; Received in revised form 17 October 2018; Accepted 18 October 2018

Available online 24 October 2018

0376-6357/ © 2018 Elsevier B.V. All rights reserved.

In addition to the representation-based theories that account for object constancy, a third theory is based on the process of transforming a novel view to a familiar view. [Shepard and Metzler \(1971\)](#) proposed that humans use a process of mental rotation to recognize rotated 2D or 3D objects, whether the stimuli are rotated in the picture plane or the depth plane. According to this theory, in order to decide whether a comparison object matches a sample object, the individual creates a mental representation of the sample object, then mentally rotates the object until it matches the orientation of the comparison object. Humans can successfully recognize new views of objects, but the reaction time for participants to make their decision increased linearly as a function of aspect angle ([Shepard and Metzler, 1971](#)). Representation and process theories differ conceptually but can be difficult to distinguish empirically. They are not incompatible, and some researchers include multiple theories to account for performance (e.g., [Jolicoeur, 1990](#)).

In visual object constancy studies, objects can be rotated in the picture plane or the depth plane. Rotating an object in the picture plane is to transform it about a fixed point along the sight line of the viewer. For example, turning a drawing of a tea cup upside down is the equivalent of an 180° planar rotation. Depth plane rotations involve rotating an object about the vertical or horizontal axis. For example, walking around a tea cup sitting on a table would afford different views of the cup equivalent to a series of depth plane rotations. For objects rotated in the picture plane, the same object features present at one view are theoretically available for all views (unlike depth plane rotations, where distinctive features can appear and disappear). The current study focuses on picture-plane rotations of 2D stimuli.

Visual object constancy studies using planar-rotated stimuli have been investigated in non-human animals in a variety of habitats. Terrestrial species that have been tested include rats ([Minini and Jeffery, 2006](#); [Sutherland, 1969](#)), ferrets ([Pollard et al., 1967](#)), sheep ([Kendrick et al., 1996](#)), dogs ([Racca et al., 2010](#)), and baboons ([Hopkins et al., 1993](#)). Arboreal species that have been examined include monkeys ([Freedman et al., 2006](#); [Kohler et al., 2005](#); [Nielsen et al., 2008](#); [Parr, 2011](#); [Parr and Heintz, 2008](#)), lion-tailed macaques ([Burmman et al., 2005](#)), and chimpanzees ([Parr, 2011](#)). Aerial species that have been investigated include pigeons ([Delius and Hollard, 1995](#); [Hamm et al., 1997](#); [Hollard and Delius, 1982](#); [Jitsumori and Ohkubo, 1996](#)) and bees ([Plowright et al., 2001](#)). Aquatic species that have been assessed include sea lions ([Mauck and Dehnhardt, 1997](#); [Schusterman and Thomas, 1966](#)), dolphins ([DeLong et al., 2017b](#)), octopuses ([Sutherland, 1969](#)), and fish ([Schluessel et al., 2014](#); [Wang and Takeuchi, 2017](#)). Some studies have tested performance on a wide range of aspect angles (e.g., [Hopkins et al., 1993](#); [Mauck and Dehnhardt, 1997](#)) whereas other studies have focused exclusively on performance on upright vs. inverted images rotated 180° (e.g., [Kendrick et al., 1996](#); [Racca et al., 2010](#)).

Few studies have explored visual object constancy in fish using planar rotations of 2D or 3D stimuli. Two studies have utilized planar rotations of 3D stimuli. Malawi cichlids were trained to discriminate between small plastic animal models such as turtles and frogs rotated in both the picture plane and the depth plane ([Schluessel et al., 2014](#)). The fish were trained to discriminate between the objects in an upright position, then presented with transfer tests in which the objects were rotated in either the picture plane or depth plane at 45°, 90°, or 180°. The cichlids were successful with planar rotations of 45° and 90° but failed with a 90° depth plane rotation. [Wang and Takeuchi \(2017\)](#) found that medaka fish trained to discriminate between images of two conspecific fish or two 3D objects failed the task when the fish images were inverted (180° planar rotation) but still succeeded on the discrimination when the two non-face objects were inverted.

A number of early studies on visual object recognition of 2D objects rotated in the picture plane in goldfish have found mixed results ([Bowman and Sutherland, 1969, 1970](#); [Mackintosh and Sutherland, 1963](#); [Sutherland and Bowman, 1969](#)). Goldfish trained to discriminate

between a horizontal and a vertical rectangle transferred to stimuli rotated up to 30°, but performance dropped with stimuli rotated 40–45° ([Mackintosh and Sutherland, 1963](#)). Goldfish that were trained to discriminate between ‘W’ and ‘V’ shapes transferred well to stimuli rotated 90° or 180°, as well as rectangular stimuli that had the same number of points or knobs as the letters at the tops of the shapes ([Bowman and Sutherland, 1969](#)). [Sutherland and Bowman \(1969\)](#) trained goldfish to discriminate between a circle and either a diamond, square, diamond with a knob on top, or square with a knob on top. In transfer tests, fish trained with the square or diamond could not recognize stimuli rotated by 45°. Those fish trained with the shapes containing knobs performed poorly in transfer tests when the stimuli were rotated +/– 135°, but half the subjects performed well when stimuli were rotated +/– 45°. Goldfish trained to discriminate between a regular square and an irregular square containing a protrusion or indentation did not recognize the regular square when rotated 45°, performed well when the irregular square was rotated 45°, but did not treat rotated irregular squares shapes as equivalent to the original stimulus for rotations of 90° or 180° ([Bowman and Sutherland, 1970](#)). The above studies show that after goldfish are trained to discriminate between two stimuli, some rotated shapes are classified as the same as the original stimuli, but some are not.

The aim of current study was to investigate the ability of goldfish to recognize planar-rotated 2D stimuli in a two-alternative forced choice task. Goldfish were selected as subjects because they have been frequently tested in studies of visual perception (e.g., [Mora-Ferrer and Neumeier, 2009](#); [Wyzisk and Neumeier, 2007](#)), and we wanted to be able to compare the results to previous experiments with the same species ([Bowman and Sutherland, 1969, 1970](#); [Mackintosh and Sutherland, 1963](#); [Sutherland and Bowman, 1969](#)). We selected an aquatic species to test because some researchers have suggested that ecological demands and habitat influence an organism’s information processing abilities, including their capacity for identifying rotated objects (e.g., [Burmman et al., 2005](#); [Delius and Hollard, 1995](#); [Mauck and Dehnhardt, 1997](#)). Aerial species and aquatic species have a horizontal plane of reference whereas a vertical plane of reference may be more important for terrestrial species. This theory predicts that aerial and aquatic species could be more likely to show viewpoint-independent performance, whereas terrestrial species should show a pattern of an increase in errors and reaction time as a function of the angle of rotation. Arboreal species, presumably, lie somewhere in between as their visual information processing system evolved to cope with the demands of their habitat ([Burmman et al., 2005](#)).

In order to assess the effect of object type on performance in the current study, the stimuli included simple geometric shapes in Experiment 1 and more complex naturalistic line drawings of turtles and frogs in Experiments 2 and 3. All previous studies with goldfish subjects that presented 2D objects used geometric shapes and other artificial stimuli such as English letters, but none used more complex stimuli such as line drawings of natural or artificial objects ([Bowman and Sutherland, 1969, 1970](#); [Mackintosh and Sutherland, 1963](#); [Sutherland and Bowman, 1969](#)). The line drawings we selected were of ecologically-relevant species that goldfish would encounter in their natural habitat. However, our goldfish were born and raised in aquaria and had never seen live turtles or frogs (or plastic models). In addition, it is unknown whether the fish would treat black and white line drawings of animals as equivalent to images of real objects or the 3D object themselves. Nonetheless, the complex stimuli did provide a richer set of features to use during discrimination. We predicted that the goldfish in the current study would have higher performance accuracy when discriminating between the complex stimuli compared to the simple stimuli.

In the current study the fish were trained to discriminate between stimuli at 0° (upright), then tested with a wide range of novel aspect angles in 45° increments (+45°, +90°, +135°, 180°, +225°/–135°, +270°/–90°, +315°/–45°). All previous studies on planar-rotated

objects in fish were limited in that they tested only two to four novel aspect angles or a very narrow range of angles (Bowman and Sutherland, 1969, 1970; Mackintosh and Sutherland, 1963; Schluessel et al., 2014; Sutherland and Bowman, 1969). Sometimes only the orthogonal angles ($+/- 90^\circ$, 180°) or only the oblique angles ($+/- 45^\circ$, $+/- 135^\circ$) are tested. Using a broader range of angles gives a more complete view of performance and may permit a better understanding of the stimulus features used by fish when recognizing rotated shapes.

Another variable we manipulated in the current study was the rotation scheme. In a two-alternative forced choice task, there are three potential rotation schemes: (1) rotate both the positive and negative stimulus ('matched rotation scheme'), (2) rotate only the positive stimulus, and (3) rotate only the negative stimulus. Many studies on fish and other non-human animals use only one scheme and tend to select the matched rotation scheme. Only one prior study on the perception of rotated shapes in fish used multiple rotation schemes (Bowman and Sutherland, 1969). All fish in Bowman and Sutherland's (1969) study succeeded in discriminating between letters with the matched rotation scheme, but one-third of the subjects failed when only one stimulus was rotated (the results do not specify whether the fish failed when it was their own positive stimulus or their own negative stimulus that was rotated). To further investigate the effect of rotation scheme on performance, we presented the fish with a matched rotation scheme in Experiments 1 and 2 and single stimulus rotation scheme in Experiment 3.

2. Experiment 1

2.1. Method

2.1.1. Subjects

The subjects were four (2M; 2F) commercially-obtained goldfish (*Carassius auratus*), 9–12 cm in total length. Individual fish were identified by their phenotypic characteristics, including coloration patterns. Fish were assigned a sex based on physical characteristics such as the appearance of their vent and body form. Two fish were obtained in 2011 and were approximately four years old at the start of the study. These fish had been tested previously in a study on shape discrimination (DeLong et al., 2018b) and a study on quantity discrimination (DeLong et al., 2017a). Two fish were obtained in 2014 and were approximately one year old at the start of the study. Subjects were housed in pairs in a 10-gallon tank (50 cm long \times 26 cm wide \times 31 cm high). Each tank contained aquarium gravel and an Aqueon Quiet Flow 10 filter unit that provided aerated and filtered water. Aqueon water conditioner was added to each tank, which neutralizes chlorine and chloramines and detoxifies heavy metals, ammonia, and other elements released from fish waste. The top of each tank contained one Aqueon Colormax 10 W mini-compact fluorescent light bulb that simulated a natural light/dark cycle. The tanks were covered on three sides by blue cellophane on the exterior walls. Water temperature was kept between 22°C–25°C. Water changes (40%) were carried out on a weekly basis and tanks were monitored regularly for pH and waste levels with Tetra Aquarium Test Strips (Tetra U.S., Blacksburg, VA). Training and testing sessions were conducted during daylight hours (morning and/or afternoon). On days with training or test sessions, subjects were food restricted since food was their reward during sessions. On weekdays when the fish were not trained or tested, they were fed twice: once with TetraFin flakes (Tetra GMBH, Melle, Germany) and once with API Premium pellets (Mars Fishcare Inc., Chalfont, PA).

2.1.2. Stimuli

Fig. 1 shows the stimuli used in Experiment 1. One stimulus was a black arrow (3.6 cm \times 2.8 cm) on a white background and one was a black half circle (1.7 cm \times 3.3 cm) on a white background. The fish had never viewed these stimuli prior to the current study (they discriminated between a full circle and a rectangle in a previous study;

DeLong et al., 2018b). Stimuli were created in Microsoft PowerPoint 2013 (Microsoft®, Inc.). The surface area of the black space in the two stimuli was equivalent (4.3 cm²). Only the 0° aspect angle was used during training sessions. All other aspect angles were used during test sessions. There were two identical exemplars for each stimulus pair (e.g., two sets of stimuli for each aspect angle), to prevent the fish from choosing a stimulus based on any minor differences between them (e.g., minute black ink spots in white space). Both sets of exemplars were used throughout training and testing. Stimuli were printed using black ink on standard white paper and then laminated. Each stimulus was centered on a 5 cm \times 5 cm square (the stimulus card) with a 0.1 cm black border. The stimulus cards were attached to a white plastic corrugated stimulus board (25.5 cm high \times 16.5 cm wide \times 0.4 cm thick) with hook and loop fasteners.

2.1.3. Experimental set-up

Fig. 2 shows the experimental set-up. During training and testing sessions, subjects were moved to individual test tanks (3.7 gallons; 36.8 cm long \times 21.8 cm wide \times 24.3 cm high). Test tanks contained about 17 cm of conditioned water and no gravel or plants. Blue cellophane covered all four exterior side walls to block external stimuli during testing. There were two identical test tanks and fish rotated between the two tanks according to a random schedule. During training and testing sessions, a stimulus board was inserted in the front of the tank and rested against the interior tank wall. On one stimulus board, a single stimulus card could be presented that was centered on the board during training stage 1 (see Fig. 2B). On a second stimulus board, two stimulus cards could be presented during training stage 2 or the testing stage (see Fig. 2C).

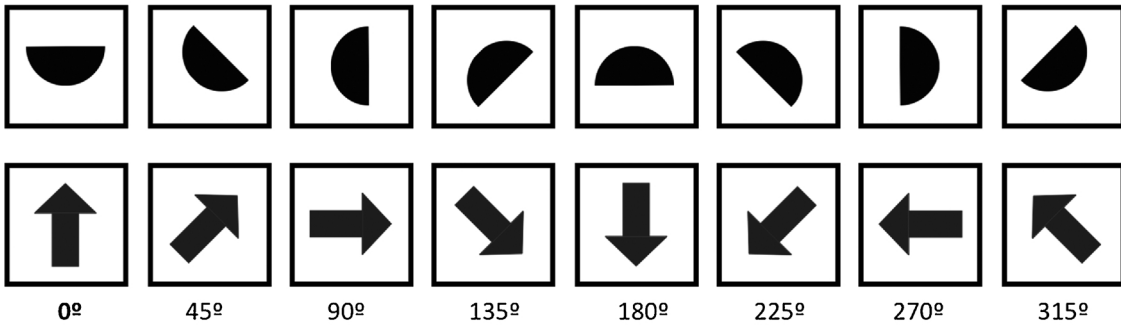
2.1.4. Procedure

Sessions were conducted once or twice per day, always around the same time of day in the morning and afternoon (this resulted in a food deprivation period of about 3–4 h between sessions on the same day). There were typically 5–10 sessions per week. Test tanks were filled with water and water conditioner, then the fish was placed into the test tank using a small net and allowed to acclimate to the tank for a minimum of 15 min. Stimulus cards were placed on the hook and loop fasteners on the stimulus board. Before the stimulus board was placed in the tank, the fish was ushered into the back of the tank by gently tapping on the water in the back of the tank (see Fig. 2A). This ensured the fish was swimming in the back of the tank when the stimulus board was placed in front of the tank at the beginning of a trial and encouraged the fish to examine both stimuli on the board before making a choice.

The fish were reinforced for correct choices with food paste; a mixture of TetraFin flakes and water (similar to Siebeck et al., 2009). Food paste was delivered to the fish using a 1.0 mL Luer-Lok tip syringe (BD, Franklin Lakes, NJ), and 0.01 mL of food paste was delivered for each correct choice. The fish had to tap (bite or push) the rewarded stimulus (S+) once before the food reward was given. The experimenter held the syringe out of sight from the fish behind the test tank wall until a correct choice was made. As soon as the subject tapped once on the rewarded stimulus, the experimenter lowered the syringe into the tank to the top center section of the S+ stimulus card. Once the syringe was positioned near the S+ stimulus card, the fish ate the food paste. If a fish tapped the incorrect stimulus (S-), it was not reinforced and the board was withdrawn. The 'non-correction' method was used where the fish was allowed to choose only one stimulus (S+ or S-) for each trial. The stimulus board was always removed from the test tank during the intertrial interval of about 10–15 s when the experimenter recorded the data for the trial.

The fish's choice (S+ or S-), the location of the choice (e.g., tap to the black area of stimulus or white background of the stimulus card), and the number of taps were recorded on each trial. A single experimenter presented the stimulus board, delivered the food reinforcement, and recorded the data during an experimental session. The

Experiment 1



Experiment 2

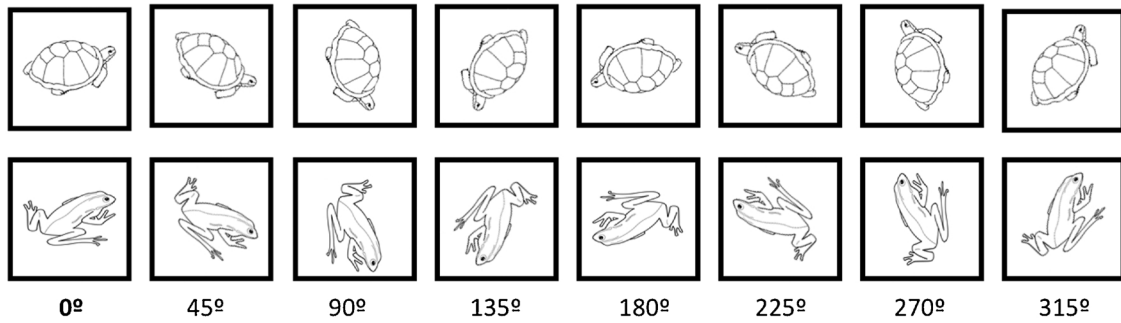


Fig. 1. The experimental stimuli for Experiments 1 and 2. During the training stage, only the 0° aspect angle was shown. During the test sessions, fish viewed all aspect angles. These experiments used a matched rotation scheme in which the S+ and S− were always presented at the same aspect angle within a trial. For example, during a 45° trial, both the S+ and S− were presented at 45°.

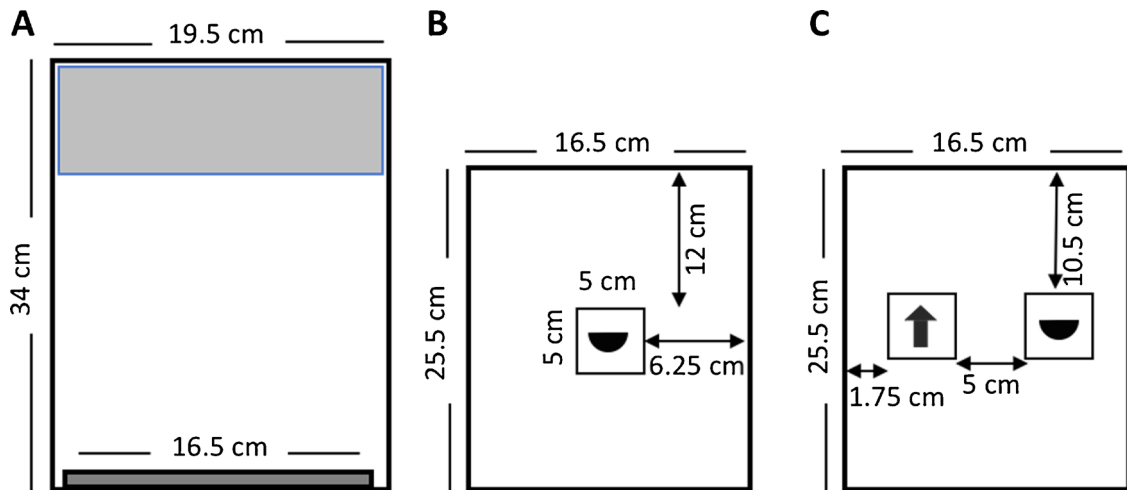


Fig. 2. Schematic diagram of the experimental set up. (A) Test tank showing the position of the stimulus board (dark gray shaded region). The fish had to be located within the light gray shaded region when the stimulus board was placed in the tank at the beginning of a session. (B) The stimulus board side used for the training stage 1 (S+ only). (C) The stimulus board side used for training stage 2 and the test sessions (S+ and S−).

experimenter stood in a position that allowed her to view the fish’s behavior and deliver the food while holding the syringe out of view of the fish. Two experimenters trained and tested the fish throughout the experiment. The total session time was recorded for each session (rounded to the nearest minute), but not individual trial times. Training sessions averaged 4.1 min (*Minimum* = 1 min, *Median* = 4 min, *Maximum* = 10 min). Test sessions averaged 4.9 min (*Minimum* = 2 min, *Median* = 5 min, *Maximum* = 11 min).

2.1.4.1. Training. Two fish were trained to select the half circle and two fish were trained to select the arrow. During the training stages, the fish were only exposed to the stimuli at the 0° aspect angle. The experimentally-naïve fish were pre-trained to acclimate to the test tanks and learn to feed from the syringe. We skipped this stage for two fish that were pre-trained in a previous study (DeLong et al., 2018b). In training stage 1, the fish had to tap its S+ to receive a food reward when only the positive stimulus but not the negative stimulus was presented (see Fig. 3A). There were 18 sessions with 6 trials per session.

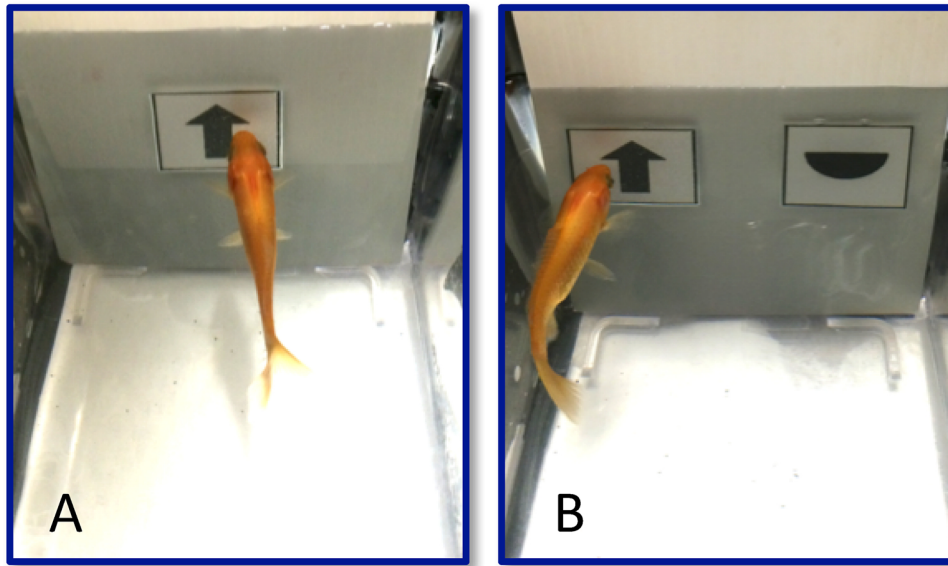


Fig. 3. Photographs of fish engaged in training and testing. (A) A fish tapping the S+ during training stage 1. (B) A fish during training stage 2 approaching its S+. After tapping the S+ the syringe was inserted from above and positioned near the fish's head. If the S- was tapped the board was removed. The set-up was the same for training stage 2 and the test sessions (both S+ and S- presented simultaneously during a session).

Two of the fish (one trained to select the half circle and one trained to select the arrow) readily tapped the S+ during all trials in the final 12 sessions and completed stage 1 in 10 days (1–2 sessions per day). Two of the fish were excluded from the experiment in training stage 1 due to a reluctance to approach and tap the S+ in a timely manner.

In training stage 2, the fish were presented with the S+ and S- in a two-alternative forced choice task (see Fig. 3B). The position of the S+ for each trial was determined using a modified pseudorandom Gellerman series (Gellerman, 1933) in which the S+ was never shown for more than two trials in a row on the same side to prevent potential side biases. The fish were prompted with the correct answer under two conditions: (1) if they did not make a choice within three minutes of the stimulus board being placed into the test tank, or (2) if they made three incorrect choices in a row. A prompt consisted of presenting the syringe in front of the S+ at the beginning of a trial so that the fish was forced to select the S+. Prompted trials were not included in the reported choice accuracy for training trials. The fish completed two blocks of training trials in stage 2 – one prior to test block 1, and one prior to test block 2.

Training stage 2 block 1 began the day after training stage 1 was completed and lasted from August 18, 2015 to February 12, 2016. There were 99 total sessions with 6 trials per session (594 trials). The high number of sessions was due to a side bias the fish developed in which they had difficulty responding correctly when the S+ was presented on the right side (please see results Section 2.2.1). Training stage 2 block 2 began five days after the completion of test block 1 and lasted from March 21, 2016 to April 22, 2016. There were 13 sessions with 6–10 trials per session (110 trials). These training sessions were used to attempt to correct a side bias and included some prompted trials.

2.1.4.2. Testing. Test sessions included both the S+ and S- in a two-alternative forced choice task like training stage 2 (see Fig. 3B). Stimulus pairs for all aspect angles were presented (including 0°). Each test session consisted of six trials. The first two trials in each session contained the 0° aspect (training aspect), with the S+ presented once on the right and once on the left. The last four trials in a session contained four of the novel aspects (45°, 90°, 135°, 180°, 225°, 270°, or 315°). As in training stage 2, the position of the S+ for each trial was determined by sequences based on a modified pseudorandom Gellerman series (Gellerman, 1933). The sequences contained no more than two appearances in a row of the S+ on the same side, and the S+ was on each side equally often within a test session (three times on the right, three times on the left). The stimulus pairs selected for

each test session were determined randomly, with the constraint that no stimulus pair was allowed to appear more than twice per session (thus there were five or six different stimulus pairs per session). The fish were rewarded for correct responses on trials containing the 0° aspect (training aspect) as well as trials containing novel aspects. This was done to provide fish with an opportunity to learn to discriminate between the stimuli when rotated to novel angles if they initially performed poorly on the task.

There were two blocks of test sessions, with each block containing 21 sessions. Each stimulus pair appeared a total of 12 times in each test block (except the 0° aspect, which appeared 42 times in each test block). Test block 1 began three days after the completion of training block 1 and lasted from February 15, 2016 to March 16, 2016. Five days after the completion of test block 1, the fish completed 13 additional sessions in training block 2 (see Section 2.1.4.1). Test block 2 began three days after training block 2 was completed and lasted from April 25, 2016 to May 25, 2016.

2.1.5. Data analyses

Statistical analyses were performed using R v3.5.0 (R Core Team, 2018). An experimental type I error level of $\alpha = 0.05$ was used. All p -values were adjusted using Holm's (1979) method to control the familywise error level at $\alpha = 0.05$ except, in the case of post-hoc comparisons, where p -values were adjusted using Tukey's WSD (1949) method for pairwise comparisons. Logistic regression modeling was used to perform inference on the impact of measured variables on discriminative accuracy as measured by a choice to the S+ (correct choice) or to the S- (incorrect choice). Model goodness-of-fit was assessed using likelihood ratio tests, inspection of residual plots, and diagnostics of leverage and Cook's distance. Tests of overall accuracy against chance (50%) performance were performed using the predicted marginal probability of a correct choice to the S+ from the logistic regression model controlling for covariates and experimental factors. Multilevel logistic regression models were considered due to the nested structure of the data, but the intraclass correlations for the session and fish levels were approximately equal to zero, so single-level logistic regression models are reported for clarity and yielded identical results.

2.2. Results

2.2.1. Performance accuracy in the training sessions

We analyzed data from training stage 2 for the two fish that completed training. In this stage, these fish both completed 99 sessions

where each session consisted of six trials for a total of 594 trials per fish and 1188 trials in total. Forty-five trials were excluded from this analysis since the fish were prompted on those trials, so we used the remaining 1143 of the original 1188 trials in the following analyses. The overall accuracy after controlling for session number was significantly better than chance (i.e., 50%), $Accuracy = 60.0\%$, $SE = 1.4\%$, $Z = 7.01$, $p < .0001$, $95\% CI [57.3\%, 62.7\%]$. The logistic regression model fit well based on a likelihood ratio test of model goodness-of-fit, .

A sequential analysis of deviance using likelihood ratio tests was performed to test the statistical significance of main effects of session number, experimenter, fish, S+ position, exemplar, and interactions between (1) session and S+ position and (2) fish and S+ position. Adjusting all p -values using Holm's (1979) method, there were no statistically significant effects of session, $\chi^2(1) = 4.97$, $p = .0772$, fish, $\chi^2(1) = 0.03$, $p = 1.0000$, and exemplar, $\chi^2(1) = 0.10$, $p = 1.0000$. However, there were significant differences in performance between the two experimenters, $\chi^2(1) = 41.45$, $p < .0001$. Performance was statistically significantly better for experimenter 2 ($Accuracy = 69.5\%$, $95\% CI [65.8\%, 73.2\%]$) than experimenter 1 ($Accuracy = 50.5\%$, $95\% CI [46.5\%, 54.4\%]$).

There was a statistically significant main effect of S+ position, $\chi^2(1) = 80.69$, $p < .0001$, which suggested that the fish exhibited a side bias during training. There was also a statistically significant interaction between fish and S+ position, $\chi^2(1) = 13.45$, $p = .0010$. Neither Fish 1 ($Accuracy = 52.7\%$, $95\% CI [45.5\%, 60.0\%]$) nor Fish 2 ($Accuracy = 41.7\%$, $95\% CI [34.5\%, 49.0\%]$) were significantly better than chance on average across all sessions when the S+ was presented on the right. However, both Fish 1 ($Accuracy = 68.1\%$, $95\% CI [61.4\%, 74.8\%]$) and Fish 2 ($Accuracy = 77.4\%$, $95\% CI [71.3\%, 83.4\%]$) were statistically significantly better than chance on average across all sessions when the S+ was presented on the left. Finally, averaged across all sessions, the performance of Fish 2 when the S+ was on the right was statistically significantly worse than the performance of Fish 1 ($Difference in log-odds = -1.14$, $SE = 0.18$, $Z = -6.32$, $p < .0001$) and Fish 2 ($Difference in log-odds = -1.63$, $SE = 0.19$, $Z = -8.50$, $p < .0001$) when the S+ was on the left.

Finally, there was also a statistically significant interaction between session number and S+ position, $\chi^2(1) = 15.00$, $p = .0005$. There was no statistically significant change in performance over sessions when the S+ was presented on the left, $Percent Change in Odds per Session = -0.41\%$, $95\% CI [-1.08\%, 0.25\%]$. Overall accuracy was significantly better than chance when the S+ was on the left at the beginning of training (i.e., session 5; $Accuracy = 76.1\%$, $95\% CI [69.6\%, 82.7\%]$). By the end of training, performance did not change significantly compared to the beginning when the S+ was on the left (i.e., session 94; $Accuracy = 69.3\%$, $95\% CI [62.4\%, 76.2\%]$). This suggested that the fish performed well when the S+ was presented on the left and that this behavior did not change during training. However, there was a statistically significant improvement in accuracy over sessions when the S+ was presented on the right, $Percent Change in Odds per Session = 1.39\%$, $95\% CI [0.78\%, 2.00\%]$. When the S+ was on the right, overall accuracy was significantly lower than chance at the beginning of training (i.e., session 5; $Accuracy = 33.0\%$, $95\% CI [26.0\%, 39.9\%]$) but ended significantly better than chance (i.e., session 94; $Accuracy = 61.0\%$, $95\% CI [54.0\%, 68.0\%]$). To summarize, there was evidence of a side bias at the beginning of training, but there was no evidence of a side bias by the end of training (see Fig. 4).

2.2.2. Performance accuracy in the test sessions

We analyzed testing data for the two fish that completed training. In the testing stage, both fish completed 42 sessions (21 sessions in each of two test blocks) where each session consisted of six trials for a total of 252 trials per fish and 504 trials in total. Each fish performed 84 trials each at the 0° orientation and 24 trials each at each of the seven novel

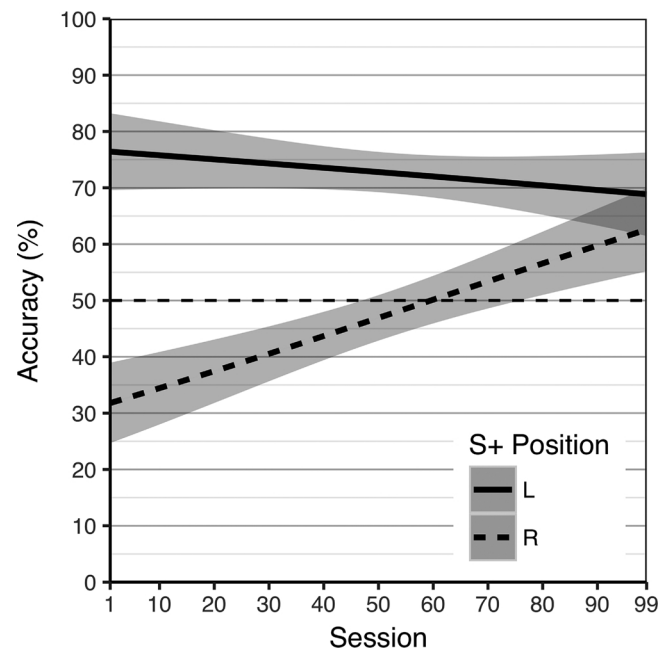


Fig. 4. The performance accuracy of the fish in the training stage of Experiment 1. A significant interaction between session number and S+ position is shown (results shown for the two fish that completed the training phase). Accuracies are shown along with 95% simultaneous confidence bands.

orientations. The overall accuracy after controlling for session number was significantly better than chance (i.e., 50%), $Accuracy = 66.9\%$, $SE = 2.1\%$, $Z = 7.04$, $p < .0001$, $95\% CI [62.8\%, 71.0\%]$. The logistic regression model fit well as suggested by a likelihood ratio test of model goodness-of-fit, $\chi^2(14) = 90.68$, $p < .0001$.

A sequential analysis of deviance using likelihood ratio tests was performed to test the statistical significance of main effects of session number, test block, experimenter, fish, S+ position, exemplar, and orientation as well as the significance of an interaction between session number and stimulus pair. Adjusting all p -values using Holm's (1979) method, there were no statistically significant effects of test block, $\chi^2(1) = 3.20$, $p = .0732$, session, $\chi^2(1) = 0.16$, $p = 1.0000$, experimenter, $\chi^2(1) = 2.13$, $p = .1458$, fish, $\chi^2(1) = 5.61$, $p = .0173$, or exemplar, $\chi^2(1) = 0.14$, $p = 1.0000$, at an overall $\alpha = 0.05$. Surprisingly, there was no statistically significant main effect of orientation, $\chi^2(7) = 7.93$, $p = 1.0000$. As shown in Fig. 5, performance was significantly better than chance for 0° ($Accuracy = 63.0\%$, $95\% CI [53.7\%, 72.3\%]$), as well as four novel aspect angles: 45° ($Accuracy = 70.8\%$, $95\% CI [54.4\%, 87.2\%]$), 90° ($Accuracy = 76.4\%$, $95\% CI [60.8\%, 92.0\%]$), 180° ($Accuracy = 67.6\%$, $95\% CI [50.3\%, 84.8\%]$), 270° ($Accuracy = 72.9\%$, $95\% CI [56.8\%, 89.0\%]$). Performance was not different from chance on three novel aspect angles: 135° ($Accuracy = 56.8\%$, $95\% CI [38.8\%, 74.8\%]$), 225° ($Accuracy = 61.8\%$, $95\% CI [44.3\%, 79.4\%]$), and 315° ($Accuracy = 65.7\%$, $95\% CI [48.6\%, 82.8\%]$). The performance of each fish at each orientation is shown in Table 1. Finally, we tested for linear, quadratic, and cubic trends in performance as a function of orientation from 0° to 315° using orthogonal polynomials with a Scheffé adjustment for multiple comparisons, but none of these trends were statistically significant, all $p = 1$.

Accuracy was higher overall when the S+ was on the left side ($Accuracy = 82.3\%$, $95\% CI [77.5\%, 87.1\%]$) than the right side ($Accuracy = 51.4\%$, $95\% CI [45.2\%, 57.6\%]$), $\chi^2(1) = 58.18$, $p < .0001$. The interaction between the fish and S+ position was statistically significant, $\chi^2(1) = 13.34$, $p = .0018$. The interaction between fish and S+ position is shown in Table 2. Performance was statistically significantly better than chance for both fish when the

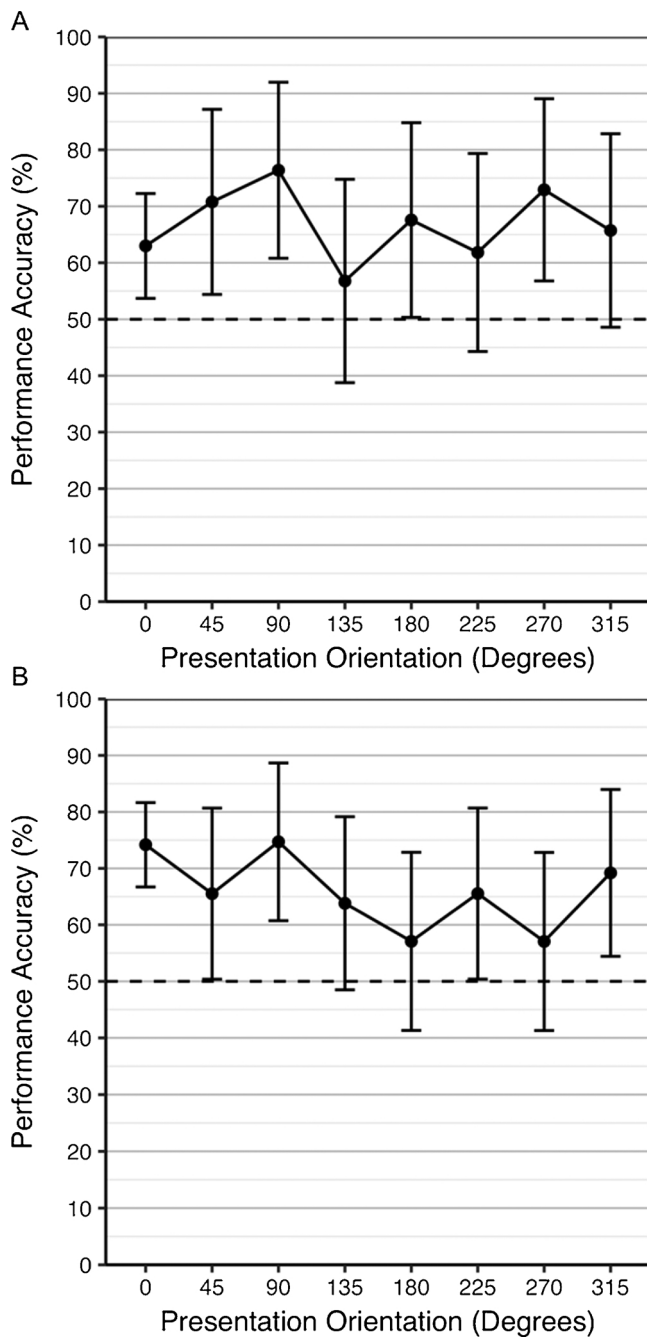


Fig. 5. Performance accuracy in the test sessions of (A) Experiment 1 and (B) Experiment 2 for each stimulus orientation. Two fish completed the test phase for Experiment 1 and three fish completed the test phase for Experiment 2. Accuracies are shown along with 95% simultaneous confidence intervals.

S+ was on the left side and for Fish 1 when the S+ was on the right side. However, performance was not significantly different from chance for Fish 2 when the S+ was on the right side (Accuracy = 38.4%, 95% CI [27.4%, 49.3%]). Overall, performance was not significantly different for the two fish and two S+ positions except for Fish 2's performance when the S+ was on right side which was significantly worse than the other three cases, *difference in log-odds for Fish 2 with S+ on right vs. Fish 2 with S+ on left* = -2.32, SE = 0.32, Z = -7.33, *p* < .0001; *difference in log-odds for Fish 2 with S+ on right vs. Fish 1 with S+ on left* = -1.88, SE = 0.29, Z = -6.47, *p* < .0001; *difference in log-odds for Fish 2 with S+ on right vs. Fish 1 with S+ on right* = -1.11, SE = 0.27, Z = -4.16, *p* = .0002. Fish 1's performance when

Table 1
Post-Hoc Contrasts for the S+ Orientation for Each Fish in the Experiment 1 Test Sessions.

Fish	Orientation (°)	Accuracy (%)	95% CI (%)
1	0	67.9	(59.7, 76.1)
	45	76.0	(63.6, 88.4)
	90	81.5	(70.4, 92.6)
	135	61.1	(46.1, 76.0)
	180	72.8	(59.4, 86.2)
	225	66.6	(52.4, 80.9)
	270	78.2	(66.2, 90.1)
	315	70.8	(57.3, 84.3)
2	0	58.1	(50.8, 65.4)
	45	65.5	(53.3, 77.7)
	90	71.2	(59.0, 83.5)
	135	52.4	(40.2, 64.7)
	180	62.4	(49.9, 74.9)
	225	57.0	(44.8, 69.2)
	270	67.7	(55.4, 79.9)
	315	60.6	(48.4, 72.9)

Note. Marginal discriminative accuracy is shown with corresponding 95% confidence intervals.

Table 2
Post-Hoc Contrasts for the Interaction Between Fish and S+ Location for the Experiment 1 Test Sessions.

Fish	S+ Location	Accuracy (%)	95% CI (%)	Group
1	Left	79.3	(70.3, 88.2)	C
	Right	64.5	(53.8, 75.2)	B
2	Left	85.4	(77.6, 93.2)	C
	Right	38.4	(27.4, 49.3)	A

Note. Discriminative accuracy is given with corresponding Tukey-adjusted 95% confidence intervals. Conditions where performance was not statistically significantly different share a group. Conditions where performance was statistically significantly different have different groups.

the S+ was on the right was statistically significantly lower than the performance of both fish when the S+ was on the left, *difference in log-odds for Fish 1 with S+ on right vs. Fish 1 with S+ on left* = -0.77, SE = 0.29, Z = -2.66, *p* = .0359; *difference in log-odds for Fish 1 with S+ on right vs. Fish 2 with S+ on left* = -1.20, SE = 0.31, Z = -3.83, *p* = .0007.

3. Experiment 2

3.1. Method

3.1.1. Subjects

The subjects were four (2M; 2F) commercially-obtained goldfish (*Carassius auratus*), 9–14.5 cm in total length. Two of these subjects were different fish than those tested in Experiment 1. Three fish were obtained in 2011 and were approximately five years old at the start of the study. These subjects had been tested previously in a study on quantity discrimination (DeLong et al., 2017a). One fish was obtained in 2014 and was approximately two years old at the start of the study. These subjects were housed in aquaria with the same equipment and fed the same food as the fish in Experiment 1.

3.1.2. Stimuli

Fig. 1 shows the stimuli used in Experiment 2. The stimuli were black line drawings of a turtle and a frog (both 4.3 cm × 2.5 cm) on a white background. The fish had never viewed these stimuli prior to the current study. The surface area of the frog and the turtle were approximately equivalent. Surface area was calculated using Sketch-AndCalc™ (Version 4.7, Icalc Inc.). Each stimulus was centered on a 5.5 cm x 5.5 cm square (the stimulus card) with a 0.1 cm black border.

As in Experiment 1, only the 0° aspect angle was used during training sessions and all aspect angles were used during test sessions. All other aspects of stimulus presentation were the same as in Experiment 1.

3.1.3. Experimental set-up

The experimental set-up was the same as in Experiment 1, except there were six identical test tanks and fish rotated among the six tanks according to a random schedule.

3.1.4. Procedure

All aspects of the procedure were the same as in Experiment 1, except that we recorded individual trial times in test block 2. We continued to record the fish's choice (S+ or S-), the location of the choice (e.g., tap to the black area of stimulus or white background of the stimulus card), the number of taps, and the total session time (rounded to the nearest minutes). Training sessions averaged 4.7 min (*Minimum* = 2 min, *Median* = 4 min, *Maximum* = 26 min). Test sessions averaged 4.9 min (*Minimum* = 0 min, *Median* = 4.0 min, *Maximum* = 14 min). A single experimenter presented the stimulus board, delivered the food reinforcement, and recorded the data during an experimental session. When individual trial times were recorded, a second experimenter used a stop watch (ProCoach RS-013) and reported the time to the first experimenter after each trial. Trial time measured the time elapsed from when the stimulus board entered the test tank to the time when a fish tapped one of the stimuli (given in centiseconds). Trial times were recorded for 335 out of 378 trials (89%) in test block 2. Trial times were not recorded for 43 trials; 42 of the untimed trials were from sessions with Fish 5 and one untimed trial was from a session with Fish 3.

3.1.4.1. Training. Two fish were trained to select the turtle and two fish were trained to select the frog. During the training stages, the fish were only exposed to the stimuli at the 0° aspect angle. Although three fish had previously been trained to feed from the syringe, we conducted 10 pre-training sessions for two of those experienced fish since many months had elapsed since they last fed from the syringe in the test tank (one fish that was experimentally-naïve was also pre-trained). In pre-training sessions, the fish simply had to approach the syringe five times and take food from it while in the test tank.

Training stages 1 and 2 were conducted in the same way as in Experiment 1. There were 11–19 sessions per fish in training stage 1 (across 6–10 days). The fish completed two blocks of training trials in stage 2 – one prior to test block 1, and one prior to test block 2. Training block 1 began the day after training stage 1 was completed and lasted from August 9, 2016 to October 18, 2016. There were 42–58 sessions for each fish. Training block 2 began seven weeks after the completion of test block 1 and lasted from January 4, 2017 to February 7, 2017. There were 25 sessions for each fish. Fish 2 was excluded from the experiment due to a pervasive side bias during training stage 2, block 1.

3.1.4.2. Testing. Test sessions were conducted in the same manner as in Experiment 1. Again, there were two blocks of test sessions, with each block containing 21 sessions. Each stimulus pair appeared a total of 12 times in each test block (except the 0° aspect, which appeared 42 times in each test block). Test block 1 began the day after the completion of training block 1 and lasted from October 19, 2016 to November 16, 2016. Test block 2 began the day after training block 2 was completed and lasted from February 8, 2017 to March 31, 2017.

3.1.5. Data analyses

The data analyses were the same as in Experiment 1.

3.2. Results

3.2.1. Performance accuracy in the training sessions

We analyzed data from training stage 2 for the three fish that

completed training. In this stage, the three fish completed 42, 50, and 58 sessions where each session typically consisted of six trials for a total of 250, 299, and 348 trials per fish, respectively, and 897 trials in total. The overall accuracy after controlling for session number was significantly better than chance (i.e., 50%), *Accuracy* = 64.2%, *SE* = 1.6%, *Z* = 8.28, *p* < .0001, *95% CI* [61.0%, 67.4%]. A logistic regression model was used to model performance, but was not significantly better than a null model based on a likelihood ratio test of model goodness-of-fit, $\chi^2(7) = 9.72$, *p* = .2052.

A sequential analysis of deviance using likelihood ratio tests was performed to test the statistical significance of effects of session number, fish, S+ position, and exemplar. Adjusting all *p*-values using Holm's (1979) method, there were no statistically significant effects of session number, $\chi^2(1) = 1.52$, *p* = .6534, fish, $\chi^2(2) = 4.38$, *p* = .5208, exemplar, $\chi^2(1) = 2.64$, *p* = .5208, and S+ position, $\chi^2(1) = 0.30$, *p* = 1.0000, at an overall $\alpha = 0.05$. There was no significant interaction between fish and S+ location, $\chi^2(2) = 0.86$, *p* = 1.0000. Fish 3 (*Accuracy* = 59.5%, *95% CI* [52.7%, 66.3%]), Fish 4 (*Accuracy* = 67.6%, *95% CI* [60.5%, 74.8%]), and Fish 5 (*Accuracy* = 65.5%, *95% CI* [59.3%, 71.6%]) each performed statistically significantly better than chance.

Overall accuracy at the end of training block 1 in Experiment 2 (67.6% at session 53) was not statistically significantly different than the overall accuracy at the end of training block 1 in Experiment 1 (65.1% at session 94), *Z* = 0.40, *p* = .6859, *95% CI for difference in log-odds*: [-0.30, 0.45]. It took the fish in Experiment 1 nearly twice the number of training sessions as the fish in Experiment 2 to achieve the same level of accuracy at the end of training. Fig. 6 shows accuracy as function of session number.

3.2.2. Performance accuracy in the test sessions

We analyzed testing data for the three fish that completed training. In the testing stage, all three fish each completed 42 sessions (21 sessions in each of two test blocks) where each session consisted of six trials for a total of 252 trials per fish and 756 trials in total. Each fish performed 84 trials each at the 0° orientation and 24 trials each at each of the seven novel orientations. Between the three fish, 252 trials were conducted at the 0° orientation and 72 trials were conducted at each of the seven novel orientations. The overall accuracy after controlling for session number was significantly better than chance (i.e., 50%), *Accuracy* = 65.9%, *SE* = 1.9%, *95% CI* [62.2%, 69.5%], *Z* = 8.00, *p* < .0001. The log-odds for the test sessions in Experiment 2 after controlling for session number was compared to the log-odds for the test sessions in Experiment 1 after controlling for session number. Overall accuracy in Experiment 2 (65.9%) was not statistically significantly different than overall accuracy in Experiment 1 (66.9%), *difference in log-odds* = -0.150, *95% CI* [-0.436, 0.136], *Z* = -1.03, *p* = .3043. The logistic regression model fit well based on a likelihood ratio test of model goodness-of-fit, $\chi^2(13) = 24.7$, *p* = .0252, and residual diagnostics.

A sequential analysis of deviance using likelihood ratio tests was performed to test the statistical significance of main effects of session number, test block, fish, S+ position, exemplar, and orientation at an overall $\alpha = 0.05$. Adjusting all *p*-values using Holm's (1979) method, there were no statistically significant main effects of session, $\chi^2(1) = 0.91$, *p* = 1.0000, test block, $\chi^2(1) = .05$, *p* = 1.0000, fish, $\chi^2(2) = 8.23$, *p* = .1141, or exemplar, $\chi^2(1) = 0.34$, *p* = 1.0000. Unlike Experiment 1, there was no statistically significant effect of S+ position, $\chi^2(1) = 0.34$, *p* = 1.0000, and no statistically significant interaction between fish and S+ position, $\chi^2(2) = 3.80$, *p* = .7471. None of the fish exhibited a side bias in this experiment.

Replicating the results of Experiment 1, there was no statistically significant main effect of orientation, $\chi^2(7) = 14.83$, *p* = .2297. As shown in Fig. 5, performance was significantly better than chance at 0° (*Accuracy* = 74.2%, *95% CI* [66.7%, 81.6%]), 45° (*Accuracy* = 65.5%, *95% CI* [50.4%, 80.7%]), 90° (*Accuracy* = 74.7%, *95% CI* [60.7%,

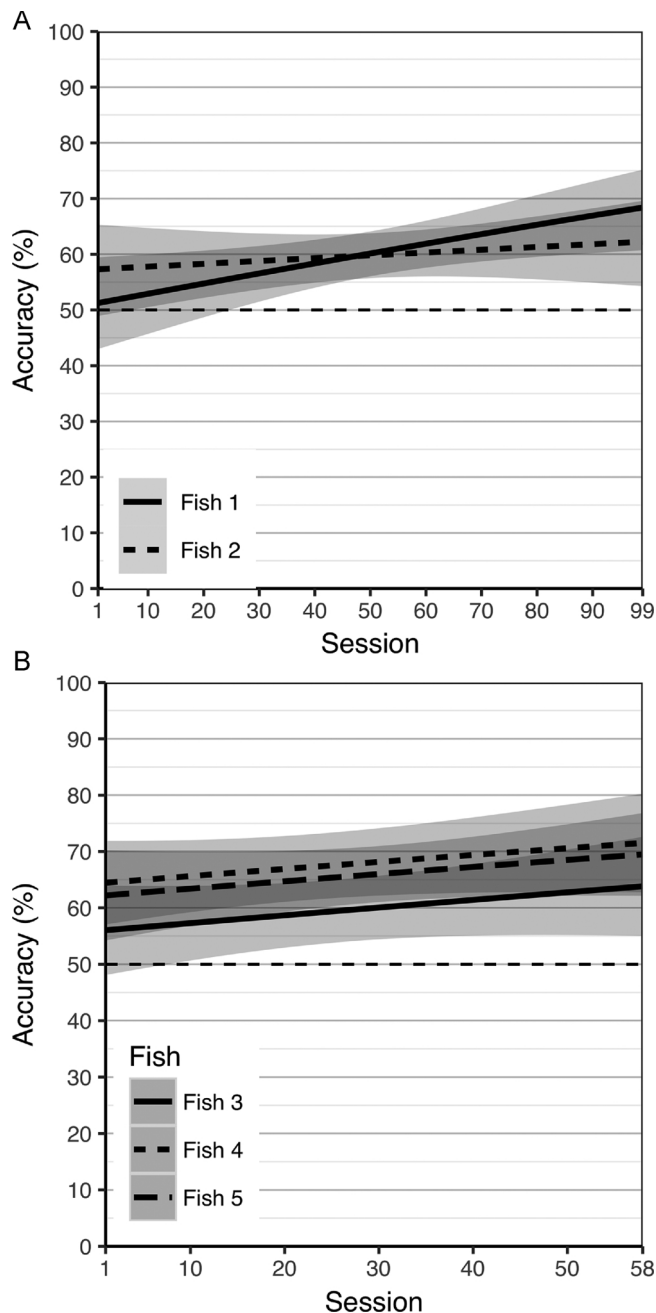


Fig. 6. Performance accuracy in the training sessions (block 1) of (A) Experiment 1 and (B) Experiment 2 shown for each individual fish that completed the training phase in each experiment.

88.7%]), 225° (Accuracy = 65.5%, 95% CI [50.4%, 80.7%]) and 315° (Accuracy = 69.2%, 95% CI [54.4%, 84.0%]). Performance was not significantly different from chance at 135° (Accuracy = 63.8%, 95% CI [48.5%, 79.1%]), 180° (Accuracy = 57.1%, 95% CI [41.3%, 72.8%]), and 270° (Accuracy = 57.1%, 95% CI [41.3%, 72.8%]). The performance of each fish at each orientation is shown in Table 3. Finally, we tested for linear, quadratic, and cubic trends in performance as a function of orientation from 0° to 315° using orthogonal polynomials with a Scheffé adjustment for multiple comparisons, but none of these trends were statistically significant, all $p > .45$.

3.2.3. Individual trial times

Trial times were recorded for 335 out of 378 trials (89%) in test block 2. Trial times were recorded for three fish (see Section 3.1.4 for

Table 3
Post-Hoc Contrasts for the S+ Orientation for Each Fish in the Experiment 2 Test Sessions.

Fish	Orientation (°)	Accuracy (%)	95% CI (%)
3	0	66.7	(52.4, 80.9)
	45	74.8	(50.2, 99.4)
	90	83.6	(62.8, 100.0)
	135	58.4	(30.6, 86.3)
	180	49.8	(21.6, 78.1)
	225	49.7	(21.4, 78.0)
	270	8.3	(0.0, 23.8)
	315	70.8	(40.4, 93.6)
4	0	88.1	(78.3, 97.9)
	45	74.8	(50.2, 99.4)
	90	67.1	(40.6, 93.7)
	135	66.8	(40.2, 93.4)
	180	58.2	(30.3, 86.1)
	225	66.4	(39.6, 93.2)
	270	58.2	(30.3, 86.1)
	315	83.5	(62.7, 100.0)
5	0	64.3	(49.8, 78.8)
	45	83.2	(61.9, 100.0)
	90	83.6	(62.8, 100.0)
	135	75.1	(50.6, 99.5)
	180	74.9	(50.4, 99.5)
	225	66.4	(39.6, 93.2)
	270	66.6	(39.8, 93.2)
	315	67.0	(40.4, 93.6)

Note. Marginal discriminative accuracy is shown with corresponding 95% confidence intervals.

the number of unrecorded trial times for each fish). Trial times were positively skewed, so the median and interquartile range are emphasized over the mean and standard deviation. The median trial time was 7.22 s and the interquartile range was 5.03 s (Minimum = 2.05 s, Mean = 8.74 s, SD = 5.88 s, Maximum = 49.65 s). As shown in Fig. 7, trial times were similar for all orientations of the stimuli with the exception of a few unusually long trial times.

Trial times were modeled using a Gamma regression model with a log link function. The model fit well based on a chi-square goodness-of-fit test, $\chi^2(319) = 281.26, p = .9371$. Differences between the three fish, between S+ locations (left vs. right), and between the eight S+ orientations were tested using main effects. Adjusting p -values using Holm's (1979) method, there were no main effects of fish, $\chi^2(2) = 4.28, p = .3528$, S+ location, $\chi^2(1) = 0.77, p = .7619$, or S+ orientation, $\chi^2(7) = 6.48, p = .7619$. These results suggest that trial times did not differ between orientations where performance was above chance (0°, 45°, 90°, 225°, 315°) and orientations where performance was at chance (135°, 180°, 270°). This suggests that the time taken by the fish to complete the discrimination task was not related to their ability to successfully discriminate between the stimuli. Furthermore, the trial times for each fish were very similar: the median trial time for Fish 5 (7.76 s, IQR = 4.32 s) was the longest, followed by Fish 3 (Median = 7.59 s, IQR = 5.27 s), while Fish 4 had the shortest median trial time (6.34 s, IQR = 6.13 s). Outliers were defined as trial times that exceeded the sum of the third quartile of trial times and three times the interquartile range (i.e., 25.35 s); there were six trials where the trial times of Fish 3 exceeded this threshold and one trial where the trial time of Fish 5 exceeded this threshold. It is not known whether these trial times in excess of nearly 30 s were indicative of unusual behavior by the fish or due to timing errors, so they were not included in the model of trial times.

4. Experiment 3

4.1. Method

4.1.1. Subjects

The subjects were four (3 M; 1 F) commercially-obtained goldfish

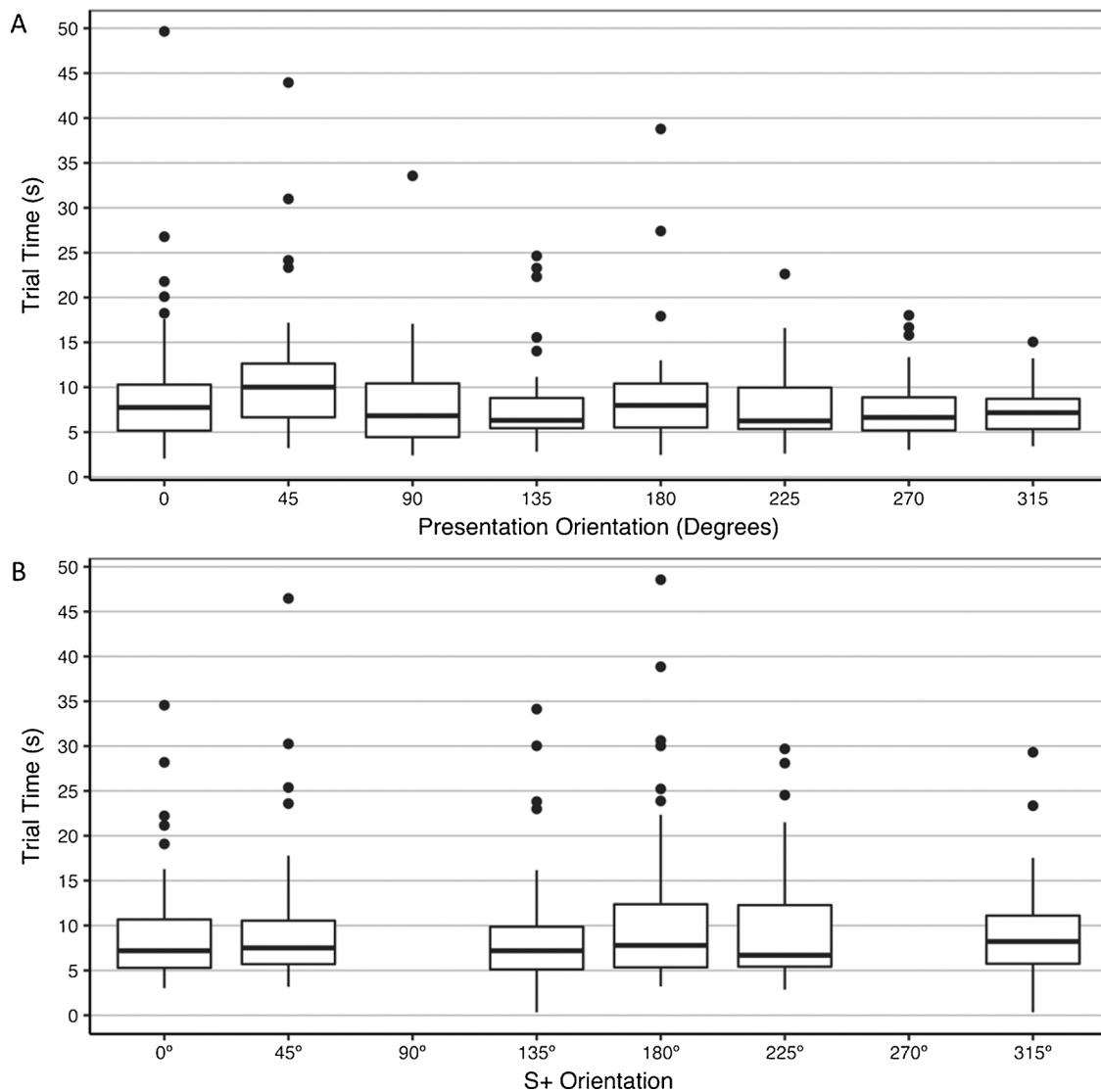


Fig. 7. Trial times in (A) Experiment 2 test block 2 and (B) all Experiment 3 test sessions. Trial times were recorded for 335 of 378 trials in Experiment 2 block 2 (89%) and 380 of 462 trials in Experiment 3 (82%). Boxplots show the median, 1st and 3rd quartiles, and the upper and lower ends of 1.5 times the interquartile range (IQR). Trial times more extreme than the 1st quartile minus 1.5 times the IQR or more extreme than the 3rd quartile plus 1.5 times the IQR are plotted individually.

(*Carassius auratus*), 12–14 cm in total length. Three subjects were tested in Experiment 2, and one was from Experiment 1. Two subjects were obtained in 2011 and were approximately six years old at the start of the study. Two subjects were obtained in 2014 and were approximately three years old at the start of the study. These subjects were housed in aquaria with the same equipment and fed the same food as in Experiments 1 and 2.

4.1.2. Stimuli

Fig. 8 shows the stimuli used in Experiment 3. Six of the stimulus pairs from Experiment 2 were used: 0°, 45°, 135°, 180°, 225°, and 315°. As in Experiments 1 and 2, only the 0° aspect angle was used during training sessions and all aspect angles were used during test sessions. The rotation scheme was different than the one used in Experiments 1 and 2. In both previous experiments, the S+ and S− were always shown at the same aspect angle (i.e., both S+ and S− would be at presented at 180° on a 180° trial). We called this the ‘matched rotation scheme.’ In Experiment 3, the S− was always shown at 0°, whereas the S+ was shown at all angles (i.e., the S− would be shown at 0° and the S+ at 180° on a 180° trial). We called this the ‘only S+ rotated scheme.’

4.1.3. Experimental set-up

The experimental set-up was the same as in Experiment 2.

4.1.4. Procedure

The procedure was the same as in Experiment 2. We recorded both total session times and individual trial times. Training sessions averaged 5.3 min (*Minimum* = 2.0 min, *Median* = 5.0 min, *Maximum* = 11.0 min). Testing sessions averaged 5.4 min (*Minimum* = 2 min, *Median* = 5.0 min, *Maximum* = 13 min). Trial times were recorded for 380 of the 462 trials in Experiment 3 (82%). There were 120 trials per fish for three fish; Fish 4 completed 102 trials due to illness. Trial times were not recorded for 82 trials; 25 of the untimed trials were from sessions with Fish 3, 21 untimed trials were from sessions with Fish 4, 18 untimed trials were from sessions with Fish 5, and 18 untimed trials were from sessions with Fish 6.

4.1.4.1. Training. Two fish were trained to select the turtle as the S+ and two fish were trained to select the frog as the S+. All training stages were conducted in the same way as in Experiment 1. We conducted pre-training and training stage 1 sessions for one fish only, who had failed to complete Experiment 1 due to slow responses during

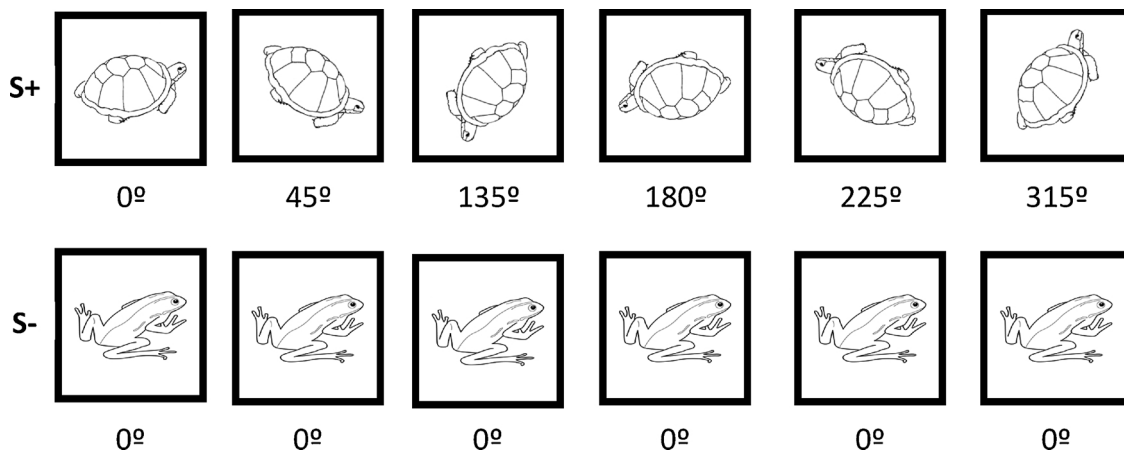


Fig. 8. The experimental stimuli for Experiment 3. This experiment used an ‘only S+ rotated’ scheme in which only the S+ was rotated and the S– was always shown at 0°. For example, on a 45° trial, the S+ was shown at 45° and the S– was shown at 0°. Although the turtle is shown here as the S+, note that the frog was the S+ for half of the subjects (thus the turtle was always at 0°).

training. In training stage 2, both the S+ and S– were at 0°. There was only one block of training trials in training stage 2 prior to testing. For the three fish that participated in Experiment 2, training trials for Experiment 3 began the day after Experiment 2 was completed. Training was conducted from March 10, 2017 to April 12, 2017, with 21–33 sessions per fish. For the other fish, training stage 2 was conducted from February 7, 2017 to April 13, 2017 (46 sessions).

4.1.4.2. Testing. Test sessions were conducted in the same manner as in Experiments 1 and 2, with a couple exceptions. There was only one block of test sessions containing 20 sessions (instead of two blocks of 21 sessions). In Experiment 3, each of the six aspect angles appeared exactly once in each session (20 total presentations of each aspect angle). The order in which the aspect angles appeared within the session was randomized. In contrast, in the two previous experiments a single test block contained 12 presentations of each stimulus pair (except the 0° aspect, which appeared 42 times in each test block and always appeared in the first two trials of each session). Testing began two weeks after the completion of training phase 2 and lasted from April 25, 2017 to June 9, 2017. Fish 4 became ill during testing and only completed 17 of the 20 sessions (his data are included with the results). After completing the testing for the ‘only S+ rotated scheme’, we intended to conduct 20 test sessions for the ‘only S– rotated scheme’. Unfortunately, the remaining fish showed pervasive side biases and overall poor performance when given 25 additional training sessions, so we did not conduct any further test sessions.

4.1.5. Data analyses

The data analyses were the same as in Experiment 1.

4.2. Results

4.2.1. Performance accuracy in the training sessions

We analyzed data from the training stage 2 for all four fish. In this stage, the four fish completed 8, 21, 22, and 41 sessions where each session consisted of six trials for a total of 48, 126, 132, and 246 trials per fish, respectively, and 552 trials in total. The overall accuracy after controlling for session number was significantly better than chance (i.e., 50%), $Accuracy = 64.1\%$, $SE = 2.5\%$, $Z = 5.37$, $p < .0001$, $95\% CI [59.2\%, 69.0\%]$. A logistic regression model was used to model performance, but was not significantly better than a null model based on a likelihood ratio test of model goodness-of-fit, $\chi^2(9) = 8.48$, $p = .4869$.

A sequential analysis of deviance using likelihood ratio tests was performed to test the statistical significance of effects of session

number, fish, S+ position, and exemplar. Adjusting all p -values using Holm’s (1979) method, there were no statistically significant effects of session number, $\chi^2(1) = 0.88$, $p = 1.0000$, fish, $\chi^2(3) = 1.72$, $p = 1$, exemplar, $\chi^2(1) = 0.52$, $p = 1.0000$, and S+ position, $\chi^2(1) = 0.30$, $p = 1.0000$, at an overall $\alpha = 0.05$. There was no significant interaction between fish and S+ location, $\chi^2(3) = 4.04$, $p = 1.0000$. Fish 3 ($Accuracy = 63.5\%$, $95\% CI [52.9\%, 74.2\%]$), Fish 4 ($Accuracy = 69.7\%$, $95\% CI [59.3\%, 80.2\%]$), and Fish 6 ($Accuracy = 63.1\%$, $95\% CI [54.7\%, 71.5\%]$) performed statistically significantly better than chance, but Fish 5 ($Accuracy = 60.3\%$, $95\% CI [41.8\%, 78.8\%]$) did not.

4.2.2. Performance accuracy in the test sessions

In the testing stage of Experiment 3, three of the four fish each completed 20 sessions while the fourth fish completed 17 sessions (due to illness) where each session consisted of six trials for a total of 102, 120, 120, and 120 trials per fish and 462 trials in total. Each fish performed 20 trials at each of the six orientations except for the fourth fish which completed 17 trials at each of the six orientations. For the four fish together, 77 trials were conducted at each of the six orientations. The overall accuracy after controlling for session number was significantly better than chance (i.e., 50%), $Accuracy = 58.1\%$, $SE = 2.6\%$, $95\% CI [53.1\%, 63.2\%]$, $Z = 3.09$, $p = .0020$. The log-odds in the test sessions of Experiment 3 after controlling for session number was compared to the log-odds in the test sessions of the Experiment 2 after controlling for session number. Overall accuracy in Experiment 3 (58.1%) was significantly lower than overall accuracy in Experiment 2 (65.9%), $Z = -2.31$, $p = .0211$, $Difference\ in\ Log-Odds = -0.330$, $95\% CI [-0.610, -0.050]$. A logistic regression model fit based on a likelihood ratio test of model goodness-of-fit, $\chi^2(21) = 43.13$, $p = .0030$.

A sequential analysis of deviance using likelihood ratio tests was performed to test the statistical significance of effects of session number, fish, S+ position, and orientation at an overall $\alpha = 0.05$. Adjusting all p -values using Holm’s (1979) method, there were no statistically significant effects of session number, $\chi^2(1) = 0.14$, $p = .8629$, or fish, $\chi^2(3) = 6.48$, $p = .4527$. Like Experiment 2, there was no statistically significant effect of S+ position, $\chi^2(1) = 2.63$, $p = 0.4527$. Replicating the results of Experiments 1 and 2, there was no statistically significant main effect of orientation, $\chi^2(5) = 6.20$, $p = .8629$. As shown in Fig. 9, performance was only significantly better than chance for the 225° ($Accuracy = 65.7\%$, $95\% CI [52.4\%, 79.1\%]$) aspect angle. Performance was not different than chance for 0° ($Accuracy = 63.1\%$, $95\% CI [49.1\%, 77.1\%]$), 45° ($Accuracy = 63.7\%$, $95\% CI [49.7\%, 77.8\%]$), 135° ($Accuracy = 56.7\%$, $95\% CI [43.0\%, 70.3\%]$), 180°

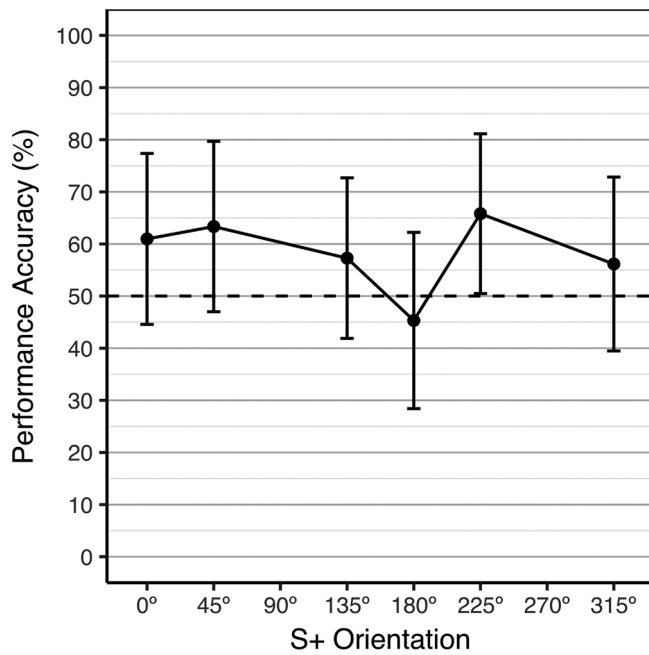


Fig. 9. Performance accuracy in the test sessions of Experiment 3 for each S+ orientation. The S− was always fixed at a 0-degree orientation. Four fish completed the test phase in Experiment 3. Accuracies are shown along with 95% simultaneous Tukey confidence intervals.

Table 4
Post-Hoc Contrasts for the S+ Orientation for Each Fish in the Experiment 3 Test Sessions.

Fish	Orientation (°)	Accuracy (%)	95% CI (%)
3	0	60.0	(38.6, 81.5)
	45	65.0	(44.2, 88.6)
	135	80.0	(62.5, 97.5)
	180	50.0	(28.1, 72.0)
	225	60.0	(38.6, 81.5)
	315	60.0	(60.0, 81.5)
4	0	67.3	(46.8, 87.8)
	45	59.8	(38.1, 81.4)
	135	61.5	(39.4, 83.6)
	180	36.2	(14.7, 57.7)
	225	87.8	(72.4, 100.0)
	315	63.6	(42.0, 85.2)
5	0	70.0	(50.2, 89.7)
	45	65.0	(44.5, 85.6)
	135	55.0	(33.7, 76.4)
	180	55.0	(33.7, 76.4)
	225	65.1	(44.5, 85.6)
	315	65.1	(44.5, 85.6)
6	0	55.1	(33.5, 76.6)
	45	65.0	(44.4, 85.7)
	135	30.0	(10.2, 49.9)
	180	50.0	(28.4, 71.7)
	225	50.0	(28.4, 71.7)
	315	45.0	(23.5, 66.6)

Note. Marginal discriminative accuracy is shown with corresponding 95% confidence intervals.

(Accuracy = 47.8%, 95% CI [33.3%, 62.3%]), and 315° (Accuracy = 58.4%, 95% CI [44.2%, 72.7%]). Performance of each fish at each orientation is shown in Table 4. Finally, we tested for linear, quadratic, and cubic trends in performance as a function of orientation from 0° to 315° using orthogonal polynomials with a Scheffé adjustment for multiple comparisons, but none of these trends were statistically significant, all $p > .90$.

There was a statistically significant interaction between fish and S+ location, $\chi^2(3) = 15.74, p = .0077$. Post-hoc analysis of simple effects

Table 5
Simple Effects for the Interaction Between Fish and S+ Location for the Experiment 3 Test Sessions.

Fish	S+ Location	Accuracy (%)	95% CI (%)
3	Left	63.4	(51.4, 75.3)
	Right	61.7	(49.6, 73.8)
4	Left	47.4	(34.5, 60.2)
	Right	78.0	(67.1, 89.0)
5	Left	53.4	(40.8, 65.9)
	Right	71.7	(60.4, 83.0)
6	Left	56.7	(44.5, 69.0)
	Right	41.7	(29.5, 53.9)

Note. Discriminative accuracy is given with corresponding Tukey-adjusted 95% confidence intervals.

for the interaction between fish and S+ location suggested that Fish 4 and Fish 5 developed side biases: Fish 4 performed statistically significantly better when the S+ was on the right (Accuracy = 78.0%, 95% CI [67.1%, 89.0%]) than on the left (Accuracy = 47.4%, 95% CI [34.5%, 60.2%]). Fish 5 also performed statistically significantly better when the S+ was on the right (Accuracy = 71.7%, 95% CI [60.4%, 83.0%]) than on the left (Accuracy = 53.4%, 95% CI [40.8%, 65.9%]). Fish 3 and Fish 6 did not exhibit significant side biases, as they performed poorly when the S+ was on the right or left. Performance was only significantly better than chance for Fish 4 and Fish 5 when the S+ was on the right side; performance for all other fish-S+ position combinations was not significantly different than chance. The interaction is shown in Table 5.

4.2.3. Trial times in the test sessions

Trial times were recorded for all four fish in 380 of the 462 trials in Experiment 3 (82%, see Section 4.1.4 for the number of unrecorded trial times for each fish). Trial times were positively skewed, so the median and interquartile range are emphasized over the mean and standard deviation. The median trial time was 7.29 s and the interquartile range was 5.67 s (Minimum = 0.34 s, Mean = 9.37 s, SD = 6.47 s, Maximum = 48.56 s).

Trial times were modeled using a Gamma regression model with a log link function. The model fit well based on a chi-square goodness-of-fit test, $\chi^2(361) = 342.50, p = .7504$. Differences between the four fish, between S+ locations (left vs. right), and between the six S+ orientations were tested using main effects. Adjusting p -values using Holm’s (1979) method, there were significant differences among the fish, $\chi^2(3) = 14.76, p = .0061$, but the main effects of S+ location, $\chi^2(1) = 0.21, p = .10000$, and S+ orientation, $\chi^2(5) = 2.36, p = 1.0000$, were not significant. These results suggest that trial times were similar for all orientations of the stimuli and that trial times did not differ between orientations where performance was above chance (225°) and orientations where performance was at chance (0°, 45°, 135°, 180°, 315°) as shown in Fig. 7. This suggests that the time taken by the fish to complete the discrimination task was not related to their ability to successfully discriminate between the stimuli.

Pairwise comparisons of the trial times for each fish found that Fish 4 ($M = 7.4$ s, 95% CI [6.3, 8.5]) was significantly faster than Fish 6 ($M = 10.0$ s, 95% CI [8.7, 11.4]), $Z = -3.71, p = .0012$, but there were no other significant differences among the fish. The average trial time was 8.3 s, 95% CI [7.1, 9.4] for Fish 3 and 8.9 s, 95% CI [7.7, 10.1] for Fish 5. Outliers were defined as trial times that exceeded the sum of the third quartile of all trial times and three times the interquartile range (i.e., 28.1 s); there was one trial where the trial time of Fish 3 exceeded this threshold and seven trials where the trial time of Fish 6 exceeded this threshold. It is not known whether these trial times near or in excess of approximately 30 s were indicative of unusual behavior by the fish or due to timing errors, so they were not included in the model of trial times.

5. Discussion

The purpose of this study was to investigate whether goldfish visually recognize planar-rotated 2D stimuli independent of their viewing angle. The fish were trained to discriminate among the stimuli with the 0° aspect, then tested with seven novel aspect angles (+45°, +90°, +135°, 180°, +225°/−135°, +270°/−90°, +315°/−45°). In the first two experiments, the fish's performance was above chance on four of the seven novel aspect angles. Performance exceeded chance on only one novel aspect angle in the third experiment. Individual trial times did not vary as a function of aspect angle. There was no significant difference in overall test performance between simple and complex stimuli, although the fish required approximately twice as many training trials to achieve the same level of accuracy for the simple stimuli compared to the complex stimuli. Finally, we compared a 'matched rotation scheme' (rotating both S+ and S-) to a single stimulus rotation scheme (rotating only the S+). The fish's performance on the matched rotation scheme was superior.

Studies utilizing planar-rotated stimuli have resulted in at least three different patterns of performance: viewpoint-independent (i.e., rotational invariance), viewpoint-dependent (a mental rotation process is often invoked), or data that does not clearly fit either pattern (Peissig and Goode, 2012). Viewpoint-independent performance has been found in pigeons (Delius and Hollard, 1995; Hollard and Delius, 1982), although Hamm et al. (1997) reported viewpoint-dependent behavior in pigeons. The performance of the goldfish in this study cannot be clearly characterized as viewpoint-independent, as they were not able to generalize to all aspect angles. In Experiments 1 and 2, the goldfish failed to recognize the stimuli at three novel aspect angles: +135° across both experiments and two other angles that varied between the two experiments (180°, +225°/−135°, +270°/−90°, +315°/−45°). However, performance accuracy always exceeded chance at +45° and +90°.

Viewpoint-dependent performance is characterized by a decrease in accuracy or increase in reaction time as the stimuli are rotated further away from 0° (the training aspect) in either direction. When trial time linearly increases as a function of aspect angle in human subjects, it is thought to provide evidence for mental rotation (Shepard and Metzler, 1971). A linear increase in reaction time with angular disparity has also been found in sea lions (Mauk and Dehnhardt, 1997), but not in baboons (Hopkins et al., 1993) or lion-tailed macaques (Burmam et al., 2005). The results of the current study do not match the pattern of a systematic decrease in performance or a systematic increase in trial time as a function of distance from the 0° aspect that would be consistent with the process of mental rotation. We found no evidence for trends in performance as a function of orientation, which would have been easier to interpret. The fish performed well on two angles close to 0° in the clockwise direction (+45° and +90°), but performed poorly on two angles close to 0° in the counterclockwise direction (on −45° in Experiment 1 and −90° in Experiment 2), and trial time was similar across all aspect angles. The performance of the fish is more characteristic of viewpoint-dependence than viewpoint-independence, but doesn't match the classic trend in performance as a function of orientation that would indicate mental rotation. Burmam et al.'s (2005) lion-tailed macaques also showed a pattern of performance that did not clearly fit viewpoint-independence or provide evidence for mental rotation. The macaques did not show a systematic decrement in performance as a function of aspect angle, but their performance was significantly better for nonrotated vs. rotated stimuli.

The goldfish in the current study were not always more accurate or faster at angles closer to 0° compared to more disparate angles. Good performance was found at some angles distant from 0° (180° in Experiment 1 and 225°/−135° in Experiments 2 and 3). These results diverge with other studies showing fish are more likely to perform well only at angles closer to the training aspect. For example, Mackintosh and Sutherland (1963) found that goldfish trained to discriminate

between a horizontal and a vertical rectangle transferred to stimuli rotated up to 30°, but performance dropped with stimuli rotated 40–45°. In another study, sticklebacks and minnows trained to discriminate between 2D triangles and squares successfully recognized those stimuli when rotated by 15° or 20° but couldn't recognize the stimuli when they were rotated at 30°, 45° or 60° degrees (Meesters, 1940 cited in Schluessel et al., 2014).

The results of Experiment 1, in which the goldfish viewed simple geometric stimuli (half circle/ arrow), agree with some past studies on the perception of rotated 2D artificial stimuli in fish. Like Bowman and Sutherland's (1969) goldfish discriminating between 'W' and 'V' shapes, our subjects performed well at 90° and 180°. Our fish were better at +45° compared to +/−135° like Sutherland and Bowman's (1969) goldfish discriminating between a circle and a diamond or square with a knob. Our results disagree with other findings. The goldfish in Sutherland and Bowman's (1969) study and Bowman and Sutherland (1970)'s study could not recognize a square rotated 45°, whereas our fish succeeded in recognizing an arrow or half circle at 45°. Further, Bowman and Sutherland's (1970) fish subjects did not treat rotated irregular squares as equivalent to the training stimuli for rotations of 90° or 180°, whereas our fish succeeded at those aspect angles. The extent to which the current results match prior studies may be a function of the exact shapes used as stimuli and/or the stimulus features utilized by the fish during the task.

The fish in the current study could have attended to local stimulus features or the global stimulus (holistic processing). Sutherland and Bowman (1969) have suggested that fish appear to attend to local features, and selectively attend to the tops or sides of objects and ignore the bottoms. In Experiment 1, the distinctive feature of the half circle could be the orientation of the straight line within the shape being horizontal, vertical, or diagonal, and the key feature of the arrow may be the direction of the arrow head, the orientation of the body of the arrow, or its presence in a certain area of the stimulus card. The fish in the current study performed poorly when the arrow head pointed down (diagonal arrow body) and the line within the half circle was diagonal (+/−135°). When the line within the circle was diagonal, but the arrow pointed up (diagonal arrow), the fish did well at +45° but poorly at −45°. The fish performed well at all orthogonal aspect angles where the line within the circle was horizontal or vertical and the arrow body was also oriented horizontally or vertically (0°, +/−90°, 180°). Thus, the angles of the lines within the stimuli may have served as diagnostic features, with the fish typically performing poorly when both stimuli contained diagonal lines. In Experiments 2 and 3, there are many potential features fish could use: the angle of the line produced by the back of the turtle or frog (straight or diagonal); the location of the head, eye, or legs within the stimulus field; or the general body shape of each animal (elongated vs. rounded). The fish's poor performance on 135°, 180°, and +270°/−90° do not yield any obvious clues as to the local features they may have used. With the naturalistic stimuli (or all stimuli in this study), it is possible that the fish used a combination of features or the holistic shape of the stimuli instead of local features. Recent studies suggest that fish may attend to visual stimuli as a whole, rather than only attending to local features of objects (Agrillo et al., 2013; Newport et al., 2016; Schluessel et al., 2012; Truppa et al., 2010).

Contrary to our prediction, the goldfish did not achieve higher performance accuracy when discriminating complex stimuli (line drawings) compared to simple stimuli (geometric shapes). There was no significant difference in overall test performance in Experiments 1 and 2, and the fish failed to discriminate between stimuli in three of seven novel aspect angles in both experiments. It is noteworthy that there were some differences in performance between Experiments 1 and 2. Peissig et al. (2005) also found differences in performance with simple vs. complex stimuli in pigeons (line drawings vs. shaded images). The fish in the current study showed some signs of difficulty when discriminating the simple stimuli vs. the complex stimuli in two ways. First, the fish required approximately twice as many training trials to

achieve the same level of accuracy for Experiment 1 (simple stimuli) vs. Experiment 2 (complex stimuli). This stands in contrast to the finding that cichlids needed about the same number of training sessions to discriminate 2D geometric symbols as they needed to discriminate 2D line drawings of fish and snails (Schluessel et al., 2012). Second, the goldfish showed evidence of a side bias throughout the training and test sessions in Experiment 1 that was not present in Experiment 2. The savings in training trials and lack of side bias in Experiment 2 cannot be attributed to a practice effect, since the fish subjects who completed Experiment 1 were different individuals than the subjects that completed Experiment 2. Disparities between the experiments may be due to individual differences between the fish subjects. Alternately, the difficulties with Experiment 1 may have been due to the fact that the simple stimuli presented fewer features to use for discrimination than the complex stimuli. The simple stimuli contained only straight, curved, and diagonal lines whereas the complex stimuli contained a variety of lines and shapes conjoined in multiple ways. As previously noted, neither of the sets of stimuli were familiar to our fish subjects. Familiarity and expertise are relevant variables for some animals when discriminating between rotated objects (e.g., Parr, 2011; Parr and Heintz, 2008; Wang and Takeuchi, 2017). A future study could explore whether goldfish show differences in performance on this task with stimuli that vary in familiarity.

In the present study, we compared a ‘matched rotation scheme’ (rotating both S+ and S-) in Experiments 1 and 2 to a single stimulus rotation scheme (rotating only the S+) in Experiment 3. It should be noted that the single stimulus rotation scheme was tested only with the complex stimuli, so our conclusions are limited to one stimulus type. The goldfish performed significantly worse in Experiment 3 compared to the first two experiments. These results agree with Bowman and Sutherland’s (1969) study in which all fish succeeded in discriminating between letters with the matched rotation scheme, but one-third of the subjects failed when only one stimulus was rotated (S+ or S-). We intended to test both single stimulus rotation schemes, but were unable to continue past the ‘only S+ rotated’ phase when the fish showed intractable side biases. Their poor performance on the ‘only S+ rotated’ phase may have been due to methodological differences. In Experiments 1 and 2, the first two trials of a test session always contained the 0° aspect but in Experiment 3, the trial containing the S+ at the 0° aspect could appear in any trial position. In addition, fish completed only 120 test trials in Experiment 3 compared to 252 trials in Experiment 2. Another potential reason for poor performance is that the fish were more familiar with the S- presented at 0°. The fish had much more exposure to the stimuli in the 0° aspect in both the training trials (where both stimuli were always at 0°) and in Experiment 2 where there were more than twice as many trials at 0° than at other aspect angles. The fish may have been selecting the S- at 0° during the test sessions in Experiment 3 because they were more familiar with that stimulus compared to the rotated S+. Perhaps the 0° stimuli gained special status as a result of overexposure.

Yet another potential reason for poor performance in Experiment 3 is that three of the four fish completed both Experiments 2 and 3, and they may have developed strategies suited to the matched rotation scheme in Experiment 2 that didn’t work well in the single stimulus rotation scheme in Experiment 3. The fish failed to discriminate between the 0° stimuli during the test sessions in Experiment 3, even though they succeeded at discriminating between the 0° stimuli during the training phase. Their inability to succeed on other aspect angles could be understood as a failure of discrimination, instead of a failure of generalization to novel aspect angles. Thus, the results from Experiment 3 are inconclusive. Further studies are needed to examine what role rotation scheme plays in the ability of fish to recognize rotated stimuli. Our results were based on comparing the rotation schemes using only the complex stimuli, so this task could be repeated with stimuli with varying levels of complexity. Separate groups of fish could be tested with these different rotation schemes to avoid the potential issues in the

current study that may have arisen from using the same subjects in Experiments 2 and 3.

Test trials with novel aspect angles were rewarded in the test phase of all three experiments to maximize the fishes’ opportunity to learn to discriminate rotated objects. If fish showed poor performance at the onset of the test phase, we wanted to find out if they could improve if given feedback. This learning opportunity (20–24 trials per fish for each novel angle) was insufficient to produce good performance across all aspect angles. It is possible that fish provided with even more rewarded trials for each aspect angle may achieve higher accuracy. There is evidence that fish provided with extensive training can achieve improved performance on a visual perception task (DeLong et al., 2017a). Alternately, no amount of practice may be sufficient to attain performance that exceeds chance at all aspect angles with 2D black and white stimuli.

Side biases were observed in two of three experiments in the present study. In Experiment 1 two fish performed better when the S+ was on the left, and in Experiment 3 two fish performed better when the S+ was on the right. Side biases are present in visual discrimination experiments with other fish species (e.g., Colwill et al., 2005; Graeber and Ebbesson, 1972; Schluessel and Bleckmann, 2005) and with goldfish (DeLong et al., 2017b). In a study of rotated 3D objects in cichlids, eight out of nine fish showed side biases at least once (Schluessel et al., 2014). Other studies with cichlids also show side biases even when overall performance is above chance level (Gierszewski et al., 2013; Schluessel et al., 2012). Side biases can come and go within and between experiments, and fish can show both left and right side biases (Schluessel et al., 2014). Side biases can also be prevalent when a fish cannot discriminate between stimuli and default to the preferred side in the absence of a successful strategy (Siebeck et al., 2008). Some studies report no side biases occurred (e.g., Siebeck et al., 2009). Laterality seems to be a widespread trait in the behavior of fishes, and may account for some side preferences (Bisazza and Brown, 2011).

We used four goldfish in each of the three experiments. We should use caution in interpreting results from few subjects because within-species differences between fish can occur (Gierszewski et al., 2013; Lucon-Xiccato et al., 2016; Miletto Petrazzini and Agrillo, 2016). However, other studies have utilized two to five fish per experiment (Frech et al., 2012; Gierszewski et al., 2013; Neumeyer, 1992, 2003; Newport et al., 2016; Wyzisk and Neumeyer, 2007). One way to compensate for having few subjects is to collect many trials per subject. In Experiments 1 and 2, each novel test angle was presented 24 times for each fish and in Experiment 3, each novel test angle was presented 20 times for each fish. Schluessel et al. (2014) and Mackintosh and Sutherland (1963) presented each novel test angle only five times in each experiment. Even though we compensated for fewer subjects with more stimulus presentations, it is still important to replicate these results with additional subjects in future research.

The current results taken together with previous studies suggest that fish do not consistently recognize 2D stimuli independent of viewing angle, but they appear to show viewpoint-invariant object recognition with 3D stimuli (Bowman and Sutherland, 1969, 1970; DeLong et al., 2018a; Mackintosh and Sutherland, 1963; Schluessel et al., 2014; Sutherland and Bowman, 1969), unless the 3D stimuli are conspecific faces (Wang and Takeuchi, 2017). Viewing 3D stimuli, but not 2D stimuli, may evoke object representations or cognitive processes that support viewpoint-invariant recognition. Fish may not recognize 2D stimuli like line drawings of animals as representations of 3D real animals. Alternately, the attributes of the stimuli used in these studies other than their identity as 2D vs. 3D objects may be responsible for the results. All studies on 2D object constancy in fish have used black and white stimuli, with all studies except the current study employing simple artificial stimuli such as geometric shapes and letters. In contrast, studies done with 3D stimuli used realistic plastic models of animals that contained rich color and texture cues (DeLong et al., 2018a; Schluessel et al., 2014). The fish may have recognized the 3D stimuli

independent of aspect angle by using color or surface texture – features that are present at nearly all aspect angles. [Schluessel et al. \(2014\)](#) did conduct an experiment where fish succeeded in discriminating all possible combinations of 10 turtles and frog models (in all different colors), so color alone was not responsible for the fish's performance. Future research efforts should include 2D stimuli that share some of the properties of the 3D stimuli (e.g., full color photos or drawings, use of realistic stimuli). Alternately, one could utilize less rich 3D stimuli (lacking color cues, less ecologically valid objects) or 3D stimuli that are more difficult to discriminate (two similarly-shaped objects). Pursuing this line of research could shed light on the conditions necessary for viewpoint-invariant performance in fish.

Some researchers have suggested that an organism's capacity for identifying rotated objects depend on ecological demands and habitat (e.g., [Burmam et al., 2005](#); [Delius and Hollard, 1995](#); [Mauck and Dehnhardt, 1997](#)). According to this theory, aerial and aquatic species should be more likely to show viewpoint-independent performance, whereas terrestrial species should show viewpoint-dependence. Only a few aquatic species have been tested, and they do not all show viewpoint-independent performance. Fish appear to show viewpoint-independent performance, but only with 3D stimuli as discussed above (with no correlation between reaction time and rotation angle; [DeLong et al., 2018a](#); [Schluessel et al., 2014](#)). Octopuses did not recognize a diamond as a rotated square ([Sutherland, 1969](#)), but more data is needed for these animals. A bottlenose dolphin's performance was above chance across all angles when viewing 2D objects rotated in the picture plane or the depth plane (no reaction time data available; [DeLong et al., 2017b](#)). A sea lion successfully identified 2D stimuli across a range of angles, but showed the viewpoint-dependent pattern of an increase in reaction time as a function of aspect angle ([Mauck and Dehnhardt, 1997](#)). Sea lions have a semi-terrestrial lifestyle and may rely on both a horizontal and vertical plane of reference, so their results may not be the same as animals with a fully aquatic lifestyle. Interpreting the results of these studies on aquatic animals as a group is difficult, given the differences in methodology and the fact that only a single sea lion and dolphin have been tested ([DeLong et al., 2017b](#); [Mauck and Dehnhardt, 1997](#)). [Peissig and Goode \(2012\)](#) suggest that stimulus type and methodology bias organisms to attend to different stimulus features, which better accounts for the data than an evolutionary/ecological explanation.

More research is needed to shed light on variables that may impact the capacity for visual object constancy in animals living in an aquatic habitat. One such variable may be related to an organism's typical movement patterns. Sea lions and bottlenose dolphins can swim upside down and do barrel rolls, and thus could potentially orient their bodies to view rotated stimuli from more typical viewing angles (e.g., [Marino and Stowe, 1997](#); [Mauck and Dehnhardt, 1997](#)). Conversely, the goldfish in our lab do not appear to swim upside down or tilt their bodies more than about 10–15° unless they are experiencing swim bladder problems. We did not video record the subjects in the current study, but we observed that they typically remained upright during the training and test phases. Thus, our goldfish likely viewed the rotated stimuli at the aspect angles we intended to present. A bottlenose dolphin and a sea lion showed superior performance with 2D stimuli compared to the goldfish in the current study, although neither the sea lion or the dolphin appeared to rotate their heads or bodies to match the rotation angle of the stimuli ([DeLong et al., 2017b](#); [Mauck and Dehnhardt, 1997](#)). The sea lion's head was in fixed upright position in a stationing hoop when viewing the sample stimuli ([Mauck and Dehnhardt, 1997](#)). The dolphin was unrestricted in his approach toward the sample stimulus and could have rotated his own body to match the rotation of the stimulus, but he maintained approximately the same body orientation for every trial and did not change his positioning in relation to the stimulus rotation angle ([DeLong et al., 2017b](#)). Comparing different aquatic species within the same study that have different underwater behaviors may be a fruitful direction for future research. For example,

some fish species swim upside down or tilt their bodies while swimming ([Blake and Chan, 2007](#); [Moore, 2002](#)). A future study could compare the performance of fish species with different swimming behaviors in terms of their ability to recognize rotated stimuli, as well as observe how they choose to orient their bodies while viewing rotated stimuli.

6. Conclusions

Goldfish trained to discriminate between 2D stimuli at 0° were not able to generalize to three of seven novel aspect angles in a two-alternative forced choice task where stimuli were rotated in the picture plane. Further, reaction time did not vary as function of aspect angle. There was no overall difference in performance when viewing simple stimuli (arrow and half circle) vs. complex stimuli (line drawings of a turtle and frog). Finally, fish performed better when both the positive and negative stimuli were rotated to the same aspect angle compared to presenting the negative stimulus at 0° and rotating only the positive stimulus. These results are comparable to some early studies on the visual perception of black and white 2D stimuli in goldfish ([Bowman and Sutherland, 1969](#); [Sutherland and Bowman, 1969](#)), but contrast with the nearly viewpoint-invariant performance reported for fish viewing ecologically relevant, full color 3D stimuli ([DeLong et al., 2018a](#); [Schluessel et al., 2014](#)). Future studies should seek to resolve this discrepancy in performance for 2D vs. 3D objects, as well as test more fish species. The ability to recognize objects independent of viewing angle is adaptive for fish since they move in three dimensions and frequently view objects from different orientations. Object constancy may be important for a wide variety of behaviors such as navigation, foraging, and identifying social partners, prey, and predators.

Ethical standards and conflict of interest

This work was approved by the Rochester Institute of Technology Institutional Animal Care and Use Committee and conducted according to the ethical standards of the United States. Animal care complied with the guidelines provided in the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 8023, revised 1978). The authors declare no conflict of interest with any of the sponsors of this study.

Acknowledgements

We thank Kathryn Gardner, Trisha Rachfal, and Colum Cross for their assistance with the experimental sessions and maintenance of the animals and the aquaria. This work was supported with two College of Liberal Arts Faculty Development Grant to CMD from the Rochester Institute of Technology.

References

- Agrillo, C., Petrazzini, M.E.M., Dadda, M., 2013. Illusory patterns are fishy for fish, too. *Front. Neural Circuits* 7 (137), 1–3. <https://doi.org/10.3389/fncir.2013.00137>.
- Biederman, I., Gerhardstein, P.C., 1993. Recognizing depth-rotated objects: evidence and conditions for three-dimensional viewpoint invariance. *J. Exp. Psychol. Hum. Percept. Perform.* 19, 1162–1182.
- Bisazza, A., Brown, C., 2011. Lateralization of cognitive functions in fish. In: Brown, C., Laland, K., Krause, J. (Eds.), *Fish Cognition and Behavior*, 2nd edn. Blackwell Publishing Ltd., Oxford, pp. 298–324.
- Blake, R.W., Chan, K.H.S., 2007. Swimming in the upside down catfish *Synodontis nigriventris*: it matters which way is up. *J. Exp. Biol.* 210, 2979–2989. <https://doi.org/10.1242/jeb.006437>.
- Bowman, R., Sutherland, N.S., 1969. The discrimination of 'W' and 'V' shapes by goldfish. *Q. J. Exp. Psychol.* 21, 69–76.
- Bowman, R.S., Sutherland, N.S., 1970. Shape discrimination by goldfish: coding of irregularities. *J. Comp. Physiol. Psychol.* 72, 90–97.
- Brown, C., Laland, K., Krause, J., 2011. *Fish Cognition and Behavior*. Wiley-Blackwell, Oxford, UK.
- Burmam, B., Dehnhardt, G., Mauck, B., 2005. Visual information processing in the lion-tailed macaque (*Macaca silenus*): mental rotation or rotational invariance? *Brain Behav. Evol.* 65, 168–176.

- Colwill, R.M., Raymond, M.P., Ferreira, L., Escudero, H., 2005. Visual discrimination learning in zebrafish (*Danio rerio*). *Behav. Process.* 70, 19–31. <https://doi.org/10.1016/j.beproc.2005.03.001>.
- Delius, J.D., Hollard, V.D., 1995. Orientation invariant pattern recognition by pigeons (*Columbia livia*) and humans (*Homo sapiens*). *J. Comp. Psychol.* 109, 278–290.
- DeLong, C.M., Barbato, S., O'Leary, T., Wilcox, K.T., 2017a. Small and large number discrimination in goldfish (*Carassius auratus*) with extensive training. *Behav. Process.* 141 (2), 172–183. <https://doi.org/10.1016/j.beproc.2016.11.011>.
- DeLong, C.M., Fellner, W., Harley, H.E., Fobe, I., Wilcox, K.T., Gardner, K., Odell, K., 2017b. Visual discrimination of rotated 2D objects in a bottlenose dolphin (*Tursiops truncatus*) and goldfish (*Carassius auratus*). Paper Presented at the Fall Meeting of the Comparative Cognition Society.
- DeLong, C.M., Gardner, K., Rachfal, H., Morrison, E., Wilcox, K.T., 2018a. Visual discrimination of rotated 3D objects in goldfish (*Carassius auratus*). Paper Presented at the 25th Annual International Conference on Comparative Cognition.
- DeLong, C.M., Keller, A.M., Wilcox, K.T., Fobe, I., Keenan, S.A., 2018b. Visual discrimination of geometric and complex 2D shapes in goldfish (*Carassius auratus*). *Anim. Behav. Cogn.* 5 (3), 300–319. <https://doi.org/10.26451/abc.05.03.05.2018>.
- Frech, B., Vogtberger, M., Neumeyer, C., 2012. Visual discrimination of objects differing in spatial depth by goldfish. *J. Comp. Physiol. A* 198, 53–60. <https://doi.org/10.1007/s00359-011-0685y>.
- Freeman, D.J., Riesenhuber, M., Poggio, T., Miller, E.K., 2006. Experience-dependent sharpening of visual shape selectivity in inferior temporal cortex. *Cereb. Cortex* 16, 1631–1644.
- Gellerman, L.W., 1933. Chance orders of alternating stimuli in visual discrimination experiments. *J. Genet. Psychol.* 42, 206–208.
- Gierszewski, S., Blackmann, H., Schluessel, V., 2013. Cognitive abilities in Malawi cichlids (*Pseudotropheus* sp.): matching-to-sample and image/mirror-image discriminations. *PLoS One* 8, e57363. <https://doi.org/10.1371/journal.pone.0057363>.
- Graeber, R.C., Ebbesson, S.O., 1972. Visual discrimination learning in normal and tectal-ablated nurse sharks (*Ginglymostoma cirratum*). *Comp. Biochem. Physiol.* 42, 131–139.
- Hamm, J., Matheson, W.R., Honig, W.K., 1997. Mental rotation in pigeons (*Columbia livia*). *J. Comp. Psychol.* 111, 76–81.
- Hollard, V.D., Delius, J.D., 1982. Rotational invariance in visual pattern recognition by pigeons and humans. *Science* 218, 804–806.
- Holm, S., 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6 (2), 65–70.
- Hopkins, W.D., Fagot, J., Vauclair, J., 1993. Mirror-image matching and mental rotation problem solving by baboons (*Papio papio*): unilateral input enhances performance. *J. Exp. Psychol. Gen.* 122, 61–72.
- Jitsumori, M., Ohkubo, O., 1996. Orientation discrimination and categorization of photographs of natural objects by pigeons. *Behav. Process.* 38, 205–226.
- Jolicoeur, P., 1990. Identification of disoriented objects: a dual systems theory. *Mind Lang.* 5, 387–410.
- Jolicoeur, P., Humphrey, G.K., 1998. Perception of rotated two-dimensional and three-dimensional objects and visual shapes. In: Walsh, V., Kulikowski, J. (Eds.), *Visual Constancies: Why Things look as They Do*. Cambridge University Press, pp. 69–123.
- Kendrick, K.M., Atkins, K., Hinton, M.R., Heavens, P., Keverne, B., 1996. Are faces special for sheep? Evidence from facial and object discrimination learning tests showing effects of inversion and social familiarity. *Behav. Process.* 38, 19–35.
- Kleerekoper, H., Matis, J., Gensler, P., Maynard, P., 1974. Exploratory behavior of goldfish *Carassius auratus*. *Anim. Behav.* 22, 124–132.
- Kohler, C., Hoffman, K.P., Dehnhardt, G., Mauck, B., 2005. Mental rotation and rotational invariance in the rhesus monkey (*Macaca mulatta*). *Brain Behav. Evol.* 66, 158–166.
- Lawson, R., 1999. Achieving visual object constancy across plane rotation and depth rotation. *Acta Psychol.* 102, 221–245.
- Lucon-Xiccato, T., Dadda, M., Bisazza, A., 2016. Sex differences in discrimination of shoal size in the guppy (*Poecilia reticulata*). *Ethology* 122 (6), 481–491.
- Mackintosh, J., Sutherland, N.S., 1963. Visual discrimination by the goldfish: the orientation of rectangles. *Anim. Behav.* 11, 135–141.
- Marino, L., Stowe, J., 1997. Lateralized behavior in two captive bottlenose dolphins (*Tursiops truncatus*). *Zoo Biol.* 16, 173–177.
- Marr, D., 1982. *Vision*. Freeman, San Francisco.
- Mauck, B., Dehnhardt, G., 1997. Mental rotation in a California sea lion (*Zalophus californianus*). *J. Exp. Biol.* 200, 1309–1316.
- Miletto Petrazzini, M.E., Agrillo, C., 2016. Turning to the larger shoal: are there individual differences in small-and large-quantity discrimination of guppies? *Ethol. Ecol. Evol.* 28 (2), 211–220.
- Minini, L., Jeffery, K.J., 2006. Do rats use shape to solve “shape discriminations”? *Learn. Mem.* 13, 287–297.
- Moore, J.A., 2002. Upside-down swimming behavior in a whipnose anglerfish (Teleostei: Ceratioidei: Gigantactinidae). *Copeia* 4, 1144–1146.
- Mora-Ferrer, C., Neumeyer, C., 2009. Neuropharmacology of vision in goldfish: a review. *Vis. Res.* 49 (9), 960–969.
- Neumeyer, C., 1992. Tetrachromatic color vision in goldfish: evidence from color mixture experiments. *J. Comp. Physiol. A* 171, 639–649.
- Neumeyer, C., 2003. Wavelength dependence of visual acuity in goldfish. *J. Comp. Physiol. A* 189, 811–821. <https://doi.org/10.1007/s00359-003-0457-4>.
- Newport, C., Wallis, G., Reshitnyk, Y., Siebeck, U., 2016. Discrimination of human faces by archerfish (*Toxotes chatareus*). *Nat. Sci. Rep.* 6 (27523), 1–7. <https://doi.org/10.1038/srep27523>.
- Nielsen, K.J., Logothetis, N.K., Rainer, G.R., 2008. Object features used by humans and monkeys to identify rotated shapes. *J. Vis.* 8 (2), 1–15.
- Parr, L.A., 2011. The inversion effect reveals species differences in face processing. *Acta Psychol.* 138, 204–210.
- Parr, L.A., Heintz, M., 2008. Discrimination of faces and houses by rhesus monkeys: the role of stimulus expertise and rotation angle. *Anim. Cogn.* 11, 467–474. <https://doi.org/10.1007/s10071-008-0137-4>.
- Peissig, J.J., Goode, T., 2012. The recognition of rotated objects in animals. In: Lazareva, O.F., Shimizu, T., Wasserman, E.A. (Eds.), *How Animals See the World: Comparative Behavior, Biology, and Evolution of Vision*. Oxford University Press, New York, NY, pp. 233–246.
- Peissig, J.J., Young, M.E., Wasserman, E.A., Biederman, I., 2005. The role of edges in object recognition by pigeons. *Perception* 34, 1353–1374.
- Plowright, C.M.S., Landry, F., Church, D., Heyding, J., Dupuis-Roy, N., Thivierge, J.P., Simonds, V., 2001. A change in orientation: recognition of rotated patterns by bumblebees. *J. Insect Behav.* 14, 113–127.
- Pollard, J.S., Beale, I.L., Lysons, A.M., Preston, A.C., 1967. Visual discrimination in the ferret. *Percept. Mot. Skills* 24, 279–282.
- R Core Team, 2018. *R: A Language and Environment for Statistical Computing (3.5.0)* [Programming Language]. Retrieved from. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Racca, A., Amadei, E., Ligout, S., Guo, K., Meints, K., Mills, D., 2010. Discrimination of human and dog faces and inversion responses in domestic dogs (*Canis familiaris*). *Anim. Cogn.* 13, 525–533.
- Schluessel, V., Bleckmann, H., 2005. Spatial memory and orientation strategies in the elasmobranch *Potamotrygon motor*. *J. Comp. Physiol. A* 191, 695–706.
- Schluessel, V., Fricke, G., Bleckmann, H., 2012. Visual discrimination and object categorization in the cichlid *Pseudotropheus* sp. *Anim. Cogn.* 15, 525–537. <https://doi.org/10.1007/s1007-012-0480-3>.
- Schluessel, V., Kraniotakes, H., Bleckmann, H., 2014. Visual discrimination of rotated 3D objects in Malawi cichlids (*Pseudotropheus* sp.): a first indication for form constancy in fishes. *Anim. Cogn.* 17, 359–371. <https://doi.org/10.1007/s10071-013-0667-2>.
- Schusterman, R.J., Thomas, T., 1966. Shape discrimination and transfer in the California sea lion. *Psychon. Sci.* 5, 21–22.
- Shepard, R.N., Metzler, J., 1971. Mental rotation of three-dimensional objects. *Science* 171 (3972), 701–703.
- Siebeck, U.E., Wallis, G.M., Litherland, L., 2008. Colour vision in reef fish. *J. Exp. Biol.* 211, 354–360.
- Siebeck, U.E., Litherland, L., Wallis, G.M., 2009. Shape learning and discrimination in reef fish. *J. Exp. Biol.* 212, 2113–2119. <https://doi.org/10.1242/jeb.028936>.
- Sutherland, N.S., 1969. Shape discrimination in rat, octopus, and goldfish: a comparative study. *J. Comp. Physiol. Psychol.* 67, 160–176.
- Sutherland, N.S., Bowman, R., 1969. Discrimination of circles and squares with and without knobs by goldfish. *Q. J. Exp. Psychol.* 21, 330–338.
- Tarr, M.J., 1995. Rotating objects to recognize them: a case study of the role of viewpoint-dependency in the recognition of three-dimensional objects. *Psychon. Bull. Rev.* 2 (1), 55–82.
- Tarr, M.J., Pinker, S., 1989. Mental rotation and orientation-dependence in shape recognition. *Cognit. Psychol.* 21, 233–282.
- Truppa, V., Sovrano, V.A., Spinozzi, G., Bisazza, A., 2010. Processing of visual hierarchical stimuli by fish (*Xenotoca eiseni*). *Behav. Brain Res.* 207, 51–60. <https://doi.org/10.1016/j.bbr.2009.09.039>.
- Tukey, J., 1949. Comparing individual means in the analysis of variance. *Biometrics* 5 (2), 99–114.
- Wang, M.-Y., Takeuchi, H., 2017. Individual recognition and the ‘face inversion effect’ in medaka fish (*Oryzias latipes*). *eLife* 6, e24728. <https://doi.org/10.7554/eLife.24728>.
- Wyzisk, K., Neumeyer, C., 2007. Perception of illusory surfaces and contours in goldfish. *Vis. Neurosci.* 24, 291–298. <https://doi.org/10.1017/S095252380707023X>.