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# Evaluating spatial learning and memory ability of juvenile Sebastes schlegelii: A case study based on the cross-shaped convertible T-maze experiment

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## Abstract

Learning behavior is an important acquired behavior in fish, and the capacity for learning and memory plays a crucial role in foraging or anti-predator behavior. In this study, we selected the juveniles of *S. schlegelii* as the experimental subjects, and set three visual color cue conditions in a T-shaped maze: no visual color cue, single green visual color cue, and double green-red visual color cues, to establish the association between visual color cues and food rewards. We quantified learning ability by measuring both the time required to obtain food rewards and task completion success rates. Moreover, the memory ability of the experimental fish was tested via memory retention tests at 3 d, 5 d and 7 d intervals. The learning and memory abilities of the experimental fish under the three visual color cue conditions were analyzed, as well as the statistical significance of the consumed time and completion rate during the improvement and stabilization processes in the experimental fish. We also determined the effects of the different visual color cue conditions on the consumed time and completion rate of the experimental fish, seeking to comprehensively investigate the differences in the learning and memory abilities of the juvenile *S. schlegelii* in the T-shaped maze while completing the same spatial task. The results demonstrated that the experimental fish exhibited significant changes in both the consumed time and completion rate and revealed a significant correlation between them. This indicated that, under the three studied visual color cue conditions, the juvenile *S. schlegelii* could acquire associative learning information through training. The juveniles exhibited optimal learning performance under single visual color cue conditions. Juveniles with different body lengths at the same developmental stage showed no significant difference in spatial learning and memory abilities. The experimental results establish a foundation for the comprehensive evaluation of spatial learning and memory abilities of *S. schlegelii* and for the in-depth understanding of the behavioral learning process.

## 1 Introduction

Spatial learning and memory are important areas of behavioral research that has attracted considerable interest in recent years, due to its broad applicability in the study of fish behavior. The main goal of this type of behavioral research is to understand how animals use learning and memory to navigate around their habitats; to help to locate food sources, shelters, and mates, and to avoid potential dangers, such as natural predators (Zeil, 1998). The most widely used experimental methods to study learning and memory in fish are maze learning and Skinner's box. In addition, behaviorists have devised a number of other experimental methods that depend on the subject and the purpose of the experiment, such as shuttle boxes or the use of simple response discrimination (left vs. right) or visual cues to mark the correct choice. As an example, researchers found that trained cichlid (*Chindongo demasoni*) juveniles learned information associatively through training in a radial maze by learning the link between food and visuospatial cues (Long & Fu, 2022).

Foraging behavior is one of the basic survival behaviors of animals, and spatial learning and memory can significantly improve foraging efficiency and survival adaptability, especially for fish living in complex habitats (Odling-Smee et al., 2008;Walton & Moller, 2010). Animals can complete spatial tasks through two distinct strategies: response strategy (learning the association between action and reward) and location strategy (learning the association between spatial position and reward) (Gibson & Shettleworth, 2005). Previous studies have confirmed that a variety of fish species can establish stable associative learning in maze tasks, with significant interspecific differences in spatial learning ability (Sison & Gerlai, 2010;Zhu, 2007). For *Sebastes. schlegelii*, existing research has found that habitat complexity significantly affects its spatial cognitive ability, with fish from barren environments showing weaker learning performance than those from complex environments (Zhang et al., 2021). Previous studies on fish spatial learning and memory are summarized in Table 1.

Table 1
Experiments related to learning and memory abilities in fish

| Date | Reference | Research target | Findings |
|---|---|---|---|
| 1951, 1971 | Aronson 1951; Aronson (1971) | *Bathygobius soporator* | When *Bathygobius soporator* is threatened, gobies jump from the tidal pools (mother pools) on which they depend to neighboring tidal pools more quickly and with significantly improved directionality. |
| 2020 | Heinrich et al. 2020 | *Heterodontus portusjacksoni* | Sharks trained with a high reinforcement frequency learned faster and passed through the correct door more frequently at the end of the trial, while reward size has a limited effect on learning speed. |
| 2015 | Zhu & Liu, 2015 | *Rhodeus ocellatus* | *Rhodeus ocellatus* have good spatial learning and memory abilities. *Rhodeus ocellatus* learns better in the presence of visual color cues. |
| 2015 | McAroe et al. 2016 | Goldfish, killifish, zebrafish, and Siamese fighting fish | Three of the fish species show a clear preference for a positional strategy in the research task, whereas zebrafish show no clear preference. |
| 2015 | Nazario et al. 2015 | Adult zebrafish | Zebrafish respond to the anxiolytic properties of CBD in a similar way to other animal models, and high doses alter their memory retention in a manner that is dependent on adenosine. |
| 2020 | Sovrano et al. 2020 | Fish | Even in the absence of reinforcement training, fish prioritize the correct corners |
| 2021 | Wallace and Hofmann 2021 | Male and female cichlids | Both sexes prefer familiar objects in a new object recognition task, and females learn the spatial task more quickly. |
| 2023 | Tsang et al. (2023) | Ram cichlid | Ram cichlid rapidly acquire CS–US associations (the experimental fish's apparently least preferred color, i.e., the conditioned stimulus or CS, paired with the food-rewarded unconditioned stimulus or US). |

Animals can solve a spatial task by learning an association between a response and a reward or they can learn an association between a given place and a reward (Gibson & Shettleworth, 2005). This has important implications for spatial learning and memory in fish. In this study, we focused on spatial learning and memory abilities of juvenile *S. schlegelii* individuals living in a complex ecological environment. In addition, previous studies have shown that body size and developmental stage significantly affect the cognitive ability of fish (Masuda & Ziemann, 2010). However, it remains unclear whether body size differences at the same juvenile developmental stage affect the spatial learning ability of *S. schlegelii*. Therefore, we set up two groups of juveniles with different body lengths to verify this scientific question. It provides an important basis for the understanding of the physiological and behavioral aspects of the learning and memory of juvenile fish. According to a study indicating that *S. schlegelii* juveniles have the capacity for color discrimination (Wu, 2023) (Neumeyer, 2003), the results of the present study are expected to improve the feeding efficiency and reduce the cost of aquaculture by highlighting the possibility to reinforce the species' memory for specific stimuli, such as the feeder's voice or color. In addition, this study could contribute to the optimization of culture technology and environmental designs, such as adjustments to the feed type, baiting time, and stocking density, in order to promote the healthy growth of juvenile *S. schlegelii* and enhance the culture efficiency and economic benefits.

## 2 Materials and Methods

# 2.1. Experimental fish

The fish were purchased from Dalian Tianzheng Industrial Co. Ltd. in June 2023, and 20 physically healthy *S. schlegelii* juveniles with a normal body color and no ulcers were selected as the experimental fish. These included 10 large-sized experimental fish (group A) with a body length of 8.23 ± 0.52 cm and 10 small-sized experimental fish (group B) with a body length of 4.03 ± 0.24 cm. They were temporarily housed in 2 round temperature-controlled tanks (diameter 80 cm, 300 L) for a period of 7 d to adapt to the new environment. The seawater consisted of sand-filtered circulating seawater, which was circulated for 24 h during the period of temporary rearing, with a water temperature of 20 ± 0.5 ℃, salinity of 31 ± 0.5, and a pH of 7.6 ~ 7.8. It continuously oxygenated for 24 h, and the dissolved oxygen level was kept above 6.5 mg/L. The fish were fed with compounded feed 3 times a day.

# 2.2. Experimental setup: T-shaped maze

The experiment was conducted using a custom-built cross-shaped convertible T-maze apparatus, which consisted of 2 cm-thick acrylic sheets, visual color cue modules, a food reward system, acrylic blocking baffles, sliding movable doors, an illumination control system, and a high-definition behavioral observation system. The entire experimental area was completely enclosed with black light-blocking cloth. Uniform LED cold light sources were installed at the four corners of the experimental shed, maintaining a photoperiod of 12L:12D (light period: 08:00–20:00) to ensure homogeneous light intensity and eliminate shadow interference across the maze.

The maze had a symmetrical cross-shaped structure, with each arm measuring 1 m in length, 0.2 m in width, and 0.2 m in height. A matching acrylic blocking baffle (0.2 m × 0.2 m × 0.2 m) was used to temporarily seal any one of the four arms, converting the cross-shaped channel into a standard T-shaped maze. This core design enabled complete randomization of the maze's absolute orientation in the experimental space, thereby eliminating confounding effects from innate left-right turning preferences of experimental fish and fixed environmental directional cues (e.g., room corners, light gradients). A sliding movable door (0.2 m × 0.2 m × 0.2 m) was installed 0.2 m from the distal end of each arm to separate the starting compartment from the main maze area. The door was lowered to confine fish in the starting compartment for acclimation and raised to allow free entry into the maze. After each trial, fish were transferred to a thermostatic holding tank (1 m × 0.3 m × 0.5 m) maintained at 20 ± 0.5°C, consistent with the acclimation environment.

## 2.3. Experimental method

This study employed a custom-built cross-shaped convertible T-maze apparatus to investigate the spatial associative learning and memory abilities of juvenile *S. schlegelii* by establishing a stable association between visual color cues and food rewards. To completely eliminate confounding factors such as innate left-right turning preference, geomagnetic field, and fixed laboratory environmental cues (lighting, corners, background noise), a triple randomization control strategy (random starting channel, random visual color cue assignment, and omnidirectional random rotation) was adopted, ensuring that the results only reflect the learning and memory abilities of fish to the preset visual color cues. The entire experiment lasted 25 d, consisting of three phases: preliminary experiment, memory acquisition experiment, and memory retention test.

Large-sized group A (body length 8.23 ± 0.52 cm) and small-sized group B (body length 4.03 ± 0.24 cm) were acclimated separately. Prior to formal experiments, fish were acclimated to the maze apparatus for 8 h per day with all movable baffles and visual color cues removed, allowing free swimming throughout the cross-shaped maze. After a 3 d preliminary experiment (no data collection, no environmental cues), all fish were numbered and transferred to individual rearing tanks. All fish were fasted throughout the acclimation period to standardize their foraging motivation.

The experiment followed the only constant rule: the channel with the green artificial aquatic plant visual color cue corresponded to the food reward end, and the channel with the red artificial aquatic plant visual color cue corresponded to the non-reward end. Food reward was exclusively and permanently associated with the green visual color cue, and their positions were randomly assigned to either of the two choice arms in each individual trial. Before each individual trial, experimental conditions were generated according to the following procedure: first, a random number was generated using SPSS 27.0 software to randomly select one of the four channels as the starting channel, and the corresponding acrylic movable baffle was inserted to temporarily convert the cross-shaped channel into a T-shaped structure; second, another random number was generated to determine the assignment of green and red visual color cues at the exact center of the two choice arms; third, the entire maze was rotated to 0°, 90°, 180°, or 270° to eliminate geomagnetic field and fixed spatial cue interference; finally, the experimental fish was placed in the starting compartment, the movable door was closed, and the trial started after 5 min of acclimation.

Three visual color cue conditions were established: no visual color cue control group (no cues on either choice arm), single visual color cue group (only green visual color cue at the center of the reward arm), and dual visual color cues group (green visual color cue at the center of the reward arm and red visual color cue at the center of the non-reward arm). All markers were made of green and red artificial aquatic plants (10 cm in width, height equal to the water depth), vertically fixed at the exact center of the channel floor using suction cups to ensure clear visibility from any depth and angle. For the no visual color cue control group, the maze was thoroughly wiped with clean seawater and randomly rotated 180° before each trial to eliminate potential directional cues from scratches, water flow, or residual odors. Additionally, 10 mL of bait odor water (1 $cm^3$ of bait homogenized in 30 mL of reverse osmosis water, supernatant collected after 15 min standing) was evenly sprayed into the maze before each trial to mask the odor location of food rewards and stimulate the foraging motivation of experimental fish.

Ten juvenile fish were tested individually per group. Each fish completed one trial under each of the three visual color cue conditions per day, with each trial lasting 20 min and a 5-min interval between trials. A total of 10 groups (30 individual trials) were conducted daily for 7 consecutive days, with a total daily experimental duration of approximately 10 h. All experimental conditions were balanced and randomized to ensure equal occurrence of each starting channel, visual color cue position, and rotation angle throughout the training period. The end of the fish's dorsal fin completely passing through the choice arm opening was used as the decision criterion, and the latency to reach the food reward end and the accuracy of the first choice were recorded. Equal amounts of bait were provided as positive reinforcement immediately upon successful arrival at the reward end. If the fish failed to make a valid arm choice within 10 min, the trial was terminated and no food reward was provided. The fish was gently guided out of the maze and returned to its holding tank. This procedure ensured that food rewards were exclusively associated with correct target arm choices, eliminating non-specific reinforcement

interference with associative learning. The apparatus was thoroughly cleaned after each trial to remove residual food odors, and all fish were returned to their original rearing tanks after daily training.

Memory retention tests were conducted on days 3, 5, and 7 after the completion of training. During memory retention tests, the end of the food reward arm from the training phase was set as the new starting compartment. This was achieved by: (1) rotating the entire maze to a random orientation; (2) repositioning the acrylic blocking baffle to reconstruct the T-shaped structure; (3) reattaching the movable door to the new starting position. The randomization rules, operational procedures, and decision criteria of the retention tests were identical to those of the memory acquisition experiment, but no additional reinforcement was provided to examine the duration and decay pattern of memory under different visual color cue conditions.

## 2.4. Data Acquisition

### 2.4.1. Collection of experimental data

(1) Time consumed: the time taken by the experimental fish to move from the starting compartment to the end of the food arm of the device was recorded in seconds (s).

(2) Completion rate: the experimental subjects' ability to complete the trial by choosing the correct direction was recorded. The behavioral trajectory of the fish in the experimental tank was calculated to obtain the completion rate, which was recorded as 1 if the fish chose the correct direction and entered the end of the correct food arm for the first time and 0 if the experimental fish chose the wrong direction and entered the end of the wrong food arm for the first time.

### 2.4.2. Analysis of the learning process

Analysis of the improvement in the time consumed and completion rate: The experimental data obtained from the beginning of the 2nd training step to the last training step were subjected to a one-sample t-test with the data from the 1st step, enabling us to compare the p-values. If the p-values showed significant differences in 4 out of 5 consecutive steps, the number of training sessions required to reach this criterion was defined as the number of acquisitions regarding the time consumed and the completion rate.

Analysis of the stabilization of the time consumed and completion rate: A one-sample t-test was used to compare the p-value from test of all of the experimental data obtained during training from the 1st step to the penultimate training step with the mean Nt of the last 3 steps. If only 1 out of the 3 consecutive steps showed a significant difference, the number of training steps required to reach this criterion was defined as the stabilization value regarding the time consumed and the completion rate.

Comparison of time consumed and completion rate under different visual color cue conditions: a one-way analysis of variance (ANOVA) was used to synthesize the three sets of experimental data, to compare the changes in the time consumed and completion rate under the three different environmental conditions, and to explore the effects of the different visual color cue conditions on spatial learning abilities of the experimental subjects.

## 2.5. Statistical analysis of experimental data

To carry out the statistical analysis of the experimental data, the mean values of time consumed and percentage correct were calculated using Excel software. The experimental data were statistically analyzed accordingly using SPSS 27.0 software. Prior to formal statistical analysis, the normality of all data was verified using the Shapiro-Wilk test, and the homogeneity of variances was verified using Levene's test. Data that did not meet the normality assumption were subjected to logarithmic transformation before analysis. One-sample t-test was used for the analysis of memory acquisition stage for consumed time and completion rate. One-way ANOVA followed by Tukey's post-hoc test was used to compare the differences in consumed time and completion rate among the three visual color cue conditions, with the significance level set at $p < 0.05$.

# 3. Results

## 3.1. Changes in time consumed and completion rate during the experiment

### 3.1.1. *S. schlegelii* juvenile group A

For Cues 0, the maximum amount of time consumed by the fish in group A was 363.817 s; the minimum amount of time consumed was 191.491 s; and the mean amount of time consumed was 274.081 ± 53.499 s (Table 2). Overall, 16 fish were rated as excellent, 68 fish were rated as good, and 16 fish were rated as medium. For Cues 1, the maximum amount of time consumed by the fish in group A was 364.264

s; the minimum amount of time was 114.615 s; and the mean amount of time was 222.224 ± 79.085 s. Overall, 18 fish were rated as excellent, 74 fish were good, and 18 fish were medium. For Cues 2, the maximum amount of time consumed by the fish in group A was 388.683 s; the minimum amount of time was 260.957 s; and the mean amount of time was 325.01 ± 44.127 s. Overall, 16 fish were rated as excellent, 65 fish were good, and 19 fish were moderate. The mean amount of time consumed by the fish in group A, in ascending order, was as follows: Cues 1 (222.224 ± 79.085 s), Cues 0 (274.081 ± 53.499 s), and Cues 2 (325.01 ± 44.127 s). The grades were ranked from high to low as follows: Cues 1, Cues 0, and Cues 2. The mean values of the completion rate for the fish in group A were, in descending order, 70% for Cues 1, 55% for Cues 2, and 49% for Cues 0.

Table 2
Comparison of the learning and memory abilities of the fish in group A under the three visual color cue conditions.

| Cues | Number of experiments | Consumed time | | | Grade | | | Select the direction | |
|---|---|---|---|---|---|---|---|---|---|
| | | Maximum(s) | Minimum(s) | Average ± error value(s) | Excellent | Good | Medium | Accuracy (%) | Error rate (%) |
| 0 | N1 | 443.03 | 233.23 | 317.36 ± 55.14 | 233.23s≦t1 < 262.22s | 262.22s≦t1 ≦ 372.50s | 372.50s<t1 ≦ 443.03s | 10 | 90 |
| | | | | | 1 | 8 | 1 | | |
| | N2 | 377.27 | 224.12 | 310.77 ± 43.76 | 224.12s≦t2 < 267.01s | 267.01s≦t2 ≦ 354.53s | 354.53s<t2 ≦ 377.27s | 50 | 50 |
| | | | | | 1 | 7 | 2 | | |
| | N3 | 366.15 | 167.23 | 280.78 ± 67.75 | 167.23s≦t3 < 213.03s | 213.03s≦t3 ≦ 348.53s | 348.53s<t3 ≦ 366.15s | 20 | 80 |
| | | | | | 2 | 6 | 2 | | |
| | N4 | 366.18 | 188.06 | 284.28 ± 55.48 | 188.06s≦t4 < 228.80s | 228.80s≦t4 ≦ 339.76s | 339.76s<t4 ≦ 366.18s | 40 | 60 |
| | | | | | 2 | 7 | 1 | | |
| | N5 | 360.04 | 201.57 | 283.23 ± 47.11 | 201.57s≦t5 < 236.12s | 236.12s≦t5 ≦ 330.34s | 330.34s<t5 ≦ 360.04s | 30 | 70 |
| | | | | | 2 | 7 | 1 | | |
| | N6 | 332.36 | 200.02 | 261.25 ± 44.72 | 200.02s≦t6 < 216.53s | 216.53s≦t6 ≦ 305.97s | 305.97s<t6 ≦ 332.36s | 90 | 10 |
| | | | | | 2 | 6 | 2 | | |
| | N7 | 395.56 | 144.37 | 246.38 ± 77.30 | 144.37s≦t7 < 169.08s | 169.08s≦t7 ≦ 323.68s | 323.68s<t7 ≦ 395.56s | 60 | 40 |
| | | | | | 1 | 7 | 2 | | |
| | N8 | 336.18 | 205.06 | 250.99 ± 44.16 | 205.06s≦t8 < 206.83s | 206.83s≦t8 ≦ 295.15s | 295.15s<t8 ≦ 336.18s | 70 | 30 |
| | | | | | 1 | 8 | 1 | | |
| | N9 | 355.22 | 163.19 | 253.62 ± 60.05 | 163.19s≦t9 < 193.57s | 193.57s≦t9 ≦ 313.67s | 313.67s<t9 ≦ 355.22s | 80 | 20 |
| | | | | | 2 | 6 | 2 | | |
| | N10 | 306.18 | 188.06 | 252.15 ± 39.52 | 188.06s≦t10 < 212.63s | 212.63s≦t10 ≦ 291.67s | 291.67s<t10 ≦ 306.18s | 40 | 60 |
| | | | | | 2 | 6 | 2 | | |
| 1 | N1 | 455.55 | 153.20 | 290.40 ± 114.20 | 153.20s≦t1 < 176.2s | 176.20s≦t1 ≦ 404.60s | 404.60s<t1 ≦ 455.55s | 20 | 80 |
| | | | | | 2 | 5 | 3 | | |
| | N2 | 492.39 | 116.04 | 239.83 ± 120.27 | 116.04s≦t2 < 119.56s | 119.56s≦t2 ≦ 360.10s | 360.10s<t2 ≦ 492.39s | 80 | 20 |
| | | | | | 1 | 7 | 2 | | |
| | N3 | 292.53 | 154.06 | 230.47 ± 49.55 | 154.06s≦t3 < 180.92s | 180.92s≦t3 ≦ 280.02s | 280.02s<t3 ≦ 292.53s | 40 | 60 |
| | | | | | 3 | 4 | 3 | | |
| | N4 | 330.56 | 127.53 | 231.10 ± 59.62 | 127.53s≦t4 < 171.48s | 171.48s≦t4 ≦ 290.72s | 290.72s<t4 ≦ 330.56s | 30 | 70 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 8 | 1 | | |
| | N5 | 388.30 | 133.08 | 228.79 ± 81.04 | 133.08s≦t5 < 147.75s | 147.75s≦t5 ≦ 309.83s | 309.83s<t5 ≦ 388.30s | 90 | 10 |
| | | | | | 2 | 7 | 1 | | |
| | N6 | 332.05 | 97.32 | 221.76 ± 76.64 | 97.32s≦t6 < 145.12s | 145.12s≦t6 ≦ 298.40s | 298.40s<t6 ≦ 332.05s | 70 | 30 |
| | | | | | 1 | 8 | 1 | | |
| | N7 | 310.29 | 121.07 | 210.19 ± 64.86 | 121.07s≦t7 < 145.33s | 145.33s≦t7 ≦ 275.05s | 275.05s<t7 ≦ 310.29s | 60 | 40 |
| | | | | | 2 | 7 | 1 | | |
| | N8 | 377.34 | 64.35 | 204.24 ± 100.38 | 64.35s≦t8 < 103.86s | 103.86s≦t8 ≦ 304.62s | 304.62s<t8 ≦ 377.34s | 80 | 20 |
| | | | | | 2 | 6 | 2 | | |
| | N9 | 355.27 | 78.26 | 192.29 ± 55.52 | 78.26s≦t9 < 136.77s | 136.77s≦t9 ≦ 247.81s | 247.81s<t9 ≦ 355.27s | 40 | 60 |
| | | | | | 3 | 5 | 2 | | |
| | N10 | 308.36 | 101.24 | 173.17 ± 68.77 | 101.24s≦t10 < 104.40s | 104.40s≦t10 ≦ 241.94s | 241.94s<t10 ≦ 308.36s | 90 | 10 |
| | | | | | 1 | 7 | 2 | | |
| 2 | N1 | 419.22 | 308.39 | 367.63 ± 34.85 | 308.39s≦t1 < 332.78s | 332.78s≦t1 ≦ 402.48s | 402.48s<t1 ≦ 419.22 | 30 | 70 |
| | | | | | 1 | 8 | 1 | | |
| | N2 | 399.26 | 306.49 | 352.04 ± 35.59 | 306.49s≦t2 < 316.45s | 316.45s≦t2 ≦ 387.63s | 387.63s<t2 ≦ 399.26s | 20 | 80 |
| | | | | | 3 | 4 | 3 | | |
| | N3 | 399.09 | 293.69 | 341.33 ± 38.40 | 293.69s≦t3 < 302.93s | 302.93s≦t3 ≦ 379.73s | 379.73s<t3 ≦ 399.09s | 50 | 50 |
| | | | | | 2 | 6 | 2 | | |
| | N4 | 389.31 | 264.29 | 328.85 ± 46.74 | 264.29s≦t4 < 282.11s | 282.11s≦t4 ≦ 375.59s | 375.59s<t4 ≦ 389.31s | 30 | 70 |
| | | | | | 2 | 5 | 3 | | |
| | N5 | 391.29 | 224.97 | 330.22 ± 54.32 | 224.97s≦t5 < 275.90s | 275.90s≦t5 ≦ 384.54s | 384.54s<t5 ≦ 391.29s | 60 | 40 |
| | | | | | 2 | 6 | 2 | | |
| | N6 | 393.16 | 261.61 | 304.29 ± 47.93 | 261.61s≦t6 < 256.36s | 256.36s≦t6 ≦ 352.22s | 352.22s<t6 ≦ 393.16s | 80 | 20 |
| | | | | | 1 | 8 | 1 | | |
| | N7 | 355.44 | 243.99 | 316.72 ± 34.76 | 243.99s≦t7 < 281.96s | 281.96s≦t7 ≦ 351.48s | 351.48s<t7 ≦ 355.44s | 70 | 30 |
| | | | | | 1 | 7 | 2 | | |
| | N8 | 351.44 | 245.21 | 309.19 ± 40.48 | 245.21s≦t8 < 268.71s | 268.71s≦t8 ≦ 349.67s | 349.67s<t8 ≦ 351.44s | 40 | 60 |
| | | | | | 1 | 8 | 1 | | |
| | N9 | 396.61 | 200.63 | 301.59 ± 72.90 | 200.63s≦t9 < 228.69s | 228.69s≦t9 ≦ 374.49s | 374.49s<t9 ≦ 396.61s | 90 | 10 |
| | | | | | 2 | 5 | 3 | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| N10 | 392.01 | 260.30 | 298.24 ± 35.30 | 260.3s≦t10 < 262.94s | 262.94s≦t10 ≦ 333.54s | 333.54s<t10 ≦ 392.01 | 80 | 20 |
| | | | | 1 | 8 | 1 | | |

*In the table, N1 ~ N10 denote daily experiments across 10 days.

In Cues 0, the experimental fish showed a significant decrease in the time consumed, as shown in Figure. 2, and an increase in the completion rate when choosing the correct direction, as shown in Figure. 3. The improvement in the consumed time was analyzed, as shown in Figure. 2A, and there was a statistically significant difference between N3 and N1 ($p < 0.05$). The improvement in the completion rate was also analyzed, as shown in Fig. 3A, and there was a statistically significant difference between N4 and N1 ($p < 0.05$). The stabilization of the consumed time was also analyzed, as shown in Fig. 2B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$). Finally, the stabilization of the completion rate was analyzed, as shown in Fig. 3B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$).

In Cues 1, the experimental fish showed a significant decrease in the time consumed, as shown in Fig. 2, and an increase in the completion rate when choosing the direction, as shown in Fig. 3. The improvement in the consumed time was analyzed, as shown in Fig. 2A, and there was a statistically significant difference between N2 and N1 ($p < 0.05$). The improvement in the completion rate was analyzed, as shown in Fig. 3A, and there was a statistically significant difference between N3 and N1 ($p < 0.05$). The stabilization of the time consumed was also analyzed, as shown in Fig. 2B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$). Finally, the stabilization of the completion rate was analyzed, as shown in Fig. 3B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$).

In Cues 2, the experimental fish showed a significant decrease in the time consumed, as shown in Fig. 2, and an increase in the completion rate when choosing the direction, as shown in Fig. 3. The improvement in the consumed time was analyzed, as shown in Fig. 2A, and there was a statistically significant difference between N3 and N1 ($p < 0.05$). The improvement in the completion rate was analyzed, as shown in Fig. 3A, with N5 showing a statistically significant difference from N1 ($p < 0.05$). The stabilization of the time consumed was analyzed, as shown in Fig. 2B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$). The stabilization of the completion rate was analyzed, as shown in Fig. 3B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$).

## 3.1.2. *Sebastes schlegelii* juvenile group B

For Cues 0, the maximum amount of time consumed by the fish in group B was 389.286 s; the minimum amount of time was 288.124 s; and the mean was 338.938 ± 34.454 s (Table 3). In total, 17 fish were rated excellent, 67 fish were good, and 16 fish were moderate. For Cues 1, the maximum amount of time consumed by the fish in group B was 398.945 s; the minimum amount of time was 269.438 s; and the mean was 328.247 ± 42.872 s. Overall, 15 fish were rated excellent, 65 fish were good, and 20 fish were moderate. For Cues 2, the maximum amount of time consumed by the fish in group B was 428.006 s; the minimum amount of time was 267.542 s; and the mean was 350.619 ± 51.936 s. In total, 18 fish were rated excellent, 65 fish were good, 17 fish were moderate. The mean values for the time consumed by the fish in group B, in ascending order, were as follows: Cues 1 (328.247 ± 42.872 s), Cues 0 (338.938 ± 34.454 s), Cues 2 (350.619 ± 51.936 s). The performance grades were ranked from high to low as: Cues 1, Cues 0, Cues 2. The mean values of the completion rate for the fish in group B, from high to low, were as follows: Cues 1 (51%), Cues 2 (50%), Cues 0 (48%).

Table 3
Comparison of the learning and memory abilities of the fish in group B under the three visual color cue conditions.

| Cues | Number of experiments | Consumed time | | | Grade | | | Select the direction | |
|---|---|---|---|---|---|---|---|---|---|
| | | Maximum(s) | Minimum(s) | Average ± error value(s) | Excellent | Good | Medium | Accuracy (%) | Error rate (%) |
| 0 | N1 | 456 | 328.18 | 374.99 ± 48.67 | 328.18s≦t1 < 326.32s | 326.32s≦t1 ≦ 423.66s | 423.66s<t1 ≦ 456s | 20 | 80 |
| | | | | | 1 | 7 | 2 | | |
| | N2 | 410.76 | 331.32 | 368.69 ± 28.46 | 331.32s≦t2 < 340.26s | 340.26s≦t2 ≦ 397.15s | 397.15s<t2 ≦ 410.76s | 40 | 60 |
| | | | | | 2 | 5 | 3 | | |
| | N3 | 408.84 | 305.3 | 351.32 ± 32.28 | 305.3s≦t3 < 319.04s | 319.04s≦t3 ≦ 383.6s | 383.6s < t3 ≦ 408.84s | 30 | 70 |
| | | | | | 2 | 7 | 1 | | |
| | N4 | 409.66 | 276.38 | 343.21 ± 41.79 | 276.38s≦t4 < 301.42s | 301.42s≦t4 ≦ 385s | 385s<t4 ≦ 409.66s | 20 | 80 |
| | | | | | 2 | 7 | 1 | | |
| | N5 | 408.42 | 267.87 | 343.77 ± 41.83 | 267.87s≦t5 < 305.94s | 305.94s≦t5 ≦ 389.60s | 389.60s<t5 ≦ 408.42s | 60 | 40 |
| | | | | | 2 | 7 | 1 | | |
| | N6 | 372.25 | 296.38 | 346.23 ± 21.76 | 296.38s≦t6 < 324.47s | 324.47s≦t6 ≦ 367.99s | 367.99s<t6 ≦ 372.25s | 90 | 10 |
| | | | | | 1 | 8 | 1 | | |
| | N7 | 417.42 | 202.27 | 309.82 ± 80.41 | 202.27s≦t7 < 229.41s | 229.41s≦t7 ≦ 390.23s | 390.23s<t7 ≦ 417.42s | 60 | 40 |
| | | | | | 3 | 5 | 2 | | |
| | N8 | 376.24 | 314.78 | 342.12 ± 23.31 | 314.78s≦t8 < 318.81s | 318.81s≦t8 ≦ 365.43s | 365.43s<t8 ≦ 376.24s | 70 | 30 |
| | | | | | 1 | 6 | 3 | | |
| | N9 | 314.91 | 295.53 | 308.94 ± 6.04 | 295.53s≦t9 < 302.90s | 302.90s≦t9 ≦ 314.91s | 314.91s<t9 ≦ 314.98s | 50 | 50 |
| | | | | | 1 | 8 | 1 | | |
| | N10 | 318.36 | 263.23 | 300.29 ± 19.99 | 263.23s≦t10 < 280.30s | 280.30s≦t10 ≦ 318.36s | 318.36s<t10 ≦ 320.28s | 40 | 60 |
| | | | | | 2 | 7 | 1 | | |
| 1 | N1 | 445.55 | 315.04 | 382.15 ± 38.23 | 315.04s≦t1 < 343.92s | 343.92s≦t1 ≦ 420.38s | 420.38s<t1 ≦ 445.55s | 10 | 90 |
| | | | | | 1 | 7 | 2 | | |
| | N2 | 400.12 | 324.19 | 364.01 ± 23.62 | 324.19s≦t2 < 340.39s | 340.39s≦t2 ≦ 387.63s | 387.63s<t2 ≦ 400.12s | 30 | 70 |
| | | | | | 1 | 7 | 2 | | |
| | N3 | 388.32 | 303.16 | 349.66 ± 36.09 | 303.16s≦t3 < 313.57s | 313.57s≦t3 ≦ 385.75s | 385.75s<t3 ≦ 388.32s | 20 | 80 |
| | | | | | 2 | 5 | 3 | | |
| | N4 | 451.28 | 275.12 | 339.55 ± 60.03 | 275.12s≦t4 < 279.52s | 279.52s≦t4 ≦ 399.58s | 399.58s<t4 ≦ 451.28s | 50 | 50 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 2 | 6 | 2 | | |
| | N5 | 385.08 | 274.11 | 330.99 ± 38.20 | 274.11s≦t5 < 292.79s | 292.79s≦t5 ≦ 369.19s | 369.19s<t5 ≦ 385.08s | 60 | 40 |
| | | | | 2 | 6 | 2 | | |
| | N6 | 419.09 | 250.5 | 303.73 ± 52.24 | 250.5s≦t6 < 251.49s | 251.49s≦t6 ≦ 355.97s | 355.97s<t6 ≦ 419.09s | 90 | 10 |
| | | | | 1 | 8 | 1 | | |
| | N7 | 355.01 | 275.13 | 313.99 ± 26.27 | 275.13s≦t7 < 287.72s | 287.72s≦t7 ≦ 340.26s | 340.26s<t7 ≦ 355.01s | 70 | 30 |
| | | | | 1 | 7 | 2 | | |
| | N8 | 398.40 | 207.47 | 316.33 ± 52.96 | 207.47s≦t8 < 263.37s | 263.37s≦t8 ≦ 369.29s | 369.29s<t8 ≦ 398.40s | 80 | 20 |
| | | | | 1 | 8 | 1 | | |
| | N9 | 379.01 | 243.34 | 297.56 ± 48.51 | 243.34s≦t9 < 249.05s | 249.05s≦t9 ≦ 346.07s | 346.07s<t9 ≦ 379.01s | 40 | 60 |
| | | | | 1 | 6 | 3 | | |
| | N10 | 367.59 | 226.32 | 284.50 ± 52.57 | 226.32s≦t10 < 231.93s | 231.93s≦t10 ≦ 337.07s | 337.07s<t10 ≦ 367.59s | 60 | 40 |
| | | | | 3 | 5 | 2 | | |
| 2 | N1 | 464.12 | 361.23 | 404.29 ± 28.37 | 361.23s≦t1 < 375.92s | 375.92s≦t1 ≦ 432.66s | 432.66s<t1 ≦ 464.12s | 30 | 70 |
| | | | | 1 | 8 | 1 | | |
| | N2 | 445.23 | 332.39 | 387.73 ± 36.87 | 332.39s≦t2 < 350.86s | 350.86s≦t2 ≦ 424.60s | 424.60s<t2 ≦ 445.23s | 10 | 90 |
| | | | | 2 | 6 | 2 | | |
| | N3 | 456.53 | 313.12 | 384.07 ± 47.70 | 313.12s≦t3 < 336.37s | 336.37s≦t3 ≦ 431.77s | 431.77s<t3 ≦ 456.53s | 40 | 60 |
| | | | | 2 | 5 | 3 | | |
| | N4 | 443.55 | 214.10 | 347.11 ± 71.94 | 214.10s≦t4 < 275.17s | 275.17s≦t4 ≦ 419.05s | 419.05s<t4 ≦ 443.55s | 30 | 70 |
| | | | | 2 | 6 | 2 | | |
| | N5 | 463.29 | 249.56 | 344.69 ± 68.92 | 249.56s≦t5 < 275.77s | 275.77s≦t5 ≦ 413.61s | 413.61s<t5 ≦ 463.29s | 20 | 80 |
| | | | | 1 | 8 | 1 | | |
| | N6 | 376.18 | 261.11 | 344.23 ± 38.04 | 261.11s≦t6 < 306.19s | 306.19s≦t6 ≦ 376.18s | 376.18s<t6 ≦ 382.27s | 80 | 20 |
| | | | | 1 | 8 | 1 | | |
| | N7 | 435.36 | 243.40 | 335.12 ± 56.88 | 243.40s≦t7 < 278.24s | 278.24s≦t7 ≦ 392s | 392s<t7 ≦ 435.36s | 70 | 30 |
| | | | | 2 | 6 | 2 | | |
| | N8 | 383.33 | 234.26 | 323.52 ± 44.73 | 234.26s≦t8 < 278.79s | 278.79s≦t8 ≦ 368.25s | 368.25s<t8 ≦ 383.33s | 90 | 10 |
| | | | | 2 | 7 | 1 | | |
| | N9 | 422.10 | 231.23 | 335.43 ± 72.55 | 231.23s≦t9 < 262.88s | 262.88s≦t9 ≦ 407.98s | 407.98s<t9 ≦ 422.10s | 70 | 30 |
| | | | | 3 | 5 | 2 | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| N10 | 390.37 | 235.02 | 300.00 ± 53.36 | 235.02s≦t10 < 246.64s | 246.64s≦t10 ≦ 353.36s | 353.36s<t10 ≦ 390.37s | 60 | 40 |
| | | | | 2 | 6 | 2 | | |

*In the table, N1 ~ N10 denote daily experiments across 10 days.

In Cues 0, the experimental fish showed a significant decrease in the time consumed, as shown in Figure. 4, and an increase in the completion rate in terms of choosing the correct direction, as shown in Figure. 5. The improvement in the time consumed was analyzed, as shown in Figure. 4A, and there was a statistically significant difference between N2 and N1 ($p < 0.05$). The improvement in the completion rate was also analyzed, as shown in Fig. 5A, and there was a statistically significant difference between N3 and N1 ($p < 0.05$). The stabilization of the time consumed was analyzed, as shown in Fig. 4B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$). Finally, the stabilization of the completion rate was analyzed, as shown in Fig. 5B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$).

In Cues 1, the experimental fish showed a significant decrease in the time consumed, as shown in Fig. 4, and an increase in the completion rate when choosing the direction, as shown in Fig. 5. The improvement in the consumption time was analyzed, as shown in Fig. 4A, and there was a statistically significant difference between N2 and N1 ($p < 0.05$). The improvement in the completion rate was also analyzed, as shown in Fig. 5A, and there was a statistically significant difference between N2 and N1 ($p < 0.05$). The stabilization of the time consumed was analyzed, as shown in Fig. 4B, and there was a statistically significant difference between N3 and Nt ($p < 0.05$). Finally, the stabilization of the completion rate was analyzed, as shown in Fig. 5B, and there was a statistically significant difference between N2 and Nt ($p < 0.05$).

## 3.2. Effects of different visual color cue conditions on spatial learning abilities of *Sebastes schlegelii* juveniles

The differences in the time consumed and completion rate between the fish in group A and those in group B in the three different visual color cue conditions are shown in Table 4. The difference in the time consumed by the *S. schlegelii* juveniles under the three visual color cue conditions was statistically significant ($p < 0.05$). The differences in the completion rates of the *S. schlegelii* juveniles under the three visual color cue conditions were statistically significant ($p < 0.05$). In this study, the *S. schlegelii* juveniles consumed the smallest amount of time, had the highest completion rate, and exhibited the best learning effect under the single visual color cue condition.

Table 4
Differences in time consumption and accuracy between group A and group B in the presence of three different visual color cue conditions.

| **Number of experiments** | **Sig.(two-tailed)** | | | |
|---|---|---|---|---|
| | **A** | | **B** | |
| | **Consumed time** | **Accuracy** | **Consumed time** | **Accuracy** |
| N1 | 0.563 | 0.088 | 0.563 | 0.239 |
| N2 | 0.025 | 0.010 | 0.327 | 0.195 |
| N3 | 0.392 | 0.000 | 0.647 | 0.106 |
| N4 | 0.873 | 0.002 | 0.375 | 0.960 |
| N5 | 0.021 | 0.005 | 0.125 | 0.803 |
| N6 | 0.563 | 0.014 | 0.769 | 0.038 |
| N7 | 0.878 | 0.002 | 0.873 | 0.594 |
| N8 | 0.166 | 0.007 | 0.563 | 0.383 |
| N9 | 0.034 | 0.011 | 0.418 | 0.245 |
| N10 | 0.034 | 0.000 | 0.612 | 0.670 |

# 4. Discussion

## 4.1. Learning and memory performance of *S. schlegelii* juveniles

The study of spatial cognition in teleost fishes has yielded diverse insights into interspecific learning capacities and memory retention patterns. Previous investigations have demonstrated substantial variability in associative learning abilities across species, often mediated by ecological adaptations and experimental paradigms. In foundational work by Sison and Gerlai (2010), zebrafish (*Danio rerio*) exhibited distinct behavioral signatures of spatial learning in plus-maze tasks, with trained individuals displaying reduced exploratory time and enhanced target orientation compared to naïve controls. This pattern aligns with findings in European bass (*Dicentrarchus labrax*) (Benhaïm et al., 2013;Covès et al., 2006;Di-Poï et al., 2007;Millot et al., 2008;Benhaïm et al., 2017), where both individual and social learning modalities facilitated efficient food acquisition, highlighting the species' notable behavioral plasticity. Subsequent research by DeRoy et al. (2020) further elucidated how intrinsic behavioral traits and navigational strategies modulate task performance in this marine species. Moreover, *Pterois volitans* individuals were trained for a period of 5 to 42 days to assess their memory retention. They showed large inter-individual learning differences. Half of the tested experimental fish learned maze navigation. In contrast, in this study, the three groups of *S. schlegelii* juveniles required significantly less time to find the food after maze training, and the rate at which they selected the correct direction was significantly higher. This suggests that the trained *S. schlegelii* juveniles were able to locate the food reward faster and more accurately. *S. schlegelii* have good spatial cognitive abilities, and they are able to establish associative memory perceptions of food rewards based on environmental visual color cues under different environmental conditions. In 2019, Mazzei et al. (2019) applied a patterning task versus a spatial task to examine *Elacatinus prochilos* in terms of habitat utilization and social behavior, seeking to determine differences in their ability to learn in two discriminative dichotomous tasks. The results indicated that most gobies performed poorly in the pattern cue task and well in the spatial cue task.

Schluessel's study demonstrated that *Chiloscyllium griseum* individuals retained spatial memory for up to six weeks without reinforcement (Schluessel & Bleckmann, 2012). In 2002, L.A. Dugatkin et al. found a relationship between male boldness and simple spatial learning (as measured through predator-checking behavior) in the species *Poecilia reticulata*. Focusing on associative learning (the association of brave and avoidant learning with foraging in experimental fish) (Dugatkin & Alfieri, 2003), their study also found that the experimental fish exhibited memory retention for up to ten days. The results of the memory retention test showed that juvenile *S. schlegelii* could maintain stable spatial memory for at least 7 days after training, which is consistent with the findings of previous studies on this species (Zhang et al., 2021).In our future studies, we will set a longer period of time for memory verification so as to extend the duration of memory retention of the experimental fish and investigate the period of time for which these abilities can be maintained. The present experiment only provides a preliminary reference for the study of learning and memory in the juvenile *S. schlegelii*.

## 4.2. Effects of visual color cues on learning and memory in *Sebastes schlegelii* juveniles

The capacity for spatial cognition in vertebrates is fundamentally mediated through two distinct neurobehavioral mechanisms: egocentric orientation (self-referenced navigation based on body axis alignment) and allocentric orientation (environment-referenced navigation using external visual cues) (Sovrano et al., 2020). Our experimental findings suggest that juvenile *S. schlegelii* may adopt different spatial cognitive strategies under different environmental conditions: they may rely on egocentric body turning strategy in the absence of visual color cues, and switch to allocentric orientation strategy using visual color cues when environmental landmarks are available. After training with the three sets of visual color cue conditions, the rate of correct direction selection among the fish increased significantly, indicating that they could associate turning to the left and right with obtaining food rewards for correct spatial recognition. In the experiment in the presence of visual color cues, although the direction of visual color cues placement was changed during training, the rate of correct direction selection among the experimental fish increased significantly, indicating that they could associate visual color cues with obtaining food rewards for spatial recognition. These results suggest that *S. schlegelii* juveniles use different spatial cognitive mechanisms under different environmental conditions. In the absence of visual color cues, they were able to determine which direction to choose by turning their bodies to the left or right; in the presence of visual color cues, they were able to determine which direction to choose based on the location of the visual color cues.

Visual color cues can have a somewhat more significant effect on the visual capacities of *S. schlegelii* juveniles than the stimulus memory of the fish itself, which has a stronger sense of direction. In the presence of one visual color cue, the final completion rate of the experimental fish was the highest among the three groups, followed by the no-cue condition, and the lowest completion rate was observed in the presence of two different visual color cue conditions. The number of training sessions required for the fish in group A to reach significant learning improvement was 2 sessions in Cues 1, 3 sessions in Cues 0, and 3 sessions in Cues 2. In this experiment, the mean completion rate in descending order was 70% for Cues 1, 55% for Cues 2, and 49% for Cues 0. For group B, the number of training sessions required to reach significant learning improvement was 2 sessions in Cues 1, 2 sessions in Cues 0, and 2 sessions in Cues 2. The mean completion rate in descending order was 51% for Cues 1, 50% for Cues 2, and 48% for Cues 0. In 2022, Long Jiaxing investigated individual spatial learning in cichlids and its effect on group decision-making regarding spatial learning (Long & Fu, 2022). The results showed that the cichlids accessed the arm of the plastic aquatic grass cue faster and with higher accuracy. The difference in the number of training

sessions required for the three groups of different visual color cue conditions in this experiment shows that the higher the mean value of the completion rate, the more training sessions required and greater stabilization is reached; meanwhile, the lower the mean value of the completion rate, the fewer training sessions required and lower stabilization is reached. Zhu (2007) showed that, after the same number of training sessions, the average correctness rate for zebrafish in the presence of one visual color cue was significantly higher than that in the presence of two visual color cues. Our results align with these findings. In Cues 2 group, the experimental fish needed to distinguish the difference between two visual color cues and make a choice. This discrimination process increased the memory load and task difficulty, so Cues 2 group showed the lowest completion rate throughout the whole learning process. In Cues 0 group, the experimental fish needed to complete the spatial task without any visual reference, so this group showed a slightly lower completion rate throughout the learning process. In this regard, the following explanations can be provided for this phenomenon. Cueing effect of visual color cues: When one visual color cue was present in the experiment, the fish could use this single visual color cue as a clear reference point, which simplified the decision-making process in the maze. A visual color cue provides a clear environmental cue that reduces the disorientation of the fish when searching for food in the maze, making it easier for them to form a conditioned reflex to remember the path and make the correct choice. This is consistent with learning theory: a single, clear cue helps experimental animals establish the link between the path and reward (food) more quickly (Zhu & Liu, 2015). The results of our experiments show that, regardless of which strategy is used for learning memory, *S. schlegelii* juveniles can achieve a relatively high level of correctness after 10 training sessions.

Fish appear to be capable of learning more complex spatial relationships. Studies have shown that *Betta splendens* Regan have the ability to recognize spatial relationships independently of any orientation preference regarding their surroundings (Roitblat et al., 1982). This allows the fish to take shortcuts or choose between alternative routes to a target. Moreover, the accuracy achieved by *Betta splendens* Regan in an eight-arm radial maze suggests that some spatial memory capacity is required in recognizing which of the eight arms have no food reward. The fact that fish are able to detect changes in their environment (Welker & Welker, 1958) and show an organized pattern of exploration when entering new environments (Kleerekoper et al., 1974) also suggests that they have the ability to memorize associations between food and cues. In this experiment, the fish consumed the smallest amount of time in the presence of one visual color cue, followed by the no-cue condition, while the most time was consumed in the presence of two different visual color cues. The results of this study indicate that these fish have the ability to associate food with visual color cues due to memory, which is consistent with the results of the *Betta splendens* Regan study. In our study, the minimum number of trials required for group A fish to reach significant learning improvement was 2 trials in Cues 1, 2 trials in Cues 0, and 3 trials in Cues 2. The mean consumed time of group A in ascending order was 222.22 s in Cues 1, 274.08 s in Cues 0, and 325.01 s in Cues 2. For group B, the mean consumed time in ascending order was 328.25 s in Cues 1, 338.94 s in Cues 0, and 350.62 s in Cues 2. Both groups showed the same trend: the shortest consumed time and fastest learning speed were observed in the single visual color cue condition (Cues 1). In the presence of one visual color cue, the juvenile *S. schlegelii* individuals possessed the strongest learning abilities and exhibited the fastest learning speeds. Zhu (2007) found that an environment with only one visual color cue present was favorable for spatial learning and memory in female *Rhodeus ocellatus*, and the present study is consistent with these findings.

Previous studies have demonstrated developmental variations in fish learning capacities. Notably, Masuda and Ziemann (2010) identified a non-linear relationship between body size and cognitive performance in *Polydactylus sexfilis*, with 50 mm and 90 mm individuals exhibiting superior learning abilities compared to smaller (22 mm, 36 mm) or larger (130 mm) conspecifics. However, our experimental results diverged from these findings. Groups A and B of *S. schlegelii* juveniles displayed comparable learning and memory retention across three distinct cue types, despite systematic differences in their body lengths. Although our experimental groups exhibited measurable size variations, all specimens remained within the juvenile developmental stage. The limited ontogenetic range in our sample likely minimized functional differences in neural development or sensory processing capacities. Nonetheless, experimental fish with larger differences should be studied in subsequent experiments for the in-depth study of their learning and memory abilities at different developmental stages. This could lead to a deeper understanding of fish's behavioral patterns, including their foraging strategies, predator avoidance behaviors, and habitat selection. The findings of this study have direct guiding significance for the aquaculture and stock enhancement of *S. schlegelii*: in industrial aquaculture, setting a single clear visual color marker in the feeding area can help juveniles quickly locate the feeding point, reduce ineffective swimming and feed waste, and improve the feeding efficiency of cultured populations. In the construction of marine ranching, setting fixed visual color landmarks in the release area may also help the released juveniles quickly adapt to the wild environment and improve the survival rate of enhancement and release. This study provides a scientific basis for the behavioral regulation of this species in aquaculture and ecological conservation.

This study has several limitations. First, the sample size of 10 individuals per group may limit the statistical power, and larger sample sizes are needed to confirm the results. Second, only green and red color cues were tested, and future studies should investigate the effects of different colors, brightness levels, and cue shapes. Third, this study was conducted under controlled laboratory conditions, and field experiments are required to validate the findings in natural environments.

## 5. Conclusions

This study suggests that juvenile *S. schlegelii* can establish stable associative learning between visual color cues and food rewards through maze training, with the best learning performance under a single clear visual color cue condition. Juveniles at the same developmental stage show consistent spatial learning and memory abilities regardless of body size differences.

Studying the spatial learning and memory characteristics of fish is of great significance for fishery stock enhancement and aquaculture management. In aquaculture production, rational use of visual marking cues can help fish quickly adapt to new environments and locate feeding areas, improve foraging accuracy, reduce ineffective exploratory behavior and feed waste, thus lowering production costs and improving economic benefits. Meanwhile, rational simplification of the culture environment and application of single clear cues can reduce the stress response of fish caused by environmental changes, and improve the survival rate of cultured individuals. This study provides a basic reference for the behavioral regulation of *S. schlegelii* in aquaculture and the habitat construction of marine ranching.

## Declarations

**Supplementary Materials:** Not applicable.

**Author Contributions:** Conceptualization, Y.Z. and X.Y.; methodology, T.T.; software, Y.Y.; validation, Z.W.; formal analysis, Y.Z.; investigation, Y.Z.; resources, Y.Z.; data curation, Y.Z.; writing—original draft preparation, Y.Z.; writing—review and editing, Q.Z. and Y.Z.; visualization, Y.Z.; supervision, T.T.; project administration, T.T.; funding acquisition, T.T. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The animal study protocol was approved by the Ethics Committee of Dalian Ocean University (protocol code 20230706-2).

**Informed Consent Statement:** Not applicable.

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## Figures

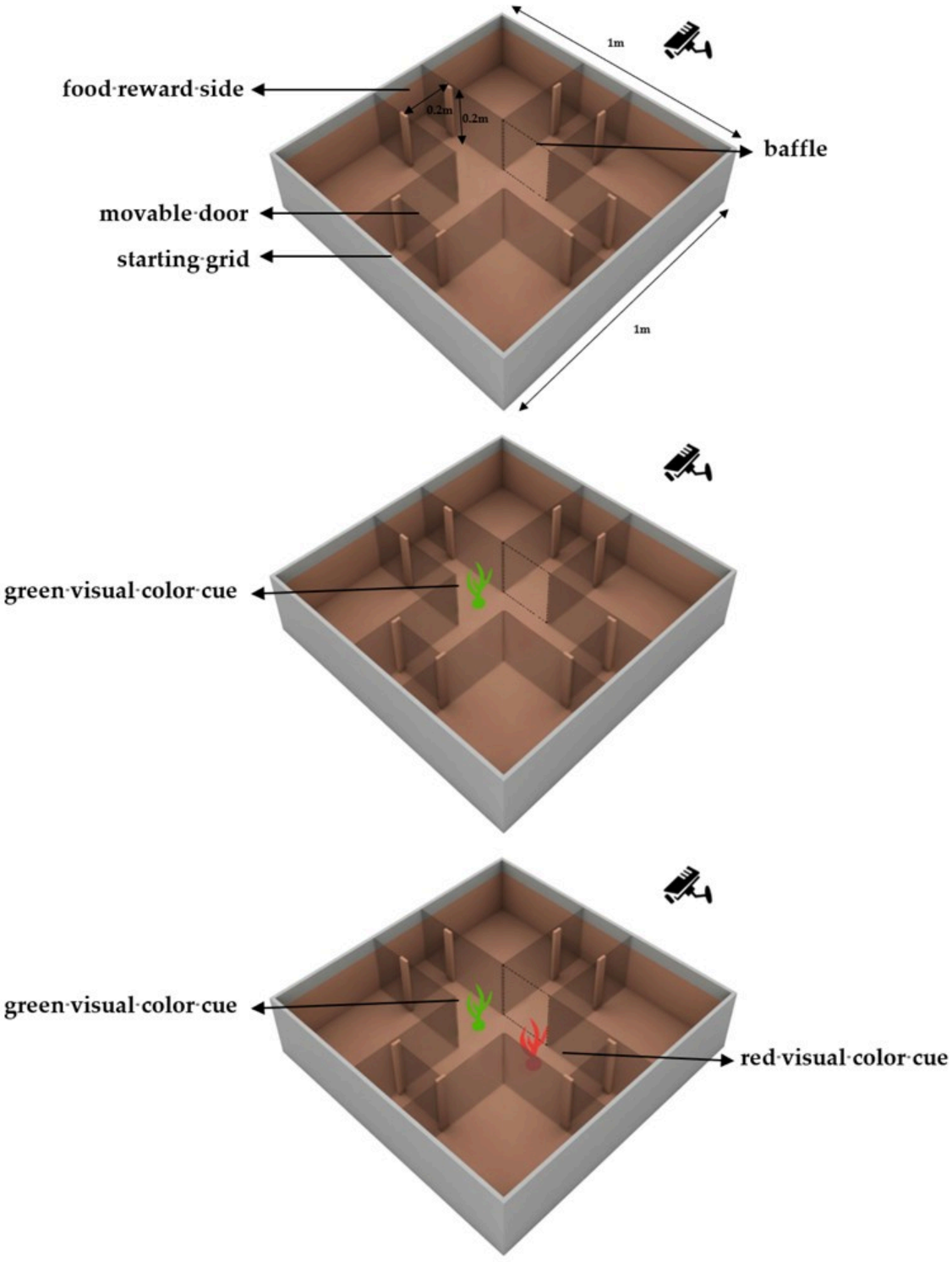



**Figure 1**

Schematic diagram of the custom-built cross-shaped convertible T-maze apparatus. Green and red artificial aquatic plant visual cues were vertically fixed at the exact center of each choice arm to eliminate visual obstruction and ensure unobstructed visibility from the starting compartment.

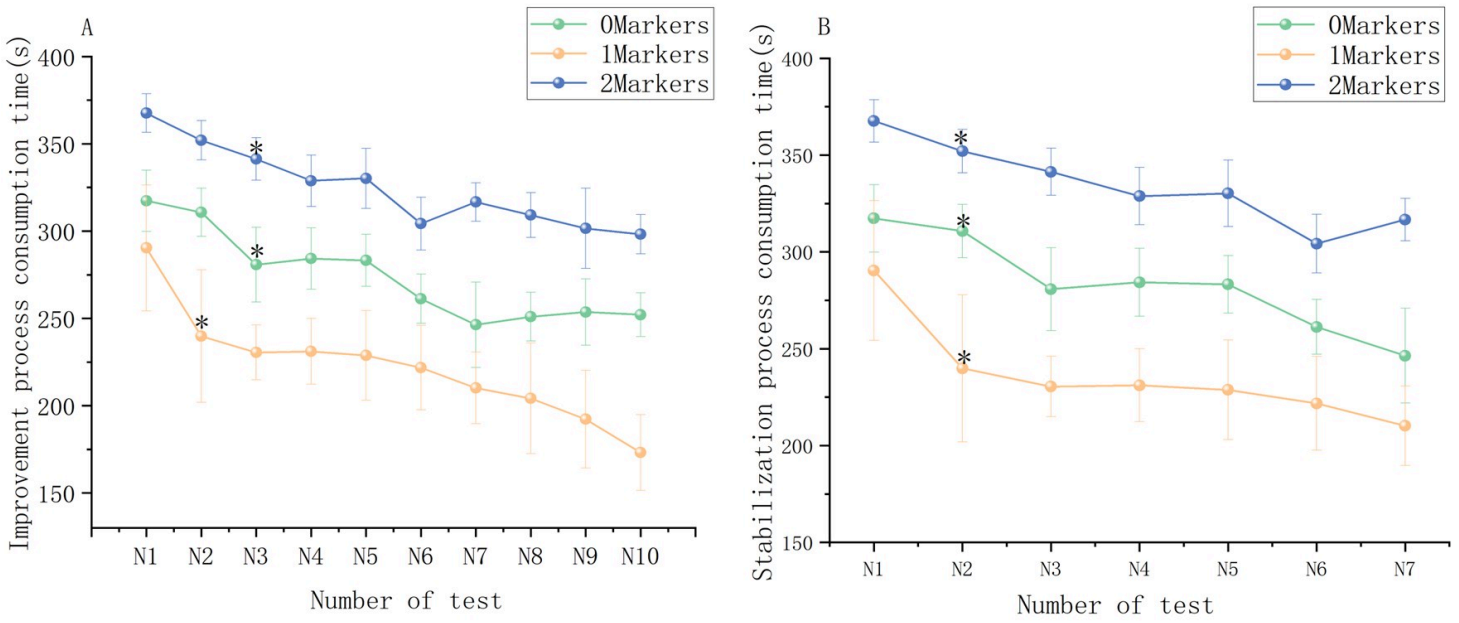



**Figure 2**

Time spent by fish in group A under the three visual color cue conditions. (A) Analysis of improvement in time consumed by group A fish; * indicates that there was a statistically significant difference between N* and N1 ($p < 0.05$). (B) Analysis of stabilization in time consumed by group A fish; * indicates that there was a statistically significant difference between N* and Nt ($p < 0.05$).

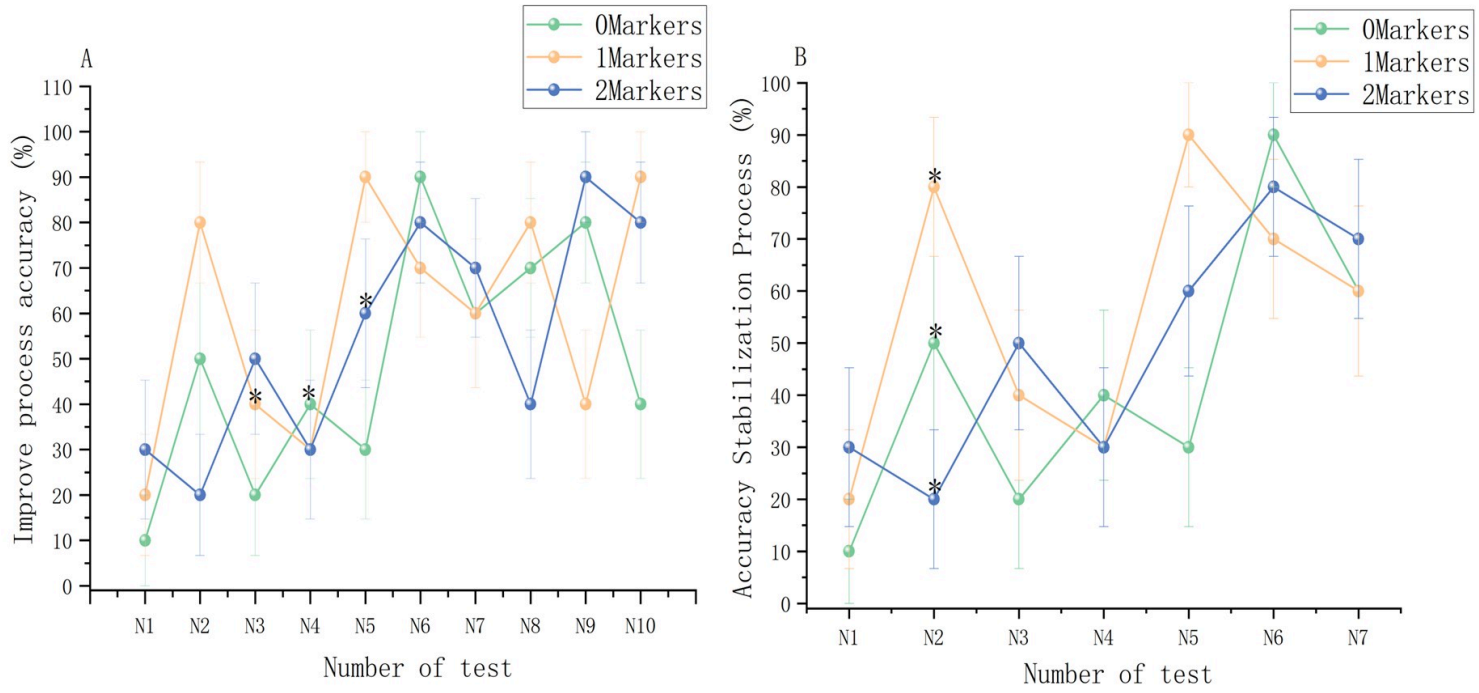



**Figure 3**

The accuracy of the fish in group A in the case of the three visual color cue conditions. (A) Improvement in the accuracy of group A fish; * indicates that there was a statistically significant difference between N* and N1 ($p < 0.05$). (B) Stabilization in the accuracy of group A fish; * indicates that there was a statistically significant difference between N* and Nt ($p < 0.05$).

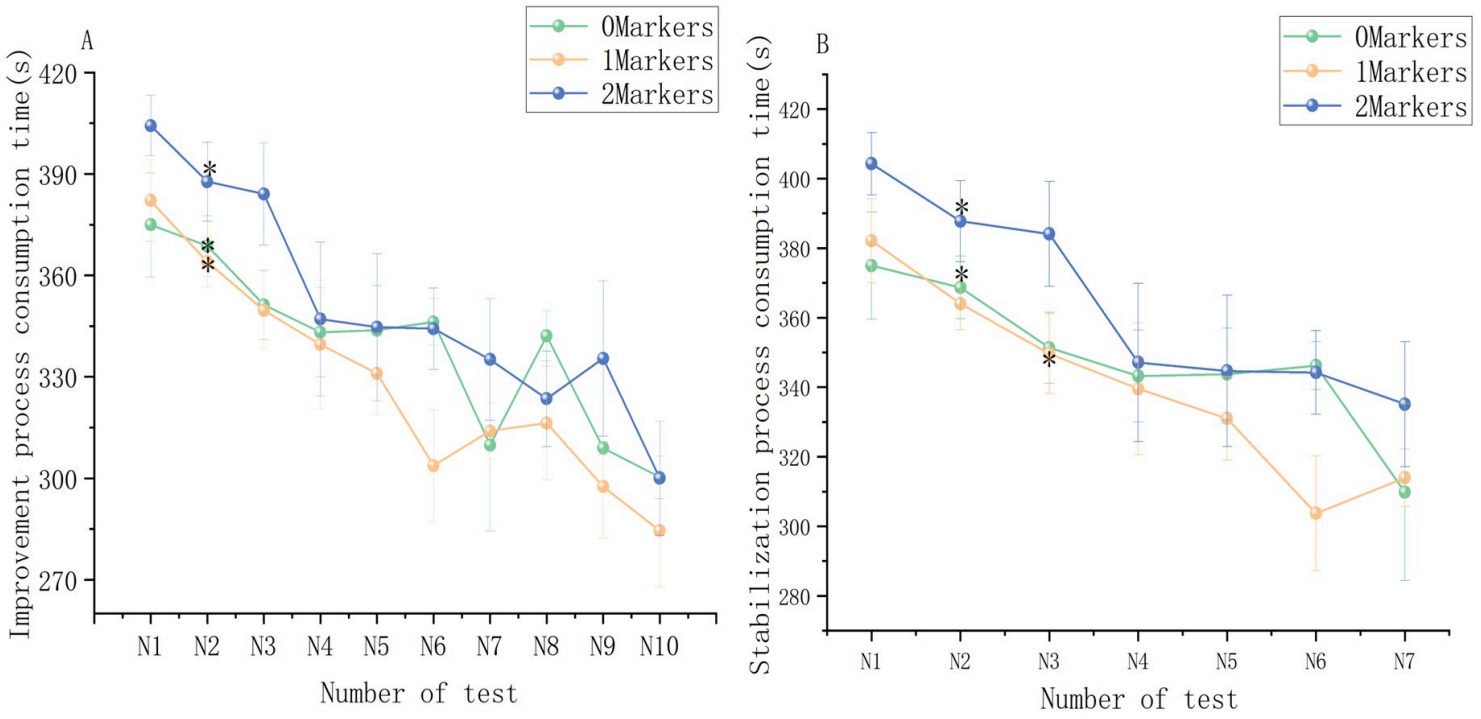



**Figure 4**

Time spent by fish in group B under the three visual color cue conditions. (A) Analysis of the improvement in the time consumed by group B fish; * indicates that there was a statistically significant difference between N* and N1 ($p < 0.05$). (B) Analysis of stabilization in time consumed by group B fish; * indicates that there was a statistically significant difference between N* and Nt ($p < 0.05$).

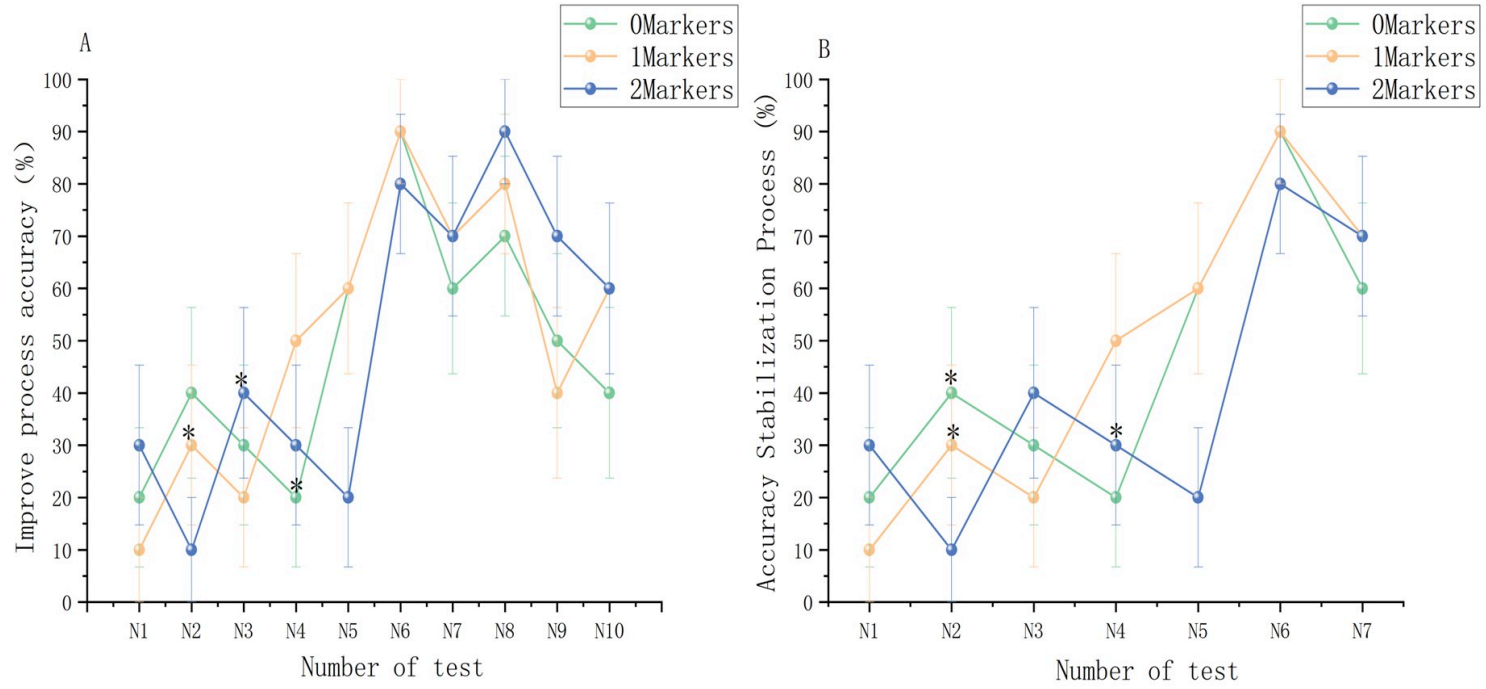



**Figure 5**

The accuracy of the fish in group B in the case of three visual color cue conditions. (A) Improvement in accuracy rate for group B fish; * indicates that there was a statistically significant difference between N* and N1 ($p < 0.05$). (B) Stabilization of accuracy rate of group B fish; * indicates that there was a statistically significant difference between N* and Nt ($p < 0.05$).

## Supplementary Files

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