

## Article

# Compatibilities of *Cyprinus carpio* with Varied Colors of Robotic Fish

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**Abstract:** Visual selection plays a fundamental role in various aspects of animal behavior, such as colony formation, maintenance, defense, and courtship. This study investigated the effect of bionic robot fish color on carp behavior based on physiological characteristics that were observed during behavioral experiments. Through computer image processing and analysis of light attenuation, we observed changes in the number and positioning of carp with bionic robotic fish of different colors (white, red, blue, green, and yellow). The results indicated that (1) the attenuation coefficient of visible light in freshwater was red > yellow > green > blue; (2) the order of the average change in the number of carp responding to different colors of robotic fish was white > red > green > yellow > blue, and carp were more sensitive and responsive to white and red robotic fish; and (3) the order of the distances between different colors of robotic fish and carp was white < yellow < blue < green < red, and white and yellow robotic fish were more attractive to carp. Therefore, the use of white or yellow robotic fish for relevant operations can reduce disturbance to fish schools.

**Keywords:** *Cyprinus carpio*; animal behavior; optomotor response; bionic robot; color; image correction



**Citation:** Huang, X.; Zhang, Y.; Chen, X.; Kong, X.; Liu, B.; Jiang, S.

Compatibilities of *Cyprinus carpio* with Varied Colors of Robotic Fish. *Fishes*

2024, 9, 211. <https://doi.org/10.3390/fishes9060211>

Academic Editor: Zhangying Ye

Received: 23 April 2024

Revised: 30 May 2024

Accepted: 30 May 2024

Published: 3 June 2024



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**Key Contribution:** Our findings suggest that different colors of robotic fish showed significant variability in their compatibility with fish schools. Therefore, using white or yellow robotic fish for these operations can reduce disturbance to fish schools.

## 1. Introduction

Color vision is a crucial characteristic of fish, which rely on color discrimination for numerous tasks [1,2]. Choice is one of the basic abilities animals have [3]. Animals perceive color through visual color selection, which plays an important role in animal foraging, reproduction, and communication [4,5].

It is known that most teleost fish have the ability to distinguish colors [6–10]. There are two types of visual cells in the retina of fish eyes: rod cells and cone cells [11]. Rod cells mainly sense light and can distinguish differences in light intensity. Cones sense wavelengths of light and can distinguish colors [12]. Most fish have more than three types of visual photoreceptors, which makes them ideal for studying color vision and color selection [13–15]. Shallow water-dwelling teleost species have a wider spectral range, with at least four cones. These include long-to-middle-wave class (LWS) cones, which are maximally sensitive to the red–green spectral region from approximately 490 to 570 nm. Additionally, middle-wave class (RH2) cones are sensitive to the green portion of the spectrum, with peak sensitivity spanning roughly 480 to 535 nm. Short-wave class

(SWS2) cones detect blue–violet light, with peak sensitivity ranging from about 410 to 490 nm. Finally, second short-wave class (SWS1) cones are sensitive to the violet–ultraviolet spectrum, peaking from approximately 355 to 440 nm [13]. Tetrachromatic vision is common in Cyprinidae-like goldfish (*Carassius auratus*) [16,17], zebrafish (*Danio rerio*) [18,19], and guppies (*Poecilia reticulata*) [20]. Tetrachromatic vision is common in Cyprinidae.

This study chose carp as the study subject because the carp is an important freshwater species with a long history as an aquaculture fish and is distributed worldwide [21,22]. In addition, this species has cone cells for distinguishing red, green, blue, and ultraviolet light [13,14,16,23], making it suitable for behavioral experiments of color selection [22].

There are differences in the choice of color by fish, and they are more inclined to be attracted to natural colors like white and blue. For example, juvenile carp showed the most obvious response to red under artificial light [24,25], whereas Nile tilapia preferred blue and green based on the preference index [26], and zebrafish preferred red [27,28]. Furthermore, the mosquitofish was more likely to be attracted to yellow robotic fish, as large amounts of yellow pigmentation in males are generally related to higher social ranks in mosquitofish populations [29]. Even within species of the same genus, there were differences in color choice. In a color selection test, *Schizothorax prenanti* was mainly distributed in the green and blue areas, whereas *Schizothorax oconnori* was mainly distributed in the green and yellow areas [30]. Most behavioral experiments on fish color vision were carried out using materials such as lighting, paint, and cardboard to change the environmental color. However, these experiments separated fish groups from stimuli, which limited the study of color choice by fish.

As an important tool to study animal behavior, in recent years, bionic robots have been widely used in the study of animal social behavior because of their controllability and the repeatability of experiments [31–37]. Compared with a traditional mobile platform and axial propeller underwater robots, bionic underwater robots have the advantages of low noise and environmental friendliness [38–41]. Bionic robots can be used in animal behavior experiments to reduce the interference of the environment and improve the accuracy of the experiment [42].

In exploring the correlation between the optomotor responses of fish and the appearance of robotic fish, we found that zebrafish and golden shiner (*Notemigonus crysoleucas*) preferred to interact with bionic robotic fish that were similar in shape and color to live fish [43,44]. Studies have shown that the closer a robot's color, pattern, and size were to that of real fish, the more attractive it was to the studied fish. The use of bionic robots for fish behavioral testing has significant advantages because they have a similar shape and structure to fish and are capable of autonomous movement; therefore, the characteristics of fish optomotor responses are more accurate. In addition, research findings on color recognition and selection preferences in fish are inconsistent. Although the optic rod and cone cells of the fish eye are capable of sensing light intensity and spectral wavelengths, further studies are needed to determine whether fish are able to accurately recognize different colors and generate corresponding behavioral responses or selective preferences. In this paper, we focused on carp preference for different colors and investigated the interaction between live fish and robotic fish by observing the responses of carp to the position and movement behaviors of robotic fish of different colors.

Previous research has demonstrated that robot-influenced behavior in fish can be observed [45–47]. Color vision experiments in fish should be performed at a distance of less than 1 m [11,48]. This study used behavioral experiments to explore animal physiological characteristics when studying the color preference of animals with the help of biomimetic robots. The robotic fish used in the experiment swims by swinging its tail fin, and white, red, green, blue, and yellow were selected as the colors of the robotic fish [26,49–54].

Using a camera to record experiments causes the pictures to have trapezoidal distortion. In this study, we used the perspective transformation formula and computer technology to correct the experimental image [55,56]. The color of the robotic fish is determined by the visible light it reflects [57]; therefore, previous studies included analyses

of the decay process of visible light underwater [11,58]. In nature, color choice is often related to several aspects such as foraging, reproduction, and peer recognition. Therefore, exploring the responses of fish to different colors not only helps elucidate their basic behavioral habits but also has important implications for ecological conservation and fishery management [33,36].

By studying the proximity of carp to robotic fish in varied colors, we could obtain the color preference of carp. This research has several application prospects. For example, in terms of aquaculture, understanding fish visual preferences can provide a better breeding environment for fish and reduce the interference to the biological living environment when using mobile equipment to monitor fish [26,59]. Furthermore, using color preference to trap a target species can increase the catch of the target fish and reduce the by-catch [60,61].

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Animals

Eighty carp (*Cyprinus carpio*) were used in the experiment, with body lengths ranging from 10 cm to 30 cm. As shown in Figure 1, in the experiments conducted in natural water bodies, the carp population exhibited a natural distribution of colors, predominantly featuring white, yellow (with wavelengths ranging from 577 nm to 597 nm), and orange (with wavelengths ranging from 597 nm to 622 nm). In addition, the fish lived in shallow open water and were in good physiological condition during the experiment. We did not observe any signs of distress, discomfort, or pain during the course of the experiment. Carp were fed special feed twice a day in the morning and evening, and each feeding consisted of 2% to 5% of the total weight of the fish.

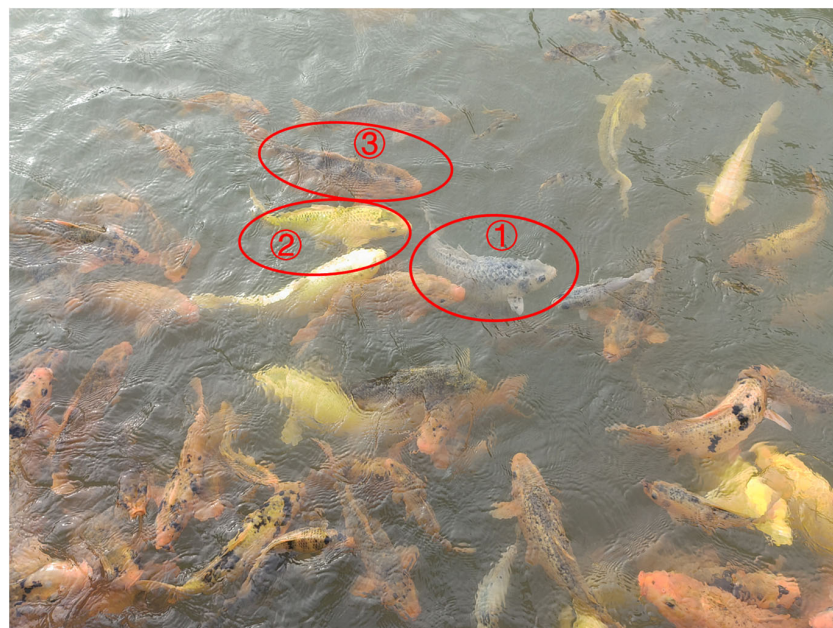


**Figure 1.** *Cyprinus carpio* used in the experiment. ① White fish, ② yellow croaker with a wavelength range of 577~597 nm, ③ orange carp with a wavelength range of 597~622 nm.

Before starting the experiment, it was crucial to consider the stress response of fish to the experimental environment. In our study, we introduced different colored robotic fish into the experimental pool for acclimation experiments. Specifically, five groups of robotic fish, each with a different color, were placed into the experimental pool. Observations started when carp showed evidence of approaching the robotic fish at a distance of approximately 2 m. Video recordings for data acquisition finished when the animal group displayed a distance of 0.5 m from the robotic fish. As shown in Figure S1, each group

of robotic fish was exposed to five acclimation experiments, and each experiment was conducted independently. During each training session, the counting process was repeated three times to ensure the accuracy and reliability of the data. There was a 5 min interval between exposures, which was used to ensure the reliability of the behavioral data and to prevent any carry-out effects from one exposure to the subsequent one. The standard error formula for calculating each group of experiments is the following:

$$\sigma = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n}} \quad (1)$$

### 2.1.2. Bioinspired Robotic Fish

The social behavior of fish is often influenced by their size [43,62]. The robotic fish designed for this experiment replicated the body characteristics of the target fish species, with a length of 34.5 cm, a height of 13 cm, and a width of 2 cm. Two AAA batteries supplied energy for the robotic fish motor. The average swimming speed in the water was 0.1 m/s, and the swing frequency of the fish tail in the air was  $1.735 \pm 0.123$  Hz.

As shown in Figure 2, the robot design included visible fish anatomy, such as a dorsal fin and a caudal fin. The robotic fish body was made of light wood, and two foam boards fit the shape of the fish on the left and right sides to maintain balance in the water. Wood and foam boards provided buoyancy for the robot fish, which allowed it to float in the water. A fin-shaped board was attached to the top of the fish body to make the movement of the robot fish in the water more similar to the movement of real fish. The board was divided into three parts that were connected by transparent plates with slight elasticity to make the tail swing more realistic. Motor operation was used to achieve a certain range of back-and-forth fish tail swinging to facilitate the swimming of the robot fish in the water.

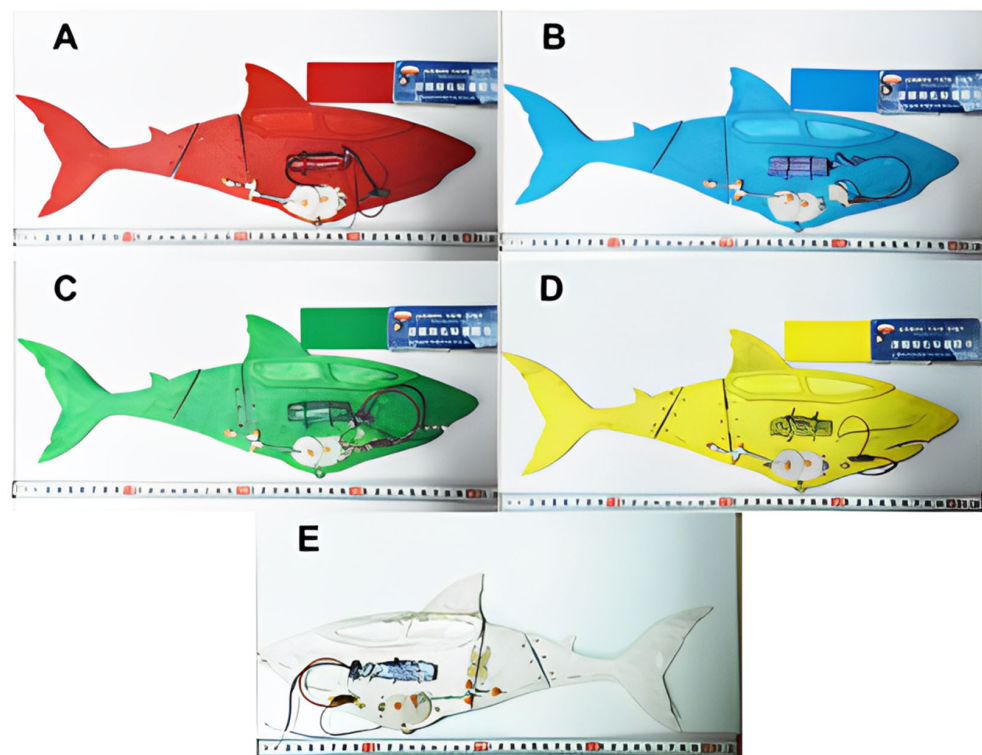


**Figure 2.** Robotic fish used in the experiment. Acrylic paint was used to paint the robotic fish. The painted robotic fish were compared with a national standard color card (GSB05-1426-2001) under natural light. (A) Bright red (R03) robotic fish; (B) light (phthalide) blue (PB06) robotic fish; (C) light green (G02) robotic fish; (D) lime yellow (Y05) robotic fish; (E) titanium white robotic fish.

### 2.1.3. Apparatus

We chose red, blue, green, yellow, and white as the colors of the robotic fish. As shown in Figure 3, we set up the camera equipment and the shooting area in advance. During the experiment, a camera (Nikon D3500) was used to record the experimental phenomena.

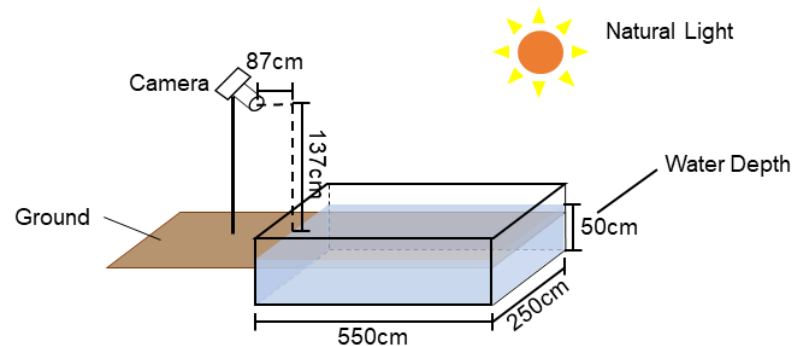


**Figure 3.** Schematic of the experimental setup. The experimental area was 5.50 m long and 2.50 m wide. The water depth was 0.5 m. The midpoint of the front end of the camera lens was 87 cm away from the experimental pool in the horizontal direction and 137 cm away from the horizontal ground in the vertical direction.

As shown in Figure 4, the advantage of choosing natural water for the experimental site was that it ensured that 80 experimental fish were in a natural state and therefore improved the accuracy of the experimental results [9].



**Figure 4.** Image of the experimental area taken with a camera (Nikon D3500).

## 2.2. Methods

### 2.2.1. Experimental Operation

Before the experiment, we randomly selected a color of robotic fish to place in the experimental pool. We allowed carp shoals 3 to 5 min to habituate to the experimental environment. Simultaneously, we measured the experimental pool water temperature, atmospheric pressure, air temperature, humidity, wind direction, and wind strength.

To maintain consistent conditions throughout the formal experiment, we conducted the experiment at 8:30–17:30 on sunny days because there was sufficient light during this period, the average ambient temperature was around 23.5 °C, the temperature did not change much, and the average water temperature was 21 °C.

When a carp school was evenly distributed in the observation tank, robot fish were placed in a fixed position on the edge of the tank, and video was then recorded. Each group of robot fish with different colors was continuously observed for 20 min. A 5 min interval was allowed between each robotic fish experiment to enable the fish to disperse and engage in free activity, ensuring the reliability of behavioral data and avoiding any lingering influences from previous exposures. To further ensure that the carp's behavior was not influenced by familiar or predictable stimuli, we limited the number of experiments with five groups of robot fish of different colors to two rounds per day. The order of colors presented each day was random to prevent the fish from exhibiting adaptive behavior to specific colors.

### 2.2.2. Image Correction

Images were affected by the tilted shooting of the camera toward a center point on the plane, and distortion occurred. To facilitate data acquisition and image analysis, we needed to correct the experimental screenshots in advance. The actual purpose of correcting the picture was to convert the obliquely shot image to a bird's-eye view [63]. The correction method selected was perspective transformation, which is also called projection mapping; this method projects the original image onto a new plane.

We used the general projection transformation formula [64] (Equation (2)):

$$\begin{bmatrix} x & y & z \end{bmatrix} = \begin{bmatrix} u & v & w \end{bmatrix} \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix} \quad (2)$$

$$A = \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix} \quad (3)$$

$$B = \begin{bmatrix} u & v & w \end{bmatrix} \quad (4)$$

$$C = \begin{bmatrix} x & y & z \end{bmatrix} \quad (5)$$

Equation (2) can be divided into three parts: Equation (3) is the projection mapping matrix, Equation (4) is the coordinates of the point on the original image, and Equation (5) is the coordinate after perspective transformation. For ease of understanding, the matrix in Equation (3) can also be divided into four parts:  $\begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix}$  represents affine transformation;  $\begin{bmatrix} a_{13} \\ a_{23} \end{bmatrix}$  represents the translation item;  $\begin{bmatrix} a_{31} & a_{32} \end{bmatrix}$  represents the transformed characteristic; and  $[a_{33}]$  is the scaling factor of the above-mentioned transformed characteristic. Because the image is a two-dimensional plane, we set  $z = w = 1$ . Additionally, to facilitate the operation, we set  $a_{33} = 0$ . To perspective transform an image, we first needed to obtain the mapping matrix A.

Through further matrix operations, the abscissa coordinates of the transformed image were obtained:

$$\begin{cases} x = \frac{a_{11}u + a_{21}v + a_{31}}{a_{13}u + a_{23}v} \\ y = \frac{a_{12}u + a_{22}v + a_{32}}{a_{13}u + a_{23}v} \end{cases} \quad (6)$$

The terms were shifted on both sides of Equation (6):

$$\begin{cases} a_{11}u + a_{21}v + a_{31} - a_{13}ux - a_{23}vx = 0 \\ a_{12}u + a_{22}v + a_{32} - a_{13}uy - a_{23}vy = 0 \end{cases} \quad (7)$$

The above formula (Equation (7)) was then expressed in matrix form:

$$\begin{bmatrix} u & v & 1 & 0 & 0 & 0 & -ux & -vx \\ 0 & 0 & 0 & u & v & 1 & -uy & -vy \end{bmatrix} \begin{bmatrix} a_{11} \\ a_{21} \\ a_{31} \\ a_{12} \\ a_{22} \\ a_{32} \\ a_{13} \\ a_{23} \end{bmatrix} = 0 \quad (8)$$

From this, it was found that a point can obtain two sets of equations about the mapping matrix. Because we set  $a_{33} = 0$ , there were 8 unknowns to be solved for the mapping matrix, which were obtained by using the coordinates of the four target points. By manually selecting four points of the original image and the corrected image, the formula for solving the unknowns of the mapping matrix can be obtained.

$$\begin{bmatrix} u(1) & v(1) & 1 & 0 & 0 & 0 & -u(1)x(1) & -v(1)x(1) \\ 0 & 0 & 0 & u(1) & v(1) & 1 & -u(1)y(1) & -v(1)y(1) \\ u(2) & v(2) & 1 & 0 & 0 & 0 & -u(2)x(2) & -v(2)x(2) \\ 0 & 0 & 0 & u(2) & v(2) & 1 & -u(2)y(2) & -v(2)y(2) \\ u(3) & v(3) & 1 & 0 & 0 & 0 & -u(3)x(3) & -v(3)x(3) \\ 0 & 0 & 0 & u(3) & v(3) & 1 & -u(3)y(3) & -v(3)y(3) \\ u(4) & v(4) & 1 & 0 & 0 & 0 & -u(4)x(4) & -v(4)x(4) \\ 0 & 0 & 0 & u(4) & v(4) & 1 & -u(4)y(4) & -v(4)y(4) \end{bmatrix} \begin{bmatrix} a_{11} \\ a_{21} \\ a_{31} \\ a_{12} \\ a_{22} \\ a_{32} \\ a_{13} \\ a_{23} \end{bmatrix} = 0 \quad (9)$$

The coordinate points obtained from the original image were  $(u(n), v(n))$ ,  $n = 1, 2, 3, 4$ . The corrected coordinate points were  $(x(n), y(n))$ ,  $n = 1, 2, 3, 4$ . After obtaining the projection mapping matrix A, we performed projection transformation on each pixel in the original image. Top-view images of the experiments were obtained using computer processing.

Perspective transformation was performed on each pixel in the original image by the Matlab program. The processed pixels were reassembled into a new image. The image comparison before and after correction is shown in Figure 5.

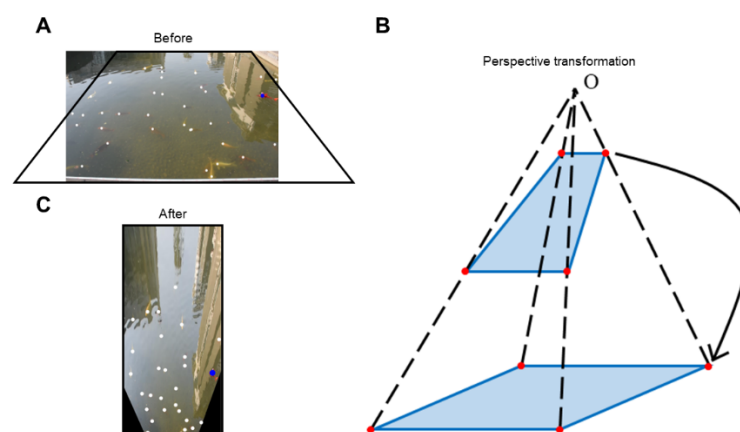


**Figure 5.** Images before and after correction. In the image, the position of the carp is represented by a solid white circle, and the position of the robotic fish is represented by a solid blue circle. (A) An original experimental image; (B) schematic diagram of perspective transformation; (C) image corrected by perspective transformation.

### 2.2.3. Experimental Data Extraction

The image data of each group of experiments were obtained from the experimental video, and the time interval of each image was 5 s. Figure 6 shows four images acquired at

5 s intervals in the experimental video. Taking the upper left corner of the processed image as the coordinate origin, the horizontal direction to the right side of the image was set as the  $x$ -axis direction, and the vertical direction to the lower side of the image was set as the  $y$ -axis direction to establish a Cartesian coordinate system. The two-dimensional positions of carp and robotic fish in the image were manually obtained. The distance between the carp and the robot fish at each position point was obtained by further calculation. A natural coordinate system was established with the robotic fish as the center. The experiment for each colored robotic fish (white, red, blue, green, and yellow) was replicated 12 times.

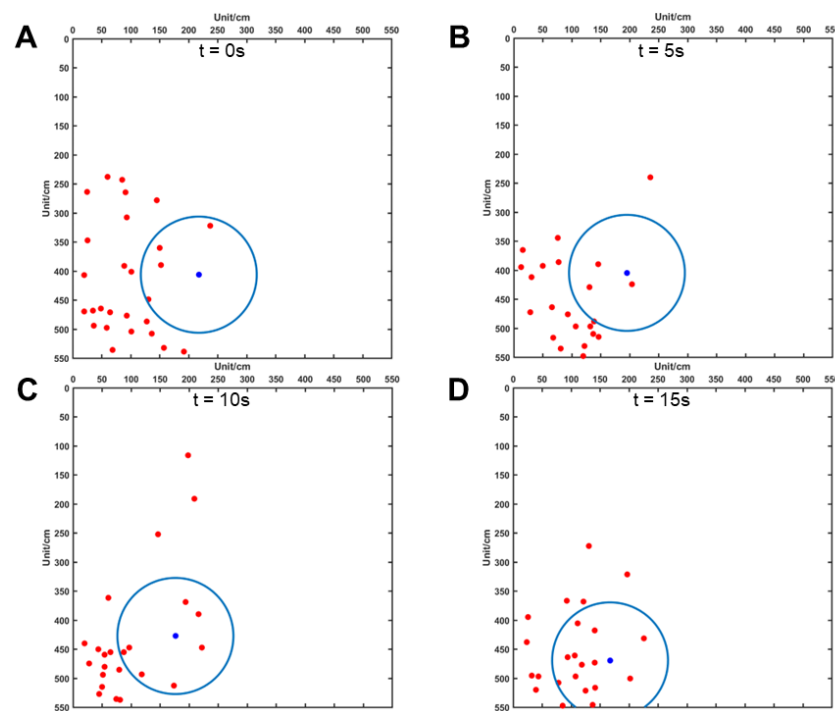


**Figure 6.** Image of acquired experimental data. Carp are represented by red solid dots, and robotic fish are represented by blue solid dots. The time interval between the four images is 5 s. The range centered on the robotic fish with a radius of 100 cm is represented by the big blue circle. Timestamps are shown in black. (A)  $T = 0$  s; (B)  $T = 5$  s; (C)  $T = 10$  s; (D)  $T = 15$  s.

According to previous studies, behavioral activities related to color selection in fish, such as courtship, should be carried out within a fixed distance of less than 1 m [11,48,59]. Therefore, in this study, the robotic fish were placed in the center, and the area with a radius of 1 m was determined as the data extraction range. Each set of experimental data included (1) the number of carp within a radius of 1 m centered on the robotic fish, and (2) the distance between carp and the robotic fish within a radius of 1 m with the robotic fish as the center.

#### 2.2.4. Visible Light Underwater Attenuation Law

The light reflected by the robotic fish had an impact on fish behavior. Different wavelengths of visible light have different attenuation coefficients in freshwater, which may lead to differences in carp color selection [65]. According to the calculation formula of the diffuse reflection attenuation coefficient  $K_w$  of visible light in natural freshwater, we can obtain the following [66]:

$$K_w \geq a_w + \frac{b_w}{2} \quad (10)$$

where  $a_w$  is the absorption coefficient in freshwater and  $b_w$  is the scattering coefficient in freshwater.

Different wavelengths and frequencies of light result in different illuminance and energy values. According to the decay law of photons in matter,

$$n(\lambda, z) = n(\lambda, 0)e^{-K(\lambda)z} \quad (11)$$

where  $n(\lambda, 0)$  is the number of photons with wavelength  $\lambda$  before passing through the material,  $n(\lambda, z)$  is the number of photons after attenuation in the material with thickness  $z$ , and  $K(\lambda)$  is the minimum visible light in water and represents the attenuation coefficient, namely  $K(\lambda) = K_w^{min}$ . We further obtained the attenuation formula of the illuminance of monochromatic light with wavelength  $\lambda$  in matter:

$$L(\lambda, z) = L(\lambda, 0)e^{-K(\lambda)z} \quad (12)$$

where  $L(\lambda, 0)$  refers to the illuminance of monochromatic light with wavelength  $\lambda$  before attenuation,  $L(\lambda, z)$  is the illuminance of monochromatic light after attenuation by a substance with a thickness equal to  $z$ , and  $K(\lambda)$  is the attenuation coefficient.

### 2.2.5. Data Acquisition of Carp Population Variation

An experimental period of  $T = 25$  s was selected to infer how carp responded to the presence of colored robot fish. Within a period, a photograph was taken every 5 s from moment 0 for a total of 6 pictures. After image correction and information extraction, coordinate positions and scatter diagrams of robot fish and carp were obtained. Then, we calculated the variation  $\Delta N$  of each group of data:

$$\Delta N = N_{max} - N_0 \quad (13)$$

where  $\Delta N$  is the variation of the number of carp within a circle with robot fish as the center and radius  $R = 100$  cm,  $N_{max}$  is the maximum number of carp within a period, and  $N_0$  is the initial number of carp within a period.

### 2.2.6. Distance Data Acquisition between Carp and Robot Fish

The images obtained in the experiment were corrected and extracted. At the same time, the distance could be obtained between carp and the robot fish in the circle of radius  $R = 1$  m ( $d \leq 1$  m) with the robot fish as the center.

To reduce the error of data processing, the weighted mean method was adopted to determine the mean distance between robotic fish and carp within a period. The specific methods of data processing were as follows. The variation in carp population was used as a weight to calculate the weighted average distance. The calculation formula of weighted average distance was

$$n_i = \frac{N_i}{\sum_{k=0}^5 N_k}, i = 0, 1, \dots, 5 \quad (14)$$

$$\bar{d} = \sum_{i=0}^5 n_i \times d_i \quad (15)$$

The number of carp in a circle with robot fish as the center within a cycle was expressed as  $N_0, N_1, \dots, N_5$ ; the corresponding distance was expressed as  $d_0, d_1, \dots, d_5$  (unit: cm).

### 2.2.7. Statistical Analysis

In the experiments, the robot fish were divided into different color groups; a total of 5 groups of robot fish, each group with one robot fish color, and 80 carp participated in the experiment. The experimental period was 6 days, and each color group of robot fish was independently tested twice a day, for a total of 12 experiments per color. To eliminate the influence of color order on the results of the experiment, the color order of the robot fish was shuffled every day before the experiment started. In the process of experimental video data extraction, data sampling was carried out at intervals of 5 s. A total of 390 sets of data were obtained. Further processing was performed according to the formulas in

Sections 2.2.5 and 2.2.6 to obtain the variation in the number of carp and the weighted average distance between carp and the robotic fish. Finally, a total of 65 sets of data were obtained through integration. A carp's choice over a short period of time was considered to represent its color preference.

The data processing was conducted using SPSS 25 software. The five different colors of the bionic robotic fish were the between-group factors, and the experimental data obtained were the within-group factors. Significance tests were performed by one-way analysis of variance (ANOVA), and the significance level was set at  $p < 0.05$ .

### 3. Results

#### 3.1. Hydrodynamic Coefficient

As shown in Figure 7, carp in the experimental pool that saw blue and red robot fish for the first time maintained a farther distance when wandering and hovered next to the robot (ANOVA,  $F [4,24] = 114.103$ ,  $p < 0.001$ ). They maintained distance but showed no tendency to approach or run away compared with machine fish of other colors. Over the course of five experiments, carp maintained this farther wandering distance, which indicated that their adaptability to the blue and red machine fish was poor. When carp saw the yellow and white machine fish, wandering time was less or they moved directly toward the yellow and white machine fish. In the following three experiments, there was no wandering phenomenon, and the adaptability to the yellow and white machine fish was good.

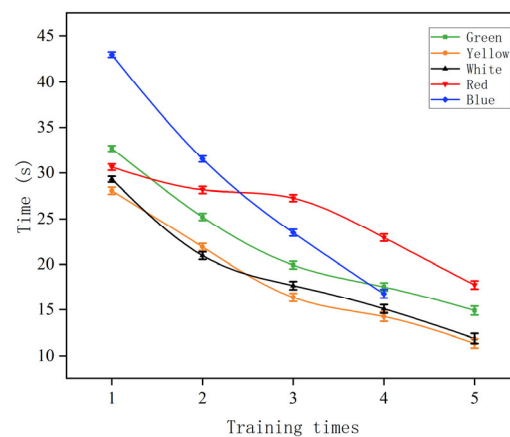


**Figure 7.** Carp acclimation to robotic fish. The results demonstrated a significant difference in the average acclimation time to the robotic fish among the five experimental groups.

#### 3.2. Changes in Light Attenuation of Different Colored Robots in Water

The minimum diffuse reflection attenuation coefficient of visible light in water,  $K_w^{min}$ , was obtained, and the attenuation rules of different colors of light in water are shown in Table 1.

**Table 1.** Attenuation law of light of different colors in water.

| Color  | Wavelength $\lambda$ (nm) | $a_w$ ( $m^{-1}$ ) | $b_w$ ( $m^{-1}$ ) | $K_w^{min}$ ( $m^{-1}$ ) |
|--------|---------------------------|--------------------|--------------------|--------------------------|
| Red    | 650                       | 0.3490             | 0.0007             | 0.34935                  |
| Yellow | 580                       | 0.1080             | 0.0012             | 0.10860                  |
| Green  | 530                       | 0.0507             | 0.0017             | 0.05155                  |
| Blue   | 460                       | 0.0156             | 0.0031             | 0.01715                  |

Note:  $K_w^{min}$  is the minimum attenuation coefficient of visible light in freshwater,  $a_w$  is the absorption coefficient in freshwater, and  $b_w$  is the scattering coefficient in freshwater.

The  $K_w^{min}$  values of the four monochromatic lights in freshwater were ranked as follows: red > yellow > green > blue, where red light had the largest attenuation coefficient in freshwater and blue light had the smallest attenuation coefficient.

For opaque objects, the color that appears in natural light is the color of the light it reflects. The illuminance value outside on a sunny day is approximately  $5 \times 10^4$  lux. According to the wavelength ratio of each color light in the visible spectrum, the illuminance of different wavelengths of monochromatic light in the experimental environment can be obtained. Using Equation (12) described in Section 2.2.4 and the attenuation coefficient of visible light in freshwater, the illuminance in the experimental water environment with a vertical depth of 0.2 m and a position of 1 m from the robotic fish in the horizontal direction was obtained. The data are shown in Table 2:

**Table 2.** Illuminance of different colors of light attenuated in freshwater.

| Color   | Wavelength $\lambda$ (nm) | Attenuation Coefficient $K(\lambda)$ ( $m^{-1}$ ) | Illuminance $L(\lambda)$ (lux) |
|---------|---------------------------|---------------------------------------------------|--------------------------------|
| Natural | *                         | *                                                 | 50,000                         |
| Red     | 650                       | 0.34935                                           | 10,889                         |
| Yellow  | 580                       | 0.1086                                            | 5371                           |
| Green   | 530                       | 0.05155                                           | 11,053                         |
| Blue    | 460                       | 0.01715                                           | 7370                           |

Note: The asterisk (\*) indicates that there is no data available.

### 3.3. Variation in Carp Numbers

As shown in Figure 8, the results indicate that the mean variation in carp population significantly differed among the five experimental groups (ANOVA,  $F [4,60] = 5.299$ ,  $p = 0.001$ ). The experimental results indicated that the order of the mean variation of carp was white > red > green > yellow > blue. The number variation in carp was highest when tested with the white robotic fish, followed by the red robotic fish and green robotic fish, and lowest when tested with the blue robotic fish.

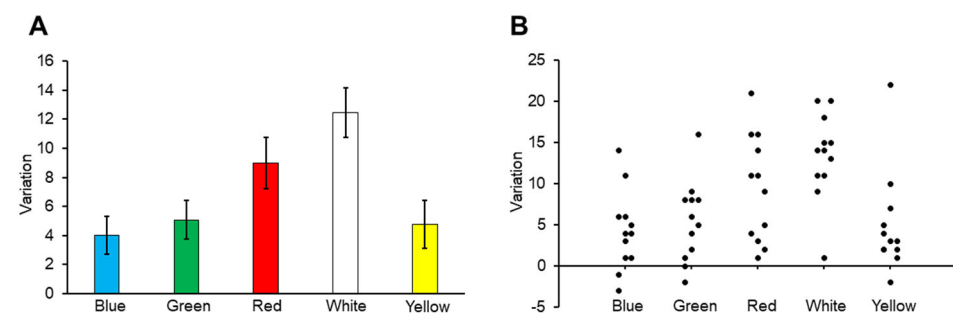


**Figure 8.** Variation in carp numbers in the presence of different robotic fish colors. (A) Mean ( $\pm$ s.d.) variation in the number of carp in blue, green, red, white, and yellow experiment groups; the experiment was independently repeated 12 times. (B) Scatter plot showing the distribution variation in the number of tested animals.

### 3.4. Distance between Carp and the Robotic Fish

As shown in Figure 9, the results indicate that the distance between carp and robotic fish among the five experimental groups was significant (ANOVA,  $F [4,60] = 6.627$ ,  $p < 0.001$ ). The distances between carp and robotic fish were sorted as follows: white < yellow < blue < green < red. The ranking results of the mean distance between carp and robotic fish of different colors revealed that the distance was closest between carp and white robotic fish, followed by the yellow, blue, green, and then red robotic fish, which had the greatest distance. This result is different from the order previously observed in the mean variation of carp.

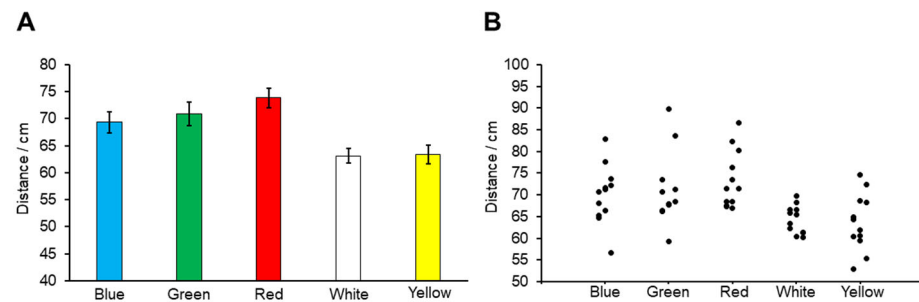


**Figure 9.** Weighted mean distance between carp and robotic fish of different colors. (A) Mean ( $\pm$ s.d.) distance between carp and robotic fish in the blue, green, red, white, and yellow experiment groups; the experiment was independently repeated 12 times. (B) Scatter plot demonstrating the distribution of distance.

## 4. Discussion

### 4.1. Changes in Light Attenuation of Different Colored Robots in Water

The observed phototactic responses of fish to different colored robots underscore their ability to discern and react to varying light stimuli. Fish exhibit directional movement toward light sources (positive phototaxis) or away from them (negative phototaxis), which indicates their sensitivity to environmental cues [58,66].

Previous studies have highlighted the optomotor response of carp to increased light intensity and emphasized that light levels should exceed 10 lux for optimal testing conditions [11,25,67]. Our findings demonstrate that carp exhibit distinct optomotor reactions to robotic fish of different colors within a 1 m radius. Despite variations in light attenuation, carp displayed observable behavioral responses to robotic fish, which indicates their ability to perceive and react to environmental stimuli.

### 4.2. Variation in Carp Numbers

The interaction between fish shoals and robotic fish revealed intriguing patterns, particularly regarding variations in carp numbers around different colored robotic fish. Carp displayed a stronger attraction to white- and red-colored robotic fish, which was potentially influenced by their contrasting colors with the aquatic environment [68,69]. The observed variations in carp numbers around robotic fish of different colors indicated a preference for certain hues and that carp possess a certain level of color discrimination ability. This phenomenon may have implications for understanding fish behavior and optimizing the design of robotic fish for future studies [70].

### 4.3. Distance between Carp and Robotic Fish

Carp followed robotic fish of different colors to varying degrees, with preferences likely influenced by visual cues and behavioral responses. Robotic fish with natural fish coloration are more likely to attract carp, leading to closer proximity [71,72].

The absence of carp with certain colors of robot fish in the experimental area indicates that fish may perceive unfamiliar colors as potential threats, which results in avoidance behavior. These findings underscore the importance of considering visual cues and environmental context when studying fish behavior with robotic stimuli.

Although our study did not account for factors such as high-frequency noise generated by robotic fish, previous literature suggested that noise reduction does not significantly impact fish responses [44]. Additionally, variations in environmental conditions may introduce experimental errors, highlighting the need for further research to refine experimental protocols and enhance data accuracy.

## 5. Conclusions and Future Work

The results of this study highlight the subtle responses of carp to robotic fish of different colors. In the experiment, carp showed different visual–motor responses and changes in behavior in the presence of differently colored robot fish.

Specifically, our observations highlight the photoinduced responses of carp to different colored robotic fish, which indicates their ability to perceive and respond to environmental cues. Carp showed a preference for certain hues, particularly white and red, which demonstrated a degree of color discrimination that could have important implications for understanding fish behavior and optimizing robotic fish designs.

In addition, carp followed differently colored robot fish to different degrees, which indicated an influence of visual cues and behavioral responses on fish school dynamics. The small concentration of carp around certain colors of robotic fish, such as blue and green, raises questions about the perception and potential avoidance behavior of unfamiliar colors, and underscores the importance of considering visual cues and environmental context in experimental design.

In future research, several key areas should be addressed to enhance the validity and scope of the findings. Firstly, the impact of noise, both from the robotic fish's tail movements and environmental background, should be mitigated to ensure that behavioral observations are not confounded by these extraneous sounds. Additionally, extending the duration of observation periods will provide a more comprehensive dataset, capturing the full range of carp behaviors under varying conditions and stimuli.

Furthermore, investigating the responses of carp with varying body lengths and colors to the specific hues presented by robotic fish will contribute to a richer understanding of fish preferences and behavioral motifs. Lastly, improvements in the design of the robotic fish, focusing on a more authentic appearance and movement dynamics, will refine the experimental setup, leading to more realistic interactions and more meaningful behavioral data.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes9060211/s1>, Figure S1: Experimental Procedure Flowchart for Carp Acclimation to Robotic Fish.

**Author Contributions:** Conceptualization, X.H., Y.Z., and B.L.; methodology, X.K.; software, X.H. and Y.Z.; formal analysis, X.H., S.J., and Y.Z.; investigation, X.K., X.H., and Y.Z.; Resources, X.H., S.J., and X.C.; data curation, X.H.; writing—original draft preparation, X.H. and B.L.; writing—review and editing, X.H., X.C., and B.L.; visualization, X.H. and Y.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Key Research and Development Program of China, grant number 2023YFD2401302, and the National Natural Science Foundation of China, grant number XTCX-KJ-2023-17.

**Institutional Review Board Statement:** The research presented in this manuscript has been conducted in accordance with the ethical standards and guidelines established by the Institutional Committee for Ethical Review of Shanghai Ocean University. The study protocol was reviewed and approved by the Committee, and any necessary permissions or waivers were obtained prior to the commencement of the research.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Acknowledgments:** We thank the College of Marine Living Resource Sciences and Management of Shanghai Ocean University for their support of this study.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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