

# Fish and robot dancing together: bluefin killifish females respond differently to the courtship of a robot with varying color morphs

P Phamduy<sup>1,4</sup>, G Polverino<sup>1,2,4</sup>, R C Fuller<sup>3</sup> and M Porfiri<sup>1</sup>

<sup>1</sup> Department of Mechanical and Aerospace Engineering, New York University Polytechnic School of Engineering, Six MetroTech Center, 11201, Brooklyn, NY, USA

<sup>2</sup> Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587, Berlin, Germany

<sup>3</sup> Department of Animal Biology, University of Illinois, 104 Shelford Vivarium, 606 E. Healey St., 61820, Champaign, IL, USA

E-mail: [mporfiri@nyu.edu](mailto:mporfiri@nyu.edu) and [rcfuller@illinois.edu](mailto:rcfuller@illinois.edu)

Received 26 February 2014, revised 20 July 2014

Accepted for publication 24 July 2014

Published 27 August 2014

## Abstract

The experimental integration of bioinspired robots in groups of social animals has become a valuable tool to understand the basis of social behavior and uncover the fundamental determinants of animal communication. In this study, we measured the preference of fertile female bluefin killifish (*Lucania goodei*) for robotic replicas whose aspect ratio, body size, motion pattern, and color morph were inspired by adult male killifish. The motion of the fish replica was controlled via a robotic platform, which simulated the typical courtship behavior observed in killifish males. The positional preferences of females were measured for three different color morphs (red, yellow, and blue). While variation in preference was high among females, females tend to spend more time in the vicinity of the yellow painted robot replicas. This preference may have emerged because the yellow robot replicas were very bright, particularly in the longer wavelengths (550–700 nm) compared to the red and blue replicas. These findings are in agreement with previous observations in mosquitofish and zebrafish on fish preference for artificially enhanced yellow pigmentation.

Keywords: animal behavior, bioinspired design, courtship, ethorobotics, killifish, robotics

(Some figures may appear in colour only in the online journal)

## 1. Introduction

Variation in animal coloration is ubiquitous in nature. Coloration is sexually dimorphic in many species, whereby males are often more brightly colored than females [1–3]; nevertheless, such phenotypic dimorphism is reversed in several species, with females that are more brightly colored than males [4, 5]. Within a population where males are frequently polymorphic in their colors, individuals exhibit discretely

different pigments [6–8]. In some species, the variation and abundance in pigmentation are tightly associated with different male behavioral strategies, and the functional significance of color patterns is well known [9–11]. However, in other systems, male color morphs do not necessarily have a unique functional significance and the ecological role of phenotypic dimorphisms is far from being fully understood [12].

Unlike bird feathers or insect cuticles [13–15], altering the coloration of fish fins is often problematic, since the dyes used in such alterations dissolve easily in water over time and

<sup>4</sup> These authors have contributed equally to the study.

are not consistent between tests [16]. Thus, researchers commonly utilize natural variants of color morphs, or different phenotypes within the same population, to study preference. For example, female choice assays often rely on the presentation of differently colored males and subsequently measure female association times with the selected morphs [17–19]. However, this approach suffers from a limited degree of consistency in the experiments. Males that exhibit different color patterns may also differ in their behavioral traits, which, in turn, reduce the ability to isolate the effects of their coloration on female mating preference.

Fish models offer an alternative solution to the canonical use of a live fish stimuli in preference studies, whereby fish behavior can be controlled and the coloration can be systematically altered. Simple fish models have long been used to examine the effects of coloration on the behaviors of fish [20–23]. These studies typically consist of controlling the motion of a fish model and examining the response of live fish. Onscreen playback of fish videos has been recently proposed as a valid alternative, whereby video monitors are placed next to the experimental tank and fish are tested for their preference toward fish images that are altered in their coloration or morphology [24–29]. The utilization of a monochromatic filter is another valid method to alter fish fin color [30], even if the filter may alter the color of the entire fish body and not only the fin.

Robotics has been recently leveraged in the study of fish behavior to deliver controllable and consistent stimuli to live subjects [31–44]. For example, robotic fish inspired by conspecifics have been used in preference tests on zebrafish (*Danio rerio*) and mosquitofish (*Gambusia affinis*) to assess the feasibility of modulating fish behavior through robots of varying degree of biomimicry [33–35, 37, 38, 40, 45]. Notably, our group [38] has demonstrated that zebrafish display a more consistent behavioral response to robotic stimuli than live conspecifics. The main benefits associated with the experimental use of robotic fish are: (i) the possibility to accurately control the morphology and motion of the stimulus, and (ii) the large degree of biomimicry of the robotic stimuli as compared to computer animations or traditional fish models.

The bluefin killifish (*Lucania goodei*) has been used as a model to investigate female mating preferences for natural variations of fin pigmentation observed in males [46, 47]. Specifically, the anal fin and the rear portion of the dorsal fin in wild adult bluefin killifish males can be red, blue, or yellow [46, 47]. Such color morphs have been consistently observed in several wild populations, and a considerable portion of such phenotypical variation has been attributed to genetic effects [48]. The relative abundance of color morphs varies with environmental lighting conditions; for example, in clear water (i.e., springs) populations, red and yellow color morphs are more copious, while blue morphs are more frequent in tannin-stained (i.e., swamps) populations [47]. A slight preference for red has been documented for females from a spring population [49, 50], but other studies have failed to confirm such a preference [12]. Conversely, Fuller and Noa [50] observed that females from a swamp population prefer

blue males when they are raised and tested in tannin-stained water, suggesting that the light environment affects female mating preferences through both its long-term effects on the visual system development and its short-term effects on the perception of visual cues due to the filtering of wavelengths through the water.

In this study, a robotic-based platform was developed to investigate female mating preferences of bluefin killifish for differently colored males. Specifically, the platform allowed for accurate control of the two-dimensional motion of bluefin killifish replicas, which were painted to resemble the color morphs that are naturally found in adult males. Although methods such as fin coloration [17–19], onscreen playback [24, 28], and utilization of color filters [30] were valid alternative methods to conduct this experiment, a novel robotics-based paradigm was developed for the possibility to selectively vary the coloration of fish replica, mimic the exact live fish size, and standardize the courting patterns. The following two questions were examined in this study: (i) do females spend time swimming with fish replicas inspired in their morphophysiology by fertile bluefin killifish males? and (ii) do females discern between red, blue, and yellow colored fish replicas? With respect to these two questions, we hypothesize that female killifish could discern between different color morphs of killifish replicas in accordance with color discrimination [50, 51] and color preference toward live males [47] observed in the species.

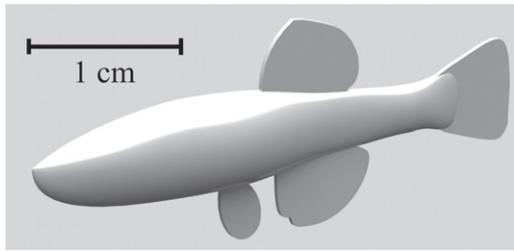
Overall, this effort seeks to determine the extent to which this robotic system can be used to infer female mating preferences in bluefin killifish and aid in the study of the behavior of this species, thus reaching beyond zebrafish [33, 35, 37, 38, 40, 52, 53], mosquitofish [34, 37], golden shiner minnows [31, 36, 39, 44], giant danios [41], and sticklebacks [32, 42].

## 2. Material and methods

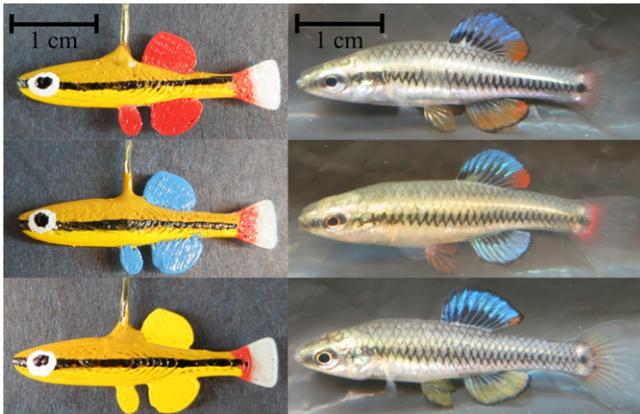
The experiment described in this work was approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC No. 0515) and the New York University Polytechnic School of Engineering (previously called Polytechnic Institute of New York University) Animal Welfare Oversight Committee (AWOC No. 2013-103).

### 2.1. Fish replica

Figure 1 shows the basic design of the fish replica. Its design included visible fish appendages, such as a dorsal fin, a pelvic fin, an anal fin, and a caudal fin. The fish replica consisted of a rigid body of solid-packing acrylonitrile butadiene styrene (ABS) material made with a rapid prototyping machine (Dimension Elite, Stratasys Ltd, Eden Prairie, MN, USA). The total length of the replica was 3.0 cm, the height was 1.2 cm, and the width was 0.4 cm. These dimensions were chosen to match the nominal scale and aspect ratio of adult killifish [47], see figure 2. Specifically, the size of the fish replicas was consistent across color morphs, and values from



**Figure 1.** Illustration of the computer aided design of the bioinspired killifish replica.



**Figure 2.** Fish replicas (left) and adult killifish males (right) with red, blue, and yellow colorations, respectively.

the literature on killifish males across multiple populations were averaged to select such as size [47].

The surface of each fish replica was painted with non-toxic pigments to imitate the three different color patterns observed in adult killifish males [48]. Each fish replica was painted yellow on the bottom half of the body, while the upper half was painted in light brown. A black line was drawn along the longitudinal length of its body, where the two main colors met. The caudal fin was partially painted in red near the peduncle, while the rest of its surface was white. The pelvic, dorsal, and anal fin of the replicas were painted consistently with the three different coloration patterns of adult male killifish [48], i.e., red (referred to as ‘Red replica’), blue (referred to as ‘Blue replica’), and yellow (referred to as ‘Yellow replica’) (see figure 2). Twelve replicas were created (four of each color) and used in our study.

## 2.2. Color reflectance comparison

To assess the ability of the models to replicate the appearance of live fish, the reflectance spectra of the anal fins of live males was compared to that of the fish replicas. The reflectance of anal fins from live males was measured in summer 2007. Fish were collected in the Wakulla River (Wakulla Co., FL, USA), Wacissa River (Jefferson Co., FL, USA), and the Everglades (Dade Co., FL, USA), and transported back to the University of Illinois. Males were euthanized with an overdose of MS-222, and the fins were removed. The animals were euthanized because reflectance measurements needed to

be performed under a microscope. This required the physical removal of the fins from the body.

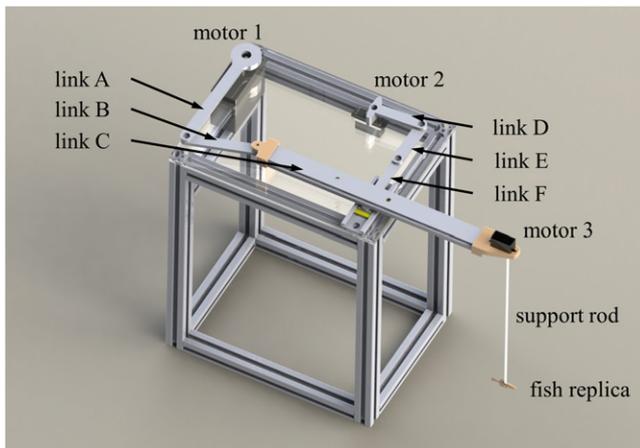
Fin reflectance was measured using a spectrophotometer (USB2000, Ocean Optics Inc., Dunedin, FL, USA) connected to a compound microscope (Axiostar, Carl Zeiss AG, Oberkochen, Germany) through a 400  $\mu\text{m}$  patch cord via the camera tube. Light was provided via a halogen lamp through two fiber optic cables positioned at approximately 45°. For each measurement, a single anal fin was placed on a glass slide with a drop of water. A piece of white teflon tape was placed on the slide to use as a white standard. Each reflectance measurement involved the following four steps. First, the objective was focused on the anal fin. Second, the stage was moved so that the lens was positioned above the white standard to record maximum brightness. Third, the stage was moved to position the lens over an empty patch of water near the fin to record the dark. Finally, the stage was moved back to position the lens above the anal fin to measure the reflectance. If the focus or the light was misdirected at any point during this the process, the process was restarted. Color reflectance measurement was collected for 6 males from the Wakulla River (3 red, 3 yellow), 6 males from the Wacissa River (2 red, 1 blue, 3 yellow), and 8 males from the Everglades population (2 red, 5 blue, and 1 yellow).

To measure the reflectance curves of the fish replicas’ fins, the same spectrophotometer connected to a reflectance probe (R200-7, Ocean Optics Inc., Dunedin, FL, USA) was used. The probe consisted of six optical fibers (through which light passed) clustered around one read fiber (that sent light back to the spectrophotometer to be counted). Hence, the light passed through the probe and bounced off the fin of the fish replica. A portion of the light bounced back to the probe and was detected by the read fiber. Light was provided from a combined deuterium-halogen lamp (DH-2000, Ocean Optics Inc., Dunedin, FL, USA). The reflectance probe was positioned directly on the fin of the fish replica at 45°. The reflectance measurements were collected for 3 red, 2 blue, and 3 yellow replicas.

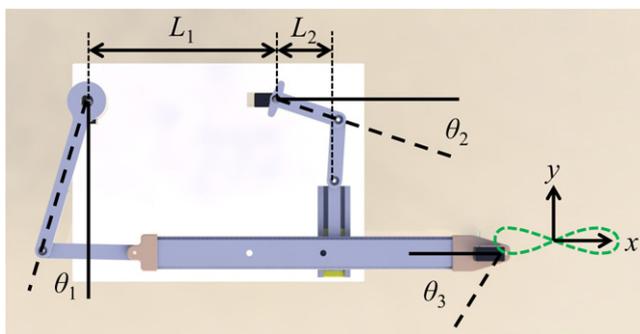
## 2.3. Robotic platform

A robotic platform was designed to move the fish replica in swimming trajectories observed in killifish males during their courtship behavior [12]. A transparent acrylic baseplate with a length of 24 cm, a width of 18 cm, and a thickness of 0.45 cm, was used as the foundation to secure the components of the robotic platform. The robotic platform was mounted above a box constructed from aluminum t-slotted framing with a height of 30 cm, a length of 24 cm, and a width of 18 cm, see figure 3.

To actuate the robotic platform, a Futaba S3306MG servo (Futaba Corporation of America, Schaumburg, IL, USA) was used as motor 1, while a Hitec HS-82MG and a Hitec HS-65MG (Hitec RCD USA Inc., Poway, CA, USA) were used as motors 2 and 3, respectively. All servo motors were controlled by a microcontroller (Arduino Uno, Arduino, Italy). Extended below motor 3, a transparent Plexiglas support rod, 20.3 cm in length, was epoxied with a cylindrical



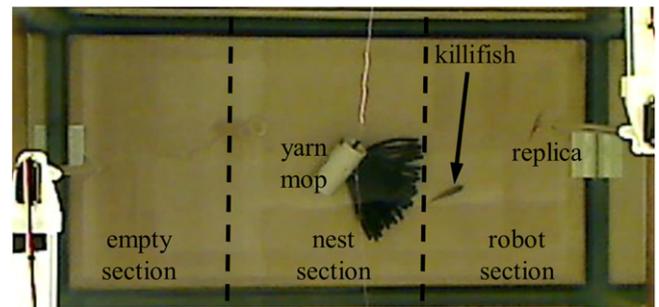
**Figure 3.** Computer aided design of the robotic platform actuated by two slider rocker mechanisms. A fish replica was attached to the bottom end of the support rod and was passively translated on a horizontal plane to mimic the courtship behavior of adult killifish males.



**Figure 4.** Illustration of the working principle of the robotic platform from a top view, depicting motor angles and origin of the end effector coordinate system. The trajectory of the replica is depicted as a dashed line and further details on its biological relevance and practical implementation are presented in the experimental procedure.

rare earth (neodymium) magnet and connected, in each experimental trial, to the opposite pole of a second magnet epoxied on the top of each fish replica (see figures 2 and 3).

The robotic platform was composed of two slider rocker mechanisms and an end effector, see figure 3. The link lengths of the slider rocker mechanisms were calculated based on the requisite motions of the end effector. MATLAB (The MathWorks Inc., Natick, MA, USA) was used to perform such analysis and run the simulations. The length of corresponding links were A, 12.7 cm; B, 7.5 cm; C, 29.1 cm; D, 5.3 cm; E, 5.0 cm; and F, 5.9 cm. The link shapes were designed in SolidWorks (Dassault Systèmes SolidWorks Corp., Waltham, MA, USA). Links A, B, D, E, and F were printed on a rapid prototyping machine (Dimension Elite, Stratasys Ltd, Eden Prairie, MN, USA), while link C was composed of a slider guide with ABS fixtures on opposite ends to connect with link B and motor 3, respectively. The links were assembled with stainless steel ball bearings and 3 mm diameter dowels at link joints.



**Figure 5.** Snapshot of the experimental setup from the webcam with overlaid nomenclature. The dashed lines depict the three virtual sections of the tank.

The slider rocker mechanisms were used to control the planar motion of the fish replica. With reference to figure 4, the length  $L_1$  from motor 1 to motor 2 was 15 cm along the  $x$ -axis. The length  $L_2$  from motor 2 to the slider was 5.17 cm along the  $x$ -axis. The angles of motor 1 and motor 2 (i.e.,  $\theta_1$  and  $\theta_2$  in figure 4) determined the position of the fish replica in the  $xy$ -plane, corresponding to the horizontal plane of the test tank, see figure 3. Differently, the orientation of the fish replica was controlled by the rotation of the Plexiglas support rod connected to motor 3 ( $\theta_3$  in figure 4).

#### 2.4. Animals and housing

Forty-six bluefin killifish (*Lucania goodei*) were used in this study. Fish were collected from the Wakulla River, which is a clear spring fed river, and acclimated for a minimum of two months in a greenhouse at the University of Illinois. In addition to natural light, the greenhouse was supplied with Xenon lamps. Lights were maintained on a 14:10 h light:dark schedule. The greenhouse was maintained between 18–27 °C at room temperature. Fish were fed frozen adult *Artemia* daily. Fish were housed in a 110 l (29 gallon) aquaria containing dechlorinated city water. Fish densities were constantly maintained at a 0.4 fish  $l^{-1}$  limit.

Experiments were performed in a fish laboratory in the basement of the Shelford Vivarium of the University of Illinois. Adult female individuals were isolated from males two days before being tested to increase the likelihood that they were gravid and ready to spawn. Thus, females were transferred into the main facility and randomly housed either 9.4 l, 19 l, or 38 l tanks in the absence of males. Each holding tank was equipped with yarn mops for shelter, and an aerator connected to a large sponge to guarantee the proper circulation and oxygenation of the water. Fish were fed with frozen *Artemia* prior to experimentation. Lights were maintained on a 14:10 h light:dark schedule. After this study, animals were returned to their stock tanks in the greenhouse and used for subsequent experiments.

#### 2.5. Experimental apparatus

Experiments were conducted in a 75.7 l (20 gallon) tank (length: 49.5 cm, height: 30.5 cm, and width: 25.5 cm), see figure 5. The tank did not have a circulation system, and the

water was placid at all times. The tank was covered with contact paper of a neutral light brown coloration on both the longitudinal walls and substrate. The fish replicas were presented to the live fish in a one-way choice test where females could choose whether or not to associate with the replica. A webcam (Webcam Pro 9000, Logitech Inc., Switzerland), interfacing with a computer via USB, was positioned 66 cm above the water's free surface in order to provide ample resolution for identifying fish use of space. The tank was virtually divided into three 16.5 cm long by 25.5 cm wide sequential same-sized sections: 'robot section', 'nest section', and 'empty section'. The amount of time females spent in each section was scored by observing the videos.

## 2.6. Experimental procedure

The robotic platform produced a trajectory that mimicked the typical courtship motion pattern that is observed in adult killifish males in the presence of fertile females [46, 50] and personal observations, [54]. The shape of the trajectory is shown in figure 4. The length of the lemniscatic trajectory in the  $x$ -axis direction was set to 10.1 cm while its position in the water column was consistently maintained at 2.1 cm from the bottom surface of the test tank. The period  $\tau$  for the completion of one trajectory's loop was 5.7 s. To produce the lemniscatic trajectory, sinusoidal commands were given to motor 1 and motor 2 such that  $\theta_1$  was oscillating at  $\tau$  with amplitude of 23.3 degrees while  $\theta_2$  was oscillating at  $\tau/2$  with amplitude of 22.5 degrees. Motor 3 was controlled such that  $\theta_3$  was set to 180 degrees when the replica was moving in the positive  $x$ -direction and 0 degrees when the replica was moving in the negative  $x$ -direction. Notably, by setting motor 3 to 0 or 180 degrees, based on its position during the period, the fish replica was always facing the direction of motion.

The time spent by adult killifish females in the section of the fish replica was measured under five different experimental treatments (control 1, control 2, Red replica, Blue replica, and Yellow replica, respectively). The illumination consisted of diffused light in the room from overhead airtight vapor lamps with natural sunlight fluorescent tubes. Control 1 consisted of recording female behavior in the absence of the fish replica, without maneuvering the robotic platform. Control 2 consisted of recording the female behavior when the robotic arm was moving without an attached fish replica. The last three treatments consisted of recording female behavior when a Red, Blue, or Yellow replica was presented. In each treatment, the fish was released in the tank starting from the nest section, i.e., the central region of the test tank where the yarn mop was positioned. The same replica was not used for all of the trials for a given color morph; instead, we rotated among the four samples for each condition involving a replica.

Once the fish was released, the webcam was turned on remotely, and the behavior was recorded for ten minutes. Females were used once for each of the three main experimental treatments and twice for the control conditions, that is, once for each control 1 and 2. Specifically, the eight fish that were used for trials of control 1, in which the rod was fixed,

were reused for trials of control 2, in which the rod was moving in a 'figure 8.' A total of 54 trials were performed in this study including 8 trials of control 1, 14 trials of control 2, 12 trials of the Red replica, 9 trials of the Blue replica, and 11 trials of the Yellow replica. At least eight trials where females left the nest area, were analyzed for each treatment. In some trials, females never left the nest section. These trials were excluded from the analysis. Trials were conducted in randomized order between 10 am and 7 pm, from May 30, 2013 to May 31, 2013.

## 2.7. Statistical analyses

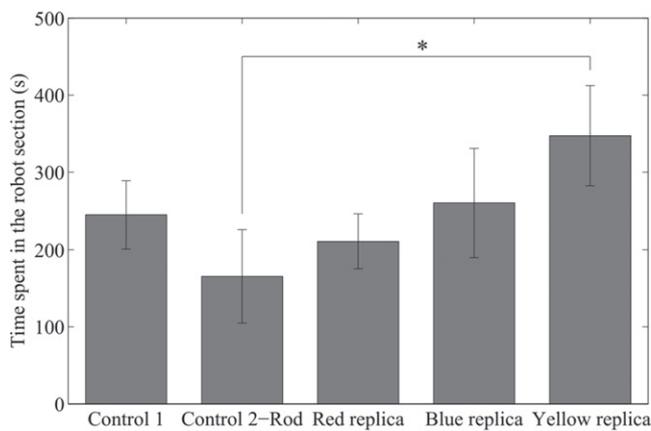
The goals of this study were to determine whether (a) females would associate with replicas painted to resemble conspecific males and (b) females would discern between males painted to resemble various color morphs found in wild *Lucania goodei* populations. Analysis of variance (ANOVA) was used to determine significant variation between the five treatments in the amount of time that females spent in the robot section of the tank. Post-hoc Fisher's Least Significant Differences was used to test the pairwise differences between experimental treatments. Tests for preference for the fish replicas in comparison to null expectations were performed using one-sample t-tests. Means and standard errors are shown throughout. All analyses were performed using R statistical software and significance level was set to  $p < 0.05$ .

## 3. Results

Females varied in their response to the robot, with some subjects spending all of the time during the trial in the vicinity of the fish replica (with the Yellow replica only) and others spending very little time associating with it (as little as 11 out of 600 s for females that left the nest substrate). While analysis of variance indicated no overall significant differences among the treatments in the time spent in the robot section ( $F_{4,35} = 1.48$ ,  $p = 0.230$ ), a pair-wise study was conducted to examine if any underlying difference existed between pairs of treatments. Pair-wise comparisons of treatments showed that females spent significantly more time in the robot section in the presence of the Yellow replica than they did with the control (see figure 6, control 2—rod present, but replica absent; Fisher's least significant difference,  $p = 0.026$ ). There was also a tendency for females to spend more time with the Yellow replica than with the Red replica, but the difference was not statistically significant ( $p = 0.094$ ).

In addition, the null expectation was that females would spend equal amounts of time (i.e., 200 s) in each of the three equal sections of the tank. Females only deviated from this null expectation in the Yellow replica treatment (one-sample t-test,  $t_7 = 2.36$ ,  $p = 0.050$ ). Specifically, t-test analysis showed that females spent more time associating with the Yellow replica than expected by chance.

Figure 7 shows the reflectance spectra for red, blue, and yellow anal fins from killifish versus the spectra of the fins from the fish replicas. A few patterns are noteworthy. First,



**Figure 6.** Mean  $\pm$  standard error of the time spent by adult killifish females swimming in the section of the replica.  $n=8$  for each treatment. A star is used to identify statistically different conditions in post-hoc analysis ( $p < 0.05$ ).

the reflectance of the Red replica's fins is more shifted towards higher wavelengths than the actual killifish. Second, the reflectance of the Blue replica shows a clear peak in the shorter wavelengths, but then declines in the longer wavelengths. In contrast, the blue of killifish anal fins rises at slightly shorter wavelengths and plateaus and remains generally high throughout the 400–700 nm range. Third, the yellow on the fins of the fish replica is red-shifted and is particularly bright compared to the real killifish.

#### 4. Discussion

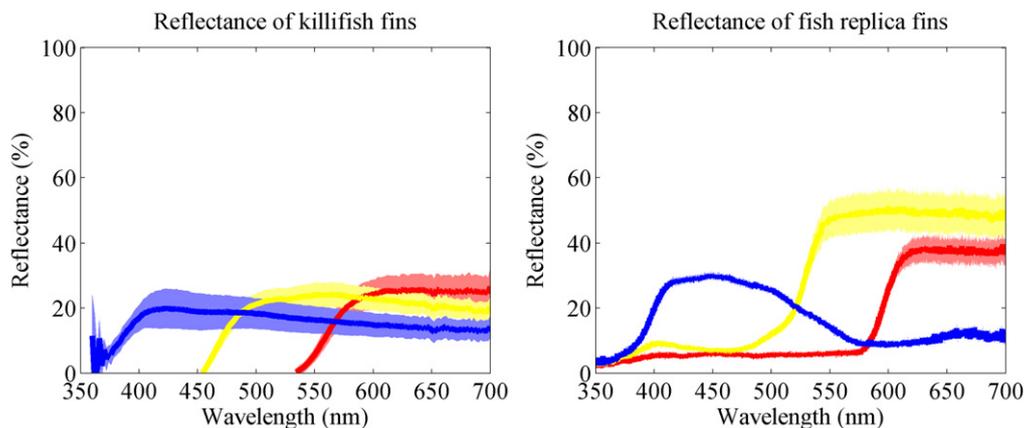
The results of this study show that the positional preference of adult female killifish was influenced by the presence of the fish replica. Specifically, adult killifish females were more likely to swim in the robot section of the tanks when exposed to some fish replicas than they were in control 2. The fish replicas were designed to offer biologically salient cues to the experimental subjects, by matching aspect ratio and body size of adult male killifish and enhancing fin colors (see figures 1 and 2). The ability of the replicas to influence killifish behavior was anticipated at the beginning of this study on the basis of our earlier studies on zebrafish and mosquitofish, which have demonstrated that visual features of a robotic fish influence the behavioral response of live subjects [33–35, 37, 38, 45]. The relevance of pigmentation in fish social behavior is widely documented by Price, Weadick, Shim and Rodd [55], and fish attraction towards yellow colorations in their conspecifics has been observed in zebrafish and mosquitofish and attributed to health conditions, reproductive maturity, and high social rank signals [28, 29].

Visual cues could be responsible for the increased time spent by females in the robot section of the tank in the Yellow replica treatment with respect to control 2. Beyond this statistically significant difference, it should be noted that the only two trials where females spent the entire observation time swimming with the robot occurred in the Yellow replica treatment. In these trials, fish actively interacted with the

Yellow replica by swimming parallel to it or swimming back and forth from the replica to the nest section. This result differs from studies where female killifish have shown a slight preference toward red morphs from a spring population [49, 50] and blue morphs from a tannin-stained water population [50]. In agreement with findings in [28] on mosquitofish, the artificially enhanced yellow pigmentation may act as a superstimulus [56]. Specifically, mosquitofish females were presented with pairs of animated stimuli [28] to investigate their preference for conspecifics altered in their coloration, aspect ratio, and swimming depth. Results presented therein have shown that enhanced yellow pigmentation significantly increases fish preference for animated stimuli in agreement with observations on the social role of yellow pigmentation in mosquitofish documented in [57]. Fish preference for the enhanced biologically-relevant features have also been observed in zebrafish [35], whereby the increased yellow pigmentation and enlarged body shape were adopted to emphasize the visual stimulation of a bioinspired robotic fish. Likely, spectra that appear as 'yellow' to humans might be perceived differently by fish with visual properties that differ from ours and this difference may account for the distinct preference for the Yellow replica.

Here, the explanation for the discrepancy of female preferences for male coloration may rely on the spectral properties of the replicas versus that of actual males. Bluefin killifish have five major cone classes. These are the ultraviolet, violet, blue, yellow and red cones, and they are maximally sensitive at 359 nm, 405 nm, 453 nm, 537 nm, and 573 nm respectively [51]. Figure 7 shows the spectral properties from live and artificial color morphs in male killifish. The Yellow replica was distinct in two respects. First, the Yellow replica most likely had high brightness because it stimulated both the yellow and red cones. Furthermore, the reflectance of the Yellow replica was doubled in magnitude from 550 nm to 700 nm compared to the natural fin reflectance in yellow male killifish and was also greater than that of the Red and Blue replicas [50]. Double cones are in high abundance throughout the retina Molstad [58] and are often thought to account for brightness perception [59, 60]. In bluefin killifish, double cones can involve the red/yellow, the blue/yellow, and the red/red cone combinations. Second, the reflectance of the Yellow replica was considerably red-shifted as indicated by a reflectance point, measured at the highest slope in the reflectance, of approximately 534 nm for the Yellow replicas compared to a reflectance point of 462 nm for natural males with yellow anal fins. In fact, the reflectance point of the Yellow replica (534 nm) was more similar to that of the preferred natural red color morphs (556 nm) than it was to the reflectance point of natural yellow males (462 nm). Similar phenomena may also explain the attraction toward yellow-colored animated images observed in mosquitofish [28]. Indeed, *Gambusia affinis* and *Lucania goodei* are both members of the order Cyprinodontiformes, and fish in this order have a similar palette of cones [48, 61–63].

In conclusion, the robotic system developed in this study constitutes a valid tool to study robot-animal interactions. Herein, adult female killifish have shown to spend



**Figure 7.** Mean  $\pm$  standard error of reflectance spectra for red, blue, and yellow killifish fins (left) and replica fins (right). Color coding corresponds to the coloration of the fins of the respective killifish or replica. For real killifish fins, sample sizes were as follows: red-8, blue-5, yellow-7. For replica fins, sample sizes are as follows: red-3, blue-2, yellow-3.

significantly more time swimming in the vicinity of yellow painted replica as compared to a control condition in which replicas were not present, offering indirect evidence that killifish are able to distinguish between color morphs. The versatility and customizability of the proposed robotic platform are expected to aid in a series of hypothesis-driven studies on mating behavior. For example, future work will focus on the role of locomotory patterns and visual feedback on killifish mating, by implementing real-time control of the replica motion in response to live female subjects. A similar interactive closed-loop control has been explored in [33], whereby zebrafish preference for a biologically-inspired robotic fish is studied. In an effort to more closely mimic locomotory patterns by enabling body undulation, future studies will also focus on the integration of miniature compliant active materials [64–68] in the replica.

## Acknowledgments

The authors would like to gratefully acknowledge Dr G Kozak for valuable help in the definition of the experimental procedure adopted in the study. This research was supported by the National Science Foundation under grant nos. CMMI-0745753, DGE-0741714, and DEB-0953716.

## References

- [1] Badyaev A V and Hill G E 2003 Avian sexual dichromatism in relation to phylogeny and ecology *Annu. Rev. Ecol. Evol. S* **34** 27–49
- [2] Messmer V, Jones G P, van Herwerden L and Munday P L 2005 Genetic and ecological characterisation of colour dimorphism in a coral reef fish *Environ. Biol. Fish.* **74** 175–83
- [3] Bell R C and Zamudio K R 2012 Sexual dichromatism in frogs: natural selection, sexual selection and unexpected diversity *Proc. R. Soc. B* **279** 4687–93
- [4] Amundsen T 2000 Why are female birds ornamented? *Trends Ecol. Evol.* **15** 149–55
- [5] Pilastro A, Benetton S and Bisazza A 2003 Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki Anim. Behav.* **65** 1161–7
- [6] Roulin A, Dijkstra C, Riols C and Ducrest A L 2001 Female- and male-specific signals of quality in the barn owl *J. Evolution. Biol.* **14** 255–66
- [7] Galeotti P, Rubolini D, Dunn P O and Fasola M 2003 Colour polymorphism in birds: causes and functions *J. Evolution. Biol.* **16** 635–46
- [8] Gray S M and McKinnon J S 2007 Linking color polymorphism maintenance and speciation *Trends Ecol. Evol.* **22** 71–9
- [9] Sinervo B, Miles D B, Frankino W A, Klukowski M and DeNardo D F 2000 Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards *Horm. Behav.* **38** 222–33
- [10] Van Gossum H, Sherratt T N and Cordero-Rivera A 2008 *The Evolution of Sex-limited Colour Polymorphisms. Dragonflies: Model Organisms for Ecological and Evolutionary Research* (Oxford, UK: Oxford University Press)
- [11] Pryke S R, Astheimer L B, Griffith S C and Buttemer W A 2012 Covariation in life-history traits: differential effects of diet on condition, hormones, behavior, and reproduction in genetic finch morphs *Amer. Nat.* **179** 375–90
- [12] McGhee K E, Fuller R C and Travis J 2007 Male competition and female choice interact to determine mating success in the bluefin killifish *Behav. Ecol.* **18** 822–30
- [13] Tibbetts E A and Dale J 2004 A socially enforced signal of quality in a paper wasp *Nature* **432** 218–22
- [14] de Heij M E, Gustafsson L and Brommer J E 2011 Experimental manipulation shows that the white wing patch in collared flycatchers is a male sexual ornament *Ecol. Evol.* **1** 546–55
- [15] Sheldon B C, Andersson S, Griffith S C, Örnborg J and Sendecka J 1999 Ultraviolet colour variation influences blue tit sex ratios *Nature* **402** 874–7
- [16] Hoefler C D and Morris M R 1999 A technique for the temporary application and augmentation of pigment patterns in fish *Ethology* **105** 431–8
- [17] Houde E D 1997 Patterns and trends in larval-stage growth and mortality of teleost fish *J. Fish Biol.* **51** 52–83
- [18] Hancox D, Hoskin C J and Wilson R S 2010 Evening up the score: sexual selection favours both alternatives in the

- colour-polymorphic ornate rainbowfish *Anim. Behav.* **80** 845–51
- [19] Beausoleil J M J, Doucet S M, Heath D D and Pitcher T E 2012 Spawning coloration, female choice and sperm competition in the redbreast dace *Clinostomus elongatus* *Anim. Behav.* **83** 969–77
- [20] Tinbergen N 1948 Social releasers and the experimental method required for their study *Wilson Bull.* **60** 6–51
- [21] Sevenster P 1949 Modderbaarsjes *Lev. Nat.* **52** 184–9
- [22] Posner G S 1982 Variation in the aggressive behavior of *Xiphophorus variatus* (Pisces: Poeciliidae) with regard to group dynamics and response towards conspecific models (New York: New York University) p 116
- [23] Rowland W J 1999 Studying visual cues in fish behavior: a review of ethological techniques *Environ. Biol. Fish.* **56** 285–305
- [24] Rosenthal G G and Evans C S 1998 Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size *Proc. Natl. Acad. Sci.* **95** 4431–6
- [25] Künzler R and Bakker T C M 2001 Female preferences for single and combined traits in computer animated stickleback males *Behav. Ecol.* **12** 681–5
- [26] Karino K, Shimada Y, Kudo H and Sato A 2010 Relative importance of the area and intensity of the orange spots of male guppies *Poecilia reticulata* as mating traits preferred by females *J. Fish Biol.* **77** 299–307
- [27] Abaid N, Spinello C, Laut J and Porfiri M 2012 Zebrafish (*Danio rerio*) responds to images animated by mathematical models of animal grouping *Behav. Brain Res.* **232** 406–10
- [28] Polverino G, Liao J C and Porfiri M 2013 Mosquitofish (*Gambusia affinis*) preference and behavioral response to animated images of conspecifics altered in their color, aspect ratio, and swimming depth *PLoS ONE* **8** e54315
- [29] Saverino C and Gerlai R 2008 The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish *Behav. Brain Res.* **191** 77–87
- [30] Seehausen O and van Alphen J J 1998 The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (Haplochromis nyererei complex) *Behav. Ecol. Sociobiol.* **42** 1–8
- [31] Swain D T, Couzin I D and Leonard N E 2012 Real-time feedback-controlled robotic fish for behavioral experiments with fish schools *Proc. IEEE* **100** 150–63
- [32] Krause J, Winfield A F and Deneubourg J L 2011 Interactive robots in experimental biology *Trends Ecol. Evol.* **26** 369–75
- [33] Kopman V, Laut J W, Polverino G and Porfiri M 2013 Closed-loop control of zebrafish response using a bioinspired robotic-fish in a preference test *J. R. Soc. Interface* **10** 20120540
- [34] Polverino G and Porfiri M 2013 Mosquitofish (*Gambusia affinis*) responds differentially to a robotic fish of varying swimming depth and aspect ratio *Behav. Brain Res.* **250** 133–8
- [35] Polverino G, Abaid N, Kopman V, Macri S and Porfiri M 2012 Zebrafish response to robotic fish: preference experiments on isolated individuals and small shoals *Bioinspir. Biomim.* **7** 036019
- [36] Marras S and Porfiri M 2012 Fish and robots swimming together: attraction towards the robot demands biomimetic locomotion *J. R. Soc. Interface* **9** 1856–68
- [37] Polverino G and Porfiri M 2013 Zebrafish (*Danio rerio*) behavioural response to bioinspired robotic fish and mosquitofish (*Gambusia affinis*) *Bioinspir. Biomim.* **8** 044001
- [38] Spinello C, Macri S and Porfiri M 2013 Acute ethanol administration affects zebrafish preference for a biologically-inspired robot *Alcohol* **47** 391–8
- [39] Abaid N, Marras S, Fitzgibbons C and Porfiri M 2013 Modulation of risk-taking behaviour in golden shiners (*Notemigonus crysoleucas*) using robotic fish *Behav. Process.* **100** 9–12
- [40] Cianca V, Bartolini T, Porfiri M and Macri S 2013 A robotics-based behavioral paradigm to measure anxiety-related responses in zebrafish *PLoS ONE* **8** e69661
- [41] Aureli M, Fiorilli F and Porfiri M 2012 Portraits of self-organization in fish schools interacting with robots *Physica D* **241** 908–20
- [42] Faria J J, Dyer J R G, Clement R O, Couzin I D, Holt N, Ward A J W, Waters D and Krause J 2010 A novel method for investigating the collective behaviour of fish: introducing ‘Robofish’ *Behav. Ecol. Sociobiol.* **64** 1211–8
- [43] Rossi C, Coral W and Barrientos A 2012 *Swimming Physiology of Fish: Towards Using Exercise for Farming a Fit Fish in Sustainable Aquaculture* ed A P Palstra and J V Planas (New York: Springer)
- [44] Polverino G, Phamduy P and Porfiri M 2013 Fish and robots swimming together in a water tunnel: robot color and tail-beat frequency influence fish behavior *PLoS ONE* **8** e77589
- [45] Abaid N, Bartolini T, Macri S and Porfiri M 2012 Zebrafish responds differentially to a robotic fish of varying aspect ratio, tail beat frequency, noise, and color *Behav. Brain Res.* **233** 545–53
- [46] Foster N R 1967 *Comparative Studies on the Biology of Killifishes (Pisces, Cyprinodontidae)* (Ithaca, NY: Cornell Univ.)
- [47] Fuller R C 2002 Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations *Proc. R. Soc. B* **269** 1457–65
- [48] Fuller R C and Travis J 2004 Genetics, lighting environment, and heritable responses to lighting environment affect male color morph expression in bluefin killifish *Lucania goodei* *Evolution* **58** 1086–98
- [49] Fuller R C and Johnson A M 2009 A test for negative frequency-dependent mating success as a function of male colour pattern in the bluefin killifish *Biol. J. Linn. Soc.* **98** 489–500
- [50] Fuller R C and Noa L A 2010 Female mating preferences, lighting environment, and a test of the sensory bias hypothesis in the bluefin killifish *Anim. Behav.* **80** 23–35
- [51] Fuller R C, Fleishman L J, Leal M, Travis J and Loew E 2003 Intraspecific variation in retinal cone distribution in the bluefin killifish *Lucania goodei* *J. Comp. Physiol. A* **189** 609–16
- [52] Butail S, Bartolini T and Porfiri M 2013 Collective response of zebrafish shoals to a free-swimming robotic fish *PLoS ONE* **8** e76123
- [53] Kalueff A V, Stewart A M and Gerlai R 2014 Zebrafish as an emerging model for studying complex brain disorders *Trends Pharmacol. Sci.* **35** 63–75
- [54] Fuller R C 2001 Patterns in male breeding behaviors in the bluefin killifish, *Lucania goodei*: a field study (Cyprinodontiformes: Fundulidae) *Copeia* **2001** 823–8
- [55] Price A C, Weadick C J, Shim J and Rodd F H 2008 Pigments, patterns, and fish behavior *Zebrafish* **5** 297–307
- [56] Tinbergen N 1953 *The Herring Gull's World: a Study of the Social Behaviour of Birds* (Oxford: Frederick A. Praeger)
- [57] McAlister W H 1958 The correlation of coloration with social rank in *Gambusia hurtadoi* *Ecology* **39** 477–82
- [58] Molstad A J 2008 *Development of Vision and the Effect of Spectral Environment on the Cone Photoreceptor Mosaic of the Bluefin Killifish Lucania goodei* (Tallahassee, FL: Florida State University) p 30
- [59] Cummings M E, Jordão J M, Cronin T W and Oliveira R F 2008 Visual ecology of the fiddler crab, *Uca tangeri*: effects of sex, viewer and background on conspicuousness *Anim. Behav.* **75** 175–88

- [60] Bybee S M, Yuan F, Ramstetter M D, Llorente-Bousquets J, Reed R D, Osorio D and Briscoe A D 2012 UV photoreceptors and UV-yellow wing pigments in *Heliconius* butterflies allow a color signal to serve both mimicry and intraspecific communication *Amer. Nat.* **179** 38–51
- [61] Flamarique I N and Harosi F I 2000 Photoreceptors, visual pigments, and ellipsosomes in the killifish, *Fundulus heteroclitus*: a microspectrophotometric and histological study *Visual Neurosci.* **17** 403–20
- [62] Körner K E, Schlupp I, Plath M and Loew E R 2006 Spectral sensitivity of mollies: comparing surface- and cave-dwelling Atlantic mollies *Poecilia mexicana* *J. Fish Biol.* **69** 54–65
- [63] Watson C, Lubieniecki K, Loew E, Davidson W and Breden F 2010 Genomic organization of duplicated short wave-sensitive and long wave-sensitive opsin genes in the green swordtail *Xiphophorus helleri* *BMC Evol. Biol.* **10** 87
- [64] Cen L and Erturk A 2013 Bio-inspired aquatic robotics by untethered piezohydroelastic actuation *Bioinspir. Biomim.* **8** 016006
- [65] Mojarrad M and Shahinpoor M 1997 Biomimetic robotic propulsion using polymeric artificial muscles *Proc. IEEE Int. Conf. Robotics and Automation (Albuquerque, NM)* pp 2152–7
- [66] Chen Z, Sharata S and Tan X 2010 Modeling of biomimetic robotic fish propelled by an ionic polymer metal composite caudal fin *IEEE ASME Trans. Mechatron.* **15** 448–59
- [67] Aureli M, Kopman V and Porfiri M 2010 Free-locomotion of underwater vehicles actuated by ionic polymer metal composites *IEEE ASME Trans. Mechatron.* **15** 603–14
- [68] Yeom S W and Oh I K 2009 A biomimetic jellyfish robot based on ionic polymer metal composite actuators *Smart Mater. Struct.* **18** 085002