And the zebrafish said: I like biomimetic robots

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Abstract—In this paper, we study the feasibility of modulating fish behavior using bioinspired underwater robots. We experimentally study the response of zebrafish (Danio rerio) to a robotic fish of varying size, color pattern, and motility in a dichotomous preference test. In this canonical experimental protocol, focal fish residing in the central compartment of a three-chambered test tank are confronted with various robots juxtaposed with the empty compartment and their position is observed over time to measure preference. A total of six experimental conditions is studied to isolate the effect of different robot design elements and provide general techniques for fostering the attraction of zebrafish.

I. INTRODUCTION

In recent years, robotics has often sought inspiration from nature to design human-centered devices for improved performance, such as smart materials for sensing and actuation, complex mechanisms for functional locomotion, and optimized algorithms for robust intelligence [1], [2]. Nevertheless, the research question on whether these biologically inspired systems can be integrated with and control the source of their inspiration remains largely untested.

In this context, the use of biomimetic robots for regulating animal behavior in laboratory and real-world environments is a particularly relevant application of biologically inspired robotics. In fact, controlled experiments leveraging the use of versatile and multifunctional biomimetic robots coupled with established genetic and pharmacological techniques have the potential to uncover many fundamental determinants of biological functions and dysfunctions. Conversely, biomimetic robots which can be deployed to aggregate, lead, or repel target species in natural environments may greatly aid animal protection, production, or control [3], [4].

Current experimental efforts have investigated the interactions between animals and robots based on a range of biomimetic features targeting several sensory cues. For example, wheeled ground vehicles have been used to influence social patterns in chickens [5], quails [6], rats [7], and ducks [8] by eliciting either social or antipredatory patterns. A similar type of motility is considered in experiments on fish interacting with rigid replicas moved in an aquarium by an external magnetic positioning system [9]. However, biomimetic locomotion is shown to be an essential cue for dogs confronted with quadrupedal robots [10], birds confronted with moving stuffed decoys designed to elicit foraging or alarm responses [11], fish swimming with a fixed robot in a water channel [12], and bees interacting with mechanisms inspired by bee dances [13]. Other ethological considerations, such as pheromone and vocal communication, are taken into account for robots developed for use with cockroaches [14] and squirrels [15], respectively. This wide range of studies has demonstrated that many species of animals of varying complexity respond to biomimetic robots with observable modifications to their behavior.

Here, we hypothesize that a biomimetic robotic fish, inspired by zebrafish in its shape, color pattern, and motility, attracts zebrafish; in other words, “zebrafish like biomimetic robots”. Zebrafish are selected based on their social behavior and their extensive use as an animal model for the investigation of functional and dysfunctional biological processes [16]–[18]. We test this hypothesis by evaluating zebrafish choice in a binary preference test wherein live subjects are offered a choice between a pair of visual stimuli in a three-chambered test tank, which physically separates fish in the central compartment and stimuli in the lateral compartments using transparent Plexiglas for visual interaction [19]–[21]. In this series of experiments, fish are confronted with an empty compartment and various robotic fish. Fish preference is measured as an individual’s proximity to one of the two juxtaposed stimuli.

The biomimetic robot is designed by drawing inspiration from natural determinants of attraction in conspecifics while compromising with the engineering need of realizing a self-propelled robot for autonomous operations. Therefore, with the constraint of a fixed payload, the robot aspect ratio is skewed towards a stocky shape resembling a fertile adult female zebrafish, which is preferred by both males and females of this species [22]. The color pattern of the biomimetic robot is selected to mimic the natural color pattern of the target species while enhancing its biological relevance [20], [23]. The robot’s tail is controlled to achieve the fastest swimming speed if left untethered [24]. To control for the effects of these design criteria, we explore the effect of variations of the robot’s features on zebrafish response by changing its aspect ratio towards either elongated or curbed resemblances, minimizing its degree of visual biomimicry by removing stripes and yellow pigment, and altering its tail...
beat frequency to slower configurations. We also conduct experiments in the darkness to test for the effect of the servomotor noise on fish behavior and establish guidelines for future design concepts.

II. MATERIALS AND METHODS

A. Experimental subjects

Zebrafish (*Danio rerio*) were acquired from two local aquarium stores (Petland Discounts, Brooklyn and New World Aquarium, Manhattan, New York City, USA) and from liveaquaria.com. The approximate age of the subjects involved in this study is six to eight months inferred by their mean body length (BL) of three centimeters. Fish were housed in either large or small glass aquaria in groups of ten or twenty animals for at least two weeks before experiments commenced to acclimatize animals to the laboratory environment. Large holding tanks have dimensions 40 cm by 20 cm by 25 cm (L × H × W) and capacity of 19 liters; small holding tanks have dimensions 30 cm by 15 cm by 20 cm and capacity of 9 liters. Water was maintained at a temperature of 26 ± 1°C and pH of 7.2 in all aquaria. The housing aquaria were illuminated by fluorescent lights for a 12 hour daily photocycle following the circadian rhythm of zebrafish, see for example [16]. Fish were fed once or twice per day with commercial flake food (Hagen Corp., Nutrafin max, USA), at least one hour before experimentation and/or after experimentation was completed. Approximately forty fish were purchased and ten fish (N = 10) were tested in each experimental condition.

B. Biomimetic robots

Four robotic fish prototypes are considered in this study to assess how the tail beat frequency, aspect ratio, and coloration of the fish affect attraction or repulsion, see Figure 1. Each robotic fish comprises a rigid acrylonitrile butadiene styrene plastic skeleton- including body shell and tail section- printed on a rapid prototyping machine (Stratasys, Dimension SST, USA) and waterproofed with rubberized plastic. A mylar caudal fin is attached to the tail section to amplify the thrust produced by the servomotor’s vibration in the case that the robot is untethered. The body shell and tail section are rigid, while the caudal fin is compliant. In all robots, the tail section and passive caudal fin represent approximately half of the robot’s total length to replicate the carangiform/subcarangiform motions typical of zebrafish. Robots are anchored to the experimental tank with a small support-rod to guarantee the repeatability of conditions. The robots’ tail sections are actuated by a servomotor (Traxxas, 2065 Sub-Micro servomotor, USA) controlled by an external microcontroller (Arduino, DueMilanove, Italy), both of which are connected to a power supply for uninterrupted operation. The microcontroller allows selecting two motor-control parameters: the tail beating frequency and amplitude.

We refer to the robot whose motility, aspect ratio, and coloration is inspired by zebrafish as the reference robot (RR). The robot dimensions are 15 cm by 4.8 cm by 2.6 cm (L × H × W), which are the minimal dimensions that can be obtained to match the desired aspect ratio resembling a fertile female given the selection of the servomotor. The robot is painted to enhance the yellow pigment and magnify the blue stripes of the target species in line with innate zebrafish preference [20], [23], see Figure 2. This color pattern is obtained by spray-painting the robot with a metallic-silver non-toxic base paint, hand-painting metallic-blue and yellow stripes, and attaching small plastic eyes. The reference robot is operated with a tail beat frequency of 2 Hz and with a displacement of the rigid tail section tip of approximately 1.5 cm. The frequency is selected to maximize the tail tip speed by approximating internal resonance of the robot’s caudal fin; the tail beating amplitude is selected as the largest possible value attainable with the selected hardware. These parameters would maximize robot terminal swimming speed if it were left untethered [24].

To control for the aspect ratio of the robot, we design two additional robots whose lengths are either elongated or compressed compared to the reference robot, referred to as elongated robot (ER) and curbed robot (CR). The elongated robot is approximately 1.5 times longer than the reference robot and the curbed robot is approximately 1.5 times shorter than the reference robot; the robots’ other dimensions are kept constant. In particular, the elongated robot is 22 cm by 4.8 cm by 2.7 cm and the curbed robot is 10 cm by 4.3 cm by 1.9 cm. Both these robots retain the visual biomimicry of the reference robot, that is, their color and stripes are identical to the reference robot, see Figure 1. To control for the effect of the robot’s color and stripes, we introduce a monochromatic replica of the reference robot that is colored using only the silver base paint of the reference robot, which we refer to as the monochromatic robot (MR). The curbed, elongated, and monochromatic robots are actuated with the same parameters of the reference robot. To control for the effect of the robot motility on zebrafish behavior, we reduce the tail beat frequency of the reference robot. We refer
to this robot as the slow robot (SLR) and we use a tail beat frequency of 0.5 Hz and a tail section tip displacement of approximately 2.5 cm. These parameters yield a more than halved tip speed as compared to the reference robot. Since the robot is fixed in all conditions, the modulation of tail beat frequency is aimed at testing zebrafish response to the perceived oscillations. However, this frequency is a determinant of the swimming speed of the untethered robot and thus, fish response to variations in this parameter may predict their amenability to robots operating with control parameters necessitated by engineering constraints.

C. Apparatus

The experimental apparatus comprises a test tank diagramed in Figure 3. In all illuminated conditions, the tank is lighted by two 25 W fluorescent strip lamps placed at 20 cm from either side of the tank to obtain homogenous illumination of the environment and a webcam (Logitech HD Webcam C270, USA) is placed at approximately 140 cm above the tank for recording fish behavior. Both the tank and lights are surrounded by fabric curtains to visually isolate experimental subjects. The dimensions of the test tank are 74 cm by 30 cm by 30 cm and its capacity is 65 liters. The test tank is partitioned into three sections along its longest axis; the two side compartments have a length equal to ten centimeters and the central region is 54 cm long. Compartments are delimited by transparent Plexiglas panels that are perforated by an array of two mm diameter holes to ensure homogeneous water conditions across the tank in the case that lateral compartments host live fish (not considered in this work). Plexiglas panels were removed for filtration of the test tank water overnight. Constant water temperature is maintained using a thermostat-controlled stick heater attached upright at the center of the long tank wall.

To study shoaling tendency in the absence of visual cues, the apparatus is modified by replacing the lateral lights with two infrared spot-lights (Wisecomm, Infrared LED night light, China) and by replacing the usual camera with a webcam (Creative Labs, VF0205, China) with its infrared filter removed to enable sensing infrared light. The modified webcam is placed at approximately 100 cm above the water’s surface to allow for a full observation of the test tank and the infrared lights are placed symmetrically around the test tank to maintain uniform illumination.

D. Protocol

For each experimental condition, a single focal fish located in the central test tank compartment is confronted with pairs of stimuli residing in the two side compartments. Stimuli pairs are taken from the empty compartment, the reference robot, the slow robot, the curbed robot, the elongated robot, and the monochromatic robot. Referring to the empty compartment as the “zero” stimulus (0), we study a total of six conditions: RR vs 0; SLR vs 0; CR vs 0; ER vs 0; MR vs 0; RR vs 0 in the dark. Each condition is tested with ten individuals and each individual is tested eight times per condition and no more than three times on a given day.

Stimuli are inverted during each condition to eliminate potential bias in the data from a preference of the zebrafish for a side of the test tank independent of stimuli. All robots except for the elongated robot are positioned at approximately 45 degrees to walls of the compartment to display their caudal fins to focal fish. The elongated robot is positioned parallel to the Plexiglas panel to avoid the mylar tail contacting with the panel during flapping. In all conditions except for that in the dark, an anchor identical to the robot’s support is inserted into the empty compartment to ensure equivalent background visual stimuli.

At the beginning of each test, the focal fish is selected at random and placed with a hand net into the central compartment of the test tank. The fish acclimates to the tank swimming freely in this compartment for ten minutes. This acclimatization period is approximately twice as long as the time for zebrafish to habituate to a new stimulus [25]; thus, any possible novelty effects influencing fish preference for stimuli is eliminated. Subsequently, the focal fish’s position in the test tank is recorded for a five-minute experimental session. After the trial, fish are separated from the general population to avoid repeated testing of the same subject.
This experimental protocol was approved by Polytechnic Institute of New York University Animal Welfare Oversight Committee AWOC-2011-101.

E. Data acquisition and analysis

Fish behavior during five-minute experimental trials is analyzed using the instantaneous sampling method [26]. Each experimental video is downsampled at a period of 30 seconds to obtain a sequence of ten snapshots per five-minute video. The two-dimensional positions of the fish are manually tracked in each snapshot and their one-dimensional components along the longitudinal axis of the tank between the juxtaposed stimuli are recorded for the analysis. The fish position is scored at the center of the apparatus if the fish is undetected; the number of observations in which the fish were undetectable was less than 5% of total observations in all conditions. An exemplary snapshot from the SLR vs 0 condition is shown in Figure 4, with circles representing the 800 fish positions acquired from that condition. We comment that a considerable portion of the experimental time is spent in the vicinity of the tank borders as illustrated in Figure 4. This behavior is referred to as thigmotaxis and is well documented in the zebrafish literature [27].

We amalgamate the data on fish position to obtain a one-dimensional position histogram of the 800 data points per condition. The one-dimensional data refer to fish position along the major axis of the central compartment of the experimental tank. The histogram bin size is one BL, which partitions the central compartment of the test tank into eighteen bins. Formally, let \( \{x_i\}_{i=1}^{800} \) be the sequence of the abscissas of fish positions in centimeters so that \( x_i, i = 1, \ldots, 800 \), are real numbers between 0 and 54. We partition the interval [0, 54] into bins of length equal to 3 cm and we count the percentage of total occurrences in each bin to obtain the histogram. The frequency of fish positions in a bin is taken as the probability of a fish to reside in that location due to the large number of data points.

To synthetically ascertain the preference of a fish for a stimulus in a given condition, we consider a further partition of the central compartment of the test tank that consists of two four-BL-long near-stimulus areas on either side of the tank and a ten-BL-long center section. For two stimuli \( A \) and \( B \), the absolute frequency of a fish’s one-dimensional position in three areas- near stimulus \( A \) (\( n_A \)), near stimulus \( B \) (\( n_B \)), and in the center- is recorded and amalgamated over all trials for the condition. We define fish preference for stimulus \( A \) by calculating the ratio of probabilities to be near stimulus \( A \) as opposed to near stimulus \( B \), and similarly for stimulus \( B \). These quantities allow balancing the preferences for the two stimuli directly by neglecting the frequency with which a fish resides in the center of the tank.

The statistical significance of fish preference is ascertained using a chi-square (\( \chi^2 \)) test where the expected distribution is taken as uniform, see for example [28]. Specifically, the chi-square test compares the number of data points in each near-stimulus region to a uniformly distributed vector with the same component sum, which indicates the event of fish having equal preference for both stimuli. We comment that the absolute number of observations enters directly into this nonparametric test. For a given condition, we compute \( \chi^2 \) by comparing the observed vector \((n_A, n_B)\) with the expected vector \(((n_A + n_B)/2, (n_A + n_B)/2)\). We find

\[
\chi^2 = \frac{(n_A - n_B)^2}{2(n_A + n_B)}
\]

and the p-value is computed from the \( \chi^2 \) distribution. The level of statistical significance for all statistical tests is set at \( p < 0.05 \).

III. RESULTS

Experimental results on fish positions are presented in Figure 5 and results on preference and its statistical significance are presented in Table I. Therein, “Preference” gives the percent of time spent by fish near the non-empty compartment relative to the total time in near-stimulus areas over all trials in a selected condition, \( \chi^2 \) and \( p \) are values from the statistical analysis on preference, and “Center” is the percentage of time spent in the central region of the test tank.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Preference (%)</th>
<th>( \chi^2 )</th>
<th>( p )</th>
<th>Center (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR vs 0</td>
<td>67.1</td>
<td>45.8</td>
<td>&lt; 0.01</td>
<td>31.3</td>
</tr>
<tr>
<td>SLR vs 0</td>
<td>67.0</td>
<td>58.5</td>
<td>&lt; 0.01</td>
<td>36.3</td>
</tr>
<tr>
<td>CR vs 0</td>
<td>56.4</td>
<td>8.7</td>
<td>&lt; 0.01</td>
<td>33.5</td>
</tr>
<tr>
<td>ER vs 0</td>
<td>55.4</td>
<td>5.4</td>
<td>&lt; 0.05</td>
<td>42.0</td>
</tr>
<tr>
<td>MR vs 0</td>
<td>53.1</td>
<td>1.8</td>
<td>= 0.12</td>
<td>40.3</td>
</tr>
<tr>
<td>RR vs 0 (dark)</td>
<td>36.1</td>
<td>36.3</td>
<td>&lt; 0.01</td>
<td>41.8</td>
</tr>
</tbody>
</table>

From a first crude assessment of the preference data in Figure 5, we find that zebrafish prefer the biomimetic robot irrespective of the tail beat frequency, they weakly prefer or are insensitive to variations in shape and color, and they avoid the reference robot in the darkness. Further insight into the statistical significance of these data can be garnered by delving into the results of the chi-square test in Table I. Specifically, we find that fish are significantly attracted by the reference robot under illuminated conditions. In addition, modifications to the robot’s flapping speed and aspect ratio do not act to hinder this preference, as fish in the SLR vs
0, CR vs 0, and ER vs 0 conditions all prefer the robot to the empty compartment. Nevertheless, coloration plays an influential role on fish preference, as fish do not significantly prefer the monochromatic robot versus the empty compartment. Among all conditions with the modified robots, fish spend approximately the same percentage of time in the central region of the experimental compartment, between 33.5% and 42.0%, which are all less than the 51% spent in the center in the RR vs 0 condition. The sound generated by the robot’s servomotor is aversive to fish in the absence of the visual stimulus, evidenced by the fish preference for the empty compartment in the reference robot versus zero trials in the dark.

IV. DISCUSSION

Based on these results, we conclude that zebrafish prefer the reference robot to the empty compartment. When the robot is modified for tail beat frequency or aspect ratio, this preference persists, which suggests that the visual stimulus of the moving, striped robot has marked attractive influence on the focal fish. This interpretation is corroborated by the lack of fish preference for the monochromatic robot juxtaposed with the empty compartment. Such results hint that the robot’s color pattern is a more influential determinant of preference than shape or tail beat frequency.

Experiments in the dark show that zebrafish are repelled by the robotic stimulus when the auditory features dominate the absent visual stimulus. Comparing this result to the lighted experiment, we determine that the zebrafish preference for the reference robot’s visual stimulus is hindered by the sound of the servomotor; thus, the observed preference in this experimental condition evidences that the robot comprises both attractive and repellant stimuli.

We find that the variations of the robot’s motility do not play an integral role in fish preference. In fact, the reference robot and its slower counterpart are not differentiated by fish preference. Since the reference and slow robots are exact replicas other than for their motion, we posit that the visual stimulus of motion, is attractive as long as its intensity is nonzero. This preference agrees with the response to moving visual stimuli observed in larval zebrafish [29]. From an engineering standpoint, this suggests that the robot’s propulsion may be driven by energy efficiency considerations rather than actual biomimicry of zebrafish, whose sociality is largely determined by visual cues [30].

Zebrafish response to modulations of the robot’s aspect ratio may also evidence species-dependent tendencies developed for social life. Although fish are never repelled by any of the modifications from the reference shape, the preference for the elongated or curbed robot is lessened compared to their preference for the reference robot. This finding supports [20], in which zebrafish are found to reject computer-animated conspecific images altered to have a similar aspect ratio to the elongated robot. In fact, the shape of the elongated robot is motivated by the morphology of native predators of this species, and indeed fish avoided such “skinny” stimuli. Nevertheless, the elongated robot is approached by fish when it is confronted with an empty compartment, which suggests that fish preference is not transitive among stimuli.

In conclusion, we have demonstrated that a robot tailored to the zebrafish species elicits a strong preference from the focal fish. That is, we have demonstrated that matching
the aspect ratio of a fertile female zebrafish, replicating its stripe pattern, and enhancing its yellow pigment are all beneficial aspects in enforcing the robot attractiveness. Modifications to the color and shape of this robot do not induce aversive responses, yet they may lessen the robot’s attraction. On the other hand, varying the robot’s motion seems to have no impact on fish preference, which posits the feasibility of tuning the robot locomotion based on engineering constraints rather than biological determinants. Future work will include integration of biomimetic actuation using noiseless artificial muscles [31] and exercising closed-loop control using visual tracking of fish position as an input for the stimulus administered by the robot [32].

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REFERENCES