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Towards Fish Welfare in the Presence of Robots: Zebrafish Case

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Abstract: Zebrafish (Danio rerio) have emerged as a valuable animal model for neurobehavioral research, particularly in the study of anxiety-related states. This article explores the use of conceptual models to investigate stress, fear, and anxiety in zebrafish induced by bio-inspired mini-robotic fish with different components and designs. The objective is to optimize robotic biomimicry and its impact on fish welfare. Previous studies have focused on externally controlled fish models, whereas this study introduces prototypes of freely actuated swimming robots to examine interactions between a bio-inspired robot and individual zebrafish. By means of analysis of behavioral responses, certain robotic components have been identified as potential causes of anxiety in fish, which have provided insights that may be applicable to other species and future aquacultural robot designs.

Keywords: underwater robotics; fish robot; robot biomimicry

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1. Introduction

The field of robotics and underwater design have directed their efforts at the development of unmanned autonomous vehicles (UUVs) [1] to carry out actions that could otherwise pose a risk to humans. This field can be directly applied to the aquaculture environment, where the working atmosphere is demanding and, in many cases, the performance of certain tasks may be challenging to human assets. Manual tasks such as cleaning, inspecting, or repairing net cages can be detrimental to workers [2]; furthermore, there are stricter regulations governing the time divers can spend underwater or the tasks they can perform in order to protect worker conditions. In recent years, the aquaculture industry has witnessed a growing trend towards robotization [3], where robots are employed to carry out various tasks. This development aims to minimize the exposure of workers and enhance the efficiency of farm production processes.

Currently, many projects are working on the development of remote-controlled underwater robots which can be used in this and other environments [4]. One project that has embraced this environment, and on which this study is based, is the ThinkInAzul project [5], which aims, as one of its objectives, to approach sustainable smart precision aquaculture by creating technology which improves inspection, maintenance, and repair operations [6], and for which there is a need to design a mimetic robot that can perform specific monitoring, inspecting, sensing, and sample-collecting tasks within the cages.

However, conventional underwater robots used to date can, in many cases, have an impact on the environment, as well as being intrusive and stressful to marine life, due to their aesthetic and/or mechanical characteristics [7,8].

In the last two decades, the emerging field of biomimetics has sought to explore the design by copying the forms of living beings found in nature, as well as their movements and forms of propulsion [9,10], in order to be friendlier and less invasive to the fauna of the marine environment in which they operate. This has led to a surge of studies in the field of biomimetics focused on the development of bioinspired fish [11]. Most of these studies have primarily concentrated on emulating fish propulsion systems and assessing their efficiency [12–14]. Traditional propellers produce currents, consume more energy, reduce propulsion efficiency, and are noisy and aggressive, while mimicking the swimming propulsion mode of fish is more efficient, less noisy, and provides better robot performance in terms of energy efficiency [15]. Additionally, in the context of aquaculture, keeping fish healthy and stress-free increases production, breeding performance, and profitability [16]. It is therefore desirable that the robot used in fish farms is respectful of the fauna and as non-invasive as possible. To optimize biomimicry and the design of underwater robots operating in fish farms, it is essential to identify and test which robotic elements are the main stressors and causes of disturbance.

To analyze the effects robotic disturbances may elicit on fish, it is imperative to understand the defensive behaviors they exhibit in response to stimuli and situations perceived as stressful threats. To date, controlled studies have been conducted to identify specific responses and consistent behavioral patterns displayed by fish in stressful situations [17]. Such studies have involved the introduction of robotic stimuli in animal behavior research, where bio-inspired fish prototypes were developed and tested to evaluate robot-fish interactions [18,19]. Nevertheless, most of these investigations have utilized robotic platforms and external mechanisms to generate the movement and trajectory of replicas. Although some recent studies have explored the potential for bidirectional interactions between robotic stimuli and live subjects in free-swimming contexts [20], such efforts have not primarily focused on identifying the stress induced by specific robotic components. Several studies have demonstrated the influence of color and/or pattern [21] on conspecific relationships, showing that fish species lack high visual acuity but have a remarkable ability to discriminate contrast. Contrasting patterns may be important cues for social mimicry in discriminating between conspecifics and predators. Animal size may also be an important factor in social interaction with conspecifics and non-conspecifics. Studies focusing on the influence of size have concluded that a larger size may result in the individual being identified as a predator or rejected as a conspecific [22]. Physical parameters like sound and light [23] can also be a source of interference, suggesting that the use of actuators capable of disturbing their acoustic and/or vision channel could be another source of stress. Finally, some studies have also focused on the robot swimming/movements using actuators to control different fins and investigating the efficiency but also the acceptance of these replicas within a group of individuals [24]. Therefore, it is crucial to understand how social and environmental interactions are managed using the senses to achieve the mimicry of bioinspired robots and assess their impact on fish behavior in aquaculture [25].

Therefore, to achieve the mimicry of bioinspired robots, it is crucial to understand how social interactions among fish are dealt with and what specific responses they exhibit in order to evaluate the impact that robots may cause. Such specific responses to challenging situations to which they are exposed are generally classified as anxiety-like behaviors (ALB). However, some researchers have suggested that it may be more appropriate to differentiate between anxiety, fear, and panic based on the perceived immediacy of the threat [26]. When fish perceive the risk as slight, they tend to display exploratory behavior. In situations where risk is perceived as moderate, escape and avoidance behaviors are observed. On the other hand, if fish perceive an imminent threat, they may respond with a defensive attack or freezing [27]. Previous studies [28] have developed models that facilitate the study of these specific behaviors and enable the identification and evaluation of various behavioral variables or endpoints (see Table 1).

Table 1. Fish behavior models and evaluative measures for reactions.

Analytical Model	Evaluation Measures for Determining the Presence of Stress
Conditioned Alarm Reaction	Time in Bottom Zone Total Path Length Freeze Time Fast Swimming
Inhibitory Avoidance	Time Until Visiting Aversive Zone
	Time in Aversive Zone
Predator Response	Burst Swimming Freeze Bottom Dwell Time Distance to Predator
Inspection of Novel Objects	Distance Between Fish and Object
	Time Near the Object

In the last decade, zebrafish (Danio rerio) have emerged as an important model organism for behavioral studies [29]. Accordingly, such behavioral model species are used to investigate the emotional effects, such as ALB, which bio-inspired fish robot prototypes can induce on individual zebrafish behavior, and to identify specific robot components as potential causes of ALB in fish.

All this previous knowledge, therefore, provides insights which enable us to optimize efficiency in the design of robotic prototypes, while also increasing mimicry to reduce robot-induced stress. These concerns are crucial for the development of robots intended to operate in environments where coexistence with fish is required without compromising their well-being. However, while some factors have been extensively studied in the literature, others may warrant further investigation. Therefore, this article is focused on exploring the implications related to stressors associated with the interaction between robots and fish. This article specifically examines various small prototypes to assess how different factors affect fish stress. It investigates the impact of the presence or absence of light, the movement or lack of movement of a body, and the oscillatory or helical movement of a conspecific's tail using different types of actuators. Statistical tests were conducted, and various variables were analyzed to determine the influence of these factors on fish stress so as to use them specifically in bioinspired robotic design for use in real-world aquacultural practices.

2. Materials and Methods

2.1. Fish and Accommodation Conditions

Ten-month-old Tubingen (Tu) zebrafish (length 3–3.5 cm) were reared under standard conditions in the facilities at the Instituto de Acuicultura de Torre la Sal. Naïve animals were acclimated to the behavioral testing room for at least 5 days and maintained at 28 °C with 14 h light/10 h dark. All experiments were performed following the guidelines of the Spanish (Royal Decree 53/2013) and the European Union Directive on the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU). The protocols applied were approved by IATS Ethics Committee (Register Number 09-0201) under the supervision of the Secretary of State for Research, Development, and Innovation of the Spanish Government.

2.2. Robots Tested

Four different types of bio-inspired robotic replicas, based on the morphology of the zebrafish, were used to classify the different types of actuators and electronic components according to the stress they could generate (Figure 1). The aim was to obtain a size and appearance (colors, line patterns and position of fins) as identical as possible to those of the live individuals to be used in the study and to enable them to swim autonomously and freely around the test area.

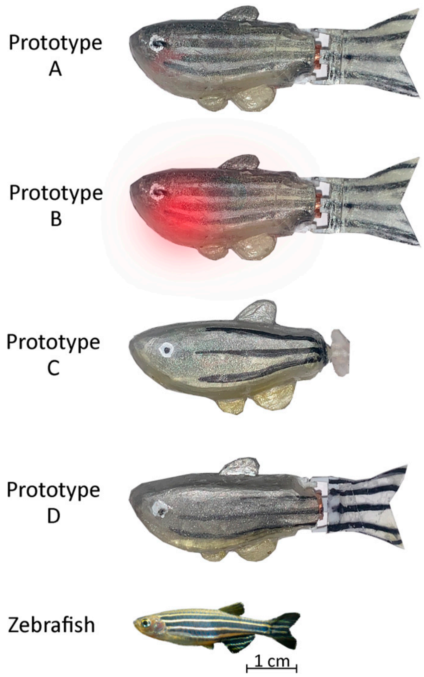
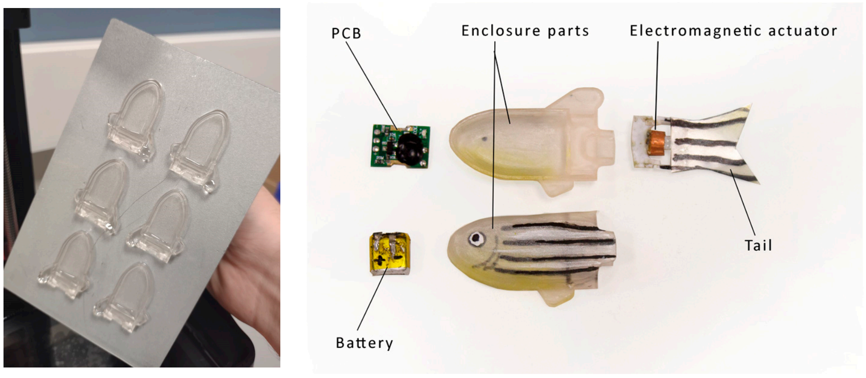


Figure 1. Real images of the four prototypes used and their comparison in size and appearance with a real zebrafish of the same size and aspect as those used in the tests.

The four prototypes were created from scratch and specifically designed for these tests using Solidworks 2020 modeling software. The housing for the prototypes was produced using a high clear ABS-like resin (Anycubic, Shenzhen, China) and an Elegoo Mars 3 (Elegoo, Shenzhen, China) printer through the process of rapid prototyping. This method was selected due to the low water absorption properties of the resin [30], the possibility of achieving smooth, non-porous surfaces, the ability to produce detailed models despite their small size (tolerance of 0.3 mm) [31], and the quality and dimensional precision, which allows for rigorous buoyancy calculations. Each prototype consists of two symmetrical halves which are sealed together using ethyl cyanoacrylate glue, creating an internal cavity where the electronics are placed (see Figure 2). To ensure waterproofing, this cavity was coated with transparent acetic silicone, offering increased resistance to impacts, UV rays, and prolonged exposure to water. The key features of each of the four prototypes are as follows:

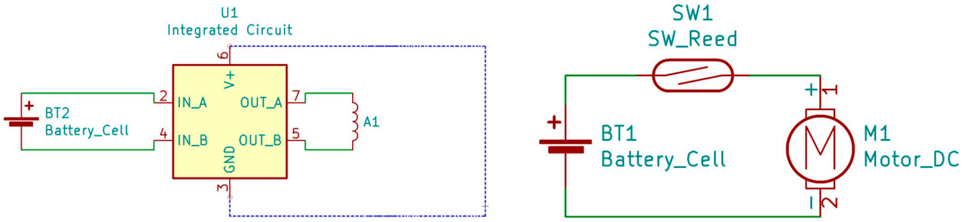
- Prototype A is powered by a 0.3 g electromagnetic actuator (provided by Shuaichi, CN), model DIY RC Aircraft, measuring 10 × 10 × 2 mm, with a resistance of 60 ohms, an operating voltage of 3.7–4.2 V, and an operating current of 55 mAh, which is connected to an acetate tail. This propulsion system generates a tail movement characterized by oscillatory beats, the frequency of which can be adjusted in advance. Consequently, changes in the robot’s speed and direction are achieved. The electronic system of this prototype includes a rechargeable 2.7 V, 30 mAh lithium battery, model 450909, and a mini-PCB (Figure 3a). The dimensions of this prototype are as follows: length—6.5 cm; height—2 cm; and thickness—1.2 cm (Prototype A in Figure 3). Upon contact with water, the circuit is automatically closed activating the prototype. However, it should be noted that Prototype A only swims on the water’s surface and is unable to go deeper than 2 cm below the surface level within the tank environment.

- Prototype B is a replica of the previous model which also includes a red LED light (Prototype B in Figure 3). This red LED is included in the mini-PCB commercially acquired and flashes intermittently at the same frequency as the tail, from within the housing, illuminating the entire body of the prototype. Given that the experiments are intended to be conducted at a maximum depth of 15 cm and a maximum distance of 35 cm, and that the wavelength of the red LED can be seen by both the cameras and individuals at these distances, this LED is used to simplify the composition of the prototypes.
- Prototype C is actuated by a planetary gear motor (provided by Zhaowei, CN), model ZWPD006006 to 420 rpm, with a weight of 1.6 g, a working torque of 40 g·cm, and a stall torque of 90 g·cm. It measures 6 mm in diameter and 21 mm in length and is linked to a 1.2 cm diameter propeller designed and manufactured following the same process as the outer housings. This propulsion system offers continuous rotation resulting in constant speed and advancement exclusively in the frontal direction. Additionally, the electronic system includes a rechargeable 4.2 V lithium battery and a magnetic switch that allows the system to be actuated by an external magnet which (Figure 3b), in turn, serves as a counterweight to achieve neutral buoyancy. The prototype measures 5.5 cm long, 2 cm tall, and 1.2 cm thick (Prototype C in Figure 3). This prototype can submerge due to the thrust generated by the propeller.
- Prototype D is identical to prototypes A and B, yet all electronic components were removed, resulting in a motionless prototype that can only float or remain stationary at the bottom, depending on its buoyancy (Prototype D in Figure 3). This model allows us to study whether the effects generated by the movement, sounds, and waves of the electronic components of the robots are significant and allows us to analyze whether the presence of a foreign object in the tank, its aesthetics, or size are influential in perceiving the prototypes as stressful.



(**a**) (**b**)

Figure 2. Manufacturing and construction of robotic prototypes: (**a**) stereolithography printing of watertight housings for the prototypes; (**b**) parts and electronic components of prototypes A and B.



(**a**) (**b**)

Figure 3. Electronic schematics of (**a**) Prototypes A and B; (**b**) Prototype C.

2.3. Behavioral Quantification

Individuals were tested in 6 L tanks measuring 27 cm × 22 cm × 15 cm (Aquaneering, San Diego, CA, USA) (Figure 4a,b). The tanks were filled with 5.5 L of chlorine-free water at the same temperature and pH as their home tank. The test lasted 7.5 min following a 60 s period of accommodation. The tests were carried out on four different days, maintaining the same testing schedule from 9:00 a.m. to 1:00 p.m. to ensure similar conditions. The activity of the fish was recorded using industrial digital cameras (IDS (UI-3240CP USB 3.0 uEye CP, IDS Imaging Development Systems GmbH, Obersulm, Germany) and/or Basler (Basler acA1280-60gc GigE camera, Basler AG, Ahrensburg, Germany) equipped with a high-quality monofocal lens (focal length 8 mm) programmed with a resolution of 640 × 426 px and a frame rate of 25 fps. Trajectory tracking was performed using EthoVision®XT v.17.0.1630 software (NoldusInc, Wageningen, The Netherlands).

Fish were recorded simultaneously using frontal and zenithal planes of the tank; therefore, three-dimensional data were obtained. To analyze the natural bottom-dwelling response, each arena was divided into two equal zones: top and bottom (Figure 4d). For the zenithal plane, the arena was divided into two parts corresponding to the center and the perimeter of the tank. The "perimeter" was denoted as the area between the tank edges and its parallel projection at 2.5 cm (Figure 4c). Individual tracking and coordinates were obtained using AnimalTA v.2.3.1 software (http://vchiara.eu/index.php/animalta, accessed on 28 May 2024). Each prototype was tested against 7 naïve fish. The locomotive behaviors of 7 fish without any prototype were used as control.

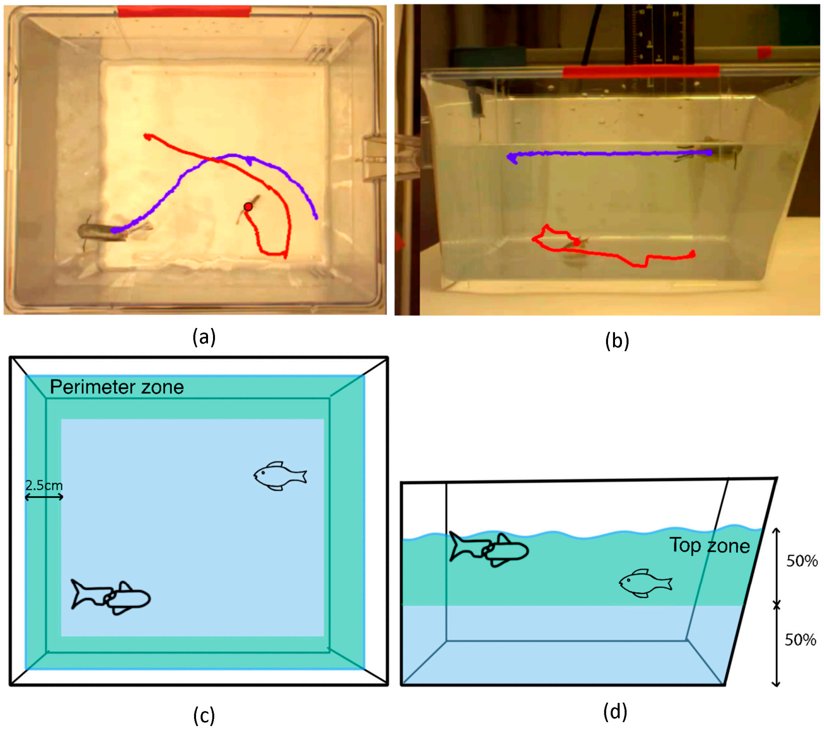


Figure 4. Recording setup of the top (**a**) and frontal (**b**) planes and tracking of the individuals using the AnimalTA software. Overhead (**c**) and frontal (**d**) views of the tank with the aversive zones marked in green: perimeter area from the top view equivalent to the space between the tank edges and its parallel projection at a distance of 2.5 cm; and top zone considered as the top half of the frontal view of the tank.

For analysis, the vision software was programmed to extract the coordinates of both individuals (the real fish and the robot), from which the degree of avoidance of the real fish towards the robot was measured. Based on the behavioral patterns analyzed in the literature [31], the following evaluation measurements calculated and examined included: (i) the proportion of time spent by the fish in the top area of the tank; (ii) the number of visits to top area; (iii) the proportion of time spent in the perimeter area (defined as a distance of 2.5 cm from the tank's edge); (iv) the distance between the robot and the fish; (v) the percentage of time the fish displayed freezing behavior; (vi) average velocity; (vii) tracking distance; (viii) velocity deviation; and (ix) acceleration deviation. These measurements were taken from the real fish in response to its interaction with the robotic fish.

2.4. Statistical Analysis

Data were analyzed parametrically using SPSS software (IBM SPSS Statistics, Version 29.0.1.1 (244)) at a 95% confidence level, with $p < 0.05$ indicating statistical significance. For comparisons between groups, the one-way ANOVA method was used for parameters that follow a normal distribution, considering "type of prototype" as a factor, and the Kruskal–Wallis method was used for samples where the assumption of normality was rejected. Furthermore, post hoc multiple comparisons tests were also used. OriginPro software (Origin (Pro), Version 2022, OriginLab Corporation, Northampton, MA, USA) was used to generate graphs illustrating the results obtained.

Initially, descriptive statistics (shown in the table in Section 3) were calculated for each group and each evaluative measurement. To ensure correct analysis of the variables, a normality test was performed to verify that the samples meet the normality requirement using non-parametric tests. Variables that meet the normality criterion are analyzed using one-way ANOVA to compare means between groups, while those that do not follow a normal distribution are analyzed using the non-parametric Kruskal–Wallis test to compare medians between groups. After conducting a one-way ANOVA on parameters exhibiting a normal distribution, a test for homogeneity of variances is performed to ensure homoscedasticity compliance and to conduct post hoc multiple comparisons, operating under a 95% confidence level. The Tukey method is employed when equality of variances is assumed, whereas the Games–Howell method is utilized when the null hypothesis is rejected.

3. Results

Initially, three parameters were evaluated, which included swimming speed, Euclidean distance between the robot and fish, and latency of the analyzed individual's position over the entire tank area. Swimming speed was relatively low and constant when test animals were exposed to prototypes A and D (Figure 5a,d), a swimming pattern consistent with that of fish swimming alone (Figure 5e). In contrast, animals exposed to prototypes B and C exhibited an erratic swimming pattern, with speeds eventually reaching peaks of up to ten times their own baseline values (Figure 5b,c).

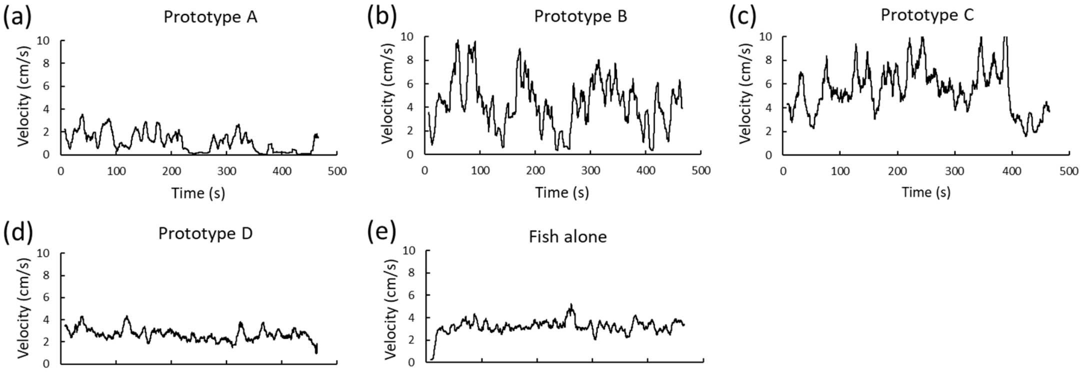


Figure 5. Reference velocities of how fish behaved when interacting with the different prototypes—(**a**) A; (**b**) B; (**c**) C; (**d**) D—and (**e**) fish alone.

The response of fish confronted with prototypes A, B, and C (Figure 6) was somewhat inconsistent as some of the tested animals exhibited freezing bouts either at the beginning of the experiment (Figure 6a/d) or during the experiment (Figure 6b/e,c/f).

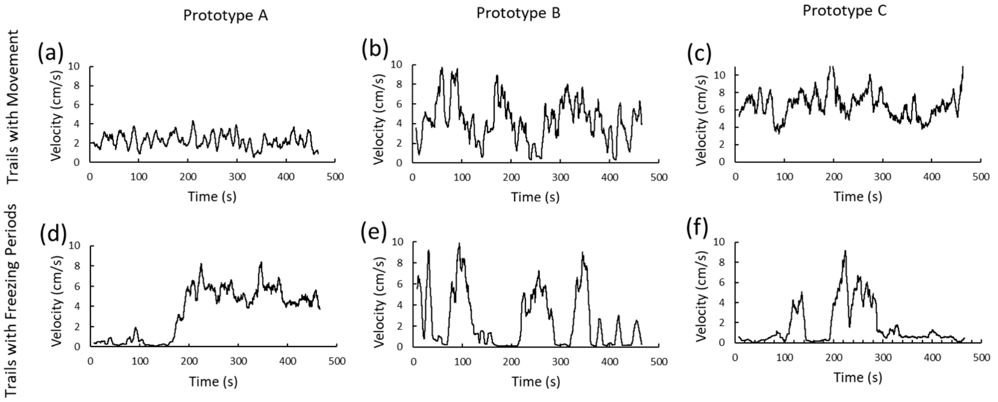


Figure 6. Reference velocities of how fish behaved when interacting with the prototypes: (**a**) Prototype A without freezing periods; (**b**) Prototype B without freezing periods; (**c**) Prototype C without freezing periods; (**d**) Prototype A with freezing periods; (**e**) Prototype B with freezing periods; (**f**) Prototype C with freezing periods.

The velocity was then plotted on the same graph alongside the Euclidean distance between the fish and the robot for each time point. This was carried out in order to analyze whether there was a direct relationship between the velocity of the fish and the distance between the fish and the prototype. This can be observed in Figure 7, where a representative case is shown for each of the evaluated prototypes. When the distance between the robot and the individual remained constant, with fewer abrupt changes over time, the fish also maintained a constant velocity. Conversely, when the distance fluctuates more erratically, the speed also fluctuates, demonstrating that the variation in distance between the animal and the prototype was related to the variation in the speed of the fish. Not only does the speed decrease when the variation in distance is more constant but also when the value of that interindividual distance is greater. Similarly, a decrease in the distance between individuals results in an increase in the velocity of the fish, thus suggesting an avoidance behavior.

In order to analyze the positioning of the fish in the tank according to the predefined "top/bottom" and "perimetral" zones, heat maps were generated to visualize the time fish spent in these different areas when confronted with each prototype. This allows a visual comparison of the prototypes and facilitates the identification of freezing behavior, exploratory activity, or areas where individuals spend more time.

Fish swimming alone showed a homogeneous distribution of the position of the individual in the tank and a maximum density of 0.032 (Figure 8e). The distribution is centered in the lower and middle region of the tank, with exploration towards the upper half without reaching the surface of the water. The remaining graphs show a less-uniform distribution compared to the fish alone, with higher densities and longer periods of positioning near the bottom of the tank, indicating potential signs of stress and/or disturbance caused by certain robotic prototypes (Figure 8a–d). However, for prototypes A, C, and D (Figure 8a,c,d), individuals tended to visit the surface by swimming from the sides of the tank rather than from the central area, whereas animals exposed to prototype B exhibited a more evenly distributed pattern across the entire "top" region. Animals exposed to prototype C spent only short periods at the top of the surface, suggesting potential escape behaviors (Figure 8c).

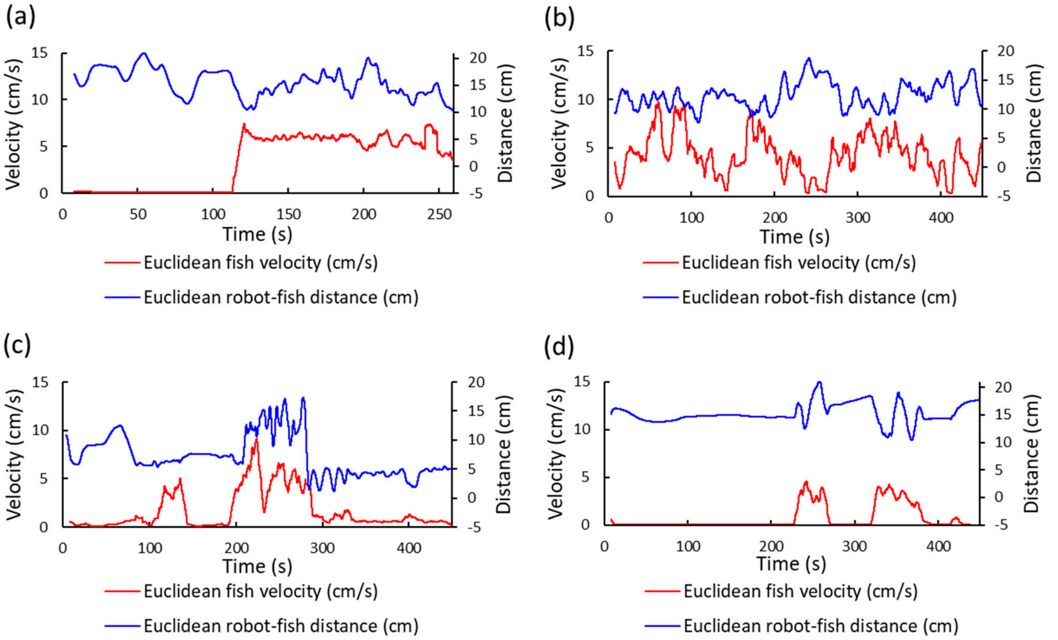


Figure 7. Relationship between velocity and Euclidean distance between the fish and the robot over time for a representative case of each of the tested prototypes: (**a**) Prototype A; (**b**) Prototype B; (**c**) Prototype C; and (**d**) Prototype D.

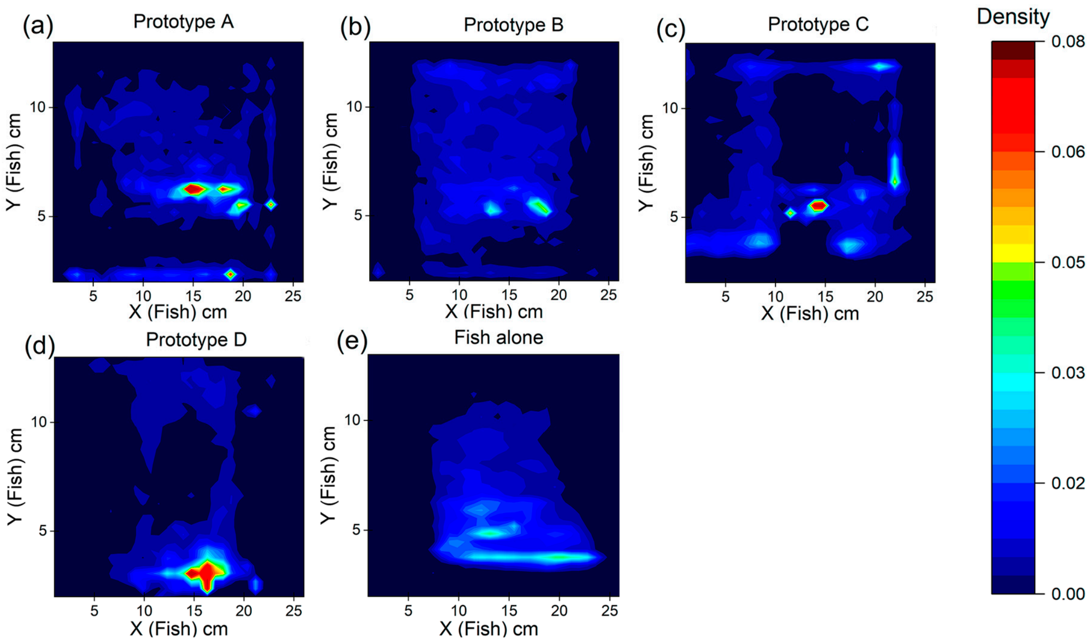


Figure 8. Representative density plots of the position of the fish from the frontal plane when (**a**) exposed to Prototype A; (**b**) exposed to Prototype B; (**c**) exposed to Prototype C; (**d**) exposed to Prototype D; and (**e**) alone. The color scale represents the cumulative time spent in each zone of the tank.

Similarly, fish swimming alone exhibited a homogeneous distribution when observed from the top (superior plane) in Figure 9. Fish swam primarily around the center of the tank, suggesting an absence of anxiety-like behavior and/or stress (Figure 9e). When fish are exposed to the prototypes, the distribution is less uniform; they remain longer in peripheral areas and present freezing episodes (Figure 9a–d).

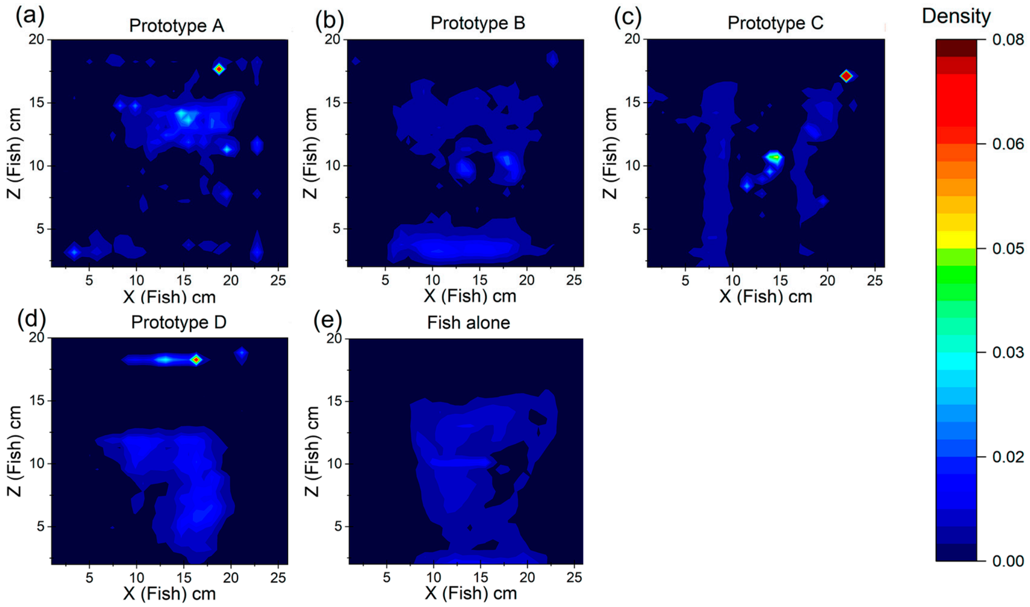


Figure 9. Density plots of the position of the live fish from the superior plane when (**a**) exposed to Prototype A; (**b**) exposed to Prototype B; (**c**) exposed to Prototype C; (**d**) exposed to Prototype D; and (**e**) alone.

Following this, Table 2 displays the descriptive statistics for each variable, in addition to normality and variance homoscedasticity studies. The variables that do not meet normality (top time proportion, number of visits to the surface, and velocity absolute deviation) criteria were analyzed using non-parametric methods (Kruskal–Wallis).

Table 2. Descriptive statistics.

				95% Confidence Interval for Mean			
Parameter	**Model**	**Mean**	**Std. Deviation**	**Lower Bound**	**Upper Bound**	**Normality Sig.**	**Homogeneity of Variances Sig. ***
Top time proportion	A	30.51	18.37	13.52	47.51	0.005	0.23
	B	56.20	20.55	37.20	75.21		
	C	28.92	30.08	3.77	54.06		
	D	22.67	29.93	−5.01	50.35		
	E	15.13	10.65	5.28	24.98		
Nº of visits to surface	A	2.81	2.21	0.77	4.86	0.002	0.440
	B	7.25	2.80	4.66	9.84		
	C	7.94	8.45	0.88	15.00		
	D	2.48	2.70	−0.02	4.98		
	E	5.33	4.43	1.23	9.43		
Perimeter time proportion	A	12.83	8.43	5.04	20.63		
	B	24.48	11.70	13.66	35.30		
	C	43.29	22.38	24.58	62.01	0.111	0.281

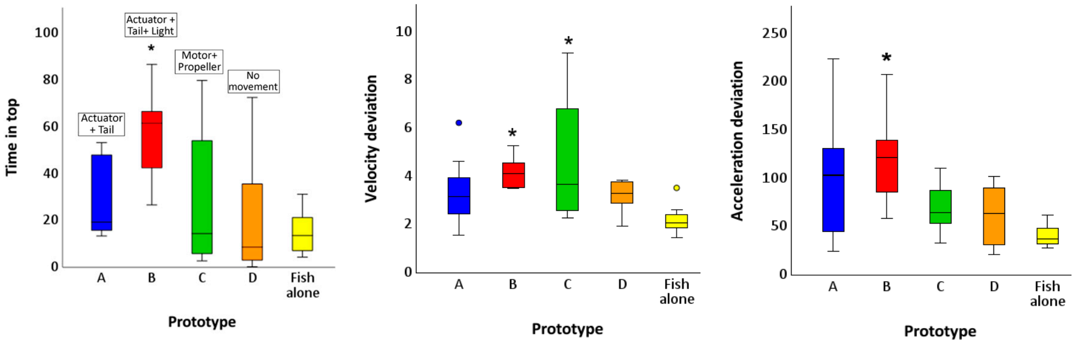
	D	23.22	15.39	8.98	37.45		
	E	31.40	10.92	21.29	41.50		
Freezing time proportion	A	21.70	18.84	4.28	39.13	<0.001	0.373
	B	6.88	14.45	−6.49	20.25		
	C	10.96	15.70	−2.16	24.07		
	D	12.52	28.67	−13.99	39.04		
	E	7.08	9.30	−1.53	15.69		
Tracking average distance	A	275.34	160.96	126.48	424.2	0.200	0.140
	B	418.24	140.34	288.45	548.03		
	C	470.95	255.39	257.44	684.47		
	D	302.38	127.81	184.17	420.58		
	E	401.09	61.83	343.91	458.27		
Velocity absolute deviation	A	3.39	1.62	1.90	4.88	0.019	0.005
	B	4.15	0.73	3.48	4.82		
	C	4.69	2.84	2.32	7.06		
	D	3.19	0.73	2.52	3.87		
	E	2.23	0.68	1.60	2.86		
Acceleration absolute deviation	A	100.41	69.72	35.93	164.90	0.056	0.020
	B	119.52	49.13	74.08	164.96		
	C	69.46	26.26	47.51	91.41		
	D	61.31	34.35	29.54	93.08		
	E	40.99	12.26	29.65	52.33		
Velocity Average	A	2.97	1.74	1.36	4.57	0.200	0.047
	B	3.2	1.55	1.76	4.63		
	C	4.97	2.9	2.55	7.39		
	D	3.55	1.84	1.85	5.26		
	E	4.42	0.93	3.56	5.28		

* Based on mean.

The statistical analyses showed significant differences between the prototypes and the fish alone for three of the parameters analyzed. These differences are indicated by asterisks in the box-and-whisker plots in Figure 10.

Performing non-parametric tests on metrics with non-normal distributions, utilizing the Kruskal–Wallis test with multiple pairwise comparisons, resulted in significant differences between the medians of prototype B and fish alone for the parameter “time in top”, with a significance value of 0.028, as shown in Figure 10a.

The Kruskal–Wallis test with multiple pairwise comparisons showed differences between the medians of prototypes B and C compared to fish alone for the parameter “velocity deviation”. Such differences were observed to be statistically significant, with significance values of 0.020 and 0.012, respectively, as depicted in Figure 10b.



(**a**) (**b**) (**c**)

Figure 10. The diagrams display the distribution and central tendency of the numerical values (through quartiles) obtained from each of the prototypes for (**a**) time on the surface; (**b**) velocity deviation; (**c**) acceleration deviation. The asterisks indicate statistical significance between the indicated prototype and the reference group (Fish alone).

However, when conducting a one-way ANOVA on parameters that exhibited a normal distribution, significant differences between group means were obtained for the evaluative measurement "acceleration deviation". The F-statistic value was calculated to be 3.803 with a significance value of 0.013, indicating that there are indeed significant differences between populations. Upon performing post hoc comparisons between groups, it was determined that there are differences in absolute acceleration deviation between prototype B and fish alone. The post hoc significance value for this comparison is reported to be 0.028 in Figure 10c.

4. Discussion

Behavioral studies have often been conducted to identify social interactions between conspecifics that promote the hierarchical allocation of individuals [32]. Such studies have often examined interactions with live stimuli and have shown that factors such as size and aesthetics play a crucial role in distinguishing leaders from predators [33,34]. In addition, research suggests that the movement patterns of replicas influence behavior, leading some researchers to use virtual representations or bio-inspired robotic replicas with closed trajectories to control specific parameters [35]. In this study, bio-replicas were meticulously designed to investigate the effects of light, noise, and propulsion mechanisms on fish locomotor behavior. Unlike previous studies, which predominantly analyzed fish behavior in a two-dimensional spatial plane with only two coordinates, this study tracked fish position throughout all three axes, yielding results which are reflective of a three-dimensional reality.

Parameters derived from existing behavioral models were employed to study fish behavioral responses (Table 1). However, some of these models were based on live stimuli without contact or on replicas with closed trajectories. Although this approach enhances experiment reproducibility and controllability, it sacrifices biomimicry and fidelity to reality, as noted by Spinello et al. [36]. Thus, the use of freely swimming robotic replicas in this study brings the experimental conditions closer to real-world scenarios.

The presence of a stationary prototype (D) in the tank was found to be less stressful compared to the presence of moving replicas, with fish perceiving D as a non-threatening entity. Accordingly, fish exposed to D exhibited similar velocity parameters (i.e., low mean velocity and animal deviation) to fish swimming alone.

The influence of the type of motion and the propulsion system of the replica was then investigated by comparing prototypes A, B, and C with the fish swimming alone. Graphical results suggested anxiety-like behaviors related to the interaction with B and C. However, the mean velocity and deviation values were more alike between the fish swimming alone and the electromagnetic-tailed prototype (A) than the propeller-driven prototype (C), which exhibited velocity spikes of up to 10 m/s. Statistical analysis confirmed significant differences in velocity and acceleration deviations between prototype C and fish swimming alone. In addition, two recordings were terminated prematurely due to fish jumping out of the tank in the presence of C, indicating an extreme escape response to a perceived imminent threat. This is consistent with the expected anxiety-like response to this robotic prototype, which can be attributed to the noisier motor and less natural, biomimetic motion associated with the propeller [25].

The effect of light was also investigated by comparing the responses of the fish to prototype A versus B, in comparison to the unstressed fish. Once more, the high velocities, peaking at 10 cm/s and irregular burst swimming patterns, which were evident in the light-presenting prototype (B), were indicative of anxiety/fear-like responses. Although the electromagnetic prototype without light (A) also exhibited differences from the unstressed fish swimming alone, including periods of freezing or less homogeneous tank positioning, these differences were exacerbated when light was added. Statistical analysis revealed significant differences between the light-presenting prototype and the fish alone, which was characterized by increased acceleration and velocity dispersion, indicative of rapid, erratic, burst-like swimming patterns associated with stress or anxiety.

In addition, prototypes B and C showed intermittent periods of freezing in some recordings throughout the trial, thus indicating stress due to a perceived imminent threat, further supporting previous observations. However, statistical support for this parameter was lacking due to non-significant results. The relationship between fish speed changes and robot-fish distance, as well as visual differences in fish tank positioning based on robot exposure, were unable to be statistically supported due to non-significant results for parameters such as “number of surface visits” or “time at perimeter”. While significance was obtained for the “time at top” parameter for B, this contradicts interpretations based on velocities and accelerations, where surface latency would indicate non-stress. These limitations hampered the conclusions of the study.

One limitation was the complexity introduced by the free-swimming nature of the robots, which, while enhancing biomimicry and realism, also increased experimental complexity due to random swimming trajectories, hindering exact repeatability and making it difficult to determine whether the robot was approaching the fish or whether the fish were habituating, perceiving the robot as less threatening and reducing inter-individual distance. Similarly, the positioning of fish within defined tank zones, particularly for B, raised concerns, with some authors [23] suggesting that zebrafish may move to the top of the tank as a predator avoidance measure rather than an accommodation response. Another limitation was the sample size, with 37 valid fish tested. Although this is within the typical range for such studies [20] high variability and deviation were observed in each model, in all likelihood due to individual fish personalities and different responses to stimuli, which, in some cases, made it difficult to obtain statistically significant results. Therefore, further expansion would be advisable for future work in order to reduce variability and to improve the accuracy of the statistical analysis.

Finally, it is important to acknowledge a potential confounding factor related to stress, arising from the discrepancy in size between the replicas and the real fish. The latter were approximately twice the size of the replicas. It is known that fish consider size to be an important factor; however, this inconsistency was necessary due to the limitations of the low-cost electronic components available, which made it impossible to further reduce the size of the replicas. To mitigate the impact of this, uniformity in size among all replicas was sought. The fact that all replicas are of the same size and therefore share the same negative implication allows us to exclude size as a differentiating factor upon comparison.

Furthermore, despite some prior demonstrations that fish are attracted to certain tail movements or similar aesthetic patterns [37,38], this study also examined the implication of the type of actuator used to generate such movement. Additionally, this was achieved using the smallest possible replica, similar to the real fish in a context of free-swimming replicas, which allows for a closer approximation of the results to the development of bioinspired robots in a more realistic context.

Such results can be applied to the design of a robot intended for use in a real aquacultural context, tailored to the specific application for which it is designed to be used. The findings are particularly useful for tasks where the robot needs to maintain a close distance to the fish, such as environmental monitoring within cages or behavioral monitoring of the fish inhabiting them. In such cases, the design can be adjusted to avoid or reduce the use of lighting as well as the use of thrusters or cease their activity when the robot is in close proximity to the fish.

As a potential future study, it would be advisable to adapt the size of the prototypes to that of the fish used. This adaptation could be achieved by incorporating larger fish species into the study and creating prototypes at an appropriate scale. This approach would allow for the study of situations and behaviors accurately resembling the real-world applications which prompted this study by offering greater design flexibility for the replicas and reducing the constraints imposed by the size limitations of the components. Additionally, to mitigate data variance and increase statistical robustness, it would be advisable to increase the sample size and re-evaluate the variables for which significant results have not been obtained. Other potential lines of research derived from this study could include exploring the implications of heat, and more specifically, the actuator noise or the magnetic fields generated by electronic components. Finally, it would be interesting to study at what distance the factors involved in stress cease to have a stressful effect and are no longer perceived as a threat by the fish. This would enable us to design recommendations adapted to specific tasks to be performed by the robot.

5. Conclusions

This study highlights discernible differences in the behavioral response of fish to different underwater robots, depending on the propulsion and lighting system. Stressful behaviors were identified via significant differences between the variation in the speed and acceleration of the fish when alone and in the presence of a robot with light or a robot with propeller propulsion. No stressful behaviors were identified via significant differences between the fish when alone or in the presence of an unilluminated, electromagnetically propelled robot. These findings have direct implications for the design of underwater robots to reduce fish stress; thus, for more effective robotic biomimicry, it is advisable to avoid the use of propellers and noisy motors and to carefully consider or limit the use of lights.

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