Acceptance Analysis of a Robotic Fish Replica, “FishBot”, by a Group of Zebrafish

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Abstract

A robot accepted by animals as conspecific is a very powerful tool in behavioral biology, particularly in studies of gregarious animals. In this paper we present the first results of acceptance of a robotic zebrafish designed for experiments on collective animal behavior. The robot consists of two modules: a replica fish fixed on a magnetic base and a miniature mobile robot guiding the replica fish from below the experimental tank. We varied several parameters of the system and used Design of Experiments methods to reduce the number of performed experiments and determined the impact of each factor on the acceptance of the robot among a small group of real fish. While a brief comparison of the mean distance of the fish to the robot tends to indicate that the fish are attracted by the mock-up fish, a linear model of the acceptance of the mock-up fish is presented. Results of this study will be used to improve the design and control of the robot for further animal-robot interaction experiments.

Keywords: Bioinspired robots, zebrafish, design of experiments, robotic fish, mixed societies

1. Introduction

One of the long-standing interests in behavioral studies is to understand relationships between stimulus and response. In order to study this mechanism, researchers often use specially designed mock-ups whose aspect and behavior can be controlled to monitor a response of the animal under test.

In recent years, as technology became more advanced and affordable, robotic devices have been introduced into animal societies to generate those stimuli. One of the first concrete example was the Leurre project, where a mixed society consisting of cockroaches and mobile robots was created [1]. These types of experiments have extended to more complex species, such as beetles [2], fish [2] or even chicken [3].

The group behavior of the zebrafish *Danio Rerio*, a fish used in more than 450 laboratories worldwide for different scientific topics [4], already raised the interest of biologists, and several examples of automated mock-up fish designed to interact with zebrafish have already appeared. For instance in [5] and [6], the zebrafish response to a robotic fish with the same ratio size as zebrafish, a beating tail and different colorations was observed. In [7], a mock-up fish attached to a support is moved using a mobile robot outside the aquarium.

In the last couple of years, we have designed a robot that can be socially integrated into a zebrafish shoal. A first version of the prototype of the robot was introduced in [8]. The last version of the robot that is used during the experiments presented in this paper can be seen in Fig. 1. One of the main advantage of our robot, compared to other solutions that exist in the literature, is its small dimensions that, adding the fact that it is wirelessly controlled, offers the possibility to have several mock-up fish able to swim next to each other, for multi-robot experiments. Furthermore, the robot has been conceived so that the mock-up fish can reach speeds and accelerations of respectively 0.5 m/s and 1 m/s^2, which are typical for zebrafish based on the available experimental results [9] and that have been observed on our own animals. Finally, the robot is powered using conductive plates that are installed on the experimental setup and thus the experiments can last several days.

In this paper, we present the results of the first experiments of acceptance of our robotic fish by a small group of real zebrafish. Several parameters will be tested: the speed of the robot, the aspect of the mock-up fish, the type of trajectory made by the robot and the continuity of the mock-up's motion. We use a Design of Experiment (DOE) analysis in order to optimize the number of experiments and to have a better understanding of the impact of the different factors on the acceptance of the robot by the fish. These experiments take part during the design phase of the robot. The main purpose is to know which part of the robot's design and control need to be improved to increase the acceptance of the robot among the fish shoal.

The paper is organized as follows: Section 2 provides a description of the robot design and the experimental setup, Section 3 describes the experiments that were made, Section 4 shows the application of the method DOE to characterize the impact of the factors on the results, Section 5 presents the results and, finally, Section 6 concludes the paper.

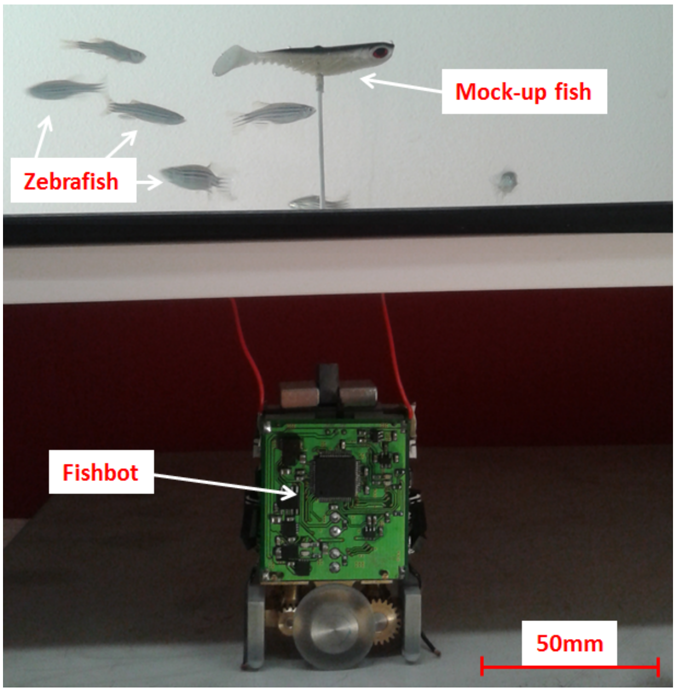

Figure 1. FishBot V3.0, the new version of our robotic system to move a mock-up fish inside an aquarium with zebrafish in the background.

2. Hardware and software

2.1. Experimental setup

Figure 2 shows the experimental setup that is used for the experiments. It consists of an aquarium of 1 m × 1 m of surface covered on the inside with white teflon plates. These plates are installed in order to avoid reflection on the glass and to have a smooth surface for the motion of the mock-up fish inside the aquarium. The tank is filled with water up to a level of 10 cm. According to [4], this level is not introducing more stress for the fish and furthermore, the mock-up fish, whose height cannot vary, will be more visible for the fish that are swimming around. The water temperature is set to 26°C, as suggested by [4]. The robot is moving under the aquarium, and the motion is transmitted to the mock-up fish using magnets. The robot is powered using two conductive plates, one glued on the bottom of the aquarium and one on the support on which the robot is moving.

2.2. Robot

The mobile robot is a differential drive mobile robot, with 2 independently actuated wheels (Fig. 3). The powering is done through electric cables (brushes) that slip against the conductive plates. A SuperCap system is used to store current in case the brushes are not in contact with the conductive plates for a short period of time. Proximity sensors are installed on the front and back of the robot in order to avoid obstacles or the border of the arena in the case when the tracking is not working. A bluetooth device is used for wireless communication to control the robot.

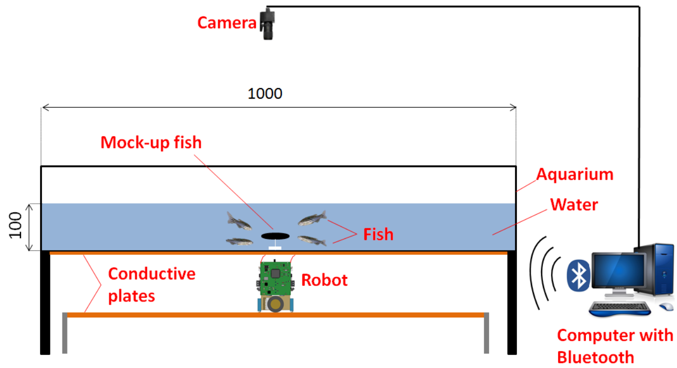

Figure 2. Experimental setup used during the experiments. The robot is moving under the aquarium and powered with two conductive plates. The mock-up fish inside the aquarium is following the robot using magnets. A camera is used to track the mock-up fish and the fish. The robot is remotely controlled with a Bluetooth link. Dimensions are in millimeters.

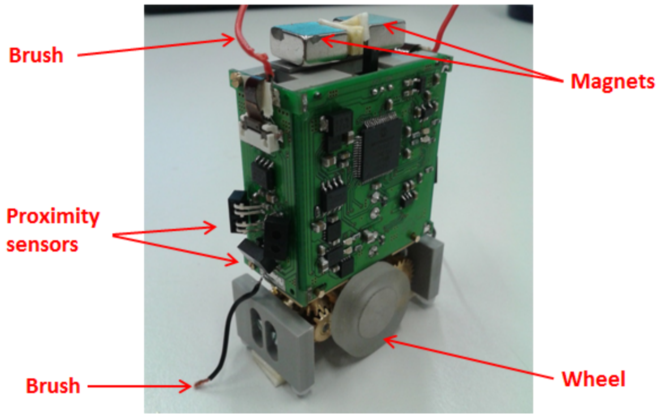

Figure 3. FishBot V3.0, the mobile robot used for the experiments.

2.3. Tracking and Control

During the experiment, a blob detection based algorithm is used to retrieve the pose (position and orientation) of the mock-up fish (see Fig. 4). A proportional controller is used to move the robot to a target. In order to make the robot follow a trajectory, the target is simply updated at a certain time step along the trajectory and the mock-up fish simply follows it with a speed proportional to the update time rate of the target. The fish are also tracked using the same method during the experiments in order to compute their position relative to the position of the mock-up fish.

2.4. Mock-up fish

The height at which the mock-up fish is swimming is 5cm and is fixed, thus the mock-up fish is swimming in the middle of the water level. Its base is painted in white in order to blend into the white background.

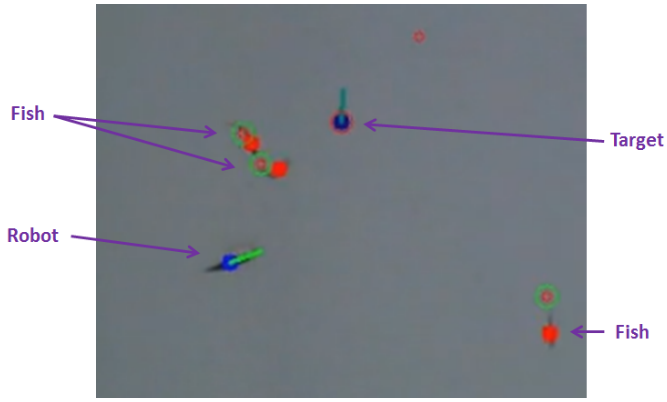

Figure 4. Graphical User Interface of the software used for the tracking and control of the robot with the mock-up fish inside the aquarium. In this case, the mock-up fish (blue dot with the green line that indicates the orientation) is moving inside the tank with three zebrafish (red dots) that are swimming next to it. The robot is following a target (dark blue dot with cyan line) with a defined position and orientation, updated at each time step.

Two different mock-up fish were used (Fig 5). The first one is an ellipsoid and the second one is a fake fish bought in a fishing shop. The goal here is to see if the shape of the mock-up fish will have an influence on the attraction of the fish.

Figure 5. Mock-up fish: ellipsoidal shape and fake fish.

2.5. *Zebrafish*

For the experiments performed, we used 10 zebrafish *Danio Rerio*, with short fins (Fig. 6). These zebrafish were bought in a pet shop, and are stored in a 60 litres aquarium when not performing any experiments. The water temperature of the housing aquarium is 26°C. The fish are fed twice a day using a food distributor with commercial food. No artifacts nor plants were put inside the housing aquarium.

Once inside the tank, the zebrafish have a tendency to follow the wall of the tank. The objective of these experiments is to have a robot that moves closer to the centre of the aquarium and sometimes even crosses the aquarium through its centre, and observe the fish reaction.

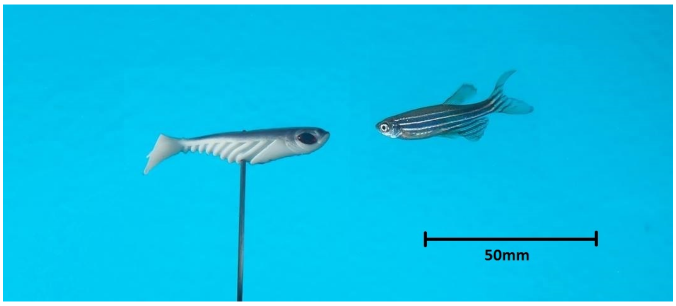

Figure 6. On the left, one of the mock-up fish used during the experiments, and on the right, one of our zebrafish Danio Rerio.

3. Description of the experiments

3.1. *Licence*

The experiments performed in this study were conducted under the authorization N.24598 delivered by the Department of Consumer and Veterinary of the Canton de Vaud (Switzerland) and Art. 18, 30 and 141 of the Swiss federal law.

3.2. *Manipulation*

For each experiment, three zebrafishes out of a shoal of ten individuals are placed inside the experimental tank. The three zebrafishes are picked randomly among the entire group in order to reduce learning possibility by the fish from the parameters.

Once inside the tank, we let them swim during 15 minutes by themselves to explore their new environment in order to resume a normal level of stress, which was raised due to the transfer. Afterwards, we place the fish replica inside and start moving the robot. The duration of each measurement is 30 minutes, thus the entire manipulation lasts 45 minutes.

3.3. *Responses and factors*

In this experiment, four factors were considered to explain the response: see Table 1 for an overview of the factors.

Factor name	Variable	Unit	Min value (-1)	Max value (+1)
Mock-up fish	X_1	-	Fake fish	Ellipsoid
Robot trajectory	X_2	-	(Half-)circles	Circles
Robot movement	X_3	-	Jerks	Continuous
Robot speed	X_4	[cm/s]	3	6

Table 1. Factors of the experiment.

The first studied factor is the shape of the mock-up fish: it can either be of ellipsoidal shape or represent a fake fish (Fig. 5). The idea here is to assess if the shape of the mock-up fish has an influence on the perception of the zebrafish. The second factor describes the different possible trajectories of the robot: it can either turn in circles in the whole aquarium or alternate between full circles and half-circles. As already mentioned, the fish have a tendency to swim along the border of the tank, as they feel more protected than in the central area. We want here to see if the robotic fish can influence the group of fish to explore the central area. The robot's movement will be included as a third factor: it can either move continuously or with forward jerks (thus mimicking more closely the fish behaviour). Finally, the robot's speed is a continuous factor, varying between 0.03 and 0.06 [m/s]. This speed range was determined by measuring the speed distribution of the zebrafish.

The responses of the experiment are twofold. The first response analysed is the mean distance between the real fishes and the mock-up fish during the experiment. By measuring this value, one can assess the acceptance of the robot fish in the fish shoal. It is measured in [mm]. The second analysed response is the number of times during the experiment a fish is situated in a radius of 10 cm around the mock-up fish. A sensitivity analysis has been conducted and the value of the cut-off distance was found not to significantly affect results.

Since the duration of the experiments is 30 minutes and the fish are randomly selected for each experiments among 10 fish, we can consider that measurements on only one run per experiment are relevant enough for the tests that we are performing.

4. Design of Experiments

4.1. Model description

The objective of this study is to understand the effect of the three discrete and one continuous factors on the response variables of the experiment. As a first step, a linear model with interactions was tested. Then, as it was not clear if the relation was indeed linear, a linear model with an additional quadratic term was compared to the first model.

The first model used in this study (linear with interactions) can be written as follows:

$$Y = a_0 + \sum_{i=1}^{4} a_i X_i + \sum_{i,j=1, j\neq i}^{4} a_{ij} X_i X_j, \tag{4.1}$$

where the X_i stand for the values of the factors, a_i are the model coefficients associated to X_i and Y is the response variable.

In a full factorial design, a total of $2^4 = 16$ experiments would be needed, as four factors explain the response variable. In order to reduce the number of experiments (which are time- and resource-consuming), a fractional factorial design (see, e.g., [10]) of type 2^{4-1}_{IV} was used. Hence, only $2^3 = 8$ experiments were needed. This reduction in the number of experiments comes at a cost, however: some of the coefficients are now aliased. This design having a resolution of IV, neither main effects a_i are confounded between each other, nor main effects with first-level interaction terms a_{ij}. However, first-level interaction terms a_{ij} are confounded between each other and main effects a_i are confounded with second-level interaction coefficients a_{ijk} (see [11]). If one neglects second-level and higher-level interaction terms, this design is able to determine main effects without bias. However, first-level interaction terms are aliased: a_{12} with a_{34}, a_{13} with a_{24} and a_{14} with a_{23}.

By using the generator $+4 = 123$, the following matrix of experiments is obtained:

$$\begin{array}{c} \begin{array}{cccc} X_1 & X_2 & X_3 & X_4 \end{array} \\ \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & 1 & -1 & -1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 \\ -1 & 1 & 1 & -1 \\ -1 & 1 & -1 & 1 \\ -1 & -1 & 1 & 1 \\ -1 & -1 & -1 & -1 \end{bmatrix} \end{array}$$

Hence, the matrix of the model is given by

$$X = \begin{array}{c} \begin{array}{cccccccc} a_0 & a_1 & a_2 & a_3 & a_4 & a_{12} & a_{13} & a_{14} \end{array} \\ \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & -1 & -1 & 1 & -1 & -1 \\ 1 & 1 & -1 & 1 & -1 & -1 & 1 & -1 \\ 1 & 1 & -1 & -1 & 1 & -1 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & -1 & 1 \\ 1 & -1 & 1 & -1 & 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 & 1 & 1 & -1 & -1 \\ 1 & -1 & -1 & -1 & -1 & 1 & 1 & 1 \end{bmatrix} \end{array}$$

5. Results

Table 2 shows the results that were obtained from the 8 performed experiments.

Run	Mean Distance ($\bar{d}$ [mm])	# of times near robot (T [-])
1	414.27	274
2	354.05	2895
3	420.90	1003
4	372.99	1706
5	401.88	1327
6	407.87	1173
7	407.57	949
8	390.47	1325

Table 2. Results obtained from the 8 experiments performed.

In order to assess if the fish seem attracted by the mock-up fish, we have compared the results obtained for the mean distance with an experiment in which only the three fish were swimming, without any mock-up fish inside the tank. We have simulated a mock-up fish that is making the same movements as the robot would make and computed the distance between each fish and this virtual mock-up fish. We have obtained a value of 465.88 mm for the average distance, which is above all the distances obtained during the experiments (see Table 2). This tends to indicate that the fish seem to be attracted by the mock-up fish that is moving inside the tank.

5.1. Results for linear model

The coefficients of the linear model have been estimated using least squares regression,

$$\hat{a} = (X^T X)^{-1} X^T Y, \tag{5.1}$$

where Y is the vector of results. As the factorial design is orthogonal, this equation reduces to

$$\hat{a} = \frac{1}{N} X^T Y, \tag{5.2}$$

where N is the number of experiments ($N = 8$ in our case).

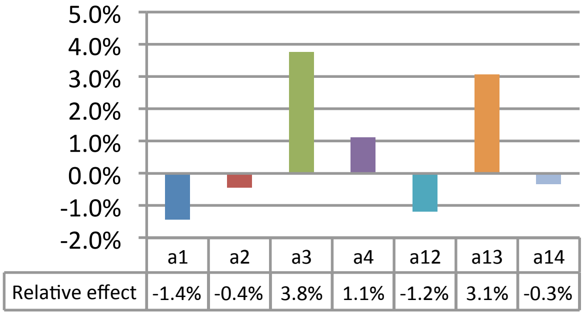

Figure 7. Relative effects of the factors on the first response, the mean distance between the mock-up fish and the zebrafishes ($\bar{d}$).

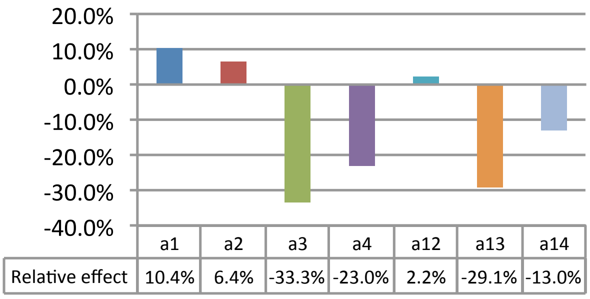

Figure 8. Relative effects of the factors on the second response, the number of times a zebrafish is situated at less than 10 cm of the mock-up fish (T).

Figure 7 shows the relative effects of the different factors on the mean distance ($\bar{d}$) between the mock-up fish and the zebrafish. It allows to obtain a first understanding about the most influential factors on the response. The robot movement (a_3) and the interaction term between the mock-up shape and the robot movement (a_{13}) seem to be the most influential factors. However, the effects are limited: none of the factors has a relative effect above 5%.

Figure 8 shows the relative effects of the different factors on the number of times (T) a zebrafish is situated at less than 10 cm of the mock-up fish. The same initial conclusions can be drawn than for $\bar{d}$: the most influential factors on the response are the robot movement (a_3) and the interaction term between the mock-up shape and the robot movement (a_{13}), followed by the robot's speed (a_4). Furthermore, all coefficients (except a_{12}) have a relative effect above 5%.

These results are coherent if one observes that in the case of $\bar{d}$, a small response value indicates a higher acceptation rate of the mock-up fish (because of a smaller mean distance), whereas in the case of T, a high acceptation rate of the mock-up fish is indicated by an elevated response value. As the relative effects of the coefficients were more significant for T, we decided to investigate further the results concerning the second response.

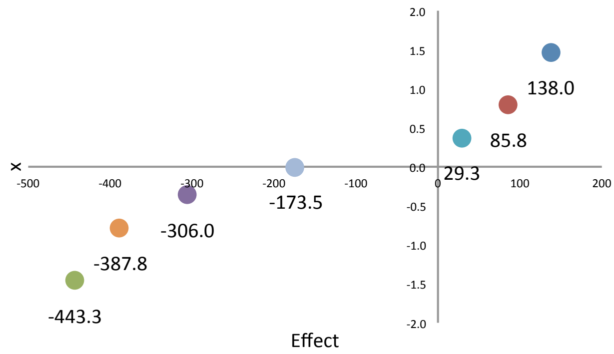

Figure 9. Normal plot for response T (the colors of the markers correspond to the colors of Figure 8).

The normal plot for response T is presented in Figure 9. It can be used to assess if the effects are normally distributed (i.e., they may be generated by a random process and therefore only represent experimental noise). In our case, no effects are clearly aligned on a straight line. It is therefore not possible to disqualify any effects based on the normal plot. It is however clearly visible in the normal plot that the main effects indicated in our first analysis (a_3 and a_{13}, green and orange points) are not normally distributed. The results seem also coherent with what can be found in the literature concerning experiments of interactions between robots and zebrafish, in which the factors that seem to influence the most is the robot motion [12].

Table 3 presents the ANOVA table of response T. In a first step, we decided to include factors with a relative effect below 5% in the residual (only a_{12} in this case). The ANOVA results show that the three main effects described earlier (a_3, a_{13} and a_4) have a probability of being random of 4.2%, 4.8% and 6.1% respectively, and can thus be considered as significant effects. The effects of the other factors are not certain because their probabilities of being random are much higher (between 10% and 20%). This uncertainty on the other effects is mainly due to the low number of experiments associated with a fractional factorial design (compared to the number of coefficients), which in turn decreases the degrees of liberty left for the residual. One solution would be to reduce the number of coefficients in order to increase the degrees of liberty left for the residual. However, as Table 4 shows, including all factors except the three main factors and the constant in the residual does not improve the p-value of the remaining factors. Finally, it was decided to keep all factors with a relative effect above 5% in the linear model for the response T:

$$Y_T = \sum_{i=1}^{4} a_i X_i + a_{13} X_{13} + a_{14} X_{14} + \text{Residual}$$

Effect	SS	df	MS	F	p
a_0	14183138	1	14183138	2072.2	0.014
a_1	152352	1	152352	22.3	0.133
a_2	58825	1	58825	8.6	0.209
a_3	1571765	1	1571765	229.6	0.042
a_4	749088	1	749088	109.4	0.061
a_{13}	1202801	1	1202801	175.7	0.048
a_{14}	240818	1	240818	35.2	0.106
Residual	6845	1	6845		
Total	18165630	8			

Table 3. ANOVA table for response T, where factors with relative effects below 5% are included in the residual.

Effect	SS	df	MS	F	p
a_0	14183138	1	14183138	123.6	0.571
a_3	1571765	1	1571765	13.7	0.168
a_4	749088	1	749088	6.5	0.237
a_{13}	1202801	1	1202801	10.5	0.191
Residual	458839	4	114710		
Total	18165630	8			

Table 4. ANOVA table for response T, where all factors except the three main factors and the constant are included in the residual.

5.2. *Addition of centre points*

Results of the fractional design showed that the robot's movement and speed have a strong impact on the number of times that the zebrafish are near the mock-up fish.

In the previous experiments, only two values were used for the robot's speed, 3 and 6 cm/s. However, as it is a continuous factor that is easily tuned, experiments can be added using another value of the speed in order to include centre points in the experimental design and thus obtain a more precise model. Furthermore, it is also possible that the increase of the speed has a non-linear impact on the fish response. We thus added a quadratic factor in our model to consider this effect. The estimation of another coefficient a_{44} in the matrix of experiments becomes thus necessary. The equation of the model including the additional quadratic factor for the robot's speed is given by Eq.(5.3):

$$Y_T = \sum_{i=1}^{4} a_i X_i + a_{13}X_{13} + a_{14}X_{14} + a_{44}X_{44}^2 \tag{5.3}$$

Since a standard Doehlaert design (see [11]) cannot be applied, it was decided to use a composite experimental design. In order to determine the number of additional experiments needed, the orthogonality and the trace of the new dispersion matrix was analyzed. Indeed, by adding the quadratic factor and additional runs, this matrix will become non-orthogonal. The additional experiments will be determined so as to minimize the trace of the dispersion matrix and maximize its orthogonality.

The trace of the dispersion matrix was computed for different number of additional experiments (see Table 5). One can observe that for an increase of the number of new runs, the value of the trace decreases. We chose to make two[1] additional runs, thus a dispersion matrix with a trace of 2.19.

Additional Runs	Trace
1	4.25
2	2.19
3	1.68
4	1.33

Table 5. Trace of the dispersion matrix depending on the number of experiments that are added.

Table 6 shows a description of the two additional runs that were carried out, as well as the results of the experiments. The three discrete factors where varied in order to minimize the trace of the dispersion matrix, and the last parameter, the average speed, was fixed at 4.5 cm/s. The three additional runs were conducted with exactly the same conditions as the 8 first ones.

Runs	X1	X2	X3	X4	$\bar{d}$	T
9	1	-1	1	0	387.66	1323
10	1	1	-1	0	382.59	1983

Table 6. Additional runs for the matrix of experiments. The factor X4 (speed) is set to 0, which means in the context of the experiment an average speed of the robot of 4.5 cm/s.

5.3. *Results with additional centre points*

In the design with additional centre points, a model with a quadratic coefficient a_{44} for the robot's speed was estimated for the response variable T. As the design is not orthogonal anymore, the coefficients have been estimated using the generic least squares formula, (5.1). The second column of Table 7 shows the result of this estimation, while the third column shows the relative effects of the coefficients. One can observe that all coefficients keep the same sign as in the linear model (compare with Figure 8). Further, the relative effects of the coefficients show that the importance of the various factors introduced in the linear model remain almost unchanged: the three main factors are a_3 (-23.7%), a_4 (-20.2%) and a_{13} (-20.1%), followed by a_{14} (-11.5%) and a_1 (9.1%). Only a_2 does not seem to be significant anymore (0.1%). The additional coefficient, a_{44}, seems to have an important relative effect, at -12.1%.

Let us now have a broader look at how well the quadratic model is performing: Figure 10 shows that for the first 8 experiments, the linear model with interactions is performing better than the quadratic model to estimate the results. Furthermore, for the two additional experiments, the quadratic model estimates the results very poorly.

[1] It was initially decided to make three additional runs, thus further decreasing the dispersion matrix trace to 1.68. However, the engine of the robot broke down at the end of the second additional experiment and could not be repaired in time to carry out more experiments.

Coefficient	Effect	Relative effect	Effect $Z1$
a_0	1515.00	-	1362.08
a_1	138.00	9.1%	168.58
a_2	1.93	0.1%	1.93
a_3	-359.43	-23.7%	-359.43
a_4	-306.00	-20.2%	-306.00
a_{13}	-303.93	-20.1%	-303.93
a_{14}	-173.50	-11.5%	-173.50
a_{44}	-183.50	-12.1%	-

Table 7. Effects, relative effects and effects of the linear model only for the additional experiments.

	SS	df	MS	F	p
Z1	23403049.38	7	3343292.77	16.70	5.76%
Z2	44896.33	1	44896.33	0.22	68.24%
Residual	400302.29	2	200151.14		
Total	23848248.00	10			

Table 8. ANOVA table for comparison between linear and quadratic model.

	Linear	Quadratic
r^2	0.9996	0.9832

Table 9. Precision of the models.

This observation can be backed up by an ANOVA analysis: see Table 8. The data is first fitted on the linear part of the model ($Z1$); the first row shows how much variance is explained by the linear model $Z1$, while the second row shows how much variance is explained by the quadratic model ($Z2$). The probability that the quadratic effect is random is very high (68.4%), thus indicating that a quadratic model is not appropriate in our case. Finally, Table 9 shows the respective precisions of the models (i.e., $r^2 = SS(Y)/SS(Y(Z1, Z2))$). The coefficient of determination is higher for the linear model with interaction than for the quadratic model, indicating once more that the quadratic model does not bring any new information.

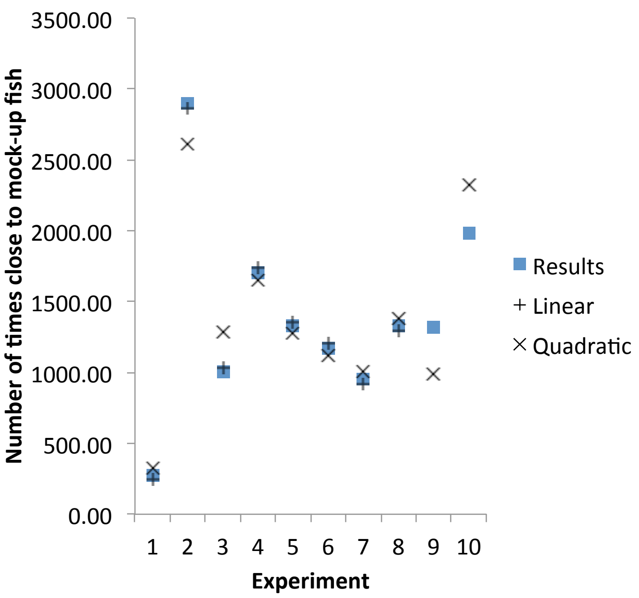

Figure 10. Comparison between models for response T.

6. Conclusion

In this paper we investigated the acceptance of a robotic fish among a group of real zebrafish while varying several parameters of the experiments, such as the shape of the robot and its motion. The design of the robotic fish was set such that it will reach the same speed and acceleration as the zebrafish and it will be possible to have several mock-up fish swimming inside the tank and forming a shoal.

We have noticed that the fish seem to be attracted by the robot, by comparing experiments with and without the mock-up fish moving inside the tank. Further statistical analyses based on DOE were performed to process the data and build a model. We used fractional factorial design to reduce the number of experiments to perform.

Two results were analyzed: the mean distance between the fish and the robot and the number of times during the experiments that a fish is at less than 10 cm from the robot. As the relative effects of the coefficients were more significant for the latter, we decided to investigate further the results concerning the second response.

Results show that among the different parameters that were varied during the experiments, coefficients corresponding respectively to the robot's movement, speed and interaction between speed and motion can be considered as significant effects, and thus included in the model. As the p-value was not improved by reducing the number of factors, it was decided to keep all the factors except the interaction term between the shape of the mock-up fish and the robot trajectory.

Finally, a quadratic factor was introduced in our model and two more experiments were performed in order to add centre points in our experimental domain. The results showed however that the quadratic factor was not significant and that a linear model with interactions is sufficient to describe the response.

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