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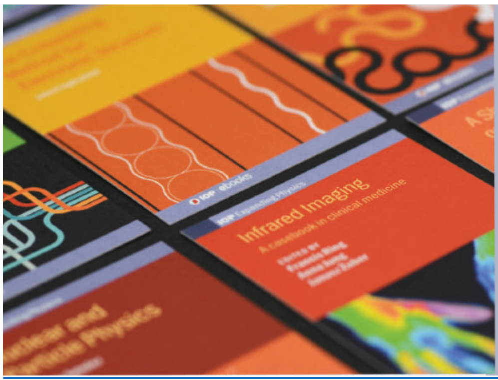

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PAPER

Live fish learn to anticipate the movement of a fish-like robot*

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Abstract

The ability of an individual to predict the outcome of the actions of others and to change their own behavior adaptively is called anticipation. There are many examples from mammalian species—including humans—that show anticipatory abilities in a social context, however, it is not clear to what extent fishes can anticipate the actions of their interaction partners or what the underlying mechanisms are for that anticipation. To answer these questions, we let live guppies (*Poecilia reticulata*) interact repeatedly with an open-loop (noninteractive) biomimetic robot that has previously been shown to be an accepted conspecific. The robot always performed the same zigzag trajectory in the experimental tank that ended in one of the corners, giving the live fish the opportunity to learn both the location of the final destination as well as the specific turning movement of the robot over three consecutive trials. The live fish's reactions were categorized into a global anticipation, which we defined as relative time to reach the robot's final corner, and a local anticipation which was the relative time and location of the live fish's turns relative to robofish turns. As a proxy for global anticipation, we found that live fish in the last trial reached the robot's destination corner significantly earlier than the robot. Overall, more than 50% of all fish arrived at the destination before the robot. This is more than a random walk model would predict and significantly more compared to all other equidistant, yet unvisited, corners. As a proxy for local anticipation, we found fish change their turning behavior in response to the robot over the course of the trials. Initially, the fish would turn after the robot, which was reversed in the end, as they began to turn slightly before the robot in the final trial. Our results indicate that live fish are able to anticipate predictably behaving social partners both in regard to final movement locations as well as movement dynamics. Given that fish have been found to exhibit consistent behavioral differences, anticipation in fish could have evolved as a mechanism to adapt to different social interaction partners.

1. Introduction

Forecasting and predicting what social partners will do in the future could be a major advantage in social interactions when one's own behavior is then changed adaptively (Wolf and Krause 2014, Poli 2019). Such an anticipatory ability (Poli 2019) forms part of broader concepts such as social responsiveness or social competence (Taborsky and Oliveira 2012, Wolf and McNamara 2013). There are several examples of sophisticated anticipatory behaviours in nonmammalian animals, especially fishes, when it comes to acting adaptively in their social environment. On a global scale, animals can predict the location of food items by observing others showing feeding behavior and then move there themselves (which is known as local enhancement learning (Brown and Laland 2003)). Animals are further able to predict a social partner's competitive strength by observing this partner interacting with others (Bierbach *et al* 2011, Hotta *et al* 2015) and adjust their own interactions with that partner. In a mate choice context, females are able to predict a male's aggressiveness during future matings, by observing male agonistic interactions and changing their own mating preferences in favor of losers by anticipating that winners will be more aggressive in future (Ophir and Galef 2003, Bierbach *et al* 2013). These examples illustrate that anticipation in a social context is a two-step process involving an initial prediction of the actions of others followed by an action by oneself that integrates the initial prediction (Poli 2019). Making useful predictions about the future actions of social partners may require animals to distinguish individual social partners and to remember their particular actions, which certainly involves learning processes (Brown *et al* 2011).

When animals move in groups, one can observe spectacular forms of coordinated behavior, with individuals showing high degrees of spatial, directional and temporal organization (Couzin *et al* 2002, Krause and Ruxton 2002). Explaining the remarkable speed at which groups can change direction of movement as a whole in a highly synchronous manner and without long inter-individual reaction time delays may point towards another, more local form of anticipation (*sensu* (Couzin 2018)). Here, individuals act based on the speed and future position of their neighbors and not merely on the instantaneous location, as exemplified in pedestrians (Moussaïd *et al* 2011, Gerlee *et al* 2017, Lü *et al* 2020). In human ball sports, players can predict the flight curve or impact location of the ball even before the ball is thrown or kicked (leaves the hand) by interpreting the first body movements and postures of the throwing/kicking player (Núñez Sánchez *et al* 2005, Diaz *et al* 2012, Maselli *et al* 2017). This anticipation ability is improved by training and professional players outperform naive ones substantially, although several aspects of this anticipation might be an innate feature of humans (Hernik *et al* 2014, Authié *et al* 2015). It seems thus reasonable that anticipation in the social context relies on innate, pre-given representations ('models') of neighbors' movements. Learning facilitated through repeated interactions and observations may then help to update initial models of social partners' behaviors. For example, familiar groups of fish reacted faster to simulated predator attacks as compared to unfamiliar ones (Nadler *et al* 2021).

Consistent differences among social partners' behaviors have been suggested to promote this kind of social learning (Wolf and Krause 2014). These consistent individual differences in behavior (aka 'animal personality' (Réale *et al* 2007)) seem to be a ubiquitous feature found even in genetically identical clonal animals (Archer *et al* 2003, Freund *et al* 2013, Bierbach *et al* 2017).

In the context of synchronously moving animals, we hypothesized that individuals anticipate their neighbors' movements to achieve minimal reaction time delays during direction changes initiated by the partners (Brown *et al* 2011). Furthermore, we hypothesized on the basis of previous local enhancement experiments that group living animals can anticipate the areas that a social partner will visit in the future.

To explore these hypotheses, we let individual live guppies—a small live-bearing fish that is facultative shoaling—swim together with a biomimetic robot that was steered open-loop on a fixed zig-zag trajectory through a large experimental tank for three consecutive trials (72 h between trials), thus giving the fish the opportunity to learn about the robot's movement behavior (regular turns to left and right with fixed acceleration and deceleration) and goal areas of the robot (e.g., corners visited along the zig-zag path). Using a robot as a social partner allowed us to provide all focal live fish with the same social stimuli over repeated trials, thus facilitating both the learning process through a high predictability of the social partner and the interpretation of the results due to the absence of variation in the provided social stimulus. We first looked at anticipation on a global scale, e.g., asking whether live fish learn to approach areas that the social partner has visited in the past even before the partner arrives there. Our prediction was that if live fish learned which corners the robot will visit, they will visit these corners even before the robot reaches them. On a more local scale, we asked whether fish can decrease their reaction times to direction changes of the robot when they gain experience with the predictable movement patterns of their robotic partners. We predicted that the fish would initially turn after the robot had turned and stayed behind the robot. With repeated interactions (e.g., in trials 2 and 3) there should be a switch in the turning times of the fish leading to their own turns executed closer to, or even before, the robot turns, and possibly also to turns in front of the robot. Both the approach towards corners before the robot and the execution of turns temporally closer to the robot turns would then be indicative of guppies anticipating their social partners' global and local behaviors.

2. Material and methods

2.1. Study animals and maintenance

In this study, we analyzed videos that had been recorded for another study but had not been analyzed for anticipatory behaviors (Lukas *et al* 2021). The study subjects were wild-caught feral guppies (*Poecilia reticulata*, figure 1(A)) caught in the Gillbach river, Germany. Fish were brought to the laboratory and housed in mixed-sex tanks (250 l) at a constant water temperature of 26 °C for the next 30 days before we started with behavioral tests. Fish were held under diurnal lighting (12:12 h light:dark cycle) and were fed twice daily *ad libitum* with commercially available flake food (TetraMin® Tetra GmbH). Feeding was halted 24 h before experiments and commenced afterwards. Offspring was removed twice a week from the holding tanks. One week prior to testing, fish were marked using VIE tagging (see (Jourdan *et al* 2014) for procedure) to allow for individual recognition throughout the behavioral assessment.

2.2. Behavioral tests

We used only adult fish in our study and included individuals if their body size exceeded 15 mm standard length (SL) and if they showed external signs of maturity (females: gravid spot, males: fully developed gonopodium). Tests were performed in a large aquarium (88 × 88 cm, 75 mm water depth, figure 1(C)) filled with aged tap water and kept at 26 °C. To reduce outside disturbances, the test arena was enclosed in black, opaque plastic and illuminated with artificial light reproducing the daylight spectrum. Behavioral observations were recorded via a camera (Ximea 4K USB 3.1 camera) mounted above the arena.

At the start of a trial, fish were introduced into a gray opaque shelter box (diameter: 100 mm) and allowed to acclimatise. After 1 min, we gently removed the sponge from the entrance (40 × 25 mm), allowing the fish to emerge and explore the test arena. A fish was scored as having emerged when its full body was visible outside of the shelter. If fish did not emerge after 5 min (15 cases in 477 trials), the lid of the shelter box was removed. If fish failed to emerge within 8 min (11 cases in 477 trials) the box was removed entirely. As these interventions could have disturbed fish and influenced subsequent measures, we confirmed that the results were qualitatively similar if we fitted models excluding these data points (results not shown).

When the fish had left the shelter (or after the removal of the shelter box if the fish did not emerge, see above) they encountered a 3D fish replica that was stationed 1 cm away from the shelter entrance. The replica, which resembled a large-sized guppy female (SL = 30 mm; see figure 1(A)), could be moved by a robot via a magnetic base (see figure 1(B), (Landgraf *et al* 2016)). Once a fish was within the distance of one body length from the shelter, the robofish was manually initiated. It then began to move along a predefined zig-zag path, following the diagonal of the tank to corner S_1 and then along the right side to corner S_2 (figure 2(A)). This path was identical during all tests and took about 50 s. After completion of the robofish's predefined path, the replica was removed immediately from the arena by hand. Once the fish resumed swimming (e.g., after a period of freezing), we recorded another 3 min to further prevent fish from associating a (negative) handling experience with the robofish through its manual removal. This would be the case if we removed the live fish shortly after robofish had been stopped or removed. This 'asocial' treatment allowed us to further assess the general activity of an individual outside a social context (see analysis below).

After the first behavioral trial, we sexed each individual and recorded its body length by photographing the fish in a water-filled Petri dish with a reference grid. Fish were transferred back to their holding tank and allowed 72 h rest before being retested following the same protocol. The order in which fish were tested was randomized for each trial. Through the repeated testing, live fish were given the opportunity to learn the robot's swimming behavior which was executed identically in all tests.

2.3. Data analysis

2.3.1. Kinematic behavioral type variables

From the video recordings of the behavioral trials we first extracted the time the fish took to leave the shelter box as our boldness score, with low latency times to emerge indicating high levels of boldness. In cases when we had to remove the shelter box after 8 min, fish were given the maximum score of 480 s. Second, we extracted the 2D positions $\vec{x} = (x, y)$ at a frame rate of 10 fps of both, robofish and live fish using the EthoVision 10 XT software (Noldus Information Technology, Wageningen, Netherlands) for both the social part (with robot) as well as the subsequent asocial part after the robot was removed. The instantaneous speed $\vec{v} = (v_x, v_y)$ and acceleration $\vec{a} = (a_x, a_y)$ vectors were computed using the positions of the robofish and live fish correspondingly. We further computed the instantaneous direction of motion by calculating:

$$\varphi = \arctan(v_y, v_x). \qquad (1)$$

All of these physical quantities ($\vec{x}, \vec{v}, \vec{a}$ and φ) were then smoothed over time using a running average with Gaussian weights. For this, we used time windows of 4 dt (dt = 1 time step = 0.1 s).

As previously established, we took the mean distance between fish and robofish during a trial as a measure of the fish's sociability as this distance is

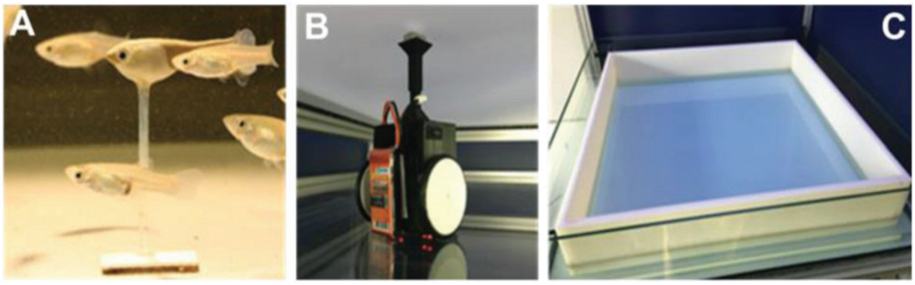

Figure 1. Robofish system. (A) Robot dummy surrounded by live guppies. (B) Robot unit below the tank on a transparent second level. (C) Test tank for behavioral observations. For more details on the setup (see Landgraf *et al* 2016).

purely influenced by the fish's decision to associate with its artificial conspecific and was further found to correlate strongly with a fish's tendency to associate also with live conspecifics (see (Bierbach *et al* 2018)). Mean velocity in the asocial part was used as a measure of general activity of a fish.

2.3.2. Anticipation variables

We hypothesized that live fish may anticipate their social partners' behavior at different spatial and temporal scales and thus performed our analysis at two different levels: on a global level, we analyzed whether fish showed anticipatory abilities with regard to their partners' ultimate goals and directions (e.g., corners reached), while on a local level we analyzed whether fish anticipate small-scale swimming patterns such as near future turns of the robot.

Global anticipation: we predicted that if live fish can anticipate the behavior of the robot, they should learn to which corners the robot will move and then move there before the robot. To test this prediction, we first defined square areas of 20 × 20 cm labeled as S_1, S_2 and S_3 that are located in the three corners of the tank that are opposite to the corner where the trials started (figures 2(A) and (B)). We explored different square-sizes ranging from 5 to 30 cm but decided to choose 20 cm as the biologically most meaningful area size although variables did not change in the range between 15 and 30 cm quantitatively (not shown). The squares S_2 and S_3 are (approximately) at the same distance to the initial position of the robofish and live fish (upper left corner in figures 2(A) and (B)), whereas the diagonal square S_1 is further away. In all trials, robofish moved diagonally to the opposite lower right corner (S_1), and afterwards to the upper right corner (S_2), while never visiting S_3 (figure 2(A) and supplementary video SI videos 1–3).

As a first analysis to confirm robofish's influence on the live fish's choice for corners, we compared the fraction of fish that visited/did not visit each of the three corners. To do so, we noted for each trial which corner a fish was visiting, and used this binary variable (visited or not) as dependent variable in a generalized linear mixed model (GLMMs, bimodal error distribution and logit link function with pairwise contrasts for post hoc comparisons; GLMM1a-c in supplementary file 'SI_Analysis_code') separate for each respective corner. We included trial, sex and body size as fixed factors and fish ID as random factor to account for the multiple testing of the same individuals. To test whether fish approached the corner S_2 more often than the equidistant corner S_3, we used *chi*^{2} tests separate for each trial.

We assumed live fish to have anticipated if they reached a corner before robofish, and thus analyzed the fraction of fish visiting corners S_1 and S_2 before robofish with a similar set of GLMMs (binary variable: 'before robot or not'; GLMM 2a,b). Further, we calculated the time difference between the arrival of the live fish and the time of arrival of the robofish:

$$\Delta t_{\text{arrival}} = t_{\text{arrival,fish}} - t_{\text{arrival,robo}} \tag{2}$$

where $t_{\text{arrival,fish}}$ and $t_{\text{arrival,robo}}$ are the arrival times to a given square. If $\Delta t_{\text{arrival}} > 0$, the live fish reached the square before the robofish, and if $\Delta t_{\text{arrival}} < 0$, the live fish reached the square after the robofish. The time differences between the arrival of robofish and the live fish at corners S_1 and S_2 was compared among trials using linear mixed models (LMMs, Gaussian error distribution, identity link and REML estimation method with LSD post hoc tests; LMM3a,b) separate for both corners and we included trial, sex and body size as fixed factors and fish ID as a random factor.

Then, we analyzed which corners fish visited first in a trial using GLMMs (binary variable: 'first corner visited or not', GLMM4a-c) separate for each corner. Similarly, we tested whether fish reached S_2 more often than S_3 first using separate *chi*^{2} tests for each trial.

In order to see whether the fraction of fish in our trials that arrived before the robot, after the robot or never at a specific corner, differed significantly from chance, we compared real trial data to numerical simulations performed using a random walk simulation—that serves as an example of unbiased motion in the tank, see supporting information: random walk—using *chi*^{2}-tests separately for each trial.

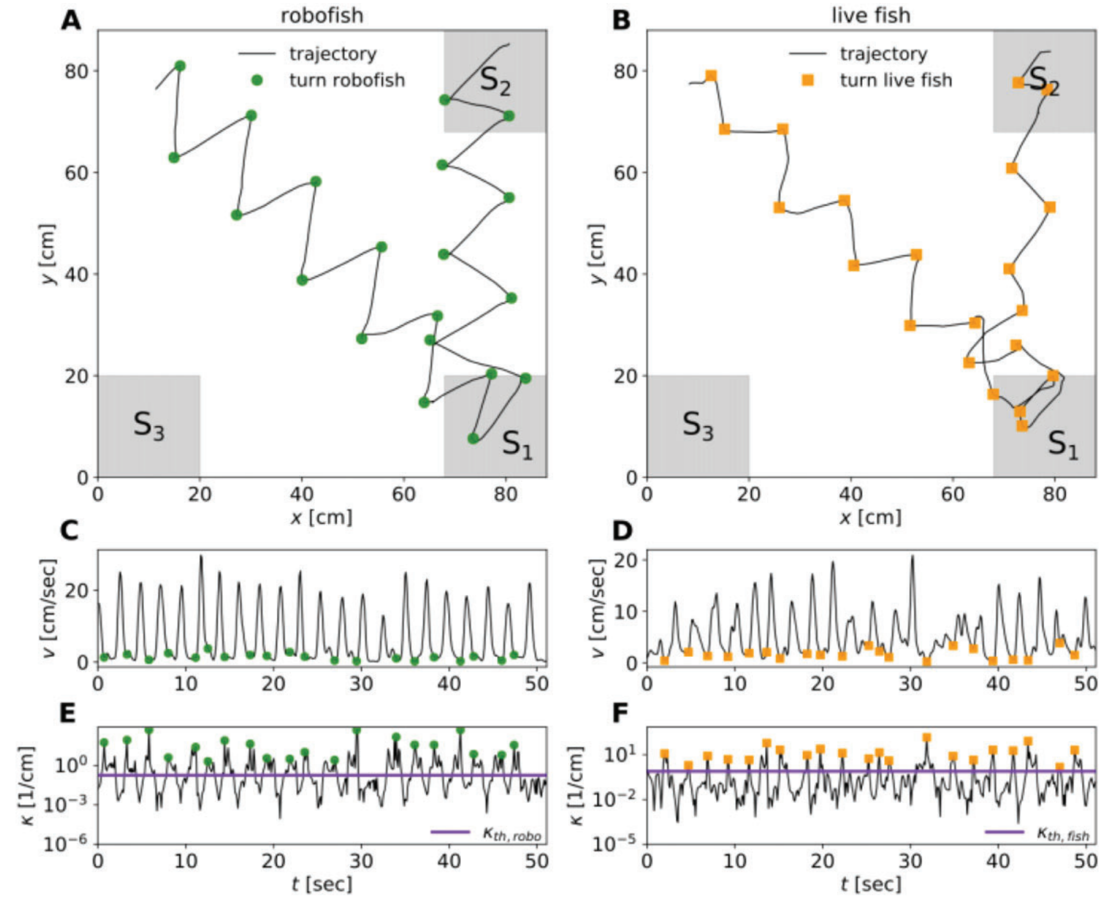

Figure 2. (A) and (B) Trajectories (black solid lines) and turns of the robofish (green circles) and live fish (orange squares). The regions marked in gray (S_1, S_2 and S_3) are the corners in the tank that we used to quantify global anticipation. Each region is a square of dimensions 20 × 20 cm. (C) and (D) Speed time series $v(t)$ for the robofish and the live fish. (E) and (F) Curvature time series $\kappa(t)$ for the robofish and the live fish. In both time series—$v(t)$ and $\kappa(t)$—the points in time associated with the turns of the robofish and live fish are marked with green circles and orange squares correspondingly.

As the tendency of fish to arrive at a corner before or after robofish could be linked to the fish's personality, we correlated the time delay to reach corners S_1 and S_2 with the boldness (time to leave shelter box), sociability (mean distance to robofish) as well as general activity (mean activity in asocial part) scores of the fish in trial 3 using Spearman's rank-order tests.

If fish developed a place preference (i.e., Clément *et al* 2020) for corners S_1 and S_2 through the repeated guidance by the robot we would further assume that they spent increasingly more time in these corners and that they spent more time in these corners than in corner S_3 that is never visited by the robot. We thus compared the fraction of time (arcsin-sqrt-transformed) spent in corners S_1, S_2 and S_3 across all three trials for the asocial part in a mixed model (LMM6) with fish ID as random factor and trial and corner ID as repeated measured fixed effects (Gaussian error distribution, identity link and REML estimation method with LSD post hoc tests). We used the last 90 s in each trial (from a total of 180 s) to allow the fish in the first 90 s to distribute freely in the tank as each asocial trial started in corner S_2 with the removal of the robot. Due to video and tracking errors, 87 cases out of 1464 could not be included.

Local anticipation: as a complement to the global anticipation analysis, we analyzed the possibility that the live fish learn to anticipate abrupt and periodic changes in the motion of the robofish at short spatial and temporal scales. For this, we used the location in space and time of the turns of the robofish and the live fish. We quantified the local anticipation by selecting each turn of the robofish and finding the 'closest' turn of the live fish in time and space. Thus, we restricted our analysis to those live fish turns that were in spatial ($\Delta x_{\mathrm{robo,fish}} \leqslant R_0 = 12$ cm = 4 body lengths (Croft *et al* 2008), where $\Delta x_{\mathrm{robo,fish}} = |\vec{x}_{\mathrm{turn,robo}} - \vec{x}_{\mathrm{turn,fish}}|$) and temporal ($|\Delta t_{\mathrm{robo,fish}}| \leqslant T_0 = 2$ s, where $\Delta t_{\mathrm{robo,fish}} = t_{\mathrm{turn,fish}} - t_{\mathrm{turn,robo}}$) proximity to the robofish turns. These spatial and temporal restrictions are meant to keep only those events (1 event = 1 turn of robofish + 1 turn of live fish) where both agents were socially interacting. The turns of the robofish and the live fish were identified using the curvature (in 2D) of the curve defined by the trajectory $(x(t), y(t))$, which is parametrized by time. We computed the curvature as follows:

$$\kappa = \left| v_x a_y - v_y a_x \right| / (v_x^2 + v_y^2)^{3/2}. \qquad (3)$$

We can find the turns of the robofish and live fish by using threshold values of the corresponding curvature time series and finding the maximum of each time interval where the curvature is larger than the corresponding threshold. The definition of the thresholds is computed using properties of the curvature signal, which means that the threshold values are, in general, different for each signal but statistically equal. The threshold used to compute the robofish turns is defined as $\kappa_{\text{th,robo}} = 0.02\langle\kappa\rangle + 0.98\kappa_{\min}$, where $\langle\kappa\rangle$ is the temporal mean of the curvature, and $\kappa_{\min}$ is the minimum value of the same signal. The threshold used for the live fish is defined as $\kappa_{\text{th,fish}} = \langle\kappa\rangle - 0.04\sigma_\kappa$, where σ_κ is the standard deviation of the corresponding time series. We used two different definitions due to the stronger fluctuations observed in the live fish trajectories compared to the rather periodic trajectories of the robofish, particularly when the individual reached the corner S_2 before the robofish. The turns of the robofish and live fish can be identified by finding the local maxima of the curvature in each set of time points where $\kappa(t) > \kappa_{\text{th,robo}}$ and $\kappa(t) > \kappa_{\text{th}}$, fish correspondingly, as seen in figures 2(E) and (F). The times at which the turns occur will be referred to as $t_{\text{turn,robo}}$ and $t_{\text{turn,fish}}$, and their positions as $x_{\text{turn,robo}}$ and $x_{\text{turn,fish}}$ correspondingly. A representative example of the trajectories and turns of the robofish and live fish can be found in figures 2(A) and (B). In this figure, we can see that the turns always happen at time points of high curvature and low speeds (figures 2(C) and (D)), as explained in (Klamser *et al* 2021).

For each selected event, we computed the time difference $\Delta t_{\text{robo,fish}}$. For the turns of the fish of each selected event, we quantified as well the relative position of the robofish at the moment of the live fish's turn by computing:

$$\Omega = ((\vec{x}_{\text{robot}} - \vec{x}_{\text{fish}}) / \| \vec{x}_{\text{robot}} - \vec{x}_{\text{fish}} \|) \times (\vec{v}_{\text{fish}} / \| \vec{v}_{\text{fish}} \|). \qquad (4)$$

The observable Ω, that takes values between -1 and 1, quantifies the location of the robofish at the precise moment when the live fish turned. A value of $\Omega = 1$ means that the robofish was located precisely in front of the fish when it turned. On the other hand, a value of $\Omega = -1$ means that the robofish was located precisely in the back. We averaged over all turns in each trial per individual to get one single value per trial and individual live fish. By doing this, we avoid a bias in the results due to closely following individuals that had many turns in the spatial and temporal interaction range.

We compared the individual averages of the time difference between a robot's turn and the fish's closest turn and the subject's relative positions among the three repeated trials using separate LMMs that included trial as fixed factor and 'fish ID' as a random factor (LMM5a,b, see supplemental information). For post hoc comparisons among trials, we used Fisher's LSD tests.

3. Results

3.1. Global anticipation

In the first trial almost 90% of all fish visited corner S_1 and the number of individuals visiting corner S_1 significantly decreased compared to trial 3 (GLMM 1a: trial: $F_{2,474} = 29.4$, $P < 0.001$, figure 3(A)). Corner S_2 was visited by roughly the same number of fish throughout the trials (GLMM 1b: trial: $F_{2,474} = 2.48$, $P = 0.09$; figure 3(B)) with females visiting this corner slightly more often than males (see SI_SPSS_code). For the corner S_3 that was not visited by the robot, we found a significant increase in the number of fish visiting it (13% for trial 1, 28% for trial 2 and 28% for trial 3, GLMM 1c: trial: $F_{2,464} = 10.9$, $P < 0.001$, figure 3(C)). In all trials, however, significantly more fish visited corner S_2 compared to the equidistant corner S_3 (*chi*^{2} tests, all $P < 0.001$, figures 3(B) and (C)). These observations indicate that with subsequent trials fish did not follow robofish to the same extent to corner S_1 but had a strongly increased tendency to swim to corner S_2 which was the final location the robot was swimming to (see figure S1 for density heat maps of live fish for all three trials).

We predicted that anticipation tendencies can be assumed when fish reached the corners visited by robofish even before robofish is actually doing so and we therefore looked at the fraction of fish that reached corners S_1 and S_2 before the robot. We found that, in all trials, roughly one third of the fish that reached corner S_1 did so before the robofish and the other two thirds arrived after robofish had arrived (figure 3(A)). In trial 2 significantly more fish arrived at S_1 before the robot as compared to trial 1 and 3 (GLMM3a: trial: $F_{2,474} = 3.18$, $P = 0.042$). The time delay between robot and fish increased from trial 1 to trial 3 with fish arriving 3.39 s after the robot in trial 3 (see figure 3(D), LMM3a: trial: $F_{2,389} = 6.40$, $P = 0.002$). In addition, females arrived significantly before males (see SI: Analysis_code). The positive time delay in arrival at corner S_1 and the stable number of fish arriving before robofish do not indicate anticipation tendencies for this corner. Also, the number of fish that arrived at corner S_1 decreased significantly from trial 1 to trial 3 (GLMM4a: trial: $F_{2,474} = 52.53$, $P < 0.001$, figure 3(F)).

Regarding the arrival at corner S_2, we found that significantly more fish arrived before the robot in trials 2 and 3 as compared to the first trial (GLMM: trial: $F_{2,474} = 15.56$, $P < 0.001$, figure 3(B)) with 52% of all fish arrived before robot in trial 3. On average, fish reached corner S_2 10.2 s in advance of robofish in trial 3 (and 6.8 s in trial 2, figure 3(E)), which is significantly earlier than in trial 1 (0.9 s in

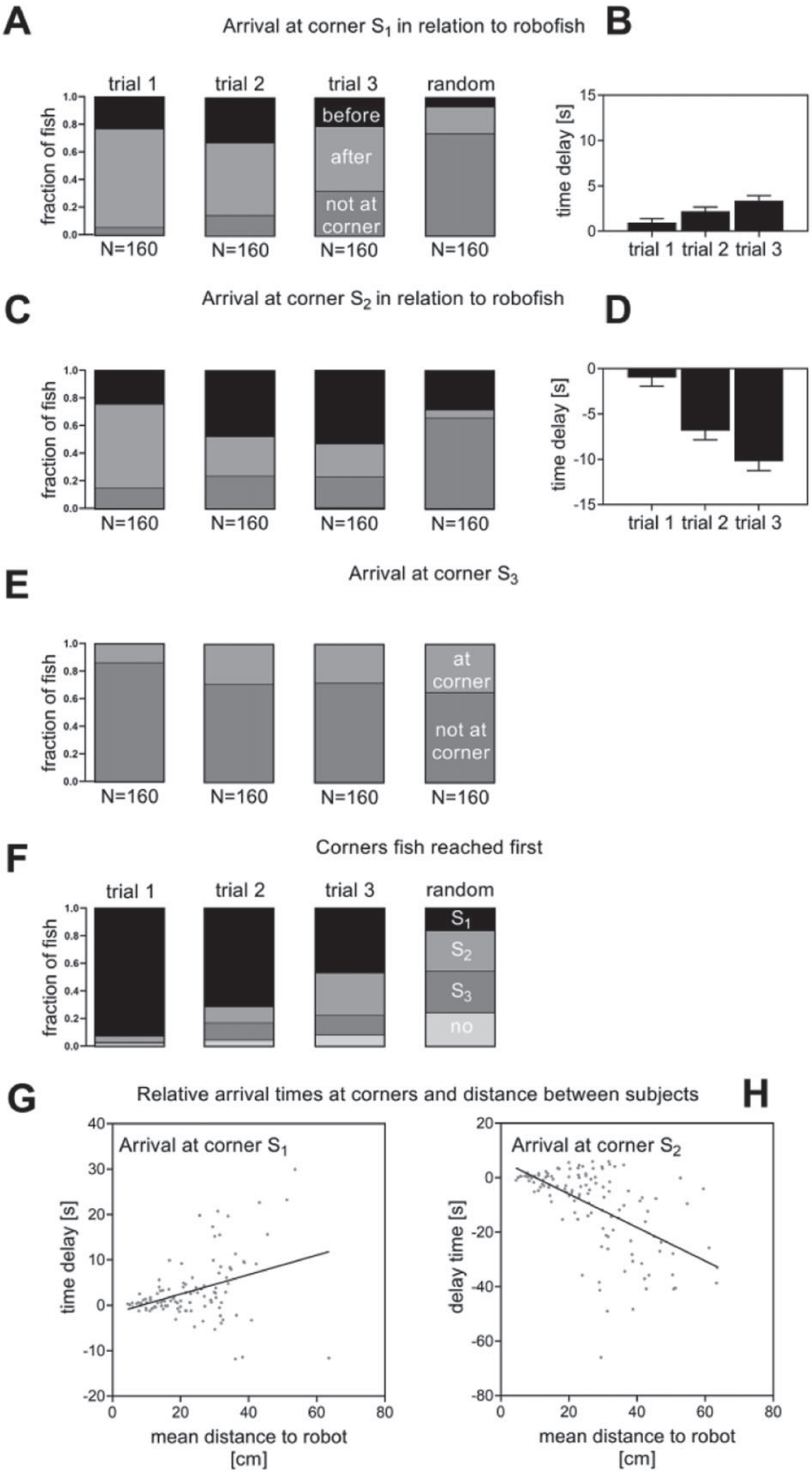

Figure 3. (A) and (B) Arrival at corner S_1 in relation to robofish. (C) and (D) Arrival at corner S_2 in relation to robofish. (E) Arrival at corner S_3. Shown are the fractions of fish arriving at the corners before and after the robot, as well as never, in each trial as well as in our random walk simulation (A), (C) and (E). Further, we show the time difference of arrivals to squares S_1 and S_2 (B) and (D) between live fish and robot with negative values (mean ± SEM), indicating that fish arrived before the robot. (F) Corners that fish visited first over all trials, as well as in the random walk simulation. We also consider those cases where individuals did not reach any of the squares (labeled as 'no.'). (G) and (H) Relative arrival time of the live fish at corners S_1 (G) and S_2 (H) in relation to the distance between live fish and robot. Please note that small distances to the robot mean high sociability of the live fish. Shown are all data points for trial 3 and a linear regression line for better visualization.

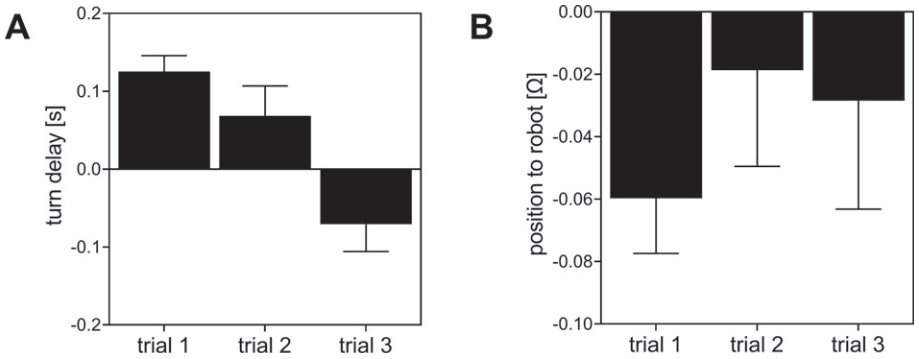

Figure 4. (A) Time of fish turn relative to the time of robot turn. (B) Relative position of the fish towards robofish when turning. Means with SEM are shown.

advance; LMM: trial: $F_{2,256} = 27.1$, $P < 0.001$). We thus conclude that a large proportion of fish learned to predict the final location of the robot and decided to swim there even before the robot arrived at this location. This is also underpinned by the increasing number of fish that swam to corner S_2 first over the course of the experiment (figure 3(F), GLMM4b: trial: $F_{2,474} = 38.39$, $P < 0.001$). In the last trial, significantly more fish arrive at corner S_2 first as compared to corner S_3 (*chi*$^2 = 7.4$, $P = 0.006$).

When these results were compared to simulated data we found that significantly more fish arrived at corner S_1 in the real trials (*chi*^{2}-test; all $P < 0.001$, figure 3(A)), and, in particular, more fish arrived before the robot in the real trials. Also, more fish in the real trials arrived at corner S_2 and also more did so before the robot compared to what was predicted by the random walk model for trials 2 and 3 (*chi*^{2}-test; all $P < 0.001$). The number of fish visiting corner S_3 differed significantly from random only in trial 1 (*chi*^{2}: trial 1 = 33.2, $P < 0.001$; trial 2 = 3.6, $P = 0.057$; trial 3 = 3.4, $P = 0.65$). Thus, the likelihood of live fish to arrive at corners S_1 and S_2 per se (as well as before the robot) is strongly increased in real trials and cannot be explained through a random movement of the fish in trials 2 and 3.

As the tendency of fish to arrive at a corner before or after robofish could be linked to the fish's personality, we correlated the time delay to reach corners S_1 and S_2 with the boldness, sociability, as well as general activity scores of the fish in trial 3. We found a positive correlation between inter-individual distance (sociability) and the time the fish took to reach corner S_1 (Spearman's *r*: 0.312, $P = 0.001$, figure 3(G)) and a negative for corner S_2 (Spearman's *r*: −0.572, $P < 0.001$, figure 3(H)). As greater inter-individual distances between the subjects indicate a reduced sociability of the live fish, this suggests that anticipation is negatively related to sociability which is the tendency to associate with conspecifics. Both boldness and general activity did not correlate with time delays reaching any of the corners in trial 3 (not shown).

In the asocial part without a robot present (used to obtain the general activity for each individual), fish did not increase their time spent in corners S_1, S_2 and S_3 from trial 1 to trial 3 (LMM: trial: $F_{21276} = 2.4$, $P = 0.084$). While fish on average spent a significantly lower proportion of time in corner S_1, no significant difference was detected in the proportion of time spent in corners S_2 and S_3 (corner: $F_{21213} = 4.8$, $P = 0.008$; mean fraction of time S_1: 0.09 ± 0.06 SD; S_2: 0.10 ± 0.08; S_3: 0.10 ± 0.09). It is thus unlikely that fish developed a place preference for corner S_2 due to the interaction with robofish.

3.2. Local anticipation

As outlined above, fish reduced to follow robofish closely over the repeated trials. Thus the fraction of turns within the defined radius R_0 and time window T_0 dropped from 78% in trial 1, down to 51% in trial 2, and to 38% in trial 3. For the cases in which fish stayed close to the robot (within radius R_0 and time window T_0), we calculated the time difference between a robot's turns and the fish's closest turns. In trial 1, the live fish had an average reaction delay time of $\Delta t_{\mathrm{robot,fish}} = 0.131 \pm 0.03$ s. This means that fish turned after the robofish (figure 4(A)). This delay decreased with subsequent testing and in trial 3, fish turned on average 0.065 s before the robofish, which is a significant change (LMM5a: trial: $F_{2,456} = 9.85$, $P < 0.001$, figure 4(A)). No such pattern was found for the relative position (Ω) of the live fish towards the robot when the robot initiated a turn (LMM5b: trial: $F_{2,307} = 0.62$, $P = 0.54$, figure 4(B)). There was no further significant correlation between reaction delay times or positions and the time delays to reach corners S_1 and S_2 in trial 3 (Spearman's *r*, all $P > 0.13$).

4. Discussion

We found that live fish that were given the opportunity to learn the local movement patterns as well as the global goals of a predictably moving, open-loop controlled fish-like robot changed from an initially close following behavior, that was characterized by fish turning after the robot and not reaching the robots' goal corners significantly before the robot, to a behavior in which they turned on average slightly before the robot turned, and even reached the final goal corner significantly before the robot did. These observations are in line with our predictions that derived from hypothesizing that fish are able to anticipate their interaction partners, at least, when the interaction partners are behaving rather consistently, and thus predictably, and when there is time to familiarize with them.

The fish in our experiment visited the last of robofish's visited corners (S_2) significantly in advance of the robot after they had the opportunity to learn the robot's behaviour, and a substantial number of the sampled fish moved to this corner first, straight from leaving the shelter box at the initialization of the experiment. In order to do this, the fish had to increase their average distance from the robot. Previous studies, including the one from which we re-analyzed the dataset for the current investigation, interpreted increasing inter-individual distances when fish were able to interact with the robofish multiple times (Bierbach *et al* 2018a, Lukas *et al* 2021) as a result of habituation and familiarization with the experimental setup (e.g., both tank and robot). However, our current study provides evidence that this effect might be driven by fish having anticipated the robot's final goal areas and moved there in the later trials before the robot, thus not staying close to the robot itself. In addition, our study revealed that fish did not randomly explore the tank as significantly more fish visited corner S_2 which was the last corner that robofish visited on its way compared to the equidistant corner S_3 which robofish never visited. Further, the number of fish visiting corner S_2 per se, and also in advance of robofish, was significantly larger than would be expected by a random walk simulation. As a side effect of moving straight to corner S_2, the number of fish that reached corner S_1 (the first corner robofish visited) during the course of the experiment dropped significantly.

Overall, our results are in line with local enhancement experiments that show fish prefer areas they saw conspecifics visiting (see (Brown and Laland 2003)), predominantly when food was provided as a reward to enforce conditional learning. Experiments where fish are trained to learn the location of a group of conspecifics without food rewards nevertheless also failed to show this kind of memorization (Kurvers *et al* 2018) and some studies did not find fish remembered the path of biomimetic robots that resembled conspecifics (Yang *et al* 2019). In our study, we hypothesized that the initial close following of the robot by the live fish, thus the direct interaction with the robot, enabled live fish to learn precisely the final goal of the robot quickly, similar to studies that found fish learn a path from pretrained conspecifics (Laland and Williams 1997, Lachlan *et al* 1998, Swaney *et al* 2001). This view is underpinned by the fact that during the asocial treatment without robofish being present, our fish did not show a preference for robofish's final corner and also did not increase their time spent in corners S_2 and S_1 with subsequent trials as would be assumed if they have been simply conditioned to those corners by the robot moving there. Future experiments should therefore focus on the distinction between direct social interactions with a demonstrator in contrast to pure observational learning.

Our analysis of the local anticipation abilities focused on the time delay between the robot's direction changes (turns) and those of the fish. We found a clear trend towards a reduction of delay times as well as even a somewhat overshooting overall reaction, as the fish turned on average 0.06 s before the robot turned in trial 3. Recently, familiarity among shoal members was found to increase reaction time and to foster reaction synchrony towards predator cues in fish (Nadler *et al* 2021). It was argued that the higher cognitive demand involved in inspecting unfamiliar schooling partners may delay the initiation of neuronal responses thus causing a longer latency to respond in unfamiliar shoals. In humans, anticipation and a resulting faster response is mediated by a change in response triggering cues after intense training (Muller and Abernethy 2012). Similarly, faster reaction times towards the familiar robofish in our trial 3 could be mediated by a switch in triggering cues. Robofish is swimming without undulating body movements, by just modulating speed and direction. The velocity of robofish peaks half way between two turns (see figure 2) while then decreasing towards almost zero during a turn. While our turn detection was based on the zero velocity phases, the onset of the decelerating phase could well have been used by the live fish as a cue to predict the upcoming turn of the robot. Here, observations at higher frame rates might enable better time resolution for the onset of the fish's responses and thus might help to identify the relevant cues used by the fish and whether those cues change during familiarization.

In summary, our experiment provides evidence that fish can anticipate a social partner's future behaviors and movement goals after repeated interactions and thus elucidates the advanced cognitive abilities of teleost fishes (Schartl *et al* 2013). Given that live fish are known to often behave predictably by showing consistent inter-individual differences in behavior (Conrad *et al* 2011, Castanheira *et al* 2013, Jolles *et al* 2017, Lukas *et al* 2021), our experiment shows that adjustments in their own behavior towards familiar individuals may play an important role in the social interactions of fishes. The ecological relevance of such an anticipatory behavior could be seen in the exploitation of the perceived higher safety of areas that are regularly visited by conspecifics (Brown *et al* 2011) as well as increased synchrony during collective movements (Couzin 2018).

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Data availability statement

The data that support the findings of this study are available in the supplemental information.

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