Any colour you like: using animal-robot interaction to unravel mechanisms promoting phenotypically heterogeneous fish aggregations

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Abstract

Fish collective behaviours provide several benefits to conspecific individuals, although mixed-species aggregations have been reported to often occur. However, the mechanisms promoting phenotypically heterogeneous fish aggregations have been poorly explored so far. Herein, the neon tetra Paracheirodon innesi was selected as ideal model organism to test the role of visible phenotypic traits in promoting fish shoaling. Robotic fish replicas of different colour (e.g. biomimetic livery, blue livery, red livery, grey livery), but with the morphology inspired to P. innesi, were developed to test the affiliation behaviour of neon tetra individuals towards fish replicas with different phenotypic traits. P. innesi individuals showed a decreasing preference in shoaling with the biomimetic replica, the blue replica, the red replica and the grey replica. This could be due to the greater visibility of the blue colour even in dark conditions in these fish. Furthermore, an increased reddening of the livery is often caused by physiological processes related to a non-optimal behavioural status. The time spent in shoaling with each fish replica was strongly influenced by different ecological contexts. The longest shoaling duration was observed when a biomimetic predator was present, while the shortest shoaling duration was recorded in presence of food. This confirms the hypothesis that heterogeneous shoals are promoted by the anti-predator benefits, and reduced by competition. Our animal-robot interaction study allowed to understand basic features of the behavioural ecology favouring heterogeneous aggregations in shoaling fish, as well as provided a novel paradigm, based on biohybridization, for the artificial life synthetic methods.

Introduction

Fish collective behaviours have long drawn attention in ethology (Pitcher, 1991), as well as they are powerful sources of inspiration in proposing computing approaches to develop artificial life coping with different real-world problems (Aguilar et al. 2014; Yazdani, et al. 2015; Fausto, et al. 2017; Li, et al. 2018; Schranz, et al. 2020). Indeed, fish aggregations (shoals and schools) provide several benefits to fish individuals, such as reduction of predation risk, improved foraging activity, efficient fluid-dynamics (Krause, et al. 2002; Ward, et al. 2011), that often outweigh crucial costs, like conspecific competition, and greater detectability by predators (Krause, et al. 2002; Thünken, et al. 2014). Although it is generally assumed that phenotypically homogeneous shoals are more advantageous (Ward, et al. 2002), mixed-species aggregations have been reported to often occur (Ward, et al. 2002; Paijmans, et al. 2019). However, little is known on the mechanisms promoting phenotypically heterogeneous fish aggregations, especially due to the difficulty in controlling different ecological contexts, and in testing separate stimuli. Recently, the use of biomimetic robots is advancing our knowledge on animal cognition and behavioural ecology (Polverino, et al. 2019; Bierbach, et al. 2020; Romano and Stefanini, 2021a). This emergent field of biorobotics and bionics is based on the interaction between living organisms and artificial agents, establishing a biohybrid system (Krause, et al. 2011; Romano, et al. 2019; Bonnet, et al. 2019). Thus, this novel bionics paradigm diverges from traditional biorobotics that uses artificial agents as surrogates of animals to study their biology (Manfredi, et al. 2013). In animal-robot interactions, artificial agents are used to evoke specific behaviours in the biological model thanks to the release of stronger life-like stimuli compared to static dummies, and video-playbacks generally used in ethology (Romano, et al. 2020; Datteri, 2020; Timbergen, 1951; Rowland, 1999), and fully controllable compared to non-focal animals. Herein, the neon tetra Paracheirodon innesi, Myers (Characiformes: Characidae) was used as model organism since it is a fish species showing robust social behaviour (Myers, 1936; Chapman, et al. 1998), and that has a colourful, easily recognizable livery consisting in a lateral blue stripe and a large red ventral area (Lythgoe and Shand, 1983). So, P. innesi is ideal to investigate the role of visible phenotypic traits in promoting fish shoaling. We developed several fish replicas, each of them presenting the same morphology of P. innesi, but with a different colour pattern. In particular, we tested the affiliation behaviour of neon tetra individuals towards fish replicas with the same colour pattern, and towards those with only one colour of the livery (e.g. blue, red, grey), as well as how this was affected by different ecological scenarios, including a feeding scenario, a predator scenario and a neutral scenario.
Material and methods

Animal rearing and general observations

*Paracheirodon innesi* subjects, bought in an aquarium store in Pontedera (Pisa, Italy) were maintained under laboratory conditions (25±1°C, 16:8 h light/dark photoperiod), in 100 L aquaria with activated charcoal filtered water that was constantly aerated by an air diffuser. A commercial diet (Tetramin® flake food) was used to feed daily ad libitum animals. The illumination of the controlled environment was ensured by an overhead fluorescent daylight tubes (Philips 30 W/33). Reflection and phototaxis were limited by using diffused laboratory lighting. These controlled conditions were also used during experiments. Test tanks and the biomimetic replicas were meticulously washed between one replicate and another (Romano, al. 2020), preventing effects due to olfactory cues from previous tests. Each animal was tested only once.

Fish replicas design and robotic apparatus

Fish replicas, whose morphology was inspired by *P. innesi* adults, were composed by four complementary elements for each body side. Each element was designed in SolidWorks (Dassault Systemes, Velizy-Villacoublay, France), and fast prototyped in a bio-compatible resin (Figure 1). Resin elements were assembled by using a chiffon fabric rectangle (18 x 3 mm) as sagittal plane.

Biomimetic replicas were painted with non-toxic pigments. In particular, one fish replica was painted similarly to the colour pattern of *P. innesi*, a second one was painted entirely in blue, a third one was painted entirely in red, and a fourth one was painted entirely in light grey (like the less coloured parts of *P. innesi*). Colour measurements (standard CIELAB colour space coordinates) were estimated by using a colorimeter (Nix Pro 2, Color Sensor). Fish replicas were silicone coated (Dragon Skin). This particular design increased the biomimcry of the fish replicas, as well as enabled passive anatomical displacements during their activation. Fish replicas were connected through a rod (⌀ 0.5 mm) with a trajectory generator (operation area ~ 400 x 200 mm, path following accuracy = 0.01 mm) that was located above the test tank (Figure 2). The trajectory generator actuated two sliding axis (i.e., x and y axes), by means of two stepper motors. A plotted trajectory was converted in G-Code (i.e., RS-274), and then sent to a microcontroller in turn connected to an external processor to manage the phases of plotting and code conversion.

Biomimetic predator

The biomimetic predator reproduced the morphology of juveniles *Crenicichla johanna* Heckel (Cichlidiformes: Cichlidae), a natural predator of neon tetras (Brejão, et al. 2013). The fabrication process of the biomimetic predator was similar to the aforementioned one for the biomimetic replicas (Figure 1). The biomimetic predator was moved on a semi-circular trajectory (radius 25 mm) by a robotic arm that was activated by a servomotor (Robbe FS 100 Servo) located above the test tank. A microcontroller was used to control the robotic arm.

Animal-robot experiments

The test tank included a robot zone (150 x 150 mm), a fish zone (150 x 150 mm), and a stimuli zone (300 x 150 mm), initially subdivided by opaque partitions (Figure 2). Neon tetra individuals were kindly transferred in the fish zone and left for 30 min to acclimatize. Once the opaque partitions were removed, and the fish were exposed to different fish replicas, and stimuli scenarios, the tests started (lasting 20 minutes). The fish replicas travelled a circular trajectory (r = 50 mm; ω = 0.2 rad/s), and for each test, the direction of the trajectory (e.g. clockwise, widdershins) was inverted to limit any spatial bias. The biomimetic predator was oscillated on a semi-circular trajectory (r = 50 mm; ω = 0.2 rad/s). During experiments the following stimulus scenarios were presented: i) food (floating flakes position); ii) the biomimetic predator; (iii) no stimuli. For each context, the time spent by fish in the robot zone of the test tank was recorded. Twenty subjects were analysed for each stimulus scenario.

Statistical analysis

Data on the impact of different stimuli on the time spent by *P. innesi* in each test tank zone were not parametrically distributed (Shapiro-Wilk test, goodness of fit *P* < 0.05). Therefore, non-parametric tests (Kruskal-Wallis test with a *P*-value threshold of 0.05), were performed to analyse data. The R software (v3.6.1) was used to analyse data. Box plots of data were generated by using MATLAB R2020b.
Results

The food scenario significantly influenced the time spent by fish with the different fish replicas ($\chi^2 = 39.98$, $d.f. = 3$, $P < 0.0001$). Fish spent a significantly longer time in the robot zone when the biomimetic replica was present than when the red replica ($Z = -3.54; P = 0.0022$), and the grey replica ($Z = -5.14; P < 0.0001$) were present. The time spent in the robot zone was shorter when the grey replica was present than when the red replica ($Z = 4.18; P = 0.0002$), and the blue replica ($Z = -4.39; P < 0.0001$) were present (Figure 3a)

When no stimuli were presented, the time spent by fish with the different fish replicas ($\chi^2 = 33.43$, $d.f. = 3$, $P < 0.0001$) was significantly different. The time spent with the biomimetic replica was longer than the time spent with the red replica ($Z = -4.23; P < 0.0001$), and the grey replica ($Z = -4.01; P = 0.0003$). The time spent with the blue replica was longer than the time spent with the red replica ($Z = -4.12; P = 0.0002$), and the grey replica ($Z = -3.7; P = 0.0012$) (Figure 3b)

The time spent by fish with the different fish replicas was not significantly different during the predator scenario ($\chi^2 = 2.8$, $d.f. = 3$, $P = 0.42$) (Figure 3c).

The time spent with the biomimetic replica was significantly affected by different scenarios ($\chi^2 = 22.73$, $d.f. = 2$, $P < 0.0001$). Fish spent a significantly longer time with the biomimetic replica in the predator scenario than in the food scenario ($Z = 4.47; P < 0.0001$), and marginally longer than in the no stimuli scenario ($Z = 2.4; P = 0.0424$). Fish spent a significantly longer time with the biomimetic replica in the no stimuli scenario than in the food scenario ($Z = 2.81; P = 0.0136$) (Figure 4a).

The time spent with the blue replica was significantly affected by different scenarios ($\chi^2 = 25.05$, $d.f. = 2$, $P < 0.0001$). Fish spent a significantly longer time with the blue replica in the predator scenario than in the food scenario ($Z = 4.34; P < 0.0001$), and in the no stimuli scenario ($Z = 2.47; P = 0.0354$). Fish spent a significantly longer time with the blue replica in the no stimuli scenario than in the food scenario ($Z = 3.61; P = 0.0009$) (Figure 4b).

The time spent with the red replica was significantly influenced by different scenarios ($\chi^2 = 32.82$, $d.f. = 2$, $P < 0.0001$). Fish spent a significantly longer time with the red replica in the predator scenario than in the food scenario ($Z = 4.88; P < 0.0001$), and in the no stimuli scenario ($Z = 4.69; P < 0.0001$) (Figure 4c).

The time spent with the grey replica was importantly affected by different scenarios ($\chi^2 = 38.4$, $d.f. = 2$, $P < 0.0001$). Fish spent a significantly shorter time with the grey replica in the food scenario than in the predator scenario ($Z = 5.33; P < 0.0001$), and in the no stimuli scenario ($Z = 5.32; P < 0.0001$) (Figure 4d).

Figure 2: Animal-robot experimental apparatus, and the fish replicas and stimuli (e.g. the biomimetic predator and flake food) used in different scenarios. The neon tetra and the fish replica are not drawn to scale in the test tank. Colour measurements (standard CIELAB colour space coordinates) of the fish replicas and of the biomimetic predator are also reported.
Discussion

The ethorobotics approach used in this study allowed to highlight significant social behavioural differences in *P. innesi* shoaling with phenotypically diverse fish replicas, that were affected by ecological contexts.
Shoaling behaviour between heterogeneous phenotypes has been reported in a wide range of aquatic species (Paijmans, et al. 2019), although it is generally assumed that aggregating with homogeneous phenotypes is a crucial factor contributing to fitness (Krause, et al. 2002).

We showed that shoaling with heterogeneous phenotypes is driven by external contexts. A decreasing preference in shoaling has been observed in P. innesi individuals interacting with the biomimetic replica, the blue replica, the red replica, and the grey replica. This trend was similar in each ecological context. The stronger preference for the blue replica compared to the red and grey replicas may depend on a greater biomimicry of the former with this species in particular conditions. Indeed, the red and grey pigments are provided by erythrophores and melanophores that in darkness are withdrawn to the centre of the chromatophores, conspicuously reducing the red and grey pigments (Lythgoe and Shand 1983), making the blue pigment more visible. Furthermore, it has been reported that a reddening of the iridescence is caused by adrenalin and pituitrin (Foster, 1937), thus the red replica can be probably perceived as a fish showing an excited or anxious behavioural status.

However, the time spent in shoaling with each fish replica was strongly influenced by different ecological contexts. The longest shoaling duration occurred when the biomimetic predator was present, while the shortest shoaling duration was observed in presence of food. This is consistent with the hypothesis that heterogeneous shoals are promoted by the anti-predator benefits that outweigh costs related to competition (Landeau and Terborgh, 1986; Krause, et al. 2002; Romano and Stefaniini, 2021b), although there are also evidences that in case of a threat, phenotypically heterogeneous shoals segregate along species divisions (Ward, et al. 2002).

Overall, our animal-robot interaction paradigm was fundamental to understand basic features of the behavioural ecology in shoaling fish, with particular reference on self-organized processes contributing to the formation of heterogeneous aggregations. The emergent field of organism-robot interaction represents a new paradigm for future artificial life studies (traditionally based on soft, hard, and wet synthetic methods) (Bedau 2007), providing a new synthetic method based on biohybridization, a promising avenue for achieving further progresses. Furthermore, this study can inspire innovative task optimisation strategies potentially applicable to develop artificial agent networks. Fish collective behaviours are increasingly used to develop new bioinspired meta-heuristic approaches for solving complex optimisation issues (Kunz and Hemelrijk 2003; Azad et al. 2014; Neshat et al. 2014; Gershenson and et al. 2018; Borg and Channon 2021). Fish individuals behave in a self-organised way when in an aggregation, and exchange information with adjacent fish without needing leaders. These simple interactions among agents make natural social systems extremely sophisticated and intelligent, such that they represent a valuable source of inspiration to develop highly robust, flexible, and accurate artificial life-based systems.

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References


