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PRIMARY RESEARCH PAPER

Fighting fish love robots: mate discrimination in males of a highly territorial fish by using female-mimicking robotic cues

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Abstract Among territorial animals, several species are characterized by males showing the same initial behaviours towards both sexes, leading to significant chances of injuries against conspecifics. In this study, we investigated how visual stimuli exhibited by a female-mimicking robotic replica can be exploited by highly territorial *Betta splendens* males to discriminate males from females. In addition, we tested the effect of light stimuli, mimicking the colour pattern of a reproductive female, on the consistence of courtship displays in *B. splendens* males. The intensity of male behaviours used in both courtship and not-physical agonistic interactions (e.g. fin spreading and gill flaring) was not importantly modulated by different stimuli. Conversely, behavioural displays used specifically in male–female interactions significantly increased when the robotic replica colour pattern mimicked a reproductive female. Furthermore, male courtship behaviours obtained in response to the robotic replica exhibiting light stimuli were comparable with responses towards authentic conspecific females. Our biomimetic approach to establish animal–robot individual interaction can represent an advanced strategy for trait-based ecology investigation, a rapidly developing research field.

Keywords Aggression · Animal–robot interaction · Bioinspired robotics · Courtship behaviour · Siamese fighting fish

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Introduction

The decision-making process in animal sexual selection is largely regulated by specific signals displayed during courtship and mating behaviour (Darwin, 1871; Sichlau et al., 2015; Benelli & Romano, 2018). The evaluation of male genetic quality and resource-holding potential by females, based on stereotyped courtship displays as well as features such as body size, colouration, and sound, is widely spread in the animal kingdom (Zahavi, 1975; Hamilton & Zuk, 1982; Bischoff et al. 1985; Höglund & Lundberg, 1987; Andersson, 1989, 1994; Rosenthal et al., 1996; Wikelski et al., 2001).

In territorial species, the initial behaviours of males towards individuals of both sexes are the same, although mate discrimination occurs in a short time (Robertson & Sale, 1975; Berglund et al., 1996; Borgia & Coleman, 2000; Patricelli et al., 2002, 2006). Accordingly, in these contexts the risk of injuries to females as well as to males is often significant (Shine et al., 2003). To achieve the maximum mating success and to minimize injuries, both males and females of these species have evolved specialized morphological features and behaviours that are important for sex recognition (Clotfelter et al., 2006).

Siamese fighting fish, *Betta splendens* Regan, 1910 (Perciformes: Osphronemidae), males are highly territorial (Simpson, 1968). In addition, *B. splendens* is a dimorphic species presenting morphological differences between males and females (e.g. coloration and fin dimensions), which are important in the recognition of the female individual by the male (Rainwater, 1967; Clotfelter et al., 2007).

During sexual selection, males advertise for females, and the latter choose the best available male (Darwin, 1871). This may hold true for *B. splendens*; however, male aggression may require that females communicate their gender and reproductive state, as well as their quality, since males heavily invest in parental care (Rainwater, 1967; Robertson & Sale, 1975), and are inclined to defend the area around their bubble nest vigorously (Simpson, 1968; Halperin et al., 1998; Doutrelant et al., 2001). Interestingly, during courtship, males display several behaviours that are identical to male–male not-physical agonistic displays (e.g. fin spreading display, gill flaring display), as well as courtship-specific displays (e.g. zigzag movements, bubbling display) (Rainwater, 1967; Simpson, 1968; Robertson & Sale, 1975; Clotfelter et al., 2006). Too aggressive males would chase the female away or would increase the risk of injuries. Conversely, too passive males would cause female loss of interest (Clotfelter et al., 2007). Therefore, in *B. splendens* the perfect balancing of aggressive and courtship-specific displays can ensure male mating success.

Robotics provides novel and advanced strategies to produce life-like, fully controllable stimuli, and to select different signals used during animal communication (Todd, 1993; Webb, 1995; Partan, 2004; Bradbury & Vehrencamp, 2011; Kopman & Porfiri, 2011; Krause et al., 2011; Abaid et al., 2012; Mondada et al., 2013; Schmickl et al., 2013; Katzschmann et al., 2018; Romano et al., 2019a). In addition, abilities performed by animals can inspire novel approaches to design robots with improved flexible and adaptive behaviours in unstructured scenarios (Ijspeert et al., 2005; Wood, 2008; Stefanini et al., 2012; Laschi, 2017; Romano et al., 2019a). A range of researches have reported successful interactions among animals and robots (Michelsen et al., 1992; Halloy et al., 2007; Gribovskiy et al., 2010; Kawabata et al., 2014; Shi et al., 2015; Romano et al., 2017a, 2019b; Batabyal & Thaker, 2018; Benelli et al., 2018a). Several studies used artificial agents to interact with different fish species (Polverino et al., 2012, 2013; Kopman et al., 2013; Spinello et al., 2013; Langraf et al., 2014, 2016; Worm et al., 2014, 2017; Bonnet et al., 2015; Ruberto et al., 2016; Donati et al., 2016). Particularly, in previous studies, we developed a biomimetic robot that was successful in adding basic knowledge to crucial aspects of territorial aggression in *B. splendens* (Romano et al., 2017b).

In this study, we developed a robotic apparatus moving biomimetic fish replicas inspired by female *B. splendens*, to investigate visual stimuli that are important for Siamese fighting fish males to discriminate females from other males. Vision has been reported to play a crucial role in communication of both aquatic and terrestrial animal species, routing conspecific size evaluation and recognition, among others (Atema, 2018; Benelli et al., 2018b; Bruce et al., 2018). We focused on the colour pattern exhibited by reproductive *B. splendens* females consisting in horizontal darker stripes along their bodies with lighter stripes in between (Rainwater, 1967). In addition, it should be considered that Robertson & Sale (1975) reported dark horizontal bars as a sign of submission in both males and females. The dark stripes could therefore be a conventional signal (Guilford & Dawkins, 1995) that is displayed by subordinate individuals independently of their sex to avoid attacks. One of our fish replicas was endowed with three bright stripes per side of its body (i.e. two dorsal, two median, and two ventral stripes), each of them obtained by using light emitting diodes (LEDs), to have two darker longitudinal areas along the body (i.e. between the dorsal and the median bright stripes and between the median and the ventral bright stripes), mimicking the colour pattern of a receptive *B. splendens* female. Besides, a further fighting fish replica developed in this study presented painted dark stripes, to investigate two potentially independent signalling mechanisms.

In this scenario, we tested two hypotheses: (a) the cues delivered by fighting fish replicas are of different attractiveness to males, e.g. (i) the robot is seen as a conspecific; (ii) the robot is seen as a non-reproductive/reproductive female (the behavioural significance of dark stripes); and (b) light stimuli reproducing the colour pattern of reproductive females increase the consistence of courtship displays in *B. splendens* males.

To address these issues, Siamese fighting fish males were exposed to a reproductive female-mimicking fish replica, exhibiting a neutral coloration, to a reproductive female-mimicking fish replica exhibiting a luminescent colour pattern, as well as to a reproductive female-mimicking fish replica with a painted colour pattern. In addition, we evaluated the degree of biomimicry of our artefacts by comparing *B. splendens* male responses to the fish replicas with those obtained during authentic male–male and male–female interactions.

Materials and methods

Ethics statement

This study complied with the guidelines reported by ASAB/ABS (2004), as well as the Italian law (D.M. 116192). No authorizations are required in Italy to conduct behavioural observations on *B. splendens* and other fish species (Donati et al., 2016; Romano et al., 2017b). Due to the high territoriality of this fish, each animal was isolated in different tanks. Injuries to the animals were carefully avoided during the experiments.

Animal rearing and general observations

Siamese fighting fish were maintained as described in our earlier study (Romano et al., 2017b). Observations were carried out from January to June 2017 in laboratory conditions (25 ± 1°C), with a 16:8 (L:D) photoperiod. The test tanks sidewalls were screened by using white filter paper (42 ashless, Whatman Limited, United Kingdom) to avoid external cues (Benelli et al., 2015a; Romano et al., 2017a). Before starting an experimental replication, the test tank was carefully washed to prevent odorant cues, as described by Romano et al. (2017b). Fifteen sexually mature *B. splendens* males were tested in our experiments. In addition, five males and five females were used as live stimuli in the experiments.

Fish replica design

The process used to fabricate the fighting fish replicas as well as the external apparatus actuating them is similar to that used in Romano et al. (2017b), with some modifications concerning the fish replicas. Fish replica design is inspired by the morphology and coloration of Siamese fighting fish females, since females of this species have less gaudy colours and shorter fins than males (Rainwater, 1967; Clotfelter et al., 2007) (Fig. 1A, B).

A liquid silicone rubber (Dragon Skin F/X PRO), mixed with a non-toxic pigment similar to the colour of a *B. splendens* female, more faintly coloured than males (Rainwater, 1967; Jaroensutasinee & Jaroensutansinee, 2001a, b), was injected in the mould, in order to cast the fish replica. In this species, the colouration of the body varies considerably among individuals (Blakeslee et al., 2009), so the colour of the fish replicas did not reproduce accurately the colour of real fish. Fish replicas were 70 mm long, with a height of 35 mm, and 13 mm wide. According to previous findings demonstrating an increased level of acceptance by real fish of robotic fish that included realistically coloured eyes (Ruberto et al., 2016, 2017; Landgraf et al., 2016), we endowed our fish replicas with two nickel-plated birdshots (diameter 2.5 mm), since they are visually similar to *B. splendens* eyes.

The colour pattern exhibited by reproductive *B. splendens* females (2 horizontal darker stripes along their bodies with lighter stripes in between (Rainwater, 1967) was reproduced in the fish replica with a luminescent colour pattern by locating 6 bright stripes (e.g. two dorsal, two median, two ventral ones), in the mould, prior to inject the silicone rubber, to have three bright stripes along each side of the fish replica body (Fig. 1C). The fish replica with LEDs off (neutral fish replica) and with activated LEDs is shown in Fig. 1D and E, respectively. This, as mentioned earlier, allows

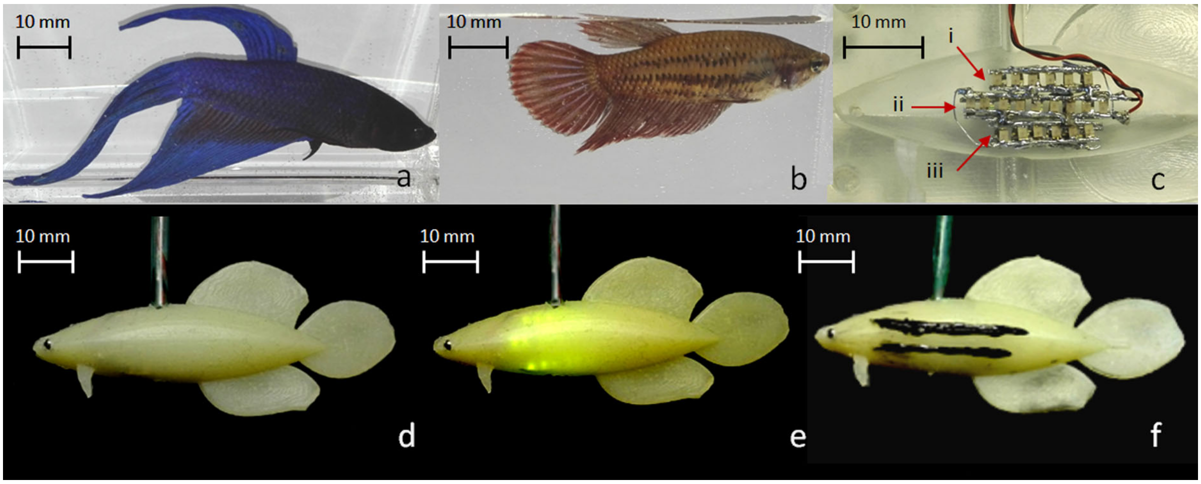

Fig. 1 Different stimuli presented to males of Siamese fighting fishes during the experiments. **a** *Betta splendens* male; **b** reproductive *B. splendens* female exhibiting horizontal darker stripes along her body with lighter stripes between; **c** dorsal (i), median (ii), and ventral (iii) bright stripes located in the mould, before casting the fish replica; **d** neutral fish replica; **e** fish replica with activated LEDs; **f** painted fish replica

us to have a darker longitudinal area between the dorsal and the median bright stripes and another darker longitudinal area between the median and the ventral bright stripes on the body of the fish replica. Each dorsal and ventral bright strip consisted of 6 LEDs connected in series. Each median bright strip consisted of 9 LEDs connected in series. The 6 LEDs stripes were connected in parallel.

In the fish replica with a painted colour pattern, two horizontal darker stripes were painted with a non-toxic pigment (Fig. 1F).

Colour measurements of the fish replicas are shown in Table 1. Spectral data were obtained using a spectrometer Ocean Optic HR2000-UV–VIS–NIR (Ocean Optics, USA) following the method recently described by Benelli et al. (2018a, b). An external microcontroller (Arduino Mega 2560) was used to activate both the servo and the LEDs.

Experimental apparatus

The tank used for the experiments (500 × 300 × 200 mm), consisted of three virtual zones: nest, middle and robot (a), (b) zone (Fig. 2). A square of bubble wrap was located in the nest zone of the tank, as described in Romano et al. (2017b), since it speeds up the bubble nest building in *B. splendens* and allows us to control the nest position (Dzieweczynski et al., 2006). Prior to perform a test, Siamese fighting fish males were individually placed in the test tank until they build a bubble nest (i.e. usually within 24–48 h) (Clotfelter et al., 2006; Dzieweczynski et al., 2006; Romano et al., 2017b).

The combination of cues (e.g. fish replicas or real fish) was placed in the centre of the robot zone (b) of the tank, which was isolated from the other zones by a one-way glass. During tests, fish replicas were individually positioned at a depth of 30 mm, in the middle of the robot zone (b) of the test tank (Romano et al.,

Table 1 Colour measurements of the *Betta splendens* fish replica body, painted stripes and incorporated LEDs; each value was a mean ± standard error of three replicates

Tested cue	L*	a*	b*
Fish replica body	47.2 ± 1.52	− 15.0 ± 2.25	0.6 ± 1.51
Painted stripe	2.11 ± 0.09	− 0.68 ± 0.32	− 0.71 ± 0.1
LEDs on	48.6 ± 3.08	− 37.1 ± 1.99	19.1 ± 3.69

L* represents the lightness component, a* (from red to green) and b* (from blue to yellow) are the two chromatic components

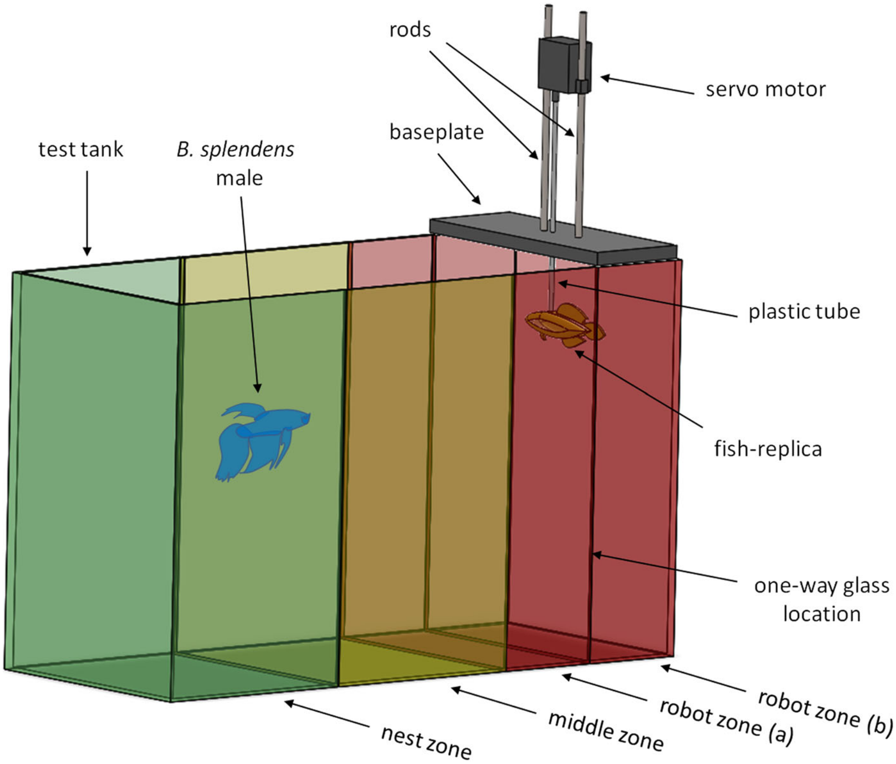

Fig. 2 Experimental setup. Different colours of the test tank indicate its virtual division in nest zone (green), middle zone (yellow) and robot zone (red). The fish replica was placed in the robot zone (b), divided from other sections of the tank by a one-way glass and an opaque partition. The experiment started once the opaque partition was removed and the *Betta splendens* male can see the fish replica through the one-way glass

2017b). The one-way glass isolates the tested combination of cues so that it cannot see the focal fish, to prevent visual feedback between the conspecifics (Ruberto et al., 2017), thus ensuring standard conditions during experiments with living or artificial stimuli.

An opaque partition (30 × 20 cm) avoided fish to view the combination of cues until the test began and was removed to allow visual contact with the combination of cues after 10 min from its insertion.

Combinations of cues presented to the tested subjects included: (i) neutral fish replica (e.g. without stripes); (ii) fish replica with activated LEDs (e.g. luminescent stripes); (iii) painted fish replica (e.g. painted stripes); (iv) female (as control); (v) male (as control). Stimuli presented are shown in Fig. 1.

In each fish replica context, the dummy displayed its body axis orthogonal to the central longitudinal line of the tank, to exhibit the lateral colour pattern of its body. In addition, the fish replica was oscillating on its longitudinal axis with an angle of 30° in amplitude and with a frequency of 0.5 Hz to emulate the decreased locomotor activity of real *B. splendens* behaving individually in a tank (e.g. a fish used as control, having no visual contact with the conspecific), as well as a female starting mate evaluation or eavesdropping (Doutrelant et al., 2001; Herb et al., 2003; Cantalupo et al., 1996; Clotfelter et al., 2006). The fish replica autonomously yawed 180° every 5 min to invert the head–tail orientation, to avoid positional bias. Fifteen sexually mature *B. splendens* males were analysed and each of them was exposed to the stimuli listed above.

Behavioural observations

Observations lasted 30 min and started when fish noted the proposed combination of cues. The sequence of tested combination of cues was randomized over the experiments. To limit prior context experience effects (Hsu et al., 2006), each fish was tested only once every 7 days with a different combination of cues (Arnott et al., 2016), since the effects of context outcomes are drastically reduced between 24 and 48 h in Siamese fighting fish (Dzieweczynski et al., 2012; Dzieweczynski & Forrette, 2013).

For each combination of cues, we noted behaviours identically displayed in both agonistic and courtship interactions, including (i) the fin spreading behaviour duration towards the combination of cues, corresponding to all fins outspreaded (Simpson, 1968); (ii) the gill flaring duration towards the combination of cues, consisting in the erection of gill covers (Simpson, 1968); (iii) time to the combination of cues, defined as the duration of *B. splendens* males swimming inside the robot zone (a).

Behaviours used only in male–female interactions, such as (iv) number of zigzag displays (the male moves away from the female in a zigzag way to magnify its colouration and body size, as described by Simpson, 1968); (v) time spent by males stopping upwards the nest and undulating their bodies in order to show the nest to females, as described by Simpson (1968); (vi) bubbling acts (intermittently work on the nest by adding bubbles to encourage the female to come closer), (Rainwater, 1967; Robertson & Sale, 1975), were recorded as well. An observer focally recorded the behaviour of *B. splendens* (Benelli et al., 2015b; Romano et al., 2017a, b).

Statistical analysis

Courtship data concerning identical displays performed in both agonistic and courtship interactions (i.e. fin spreading duration, gill flaring duration and time to the combination of cues) as well as courtship data related to specific male–female interactions (i.e. number of zigzag displays, upwards the nest duration and bubbling acts) were analysed by JMP 9 (SAS) using the general linear mixed model (GLMM) described by Romano et al. (2017b). We used a GLMM with a fixed factor (i.e. the tested cue/combination of cues), which also considered ID_w as the w-th random effect of individual over repeated testing phases. Averages were separated by Tukey's HSD test. A probability level of $P < 0.05$ was used to test significance of differences between means.

Results

Visual cues produced by different agents (e.g. living agents and artificial ones) marginally modulated male displays used both in agonistic and courtship interactions (Fig. 3). Fin spreading duration was not affected by different combinations of cues ($F_{4,56} = 0.1309$; $P = 0.9705$). The duration of the fin spreading behaviour was not significantly different in male–male and male–female contexts as well as in contexts involving the neutral fish replica, the fish replica with activated LEDs and the painted fish replica (Fig. 3a).

Duration of gill flaring display was marginally influenced by different combinations of cues ($F_{4,56} = 4.5939$; $P = 0.0028$). Gill flaring was performed slightly longer towards conspecific males, compared to conspecific females and to the fish replica with activated LEDs. Gill flaring duration was shorter in contexts involving the neutral fish replica and the painted fish replica (Fig. 3b).

Time spent by males in the robot zone (a) was not significantly affected by the different combinations of cues that were proposed ($F_{4,56} = 1.4324$; $P = 0.2353$), as in Fig. 3c.

Visual cues produced by different agents (e.g. living agents and artificial ones) significantly affected specific courtship displays performed by *B. splendens* males (Fig. 4).

The number of zigzag displays was significantly affected by different cues ($F_{4,56} = 46.9644$; $P = 0.0001$). The number of zigzag displays performed by males during male–female interactions was comparable with those performed in contexts involving the fish replica with activated LEDs. A significantly lower number of zigzag displays were performed in contexts involving the painted fish replica. The number of zigzag displays was significantly lower in contexts involving the neutral fish replica and in male–male contexts (Fig. 4a).

Time spent by males upwards the nest was significantly influenced by the different combinations of cues ($F_{4,56} = 39.4586$; $P = 0.0001$). Males spent a longer period upwards the nest in contexts including a female conspecific as well as the fish replica with activated LEDs compared to a context involving the painted fish replica. In addition, the neutral fish replica and male conspecifics produced shorter periods spent upwards the nest (Fig. 4b).

The number of bubbling acts was significantly affected by different agents ($F_{4,56} = 27.2202$; $P = 0.0001$). *B. splendens* males displayed a comparable number of bubbling acts during interactions with a female conspecific and the fish replica with activated LEDs (Fig. 4c). The painted fish replica evoked a significantly lower number of bubbling acts in Siamese fighting fish males, compared to other agents. An almost absent response, concerning the number of bubbling acts, has been recorded in male–male

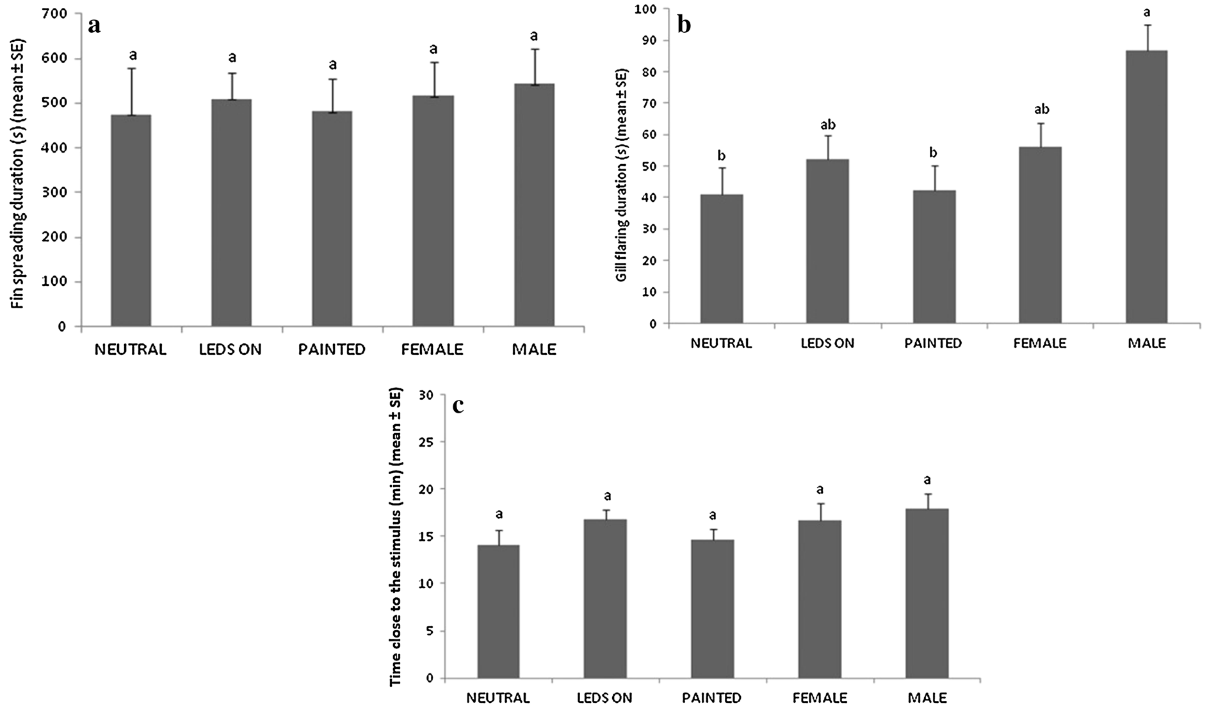

Fig. 3 Duration of *Betta splendens* **a** fin spreading, **b** gill flaring, and **c** swimming in the robot zone **a** evoked by different agents. NEUTRAL = neutral fish replica (without stripes). LEDs ON = fish replica with activated LEDs (luminescent stripes). PAINTED = painted fish replica (painted stripes). FEMALE = female conspecific. MALE = male conspecific. Same letters above each column indicating not significant differences ($P > 0.05$). T-bars are standard errors

interactions as well as in interactions involving the neutral fish replica.

Discussion

The robotic system presented in this study provided a relevant contribution in unveiling the decision-making process of *B. splendens* males during sexual recognition and courtship behaviour. The robot reliably induced stereotyped behaviour patterns that *B. splendens* males use during agonistic interactions, and thus it was treated like a conspecific. In addition, courtship-specific behaviour was almost exclusively displayed in response to colour signals, with LEDs being consistently more effective than painted stripes.

In a wide number of researches, biomimetic robots were used to study social behaviour in fish (Polverino et al., 2012, 2013; Butail et al., 2013; Kopman et al., 2013; Spinello et al., 2013; Langraf et al., 2014, 2016; Worm et al., 2014, 2017; Bonnet et al., 2015; Ruberto et al., 2016; Romano et al., 2017b). Concerning courtship behaviours in fish, Phamduy et al. (2014) investigated female mating preferences of bluefin killifish for differently coloured male-mimicking robotic fish.

However, in highly aggressive species, how male courtship displays are elicited by receptive female signals is a poorly explored issue, which can greatly profit from using robots, performing highly reliable and standardized behaviours.

In Siamese fighting fish, mating success is ensured by the rapid recognition of the mate that is mainly affected by specific visual cues delivered by the two mating fish, and this avoids the risk of injuries as well (Rainwater, 1967; Simpson, 1968; Robertson & Sale, 1975; Clotfelter et al., 2006).

Our results showed no significant differences in the fin spreading duration towards males, females and female-mimicking agents (Fig. 3a). Generally, larger males are socially dominant and they build larger nests (Bronstein, 1984; Jaroensutasinee & Jaroensutasinee, 2001b). Thus, fin spreading could be a strategy used by males to appear larger to threaten conspecific males,

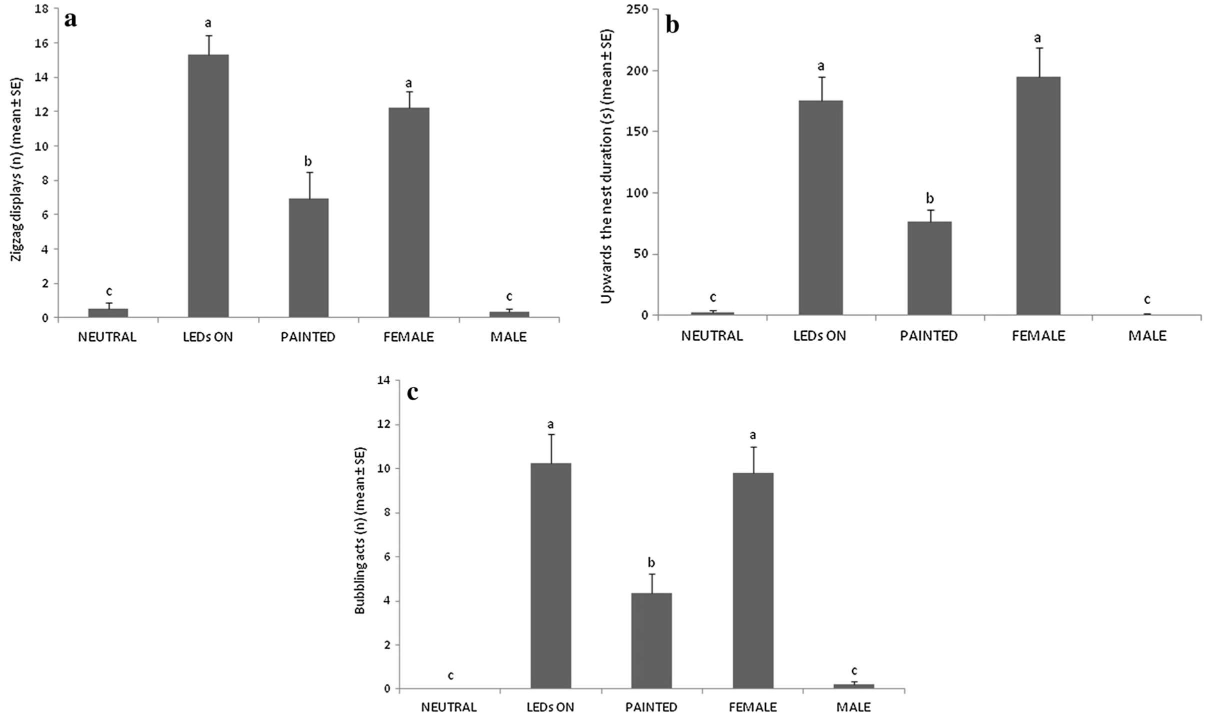

Fig. 4 Number of *Betta splendens* **a** zigzag displays, **b** duration upwards the nest, and **c** number of bubbling acts evoked by different agents. NEUTRAL = neutral fish replica (without stripes). LEDs ON = fish replica with activated LEDs (luminescent stripes). PAINTED = painted fish replica (painted stripes). FEMALE = female conspecific. MALE = male conspecific. Different letters above each column indicated significant differences ($P < 0.05$). T-bars are standard errors

and at the same time to attract conspecific females during courtship (Simpson, 1968; Robertson & Sale, 1975).

The gill flaring display was marginally longer in male–male compared to the case of male–female and male-fish replicas with activated LED interactions, and significantly longer compared to the interactions involving the painted and the neutral fish replica (Fig. 3b). During this display, oxygen extraction from water is drastically limited in fish (Abrahams et al., 2005).

However, Siamese fighting fish have evolved a particular organ (i.e. the labyrinth organ), which acts functionally like a lung (Tate et al., 2017). This enables *B. splendens* and the other anabantoids, a group of air-breathing fishes living in Africa and south Asia, to persist in extremely hypoxic situations where gill breathing would be ineffective anyways (Rüber et al., 2006). The longer persistence of gill flaring in male–male interactions suggests that gill flaring behaviour is more cost efficient as not-physical aggressive display in defending the nest from other intruders than as courtship behaviour. Indeed, the gill flaring behaviour as a courtship display seems to be not correlated with male parental quality but with tolerance to hypoxia, and the relevance of this to female reproductive success is unknown (Abrahams et al., 2005; Clotfelter et al., 2006). Likely, males performed gill flaring significantly shorter towards the painted fish replica and the neutral fish replica because they do not mimic a receptive female enough. However, the similarity of the intensity of male behaviours used both in courtship and agonistic interactions can be explained by *B. splendens* female selection of males that are aggressive and large enough to protect the offspring (Bronstein, 1984).

The intensity of courtship-specific behaviours (e.g. zigzag displays, time spent by males upwards the nest, bubbling acts), significantly increased in the following combination of cues: conspecific males, neutral fish replica, painted fish replica, fish replica with activated LEDs and conspecific females (see Fig. 4a, b, c). The extreme difference of male courtship-specific responses displayed to female conspecifics compared to other male conspecifics allows us to use combinations of robotic cues to understand which stimuli are displayed by females to be considered potential sexual mates.

The horizontal darker stripes along the reproductive *B. splendens* females body (Rainwater, 1967) have also been reported as a submission signal in both males and females (Robertson & Sale, 1975), potentially behaving as a conventional signal that is displayed by subordinate individuals of both sexes to avoid attacks. Such robotic approach could be exploited in future researches to verify this hypothesis. Indeed, conventional signals are signals whose level can or cannot be connected with underlying quality, and are not explained by physical or physiological reasons (Guilford & Dawkins, 1995; Hurd & Enquist, 2005; Bachmann et al., 2016). However, the display of courtship-specific behaviours towards the painted fish replica and the fish replica with activated LEDs highlighted the role of the darker stripes during the courtship and mating behaviour of *B. splendens*. In particular, the painted fish replica always evoked courtship-specific behaviours, indicating the pivotal role of the longitudinal stripes in triggering these highly selective responses.

Noteworthy, the fish replica with activated LEDs evoked a significantly higher intensity of these responses compared to the painted fish replica, and triggered similar reactions in males to those evoked by conspecific females, revealing its closer biomimicry with a *B. splendens* female. This novel animal–robot interaction paradigm based on a light emitting communication strategy was recently proposed in a study involving *B. splendens* males during agonistic interactions towards a biomimetic robot (Romano et al., 2017b). Concerning the role of light stimuli in the courtship responses produced in *B. splendens* males, although the fish replica with activated LEDs has less marked dark stripes compared to the painted fish replica, the former, in addition to dark stripes, also exhibits a brighter appearance that can be perceived by males as an indication of a healthy female (Vershinin, 1999). For instance, carotenoid pigments, procured by fish through foraging, accomplish several physiological roles (Vershinin, 1999; Clotfelter et al., 2007; Svensson & Wong, 2011). In addition, carotenoids have an important role in animal communication, in the context of carotenoid-based signals (Svensson & Wong, 2011). Fish that have high levels of carotenoids in their diet display a brighter colouration, and are perceived as high-quality subjects (Clotfelter et al., 2007; Svensson & Wong, 2011); thus, carotenoids are importantly involved in intraspecific sexual selection in several species (Maan et al., 2006; Svensson & Wong, 2011). However, further efforts are needed to understand to what extent the wavelengths of the LEDs actually mimic carotenoids and to what extent real females use them to communicate fitness. In addition, Hinow et al. (2017) proposed an interesting mathematical approach to investigate pheromone communication, arguing that the ratio of the individual chemical compounds provides the sense of the distance along the trail of pheromone, and shifts in this ratio describe the direction of the source. A similar approach could be applied for visual communication to modulate the ratio of the red–green–blue (RGB) components to obtain a closer biomimetic interaction involving LEDs incorporated in the robotic fish.

Overall, our study firstly reports which combinations of cues, produced by an artificial agent, are important in eliciting courtship behaviours in a high territorial species such as *B. splendens* during sexual recognition. In addition, we demonstrated that light stimuli, mimicking the colour pattern of reproductive females, boost the consistence of courtship displays in *B. splendens* males, probably because they indicate a female that is a good forager as well as a parasite-free individual, likely producing a high number of top-notch quality eggs.

Our robotic approach to establish bio-hybrid individual interactions can represent an advanced tool for trait-based ecology that is a rapidly developing context of ecology merging evolutionary with traditional population and community ecology (Kiørboe et al., 2018).

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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