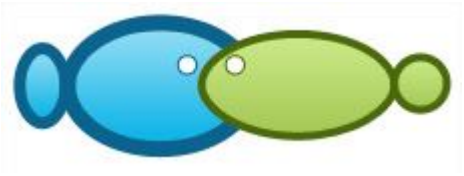

# Male Siamese fighting fish (*Betta splendens*) recognize resource holding power of opponents

[1]Nasim Z. Polgardani, [2]Soudabeh Rouzbehani, [2]Mohadeseh Parsaiyan

[1] Faculty of Marine Sciences, Tarbiat Modares University, Mazandaran, Iran; [2] Department of Aquatic Science and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C., Evin, Tehran, Iran. Corresponding author: N. Z. Polgardani, zarei_nasim@yahoo.com

**Abstract**. There has been a growing trend towards examining interactions in the context of communication networks consisting of many individuals rather than isolated pairs of signaling animals. An important question in state-dependent behavior is how multiple influences on state are integrated to determine current behavior. The goal of the following study was to investigate whether the availability of female, both in terms of density and accessibility, affects male-male interactions in male Siamese fighting fish, *Betta splendens*. For this purpose, direct male-male fighting trials were performed considering 12 different scenarios. The findings indicated that it is the physical isolation and/or presence of an escorting male in the community, not female density, which modifies male-male interactions. These results suggest that the resource holding power (RHP) in a specimen can be innately recognized by other opponents in bettas.

**Key Words**: Siamese fighting fish, *Betta splendens*, resource holding power (RHP), escort male, agonistic behavior.

**Introduction**. Aggressive behavior, occurring frequently in the animal kingdom, is believed to play a significant role in the fitness of animals (Popova 2006). In general, animals seem to use aggression to defend themselves and their progeny against an attack from other predators, to fight for females, to feed, and to maintain the social hierarchy. Fighting ability, or in the other term resource holding power (RHP), is a crucial factor of fight sequel and dynamics (Maynard-Smith 1982). Nevertheless, the study of aggressiveness is a complicated case because such behaviors are not usually presented as a unitary trait (Popova 2006). The Siamese fighting fish, *Betta splendens* Regan, 1910, is a nonsocial freshwater fish indigenous to standing waters of flood plains, canals, rice paddies and medium to large rivers in the Mekong basin in Southeast Asia (Taki 1978; Rainboth 1996). In nature, they establish disperse populations of 1.7 fish $m^{-2}$ stabilized by male territorial behavior (Jaroensutasinee & Jaroensutasinee 2001). Aggressiveness in *B. splendens* is shown to be an honest indication of male condition and/or motivation (Simpson 1968), and is classified to traits pertaining to fighting capacity as well as traits elucidating energetic and reliable signals of male quality (Evans 1985). As one of the most common traits, the opercular display is believed to be used in mate selection through conveying accurate information about potential mate quality (Abrahams et al 2005). However, it has recently been argued that agonistic behavior fits to the context of the broader social environment instead of dyadic interactions (Snekser et al 2006) and phenomenon known as communication networks (i.e. the selective environment of networks of signaling and receiving individuals (McGregor 1993)) has been successfully discussed in *B. splendens* in terms of eavesdropping and its subsequent effects on intra and/or intersexual interactions (eg. Oliveira et al 1998; Doutrelant et al 2001; Herb et al 2003; Matessi et al 2010). Generally, it has been accepted that female *B. splendens* chooses the winner of male-male aggressive interactions based upon information she obtains from eavesdropping (Snekser et al 2006). In this study, the effects of female (as a source) availability, in terms of density and accessibility, on

representative aggressiveness of *B. splendens* males living in close contact were examined. Although in many research on aggression in fish, mirrors are used instead of a live opponent (Wilson et al 2011; Balzarini et al 2014), recent studies have shown that aggressive behavior towards a mirror have slower pace than to a real opponent (Elwood et al 2014; Arnott et al 2016). The key question probed to be answered in the present study was whether males like females can also act as receiver organisms and use data on situational source characteristics to modulate agonistic actions innately.

## Material and Method

***Subjects***. One hundred and fifty individually reared 200-day-old sexually mature *B. splendens*, of blue color morph, including 75 males and 75 females, of the same size (standard length = 3.5+0.68 cm, mean+SE) were obtained from a local dealer in November 2015. The fish were maintained individually for two weeks in opaque plastic containers (15 × 15 × 15 cm; containing approximately 2 L tap water) housed within a larger (200 L) tank. During the study, the fish were fed 'ad libitum' using frozen *Tubifex* and 15% water exchange was performed on a weekly schedule.

All tests took place in five opaque polyethylene circular tanks (radius: 12.5 cm; height: 25 cm) divided to a peripheral chamber (PC) and a central chamber (CC) using a transparent balloon glass (radius: 7 cm) held in submerged position and lined with water level. Test tanks were filled to 20 cm with dechlorinated fresh tap water at 25$^{o}$C and artificial weeds were placed on the bottom of the test tanks as a refuge. Full-contact fighting trials were used to compare the post-exposure rate of aggressiveness of resident males towards an intruder male. Total of 13 scenarios were designed considering the density (one, two, or three fish) of resource (female fish) and its social context (joined (Jo): when focal males could interact freely with stimulus females; isolated (Iso): when stimulus female(s) was/were presented inside the balloon glass, so direct physical interaction between focal male and stimulus female(s) was ruled out; or isolated and escorted (Iso-E): when isolated female(s) was/were accompanied with another male considered as an escort male) as well as control trials of no female scenario as well as dead escort male scenario (Iso-D; euthanized with 150 mg $L^{-1}$ clove oil by 30 min exposure) to evaluate innate territorial aggressiveness of examined fish. Each trial was conducted in three proceeding steps. In the first step, in order to let the establishment of social order with little probable aggression at the time of presentation of the stimulus to the focal fish, female(s) as well as the escort male (when present) were maintained in the test arena for one hour. This was followed by the second step during which the focal male was introduced to the peripheral chamber of the test tank and allowed to receive visual stimuli from the established social environment for 10 min. During the last step females as well an escort male (when present) were removed from the test arena and an intruder male was released into the peripheral chamber and a 10 mins fighting session were recorded (Figure 1). The rate of aggressiveness was calculated by dividing the number of focal male opercular gill flarings (OGFs) by 10 (modified from Karino & Someya 2007). Opercular gill flaring is considered as first sign of territorial aggression in male Siamese fighting fish (Simpson 1968; Evans 1985; Karino & Someya 2007). The display is costly due to gill ventilation limitations and it is likely to be an honest signal of aggression (Abrahams et al 2005). In those circumstances when the focal males or an intruder male retreated or fled from the opponent for five consecutive times the fighting session was stopped and repeated by other fish. All observations were recorded from 8:00 am to 4:00 pm in the daytime. Each trial was repeated for 8 times using experimentally naïve fish. Group balancing of the males were done before the experiment according to Karino & Someya (2007). For this purpose male fish were assessed for the density of performed OGFs during 20 min exposure to a mirror and those males with the same estimated OGF counts constituted the experimental fish. Testing and maintenance sessions were conducted under nearly fixed temperature (25-27°C), oxygen content (oxygen= 7.5-8.0 mg $L^{-1}$), pH (6.5-6.8), and photoperiod (12:12 h light:dark cycle). After the experiment, the animals were retained for further educational research.

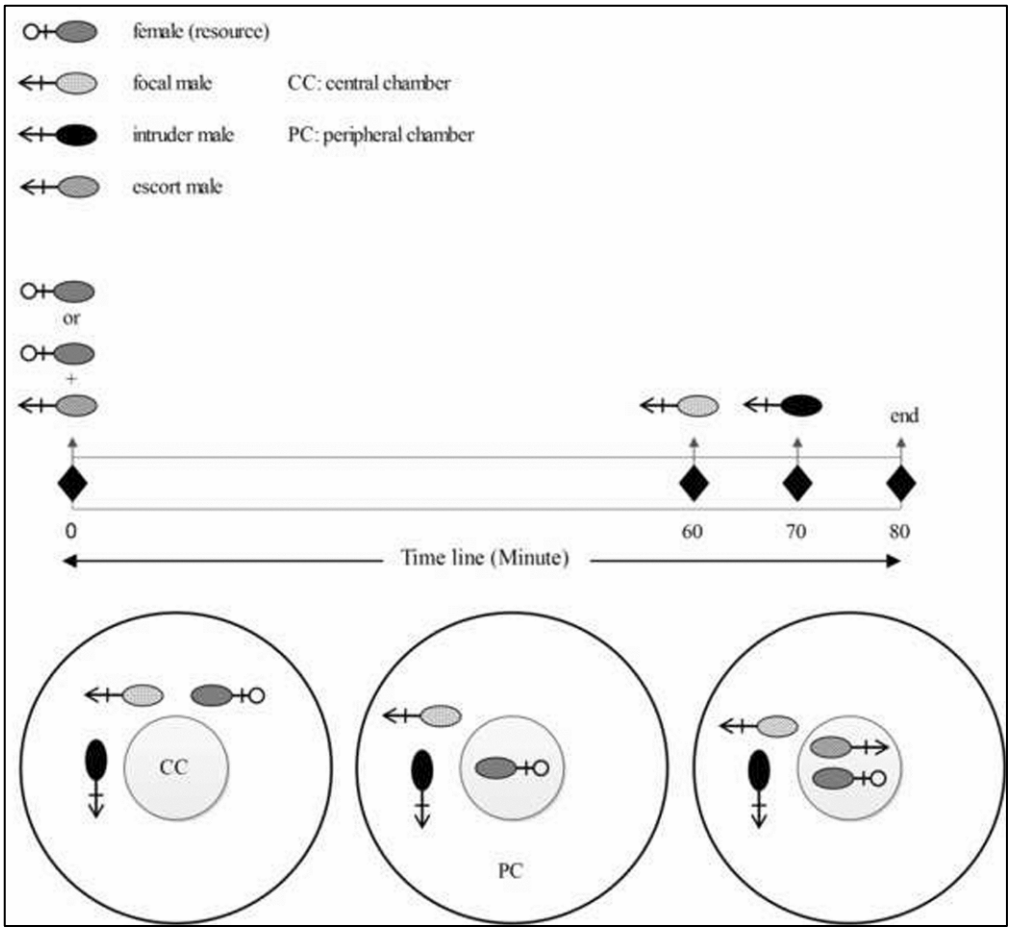


Figure 1. Time lined schematic view of the general set-up of the experimental trials.

***Data analysis***. Data means were assessed for homogeneity of variance using F-test. Means at each stimulus choices were compared with means of a no-stimuli condition using two-way ANOVA followed by Honestly Significant Differences (HSD) multiple comparison procedure, respectively (Zar 1999). Data were analyzed with Minitab 16.2.0.0.

***Ethical note***. Since the study design consisted of jointed/isolated sections, direct fights were inevitable. Hence, during the test sessions, biting was observed in male-male interactions especially when no females were present. Bites were usually targeted at the opponent's belly portions, but fin pinching was also recorded two times. In order to minimize injuries, artificial weed was placed at the periphery of the circular tanks as a refuge. In 23 out of 30 trials, the first biting action was accompanied by subsequent retreat of defeated specimen and, at that point, the outcome of the fight was considered determined. Afterwards, the test fish were removed out of the tank and the trials were repeated for other seven cases in which retreatment did not occur after the first bite. Finally, after the experiment was done, the animals were retained for further educational research. It should be noted that the experimental protocol was approved by the local ethical committee for experiments on animals (Shahid Beheshti University, Iran, Code: MB-0846).

**Results**. All fish subjected to close contact fights survived the experiment. Of total of trials, 18 cases of focal male retreats were recorded with the highest occurrence rates (~53%) when a single female was to be escorted by an escort male. Intruder male retreats were occurred in 6 cases all of which were observed when three female fish were joined. Types of dyadic agonistic interactions observed during the study included OGF,

tail beating (TB), belly bending (BB), and biting (B) between focal male and intruder male; OGFs, and BBs in female-female conflicts; and OGFs, TBs, and BBs between focal male and escort male occurring when three females were to be escorted. In general, when no female fish were present, significantly ($F = 182.82$, $p < 0.001$) more OGFs were performed by focal males toward an intruder male but the density of female fish was not found to be significantly effective ($F = 0.84$, $p = 0.448$) (Figure 2). On the other hand, the rate of OGFs significantly ($F = 26.60$, $p < 0.001$) differed among varied social environments and reduced level of aggression was found when isolated females were escorted by a living male (Figure 2). Significant ($F = 7.89$, $p = 0.01$) interactive effects of density and social context was also found.

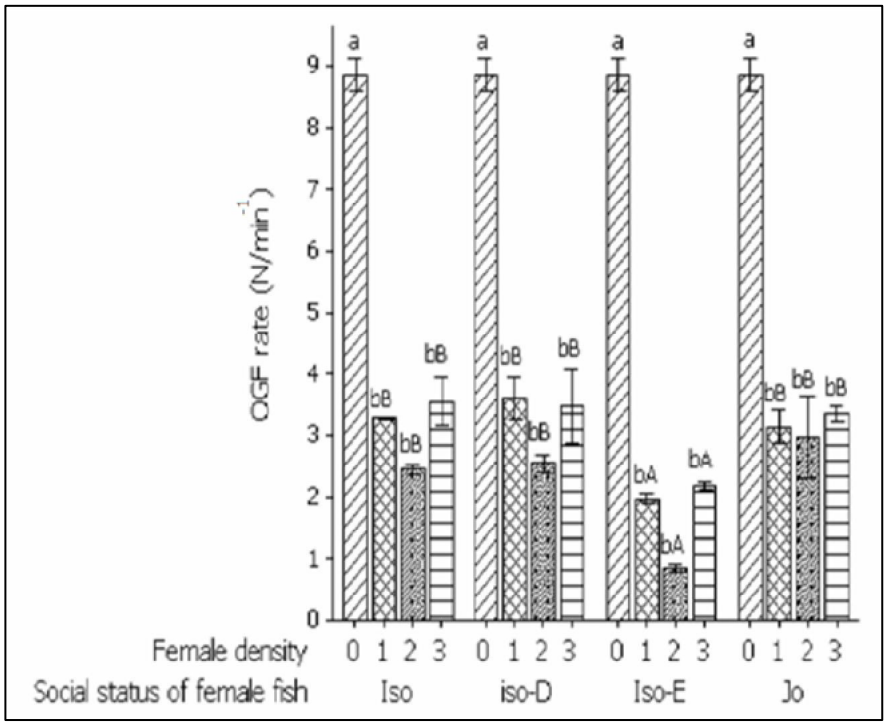


Figure 2. Comparative effects of the density and social status of reproductive resource (female fish) on mean rates of opercular gill flaring (OGF) performed by the focal male (N = 6) against an intruder male. Note: different capital letter show significant difference ($p < 0.05$) among different social communities; different lower case letter show significant difference ($p < 0.05$) among different densities of a reproductive resource (female fish).

**Discussion**. The influence of the by-standing behavior of a pair of communicating individuals (audience effect; Zajonc 1965), is considered as one of the key regulators of male-male interactions in *B. splendens* (Evans & Marler 1984; Dzieweczynski et al 2005). Previous studies have illustrated that the intensity of the aggression of the host males is impacted by the sex of the intruder (Forsatkar et al 2017). In general, our results show that the degree of male Siamese fighting fish responses to intrusions varies after instantaneous visual exposure to social environments representing different context and status of the competitive resource (female fish) with the modest territorial aggression occurred when previous possession (escorting) of the resource is perceived. According to the "stable evolutionary theory", when conflicts between interacting individuals exist, strategic decisions are made through a prior assessment of the value of the potential resource and the cost of the fight, if both combatants continue to escalate following their first encounter (Parker & Rubenstein 1981). In this case, the escorted females may be considered as seized and inaccessible resource by focal males leading to low investments in territory holding. This can be confirmed by common occurrences of resident male retreat actions in those cases when females were being escorted. On the other hand, focal male-escort male aggressive interactions were only observed when number of escorted females was max. This may be due to the fact that evaluation of the density of the holding resource may come in the next order during the process of possession power

assessment. It was suggested that when individuals can estimate their opponent's fighting abilities, strict linearity hierarchies should emerge and that within these hierarchies, aggressive interactions have the form of 'attack-retreat' rather than the form of 'fight' (Dugatkin & Dugatkin 2007). Moreover, the case of intruder male retreats when three female fish were joined in our study may be due the fact that in dyadic conflicts between residents and intruders, fight winning probability of the residents is seemed to be greater in structurally diverse and resource-rich environment than in structurally poor environments without resources (Nijman & Heuts 2000).

**Conclusions**. The current experiment may reveal that not only female existence, but also accessibility of the existing females to competing males can bias the degrees of aggressiveness in male-male interactions in *B. splendens*. Considering the modest levels of induced aggression observed when interactive males faced with isolated and escorted females, it seems that the behavior is regulated in a cognitive perspective and the results of the current study may add a new dimension to communication networks in terms of resource holding power (RHP).

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Authors:
Nasim Zarei Polgardani, Faculty of Marine Sciences, Tarbiat Modares University, P.O. Box: 64414-356, Noor, Mazandaran, Iran, e-mail: Zarei_Nasim@yahoo.com
Soudabeh Rouzbehani, Department of Aquatic Science and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G. C., P.O. Box: 19839-4716, Evin, Tehran, Iran, e-mail: sou.rouzbehani@gmail.com
Mohadeseh Parsaiyan, Department of Aquatic Science and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G. C., P.O. Box: 19839-4716, Evin, Tehran, Iran, e-mail: M_parsaiyan@yahoo.com

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