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Short report

Fish adjust aggressive behavior to audience size with limited information on bystanders' fighting ability

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ABSTRACT

In a social environment, individual behavior is modulated by surrounding observers (a phenomenon known as the audience effect). Here, we used mirrors to test the effect of two audience sizes (one virtual bystander *vs.* three virtual bystanders) on the aggressive behavior of a focal fish when bystander's fighting ability was not clear (*i.e.*, information about the ability of virtual conspecifics limited by their mirror images). We found that the Nile tilapia, a cichlid fish, responds to its image as an audience by reducing overt aggression in the presence of larger audience.

1. Introduction

Social animals interact with conspecifics in unpredictable social environments, and a given individual's behavior is often shaped by the behavior of others conspecifics' (Taborsky and Oliveira, 2012). Information is acquired either directly (when a signaler and receiver are involved) or indirectly, through observation of bystanders' behavior (McGregor and Peake, 2000). However, while bystanders acquire information within a communication network, signalers and receivers can also detect the presence of a bystander and change the way they interact, a phenomenon known as audience effect theory (Taborsky and Oliveira, 2012). According to this, behavioral adjustments in aggressive interactions depend on the presence of bystanders (Cruz and Oliveira, 2015), on previous knowledge of bystander's fighting ability (McGregor and Peake, 2000), and on the social context (Dzieweczynski et al., 2014). Behavioral modulation in natural environments also depends on the number of individuals within a group, and sometimes information on an individual's ability is not available. This will require fast decisions that can avoid physical damage and other negative consequences from competitive contexts. The higher the probability of a fight, the higher the probability for energy expenditure; therefore, aggressive behavior can change accordingly. Thus, we hypothesized that higher-cost aggressive behavior may decrease. Here, we tested the effect of audience size on the aggressive behavior of the Nile tilapia, a cichlid fish whose males defend territory in a lek system (Lowe-McConnell, 1958) in which they are surrounded by an audience of other males. We predicted that fish would reduce the frequency of attacks (high-cost fights), and increase displays (low-cost aggression) in the presence of a larger number of bystanders (*i.e.*, increased probability of fighting), when information on the bystanders' fighting ability is limited.

2. Material and methods

Adult male Nile tilapia *Oreochromis niloticus* (L.) were isolated and tested in two virtual bystander's treatments using aquariums with one or three mirrors (Fig. 1), so that fish could see either one or three virtual bystanders (18 replicates each). It is well documented that several fish species fight against their images, thus indicating they do not recognize themselves in the mirror, including Mozambique and Nile tilapia (Oliveira et al., 2005; Barreto et al., 2009). Therefore, two sets of mirrors were used, one of which was used to manipulate the number of bystanders, while the second set was used to test for the aggressive response of the isolated (focal) fish. The first set was formed by one or three mirrors placed at one end of the aquarium, and a glass partition placed in the center (Fig. 1) to prevent focal fish from reaching the mirror(s); thus, the fish could see the mirror images without fighting. The three mirrors were arranged at a 115° angle, which enabled the focal fish to see three images simultaneously. The second set was formed by only one mirror (hereinafter referred to as a fight mirror) placed in the focal fish's compartment, and opposite to the first mirror set, thus allowing each fish to fight against its own image. Here we use the term "fight" to designate a direct interaction with the mirror image,

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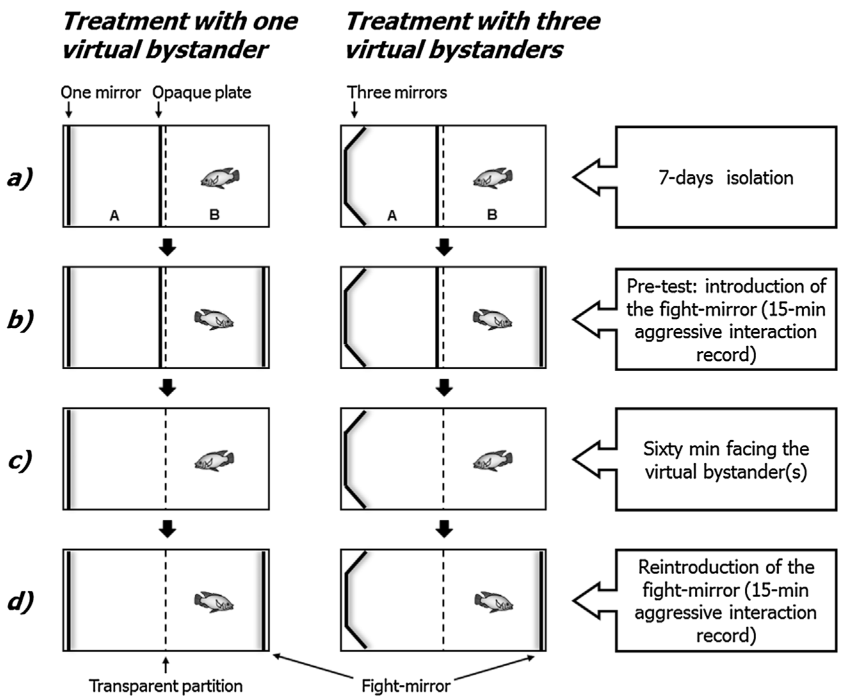

Fig. 1. Schematic view of experimental setup from above showing the aquarium's compartments and the focal fish. In compartment A, one or three mirrors were used to manipulate the number of bystanders; in compartment B, one mirror was used to test aggressive responses of the focal fish.

both by displays near the mirror, and also mouth and tail biting directly on the mirror. In this way, we created a virtual social environment in which a focal fish would have limited access to bystanders' fighting ability (mirror images do not interact among themselves), but in which the fish's individual aggressive response could be evaluated. We considered as limited access because the image from focal fish could provide some cues about its own fighting ability though Nile tilapia change its body color according to social rank (Falter, 1987; Volpato et al., 2003). By using a mirror, we also controlled for variables such as opponent's size or sex, which could bias the aggressive responses of the focal fish.

Tests were run in glass aquaria (40 × 30 × 40 cm; ca. 48 L) in which each fish was isolated for 7 days to minimize the effect of prior social experiences (e.g. Oliveira et al., 2001). First, the mirrors remained hidden by an opaque plate attached to the glass partition (Fig. 1a). On the eighth day, the fight mirror was introduced opposite to the hidden mirrors (Fig. 1b), and focal fish behavior was recorded for 15 min in a pre-test. Then, the fight mirror and the opaque plate were removed from the aquaria (Fig. 1c), revealing the virtual bystander(s). After one hour of fish exposure to bystanders, the fight mirror was reintroduced into the aquarium (Fig. 1d), and the fish that fought against their mirror images were video recorded (15 min). Aggressive behavior was labeled as displays (lateral and frontal threats) or attacks (biting and tail beating), based on an adapted ethogram for Nile tilapia (Carvalho et al., 2012). Attacks are types of overt fights and demand more energy to be performed, while displays are examples of low-cost aggression (Ros et al., 2006).

The fish in the experiment with one virtual bystander (SL: 11.38 ± 0.51 cm; weight: 47.68 ± 6.43 g) were compared to those in the experiment with three virtual bystanders (SL: 11.14 ± 0.51 cm; weight: 48.59 ± 5.12 g). The fish standard length and weight were similar between the two treatments (Student's *t*-test, $P > 0.66$) in terms of Mean ± SE. Water temperature was set at 27 °C on a 12 L:12 D cycle. Water oxygen, pH, ammonia, and nitrite were controlled at optimal levels using external biological filters. Fish were fed standard tropical fish food twice a day (3% of fish mass). Behavior was recorded between 2:00pm and 4:00pm.

2.1. Data analysis

The number of displays and attacks were tested for outliers using Grubbs' test. Data normality was checked using the Shapiro Wilk test, and homoscedasticity was checked using Bartlett's test. Data were square-root transformed to fit parametric assumptions. We tested for differences between treatments and within observation sessions using Mixed Model ANOVA, in which treatments were considered as independent factors (between treatments) and the sessions of aggressive behavior against the mirror, as repeated measures (within subject). Data were also tested by Fisher-LSD post hoc test to make comparisons between and within subjects. Statistics were run in the Statistica software (license number 134-810-523).

This study was approved by the Ethics Committee on Animal Testing of the São Paulo State University (UNESP), under permit number 082/2013.

3. Results

As shown in Fig. 2a, the number of attacks differed between treatments ($F_{(1,34)} = 4.61$; $p = 0.039$) and was marginally different within observations ($F_{(2,68)} = 3.99$; $p = 0.053$). Statistical interaction was no significant ($F_{(1,34)} = 1.29$; $p = 0.26$). Fisher-LSD post hoc test showed a similar number of attacks between treatments before fish exposure to virtual bystanders ($p = 0.34$). However, the number of attacks was lower only after fish exposure to three virtual bystanders ($p = 0.019$). Attacks were similar within one mirror treatments ($p = 0.55$) and different within three mirrors one, decreasing after virtual bystander exposure ($p = 0.03$). Displays were similar (Fig. 2b), both between treatments ($F_{(1,34)} = 0.96$, $p = 0.33$) and within treatments ($F_{(1,34)} = 0.56$, $p = 0.46$).

4. Discussion

These experiments found that the Nile tilapia adjusts its aggressive behavior to audience size and reduces the number of overt fights (attacks) when the information about fighting ability of the audience is limited. The results herein allowed us to demonstrate that mirror images can serve two functions, representing fish opponents and also

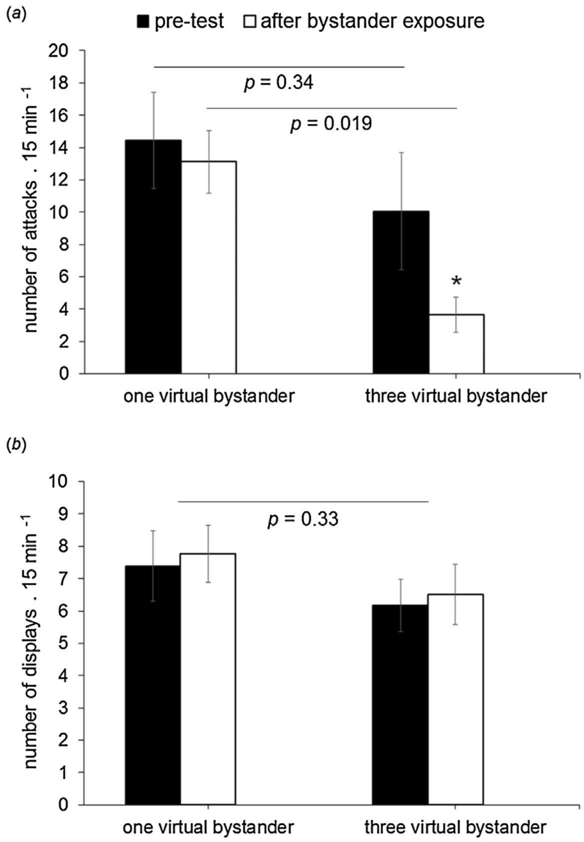

Fig. 2. Number of attacks (a) and displays (b) in the treatments with one and three virtual bystanders. Asterisk indicates differences within treatments. Data are showed as mean ± SE.

bystanders. The Nile tilapia signals social rank using visual and chemical signals (Gonçalves-de-Freitas et al., 2008). Therefore, in our study, fish may have displayed aggressive behavior to their images in the experiments, but fights did not ensue because the mirrors were distant, thus producing images that served as an audience. Furthermore, the focal fish had no information on the bystanders' fighting ability, because there was no real fighting among virtual bystanders. Some methods without a real fish, as dummies (Dzieweczynski et al., 2006) and video playback (Makowicz et al., 2010), have been used in previous studies to control for individual characteristics in audience effect experiments. Here, we found mirrors to be a simple and effective method for an audience experiment.

In addition to the visual communication demonstrated herein, animals in their natural habitats can take information from surrounding individuals using sound, touch, and olfactory signals (Damsgård and Huntingford, 2012). However, visual information can help individuals to quickly make decisions in a social environment. Though mirror images preclude multiple communication channels, this is an effective method for obtaining information on aggressive motivation in fish (Oliveira et al., 2005; Barreto et al., 2009).

Aggressive displays are commonly shown for individual evaluation (before fighting escalating), and also to signal social rank with small cost as the social hierarchy is established (Ros et al., 2006). In this study, the number of displays was similar between the treatments; this similarity likely occurred because rank could not be determined, as reported in a study on the Mozambique tilapia (Oliveira et al., 2005). However, fish that observed a larger audience reduced overt fights. Thus, the larger the audience, the more careful the individual's aggressive response should be. Different responses to different number of bystanders require the fish species to possess numeric skills, which has, in fact, been found in guppies (Agrillo et al., 2012) and angelfish (Gómez-Laplaza and Gerlai, 2011). This ability may increase fitness, for instance, by shaping adaptive decisions when a competitor's fighting ability is limited known.

5. Conclusion

We conclude that aggressive interactions among cichlids is affected by audience size, which reduces costly aggressive behavior, and that this change is independent of a complete knowledge on the opponent's fighting ability.

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