

Original article

Unusual effect of chemical communication on social aggression in juvenile cichlid fish *Cichlasoma paranaense* (Cichliformes: Cichlidae)

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Some fish species are socially organized and show a social rank order which is achieved through aggressive interactions. After hierarchy is settled, such species communicate their ranks through several sensorial cues; this communication is adaptive because it reduces detrimental effects from physical contests. Cichlid fish are socially organized and signal their social ranks through visual, acoustic and chemical communication. The response to signaling may vary according to the species and environment; the knowledge of different species is fundamental to understand the evolutionary forces upon their social communication. We tested the effect of chemical signaling on social groups of juvenile cichlid *Cichlasoma paranaense* by renewing the water in the aquarium, a procedure that washes away chemical information and increases aggressive interactions in other cichlid species. Two treatments were designed: 50% and 0% water renewal. Aggressive interactions were video-recorded immediately before water renewal, 1min, 1h, 2h, and 24h after water renewal. The treatment with the water renewal did not increase aggressive interactions within the group. The 50% water renewal apparently reduced aggressive interactions in this species, indicating an interspecific difference on the aggressive response to chemical variation in the social environment.

Keywords: Agonistic behavior, Social behavior, Social environment, Social signals, Water renewal.

Algumas espécies de peixes são organizadas socialmente e apresentam uma ordem de rank social que é alcançada por meio de interações agressivas. Após o estabelecimento da hierarquia, essas espécies comunicam seu rank por diversas pistas sensoriais, essa comunicação é adaptativa, pois reduz os efeitos prejudiciais das lutas físicas. Peixes ciclídeos são socialmente organizados e sinalizam o rank social por comunicação visual, acústica e química. A resposta à sinalização pode variar de acordo com a espécie e o ambiente; o conhecimento sobre diferentes espécies é necessário para entender as forças evolutivas sobre sua comunicação social. Nós testamos o efeito da sinalização química em grupos sociais de juvenis do ciclídeo *Cichlasoma paranaense* por meio da renovação de água do aquário, um procedimento que dilui informações químicas e aumenta a interação agressiva em outras espécies de ciclídeos. Dois tratamentos foram realizados: 50% e 0% de renovação da água. A interação agressiva foi registrada imediatamente antes da renovação da água, 1min, 1h, 2h e 24h após a renovação da água. O tratamento com renovação não aumenta as interações agressivas dentro do grupo. A renovação de 50% da água do aquário aparentemente diminui as interações agressivas nessa espécie, indicando uma diferença interespecífica na resposta agressiva a variação química no ambiente social.

Palavras-chave: Ambiente social, Comportamento agonístico, Comportamento social, Renovação de água, Sinais sociais.

Introduction

Social communication in fish occurs through several sensorial modalities, such as visual, acoustic and chemical; the latter is remarkably widespread in their communication (Sorensen, Stacey, 2004; Huertas *et al.*, 2008). In cichlid fish, a group of species with a rich behavioral repertoire,

chemical communication is used in several social contexts (Keller-Costa *et al.*, 2015), such as reproduction (see Giaquinto *et al.*, 2010; Huertas *et al.*, 2014), alarm (see Barreto *et al.*, 2010, 2013), recognition of conspecific, both among adults (see Plenderleith *et al.*, 2005; Thünken *et al.*, 2009), and among young-adults, influencing parental care (see Wisenden, Dye, 2009; Wisenden *et al.*, 2014).

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The use of chemical signals to show social rank, either as dominant or subordinate individuals, has been studied mainly in African cichlids, including, *Oreochromis mossambicus* (Peters, 1852), *Oreochromis niloticus* (Linnaeus, 1758) and *Astatotilapia burtoni* (Günther, 1894). Barata *et al.* (2007) showed that Mozambique tilapia males (*O. mossambicus*) store urine and use it to signal their dominant position. This behavior modulates aggressiveness in rival males, contributing to the stability of the social hierarchy. According to Giaquinto, Volpato (1997), the absence of chemical signals prevents or delays the establishment of social hierarchy in juvenile Nile tilapia (*O. niloticus*). Additionally, renewing the water in aquaria increases aggressive interactions because it dilutes chemical signals used for social rank recognition thus disturbing the hierarchy stability in juvenile Nile tilapia (Gonçalves-de-Freitas *et al.*, 2008). Maruska, Fernald (2012) demonstrated that dominant *A. burtoni* males increase the frequency of urine releasing in the presence of another male, thus showing that chemical signals are used to inform the individual's dominance status. Altogether, these studies have shown that chemical communication is adaptive because it signals social rank, thus reducing the detrimental effects of physical contests.

The ecology and evolution of African cichlids have been well documented. However, little is known about this mechanism on social rank signaling in Neotropical cichlids. Some differences in social signaling among them are expected, since the evolutionary mechanism favored differences in social behavior between these two large groups (Keenleyside, 1991; Barlow, 2000). In a recent study with juvenile Amazonian cichlid, *Pterophyllum scalare* (Schultze, 1823), Gauy *et al.* (2018) found that chemical information is important to keep social stability within the group. For instance, the greater the renewal of aquarium water (and the more diluted chemical information), the greater the overt aggression in aggressive interactions, and longer time was needed to restore the baseline levels of aggression in the group. This finding suggests that the response to chemical cues seems to follow a pattern for cichlid's social communication.

In previous studies in our laboratory, however, we observed some differences in the aggressive behavior patterns for *Cichlasoma paranaense* Kullander, 1983 and those expected for cichlids (personal observations). After setting social hierarchy, cichlids (and other animals, for example, the red deer, see Clutton-Brock, Albon, 1979) reduce overt aggression and increase restrained aggression as a way to keep social hierarchy with a minimum risk (Johnsson *et al.*, 2006). In *C. paranaense*, restrained aggressions are expected to be less frequent than overt aggression, even after social rank settlement (see Brandão *et al.*, 2018). Since restrained aggressions are typically displayed by visual signs, chemical information would be a key element to signal social hierarchy in this species. Herein, we tested the effect of chemical

signaling in juvenile *C. paranaense* social groups by renewing the water in aquarium, a procedure that washes away chemical information and increases aggressive interactions in other cichlid species. The family Cichlidae consists of diverse group of species varying in colors, behaviors and niches, which probably reflects a variety in chemical communication mechanisms (Keller-Costa *et al.*, 2015). Comparing species within this group can be a way to provide more comprehensive information on their evolutionary forces driving social behavior.

Material and Methods

Fish housing. Fish were collected from water bodies in the city of Frutal, MG, Brazil (20°03'37.40" S and 49°12'16.09" W), and acclimated in the lab for 20 days in 500-L polypropylene tanks (ca. 1 fish / 10L) with water at 27 ± 1°C and a 12 h light/dark light regime (from 07:00 to 19:00 h). Fish were fed with commercial food (Fri-Ribe® Tropical Fish, 28% crude protein) twice a day (8:00 a.m. and 6:00 p.m.) to apparent satiation. Water quality was maintained through Canister biological filters (filtering 400 L / h) and constant aeration.

There was not evident external sexual dimorphism in *C. paranaense*. Juvenile fish were used to avoid the effect of sex on aggressive interactions, since cichlid males appear to show higher androgen levels than females in adult life (Oliveira, Almada, 1998; Oliveira, 2004). In addition, juveniles *C. paranaense* are known for their aggressive interactions and establish social hierarchy (see Brandão *et al.*, 2015, 2018). Thirty-one individuals of similar size (mean ± S.E. = 68.1 ± 0.08 mm), weight (mean ± S.E. = 14.14 ± 0.47 g) and age were euthanized to examine the gonadal development using a microscope, confirming that individuals were indeed juveniles (classification according to Babiker, Ibrahim, 1979). A voucher specimen was placed in UNESP's fish collection, São José do Rio Preto, SP, Brazil, (DZSJRP-Pisces 13046).

Experimental design. The effect of water renewal on aggressive interactions was tested in groups of three *C. paranaense* without sex identification assigned to one out of two treatments: 1) 0% [T0%] water renewal (control); and 2) 50% [T50%] water renewal (amount of water renewal that has the greatest effect on other cichlids; see Gonçalves-de-Freitas *et al.*, 2008; Gauy *et al.*, 2018). We used 15 social groups of each treatment (total of 90 fish). In both treatments, 50% of the water volume of the aquarium was removed through a plastic tube attached to the inner side of the aquarium. In T0%, the same water taken from the aquarium was returned to it, as a control. In T50%, 50% fresh water was replaced. The "new" water was collected from a similar aquarium to those used in the tests, with the same biological filter, aeration and temperature, but without fish. The water was carefully placed in the aquarium by using a beaker, so that interference in fish's behavior was minimized.

The fish were grouped for five days in the experimental aquaria. The first three days were used to set the hierarchy of the group. On day 4, aggressive behavior was recorded (10 min) before renewal (baseline), 1 minute, 1 hour, 2 hours and 24 hours after water renewal to evaluate the possible effects of the chemical signal dilution on the fish aggressive interactions, and effects on the time for recovering aggressive interactions to the basal levels (before water change). Behavioral records were made between 14:00 and 18:00 to avoid possible circadian influences. This period was chosen because it was distant from the first feeding, thus avoiding the influence of food competition (Gómez-Laplaza, Morgan, 2003; Grobler, Wood, 2013).

Aggressive interactions. The number of aggressive behaviors showed by each individual was quantified based on the ethogram previously described for *C. paranaense* (Brandão *et al.*, 2015; Fig.1). Aggressive behaviors were labeled as overt aggression (chasing, lateral fighting, biting, pushing, pulling fins, mouth fighting) and restrained aggression (lateral display, lateral threat, frontal threat and perpendicular threat). Overt aggressions are aggressive behavioral units that involve direct physical contact and are often followed by high energy expenditure, whereas restrained aggressions are aggressive units that involve displays, with no physical contact, and are usually followed by reduced energy expenditure (Haller, Wittenberger, 1988; Ros *et al.*, 2006).

Social rank. The individual's social rank was inferred by the dominance index (DI = the number of aggressive interactions of an individual/ the number of aggressive interactions of the group), as used by Gonçalves-de-Freitas *et al.* (2008) for Nile tilapia and Gauy *et al.* (2018) for angelfish (*P. scalare*). DI ranges from 0.0 to 1.0; the highest DI characterizes the dominant fish, whereas the lowest DI, the more subordinate ones. We used three individuals and they were ranked as alpha, beta and gamma fish.

DI tends to be similar among individuals before social rank establishment, increasing in the dominant and reducing in the subordinate (Oliveira, Almada, 1996; Gonçalves-de-Freitas *et al.*, 2008; Gauy *et al.*, 2018). DI was checked for each social rank during the first three days of grouping and also on the day 4 before water renewal (baseline session) to confirm that social rank was settled before water manipulation.

Experimental details. Before grouping, fish were anesthetized with tricaine methanesulfonate (MS222 - Sigma Aldrich, China; 20 mg / L; see Brandão *et al.*, 2015), weighed and measured. Their standard length and weight were respectively: (mean \pm S.E.); T0% = 68.5 \pm 0.8 mm; 14.13 \pm 0.55 g; T50% = 68.0 \pm 0.6 mm; 14.11 \pm 0.44 g. There was no significant difference between treatments for the length (Independent t-test, $t_{(28)} = -0.54$; $p = 0.59$) or

weight ($t_{(28)} = -0.02$; $p = 0.98$). The intragroup coefficient of variation was also similar between treatments for the length: (mean CV \pm S.E.); T0% = 0.04 \pm 0.005 mm; T50% = 0.02 \pm 0.004 mm ($t_{(28)} = -1.61$; $p = 0.12$), and weight: T0% = 0.1 \pm 0.01 g; T50% = 0.1 \pm 0.01 g ($t_{(28)} = -0.18$; $p = 0.85$).

The fish were individually identified through VIE tags - Visible Implant Fluorescent Elastomer (see Ang, Manica, 2010; Brandão *et al.*, 2015) before being assigned to one of the two treatments. During the procedure, fish were kept on moist cloths to preserve their skin mucus. The elastomer was inserted under 3 scales on each side of the fish. Fish were observed in glass aquaria (400 x 300 x 400 mm, ca. 48 L) with three side walls covered by blue plastic to avoid visual contact between fish of adjacent aquaria, and only the front wall uncovered to allow video-recording. Blue color was chosen because it prevents stress in other cichlid species (Maia, Volpato, 2013). The water temperature was 27 \pm 1° C, and the photoperiod was 12 h light/dark (7:00 a.m. to 7:00 p.m.). Water oxygen was 8.24 \pm 0.04 ppm and the pH was 7.0 \pm 0.1 (measured with the electronic device - Hanna HI9146 and Hanna HI98127, respectively). Ammonia and nitrite levels were measured with commercial kits (LabconTest) and were respectively: T0% = 0 ppm; 0.3 \pm 0.24 ppm; T50% = 0 ppm; 0.15 \pm 0.1 ppm. Fish were fed the same food used during acclimation, corresponding to 3% of the biomass, offered twice a day (8:00 a.m. and 6:00 p.m.).

Statistical analysis. Data were tested for normality by Skewness and Kurtosis; F max test was used to test homoscedasticity (Zar, 2010; Ha, Ha, 2011). DI values for each social rank were compared on the days before water changing through Mixed Model ANOVA, in which social rank was a categorical factor, and behavioral sessions the repeated measures. A Mixed Model ANOVA was used to compare the number of overt and restrained aggressions before renewal at 1 min, 1h, 2h and 24h after water renewal; in which the treatments were the categorical factors and observation sessions the repeated measurements. We used Fisher-LSD as a *post hoc* test and p was statistic significant at ≤ 0.05 .

Results

Social hierarchy was established within the four days preceding water manipulation, and social rank emerged since the first day. There was statistically significant interaction between the social rank and the observation session for DI ($F_{(6,144)} = 10.61$; $p < 0.0001$; Fig. 2). Alpha fish showed a higher DI than beta ($p < 0.0001$) and gamma fish ($p < 0.0001$), and beta fish had higher DI than the gamma one ($p < 0.0001$). DI increased from the first to the fourth day for the alpha fish ($p < 0.0001$), reduced for the beta ($p = 0.0001$), and remained the same throughout the days for the gamma individual ($p = 0.16$) (Fig. 2).

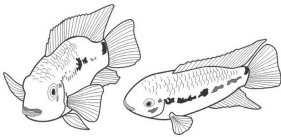
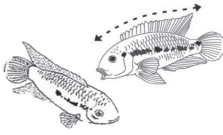
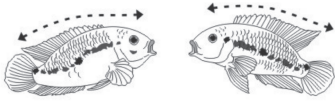
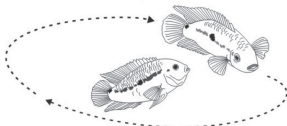

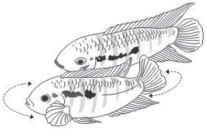




Observed Behavior	Description of behavior
 <p data-bbox="517 368 652 395">Lateral display</p>	<p data-bbox="831 271 1158 348">A fish stands close to the opponent, opens the fins and opens the opercula exposing the lateral portion of his body.</p>
 <p data-bbox="523 549 646 576">Lateral threat</p>	<p data-bbox="847 464 1142 520">A fish approaches quickly toward its opponent that swims away.</p>
 <p data-bbox="523 700 646 727">Frontal threat</p>	<p data-bbox="815 592 1174 696">Two fish stand face to face without opening the opercula, each one with its mouth open, its body inclined (about 45°) and head up. There is no physical contact.</p>
 <p data-bbox="491 876 678 903">Perpendicular threat</p>	<p data-bbox="807 754 1190 882">A fish stays in the center of the aquarium while the other swims in a circular path around it. The fish in the center opens the opercula and accompanies with the head the trajectory of the opponent without leaving the place.</p>
 <p data-bbox="555 1021 630 1048">Chasing</p>	<p data-bbox="815 938 1174 994">A fish swims in the direction of its opponent, causing the opponent to flee.</p>
 <p data-bbox="515 1197 657 1224">Lateral fighting</p>	<p data-bbox="799 1100 1190 1176">A fish intensively undulates its body against the opponent sometimes touching and displacing the opponent.</p>
 <p data-bbox="560 1363 619 1390">Biting</p>	<p data-bbox="815 1280 1174 1336">A fish approaches its opponent and touches its body with its mouth</p>
 <p data-bbox="552 1539 622 1566">Pushing</p>	<p data-bbox="799 1446 1190 1522">A fish with its open mouth nips the lateral flank of its opponent, sometimes displacing the opponent.</p>
 <p data-bbox="536 1705 643 1732">Pulling fins</p>	<p data-bbox="823 1632 1166 1688">A fish bites its opponent's fin, moving the opponent away by pulling the fins.</p>
 <p data-bbox="520 1881 654 1908">Mouth fighting</p>	<p data-bbox="839 1777 1158 1881">Two fish approach one another and simultaneously bite their jaws. They may swim in a circle while keeps their mouth attached.</p>

Fig. 1. Ethogram of the aggressive interactions in *Cichlasoma paranaense* based on Brandão *et al.* (2015).

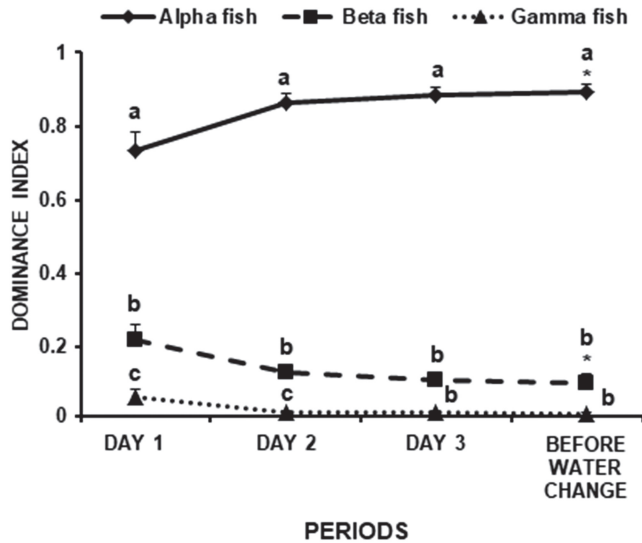


Fig. 2. Establishment of social rank. Dominance index (mean \pm S.E.) by rank before aquarium water changing. Data were collapsed across treatments. Letters compare means between ranks within each observation session. Different letters indicate statistical significance among ranks. Asterisks indicate significant differences within rank between the first and the fourth observation session (Mixed Model ANOVA followed by Fisher's LSD *post hoc* test).

Overt aggressions. The number of overt aggressions was similar both between and within treatments; however, 2 hours after water manipulation it was lower (interaction between treatments and observation sessions: $F_{(4,112)} = 3.03$, $p = 0.02$, Fig. 3a). In T0%, the number of overt aggressions in all observations after water renewal was similar to the baseline ($p > 0.08$). The number of overt aggressions was also similar to the baseline in most observations after water renewal in T50% ($p > 0.41$), but it reduced after 2h ($p = 0.04$). In addition, the number of overt aggressions was similar between treatments in most observations ($p > 0.18$), except 2h after the renewal, when it was lower in T50% ($p = 0.009$).

Restrained aggressions. A significant interaction between treatments and observation sessions was found for the number of restrained aggressions ($F_{(4,112)} = 2.59$, $p = 0.04$, Fig. 3b). In T0%, the number of restrained aggressions was similar to the baseline in most observation sessions after water renewal ($p > 0.31$), but higher after 2h ($p = 0.008$). In T50%, the number of restrained aggressions was higher than the baseline right after the renewal ($p = 0.01$) and remained similar to the baseline throughout the other observation sessions ($p > 0.08$). In addition, the number of restrained aggressions was lower for T50% than for T0% after 2h ($p = 0.005$) and similar between treatments in the other observation sessions ($p > 0.19$).

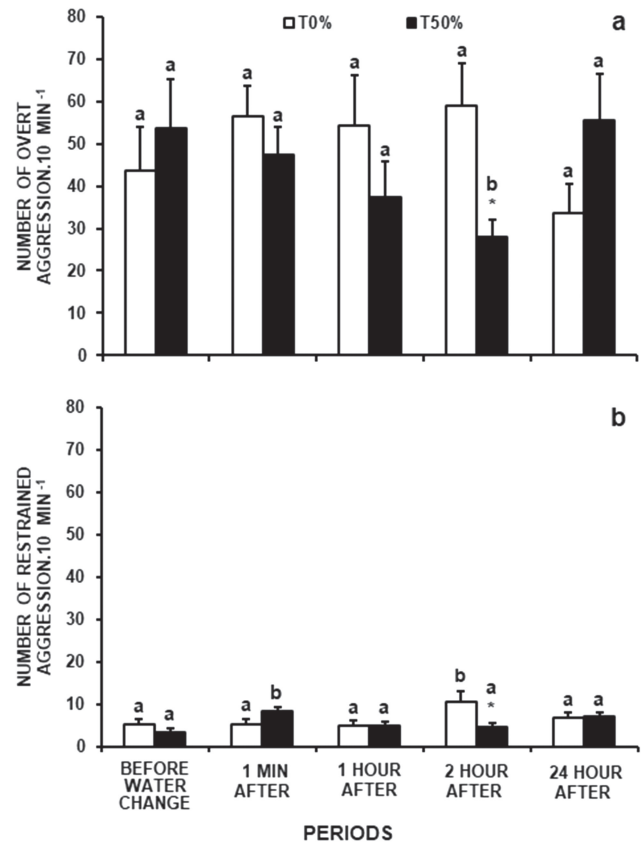


Fig. 3. Number (mean \pm S.E.) of **a.** overt aggression and **b.** restrained aggression following water renewal in each treatment. Letters indicates a significant difference in relation to observation before water change (baseline) within each treatment. Different letters indicate statistical significance. Asterisk compares the observations between treatments. (Mixed Model ANOVA followed by Fisher's LSD *post hoc* test).

Discussion

This study showed that the aquaria water renewal does not increase aggressive interactions in juvenile *C. paranaense*, as it has been observed in other cichlid species. On contrast, 50% water renewal apparently reduces the aggressive interactions in the group, rejecting our hypothesis. However, the results highlight the differences in social interactions among cichlid species.

There was no difference between the number of overt and restrained aggressions in the observation session before water renewal between treatments, indicating that the first three days were enough to establish social hierarchy in the group. DIs remained similar on the 3 days leading up to water renewal. While this initial condition was a baseline, it shows that social groups in both treatments started at the same level of aggression. After water renewal (T50%), we observed an increase in restrained aggression 1 minute after water change within T50%, but it was similar to the control. In the control, restrained aggression increased after 2 hours, suggesting that

these variations in restrained aggression can be an effect of water changing. With caution, this result might indicate that aggressive interactions did not increase within the *C. paranaense* social group thus revealing a different response pattern to chemical dilution compared to other cichlids.

Juvenile angelfish, for example, had increased overt aggression and reduced restrained ones in 50% water renewal, indicating that chemical communication is an important cue for decreased contests within the group (Gauy *et al.*, 2018). In juvenile Nile tilapia males, chemical communication also reduces aggressive interactions (Giaquinto, Volpato, 1997), whereas water renewal increases interactions and destabilizes social hierarchy (Gonçalves-de-Freitas *et al.*, 2008). It was also evidenced that in adults, such as dominant Mozambique tilapia, males increase the frequency of urine release during aggressive interactions, signaling their social rank (Barata *et al.*, 2007, 2008) and reducing aggressive interactions (Keller-Costa *et al.*, 2016). Another species that shows signs of social status through urine is *A. burtoni*, which increases the frequency of urine releasing in the presence of a rival male (Maruska, Fernald, 2012). Unlike these species, our results showed that the water (and supposedly chemical signals) dilution does not increase aggressive interactions in the *C. paranaense* social group. We can assume this effect is due to fish age, since we used juveniles. Previous studies using other species of cichlids suggest that chemical signals are an important part of social communication, even in juveniles, such as in Nile tilapia (Gonçalves-de-Freitas *et al.*, 2008) and angelfish (Gauy *et al.*, 2018). However, because aggressive interactions can change after fish become adults (Damsgård, Huntingford, 2012), we cannot overlook this possibility.

The number of overt aggressions of *C. paranaense* throughout the study was always higher than the number of restrained aggressions. The aggressive behavior of *C. paranaense* did not follow the pattern of ritualized fighting, which has been observed in other cichlids (Johnsson *et al.*, 2006). This pattern of aggressive behavior in *C. paranaense* (high overt aggression frequency and reduced restrained aggression frequency) was observed in another study, which was performed under similar experimental conditions (Brandão *et al.*, 2018). Such aggressive behavior seems to be characteristic of this species.

The decreased of overt aggression observed after 2 hours of water change indicates that the conspecific scent stimulates fights instead of reducing them. This tendency can be interpreted as a generalized response of behavior change due to an alteration of the environment. For example, juveniles of matrinxã *Brycon amazonicus* (Agassiz, 1829), decrease their aggressive interactions in a new environment as a sign of alert (Serra *et al.*, 2015). Even though *C. paranaense* has a high aggressiveness level, these individuals are more exposed to predation in the natural environment; thus, a change in the environment may cause a decrease in aggressive interactions, thus decreasing the animal's vulnerability to predators. The cichlid *Crenicichla lepidota* Heckel, 1840, for example, shows a higher latency and a lower number of overt aggressions in

disturbed environments due to tourist visitation than in places without tourism activity (Bessa, Gonçalves-de-Freitas, 2014). This decreased territorial response was attributed to a fear reaction (Yue *et al.*, 2004; Martins *et al.*, 2011).

We recognize that the amount of water changed could not be enough to destabilize social hierarchy. In fact, the amount of chemicals in water can be perceived for some fish species in highly diluted solutions (Levesque *et al.*, 2011). The dilution of 50% used in this study could not be enough to abolish the social chemical perception. We also speculate that chemicals in the environment increases overt aggression instead of reducing it in *C. paranaense*. As odors and chemicals could accumulate again after 24 hours, this accumulation would increase overt aggression, which is an uncommon effect for cichlids. In this sense, the effect of chemical communication would not support our predictions. Nevertheless, further studies are needed to test all these hypotheses, as well as to better understand the social communication process in *C. paranaense*. This study demonstrates, for the first time, an important interspecific difference in the mechanism of social communication in cichlids, as well as the need to compare several species to understand the evolutionary forces which drive social communication in Neotropical fishes.

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