

Original article

Water temperature affects aggressive interactions in a Neotropical cichlid fish

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Changes in water temperature may affect the aggressive behavior of aquatic organisms, such as fish, either by changing some physiological mechanisms or by increasing the probability of encounters between individuals as a result of variation in their swimming activity. In our study, we evaluated the influence of increasing and decreasing temperature on the aggressive behavior of the Neotropical cichlid fish *Cichlasoma paranaense*. Firstly, we tested the critical thermal maximum (CTMax) tolerated by this species. Then, we tested the effect of decreasing or increasing the water temperature in 6° C (starting at 27° C) on the aggressive interactions of fish under isolation or housed in groups. We found a CTMax value of 39° C for *C. paranaense*. We also observe that a 6° C decrease in water temperature lowers swimming activity and aggressive interactions in both isolated and group-housed fish, as expected. On the other hand, the increase in temperature had no effect on the fish's aggressive behavior, neither for isolated nor for grouped fish. We concluded that *C. paranaense* shows high tolerance to elevated temperatures and, in turn, it does not affect aggressive behavior. Nevertheless, we cannot dismiss possible effects of elevated temperatures on aggressive interactions over longer periods.

Keywords: Agonistic behavior, Critical thermal maximum, Social hierarchy, Social isolation, Thermal environmental changes.

Mudanças na temperatura da água podem afetar o comportamento agressivo de organismos aquáticos, como peixes, seja alterando alguns mecanismos fisiológicos ou aumentando a probabilidade de encontros entre os indivíduos como um resultado da variação na sua atividade natatória. Em nosso estudo, nós avaliamos a influência do aumento e da diminuição da temperatura sobre o comportamento agressivo do peixe ciclídeo neotropical *Cichlasoma paranaense*. Primeiramente, nós testamos a temperatura crítica máxima (CTMax) tolerada por essa espécie. Depois disso, nós testamos o efeito de reduzir ou aumentar a temperatura da água em 6° C (a partir de 27° C) sobre as interações agressivas de peixes sob isolamento ou alojados em grupos. Nós encontramos um valor de CTMax de 39° C para *C. paranaense*. Nós também observamos que uma redução de 6° C na temperatura da água diminui a atividade natatória e as interações agressivas tanto em peixes isolados quanto agrupados, como esperado. Por outro lado, o aumento na temperatura não teve efeito no comportamento agressivo dos peixes nem isolados, nem agrupados. Nós concluimos que *C. paranaense* apresenta alta tolerância a temperaturas elevadas, e que isso, por sua vez, não afeta o comportamento agressivo. No entanto, nós não podemos descartar possíveis efeitos de temperaturas elevadas sobre interações agressivas durante longos períodos.

Palavras-chave: Comportamento agonístico, Hierarquia social, Isolamento social, Mudanças ambientais térmicas, Temperatura crítica máxima.

Introduction

Environmental changes alter the physiology and behavior of animals, which allow them to deal with these new environmental conditions (Hofmann, Todgham, 2010; Wingfield, 2013). Such disturbances may be unexpected and demand immediate responses from individuals. These

responses are part of what we call emergency life-history strategies (Wingfield, 2003), selected to help individuals cope with fluctuations in the environment (Wingfield, 2003). However, when some environmental changes surpass the individual's capacity of adjustment to deal with them, animals may collapse. Therefore, research concerning the effects of environmental changes on animal

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behavior, especially those caused by climate change and anthropic actions (Walther *et al.*, 2002; Bærum *et al.*, 2013), has been increasing over the last years.

Riparian degradation, for example, is a type of disturbance with immediate effects on aquatic environments, causing a rise in water temperature, light incidence, evaporation, and siltation (Matthews, Marsh-Matthews, 2003). Changes in temperature may quickly affect the physiology and behavior of aquatic animals such as teleost fish, changing their metabolic rates (Kochhann *et al.*, 2015; Campos *et al.*, 2017), impairing their reproduction and modifying the individuals' personalities (Biro *et al.*, 2010).

Therefore, upper and lower thermal tolerance limits are selected and fish will spend most of their lives in an optimal temperature range which allows the proper functioning of their physiological processes (Pörtner, 2010), and avoid temperatures outside their optimum range which can damage their cells and tissues and impair the individuals' health and survival (Motani, Wainwright, 2015).

Actually, temperatures close to the thermal tolerance limit may affect fish's aggressive behavior (*e.g.*, the marine fish *Tautoga onitis* (Linnaeus, 1758); Olla *et al.*, 1978). Elevated temperatures, for instance, may increase aggressive interactions, by raising their metabolic rate (Campos *et al.*, 2017) and swimming activity (Beamish, 1978; Claireaux *et al.*, 2006), which in consequence, increases the probability of aggressive encounters among some species of fish (Ratnasabapathi *et al.*, 1992; Sakakura, Tsukamoto, 1997; Kochhann *et al.*, 2015).

Thus, our aim was to test the effect of water temperature on aggressive interactions in a social Neotropical fish, *Cichlasoma paranaense* Kullander, 1983, to better understand how individuals cope with changes in the abiotic and social environment. We chose a fish from the Cichlidae family, comprised by social species which are socially ranked through aggressive interactions (Barlow, 2002; Shumway, 2010; Brandão *et al.*, 2015); and also because *C. paranaense* inhabits Brazilian streams which are at deforestation risk and, consequently, undergo fast temperature variations. Therefore, we designed two studies; the first one aimed to test the critical thermal maximum (CTMax) tolerated by *C. paranaense*, under isolated and grouped conditions. In the second study, we tested the effects of elevated and reduced temperatures on the swimming activity and aggressive interactions of isolated and group-housed fish. Although it is a social species, we aimed to test isolated and grouped *C. paranaense* to understand how fish deal with environmental changes in the presence and absence of social group interferences. We predicted that high temperatures would increase the individuals' swimming activity and it would indirectly increase aggressive encounters among animals, whereas a decrease in water temperature would have an opposite effect, reducing aggressive interactions.

Materials and Methods

Animals and experimental conditions. *Cichlasoma paranaense* juveniles of unidentified sex (members of this species are sexually monomorphic) were captured from water bodies in the city of Frutal, MG, Brazil (20°03'37.40" S and 49°12'16.09" W), and held in outdoor tanks at the Instituto de Biociências, Letras e Ciências Exatas, IBILCE/UNESP, São José do Rio Preto, SP, Brazil. We chose to use sexually immature juveniles as it is known from another study with cichlid fish that males and females may fight differently when adults (Arnott, Elwood, 2009). Therefore, in order to avoid the effect of sex steroid hormone surges on aggressive behavior, we decided to study juvenile fish. A voucher specimen was deposited in the UNESP fish collection, in São José do Rio Preto, SP, Brazil, (DZSJRP-Pisces 13046).

The fish were acclimated to laboratory conditions in 500 L indoor tanks (*ca.* 1 fish/5 L; 27° C and 12 h light/dark cycles) for 15 days. Biological filters and constant aeration ensured water quality. Fish were fed twice a day to satiation with a commercial diet for tropical fish, Pirá® 28% protein.

For both studies, fish were isolated in glass aquaria (40 × 30 × 40 cm; *ca.* 48 L) which had their side and back walls covered with blue plastic to prevent visual contact between replicates; blue plastic was chosen because this color is known to prevent stress in *Oreochromis niloticus* (Linnaeus 1758), which is also a cichlid fish species (Volpato, Barreto, 2001). Before the experiments, each fish was anaesthetized with tricaine methanesulfonate (MS222; Sigma-Aldrich, China; 20 mg/L) to avoid stress during manipulation and, then, weighed and measured. After experiments, individuals were killed with an anesthetic overdose and dissected to confirm the gonadal stages macroscopically and confirm whether the fish were indeed immature.

Study 1 - Definition of a CTMax for *Cichlasoma paranaense*. In order to test the critical thermal maximum (CTMax) for *C. paranaense*, we conducted an experiment to identify the thermal point at which animals may lose motor capabilities, showing postural loss, which indicates an inability to escape from thermal situations that may lead to individuals' death (Cowles, Bogert, 1944). Fish were analyzed under both isolation and group-housed conditions.

CTMax for isolated fish. Isolated juveniles (standard length and weight of 8.24 ± 0.5 cm and 24.1 ± 7.45 g, respectively; n = 5) were allocated individually in an aquarium, where they remained for two days at 27° C for habituation before beginning the temperature manipulation. *C. paranaense* inhabits the Paraná basin, whose temperature varies depending on the region and period of the year. Because there is no information regarding the ideal temperature for *C. paranaense*, we used the mean temperature considered for other cichlid species, which is 27° C (*e.g.*, Figler, Einhorn, 1983; Schofield *et al.*, 2010; Brandão *et al.*, 2015).

The water temperature was increased in 1° C per day with the use of heaters and thermostats. This increase in water temperature continued until we found that the fish had lost their corporal posture, revealing impaired swimming activity and, therefore, loss of an appropriate response to escape from a dangerous situation; we considered this to be the end point of the individuals' CTMax (Lutterschmidt, Hutchison, 1997). Behavioral quantification was carried out using videos (10 min each) recorded every two days to quantify their swimming activity. When fish started to lose their body posture, individuals were killed with an anesthetic overdose and dissected to confirm their immature gonadal stage.

CTMax in group-housed fish. After determining the CTMax for isolated fish, we grouped 3 fish (SL and weight of 7.6 ± 0.71 cm and 24.1 ± 7.45 g, respectively; $n = 11$, total of 33 fish) under the same conditions of housing and manipulation described above. These individuals were addressed to the same temperature manipulation described for isolated ones.

Study 2 - Effect of temperature on aggressive behavior of isolated and group-housed fish. In the second study, we tested the effect of temperature variation on aggressive behavior in grouped and isolated fish, by means of three treatments: 21° C (decreased temperature, DT), 27° C (control temperature, CT) and 33° C (increased temperature, IT) ($n = 12$ each treatment), so that DT and IT were 6° C below or above the control, respectively. A recent study with several families of freshwater fish showed an average CTMax for these species of about 28.5° C; the described cichlids support a maximum of 33.5° C average (Comte, Olden, 2017). Another study with a Neotropical cichlid [*Amatitlania nigrofasciata* (Günther, 1867), according to Ratnasabapathi *et al.*, 1992] showed that a change of 4° C in water temperature was enough to affect their aggressive behavior. Based on these studies and the results from our first study regarding thermal tolerance, we chose a 6° C variation in water temperature starting from 27° C. The water temperature was controlled using heaters and thermostats manipulated in 2° C per day after the third day of experiment. All experiments were conducted in a laboratory equipped with an air conditioner, allowing us to achieve the DT as well.

Before experiments began, the oxygen concentration was measured daily using an oximeter to monitor whether the treatments DT, CT and IT would present low oxygen concentrations, resulting in a hypoxic environment for the fish, which could affect their aggressive behavior (Kochhann *et al.*, 2015). We conducted this oxygen monitoring as a baseline for the experiments, and it was carried out in tanks filled only with water. The mean oxygen concentrations (partial pressure of oxygen, PO₂) on the last day of experiment for each treatment were: 166 mmHg (DT), 132 mmHg (CT) and 118 mmHg (IT). All treatments corresponded with oxygen concentrations considered as

normoxia for cichlid fish (*e.g.*, Chippari-Gomes *et al.*, 2005; Schofield *et al.*, 2009; Kochhann *et al.*, 2015). Moreover, during the experiments, tanks were supplied with artificial aeration in all treatments.

Swimming activity and aggressive interactions in isolated fish. Twelve individuals were isolated for 3 days at CT for habituation under experimental conditions (SL and weight of 7.15 ± 0.32 cm and 18.11 ± 1.77 g, respectively). After this period, the temperature was gradually decreased to the DT (2° C/day), maintained at the CT or raised to the IT (2° C/day). After achieving the three experimental conditions, the fish remained isolated for two more days.

We evaluated the effects of temperature on the swimming activity of isolated individuals. During the third and eighth days of temperature manipulation, the latency of their swimming activity was quantified through 10-minute video recordings (Olla *et al.*, 1978). We compared the swimming activity on the third and eighth days of experiment. The third day was the last day at control temperature and the eighth day was the last day after temperature was manipulated for all the treatments.

Aggressive behavior exhibited towards a mirror (*e.g.*, mirror-elicited fights: Barreto *et al.*, 2009; Falsarella *et al.*, 2017) were recorded in two 15-minute videos; the first recording was made on the third day of the experiment, with all treatments at CT, and the second recording was made on the eighth day, when final temperatures had been reached. As we did for the swimming activity, we compared these two observations to determine whether there was a difference in aggressive interactions among the treatments after the change in temperature. Here, we chose to use isolated fish confronted with their mirror image to determine individual aggressive responses when faced with temperature changes, without any interference from the social group. Aggressive interactions were observed after we introduced a mirror (30 × 30 cm) to one side of the aquarium during the video recordings. The frequency of aggressive interactions was achieved by means of mirror-elicited fights as it has already been done for other fish species (Oliveira *et al.*, 2005; Scherer *et al.*, 2016; Ivy *et al.*, 2017). Fish cannot recognize their own image reflected in a mirror, therefore they will fight with their image as it were a real opponent (Falsarella *et al.*, 2017). Thus, such mirror test is an efficient way to control for individual responses to variables such as the opponent's sex, size and previous social ranks (Balzarini *et al.*, 2014), which could bias the focal fish's aggressive responses. The aggressive interactions observed here were based on an ethogram developed for *C. paranaense* (Brandão *et al.*, 2015) and adjusted for mirror interactions, as described for the cichlid *Astronotus ocellatus* (Agassiz, 1831) by Gonçalves-de-Freitas, Mariguela (2006). Four types of aggressive behaviors were observed: frontal exhibition, lateral exhibition, butting and undulation. These behaviors were then grouped into overt aggression and restrained aggression. Generally, restrained aggressions are

interactions with absence of physical contact, involving only displays of a hierarchical rank, whereas overt aggressions are physical encounters that demand a higher energy cost and may result in body injuries (Ros *et al.*, 2006; Hellmann, Hamilton, 2014). Thus, behaviors such as frontal and lateral exhibition were considered restrained aggression, whereas butting and undulation were considered overt aggression.

Aggressive interactions in group-housed fish. We evaluated the aggressive interactions among 3 grouped fish of homogeneous size (standard length and weight of 7.62 ± 0.86 cm and 24.12 ± 8.91 g, respectively). We maintained the same experimental conditions as used for isolated fish: treatments DT (21° C), CT (27° C) and IT (33° C), with $n = 12$ each (total of 108 fish used). In this experiment, aggressive interactions were quantified using two 10-minute video recordings, one on the third day and another on the eighth day after fish were grouped.

We quantified the same aggressive behaviors described for isolated fish; however, here the behaviors were displayed against a real opponent. We classified lateral exhibition and lateral threat as restrained aggression, and undulation and lateral attack as overt aggression.

Statistical analysis. Data were tested for normality by Skewness and Kurtosis and for homoscedasticity by the F max test (Zar, 2010; Ha, Ha, 2011) and were log-transformed to fit into the parametric tests. Both the swimming activity and the frequency of aggressive interactions of isolated and group-housed fish were tested by Mixed Model ANOVA, to compare between (21° , 27° and 33° C) and within (third and eighth days) treatments, completed by Fisher LSD post hoc test. We assumed $p < 0.05$ for statistical significance.

Results

Study 1 - Definition of a CTMax for *Cichlasoma paranaense*. CTMax for isolated fish. All the isolated individuals lost corporal posture at the temperature of 39° C, while at 41° C, 66% of them were assumed as dead, with no opercular movements, as stated by Carter *et al.*, 2011. Therefore, we settled 39° C as the CTMax for this species when individuals were isolated.

CTMax and aggressive interactions in group-housed fish. When the water temperature reached 37° C, 66% of replicates had at least one dead fish. The dead fish had several body and head injuries, suggesting that they died after suffering overt aggression from the other individuals in the group. Thus, although we observed that CTMax was 39° C for isolated individuals, the social group was affected by aggressive interactions. Postural loss was never observed for the remaining fish, thus such a criterion could not be used to infer CTMax. Therefore, aiming to avoid individuals' mortality during this phase, we chose to maintain the fish group at 35° C as a safe margin for individual survival.

Swimming activity and aggressive interactions in isolated fish. For the first three days of experiment, the mean \pm SE water temperature was $26.9 \pm 0.2^\circ$ C in all treatments. For the last three days, each treatment presented the mean temperature as proposed in the experimental design ($20.8 \pm 0.1^\circ$ C, $26.9 \pm 0.1^\circ$ C and $33.1 \pm 0.2^\circ$ C for DT, CT and IT, respectively). The results were evaluated as follows:

The comparison of the third and eighth days of the swimming activity was significant among treatments ($F_{2,33} = 15.04$, $p < 0.0001$; Fig. 1). There was a significant reduction in the swimming activity at DT compared to CT ($p = 0.01$) and IT ($p < 0.0001$). The swimming activity was also higher at IT when compared to CT ($p < 0.001$) (Fig. 1). When compared within treatments, both CT ($p = 0.03$) and IT ($p < 0.0001$) showed an increase in the individuals' swimming activity over the days of experiments.

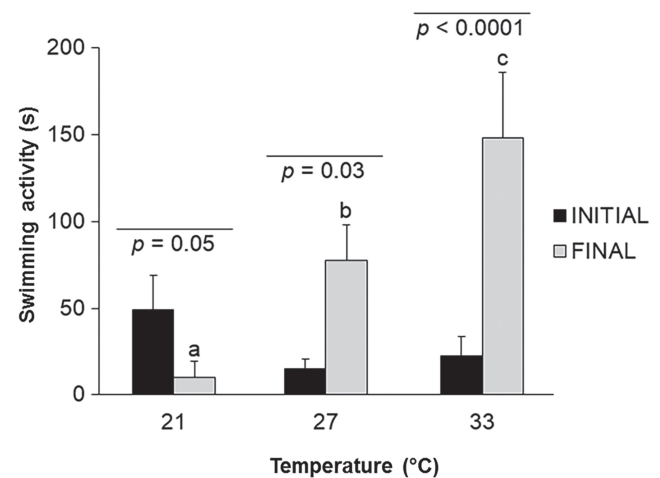


Fig. 1. Mean \pm SE of initial (third day) and final (eighth day) latency of swimming activity of isolated fish *Cichlasoma paranaense*. Different letters indicate statistical significance among treatments. Mixed Model ANOVA completed by Fisher-LSD post hoc test.

There were differences among and within treatments when the frequency of restrained aggression in the final observation (eighth day) was compared to that in the initial one (third day) ($F_{2,29} = 30.78$; $p < 0.001$; Fig. 2a). Comparing initial observations, the DT did not differ from the CT ($p = 0.34$) or the IT treatments ($p = 0.2$), but CT and IT had lower frequencies of restrained aggression during the initial observation ($p = 0.03$) (Fig. 2a). The restrained aggression noticed in the final observations was lower for DT when compared to CT ($p = 0.04$) and IT ($p = 0.04$), whereas the frequencies of restrained aggression performed during the final observations were similar between CT and IT ($p = 0.9$) (Fig. 2a). There was an increase in the frequency of restrained aggression over the experiment days at CT ($p < 0.001$) and IT ($p = 0.01$), but there was no difference at DT ($p = 0.06$) (Fig. 2a).

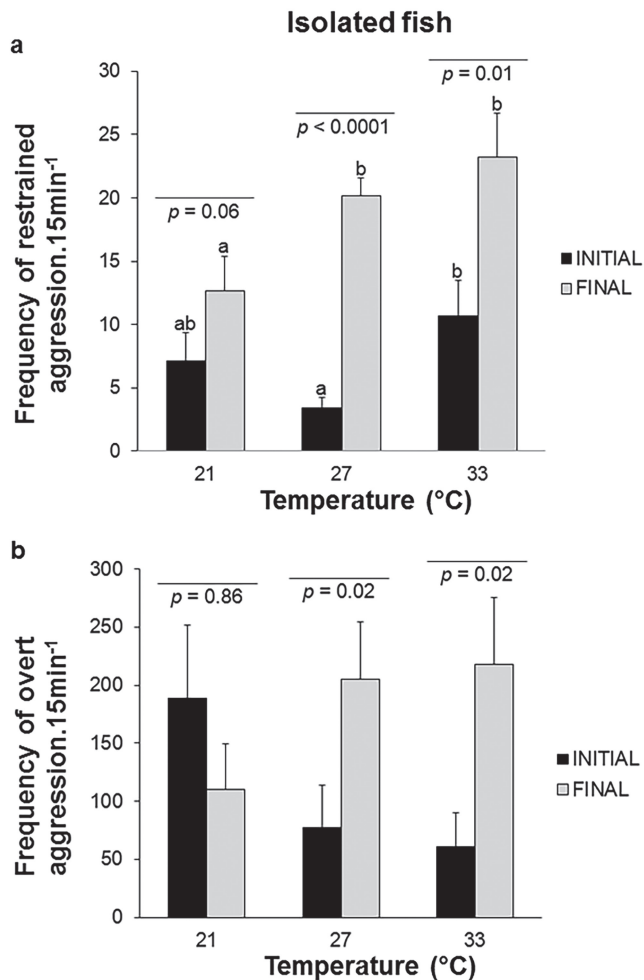


Fig. 2. Mean \pm SE of initial (third day) and final (eighth day) frequencies of **a.** restrained aggression and **b.** overt aggression of isolated fish against the mirror for *Cichlasoma paranaense*. Different letters show differences among treatments. Mixed Model ANOVA completed by Fisher-LSD post hoc test.

We also found significant differences when comparing the frequencies of initial and final overt aggression between and within treatments ($F_{2,29} = 6.93$; $p = 0.01$; Fig. 2b). The frequency of overt aggression was similar when comparing the initial and final observations between treatments (DT and CT, $p = 0.3$ and $p = 0.1$, respectively; DT and IT, $p = 0.3$ and $p = 0.08$; CT and IT, $p = 0.9$ and $p = 0.75$) (Fig. 2b); however, we detected more overt aggression when the initial and final observations were compared within CT ($p = 0.02$) and IT ($p = 0.02$).

Aggressive interactions in group-housed fish. For the first three days of experiment, the mean \pm SE water temperature was $27.1 \pm 0.2^\circ\text{C}$. For the last two days, each treatment had the proposed values, that is, water temperatures were at $21.8^\circ \pm 0.2^\circ\text{C}$, $27.2^\circ \pm 0.1^\circ\text{C}$ and $32.8^\circ \pm 0.2^\circ\text{C}$ for DT, CT and IT, respectively.

There were significant differences among and within treatments when we compared the frequency of restrained

aggression in the final (eighth day) and initial observations (third day) ($F_{2,33} = 9.69$; $p = 0.003$; Fig. 3a). There was no difference in the initial observation of restrained aggression at DT in relation to CT ($p = 0.65$) or IT ($p = 0.07$). Nevertheless, there was a decrease in restrained aggression in the final observations of DT when compared to CT ($p = 0.01$) and IT ($p < 0.001$) (Fig. 3a). CT and IT were not different concerning the initial ($p = 0.18$) and final ($p = 0.23$) observations of restrained aggression (Fig. 3a).

We also observed differences when comparing the initial and final frequencies of overt aggression between and within treatments ($F_{2,33} = 8.68$; $p = 0.005$; Fig. 3b). There were no differences in initial observations between DT and CT ($p = 0.38$), nor between DT and IT ($p = 0.37$); however, there were lower frequencies of overt aggression during the final observations for treatments DT and CT ($p = 0.005$), and DT and IT ($p = 0.001$) (Fig. 3b). CT and IT did not differ from each other in either the initial observations ($p = 0.08$), nor in the final ones ($p = 0.69$) (Fig. 3b).

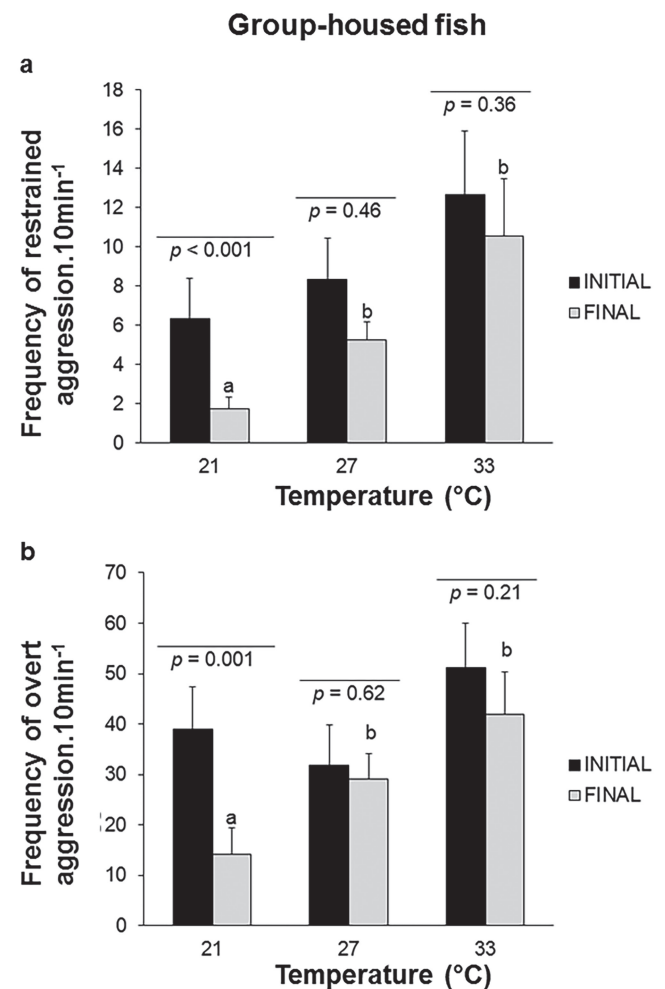


Fig. 3. Mean \pm SE of initial (third day) and final (eighth day) frequencies of **a.** restrained aggression and **b.** overt aggression of group-housed fish. Different letters show differences among treatments. Mixed Model ANOVA completed by Fisher-LSD post hoc test.

Discussion

In this study, we found CTMax for *Cichlasoma paranaense* to be 39° C for isolated fish. For group-housed fish, however, we found that 37° C increases mortality within the group due to aggressive interactions, thus suggesting that an increase in both temperature and social aggression will increase mortality before fish can reach a CTMax under such conditions. As expected, the different water temperatures affected their swimming activity at both DT and IT for isolated fish. In relation to study 2, we observed stabilized aggressive interactions at DT when individuals were isolated, whereas CT and IT showed increased aggressive interactions on the final days of the experiment. For group-housed fish, decreased temperatures indeed reduced aggressive interactions, whereas at increased temperatures aggressive interactions were the same on the first and last days, as we also observed for the control treatment.

Cichlasoma paranaense seems to tolerate increased temperatures well. In our first study, isolated individuals of *C. paranaense* presented a CTMax of 39° C, at which 100% of the fish showed a loss of corporal posture. When we tried to use the CTMax of 39° C for group-housed fish, however, we observed that 66% of the replicates had at least one fish that died at a water temperature as low as 37° C. As we manipulated 1° C each day, but recorded aggressive interactions only after two days of this manipulation, we could not determine fish mortality at 36° C, thus, as a safety margin, we chose to settle the temperature at 35° C to observe the behavior of group-housed *C. paranaense*. Therefore, we could not determine CTMax for group-housed fish, as we did not observe any postural loss in this experiment. Nevertheless, CTMax found for isolated individuals shows that *C. paranaense* is a very tolerant species to increased water temperatures when compared to other freshwater fish (e.g., average CTMax of 28.5° C for several fish species; Comte, Olden, 2017). This was also found for other cichlids such as *Pterophyllum scalare* (Schultze, 1823), *Etioplos suratensis* (Bloch, 1790) and *Geophagus brasiliensis* (Quoy & Gaimard, 1824), which show a CTMax of 40° C (Pérez *et al.*, 2003) and 35° C (Comte, Olden, 2017), and a lethal temperature of 32° C (Rantin, Petersen, 1985), respectively.

Concerning study 2, the treatments 21° C (DT), 27° C (CT) and 33° C (IT) for isolated fish resulted in the same frequencies of restrained aggression in the initial observations, showing the same performance to display these behaviors. However, after the water temperature manipulation, on the final day of observation, fish showed the same performance when fighting at DT as on the first days, whereas at CT and IT, the individuals increased their aggressive interactions. When isolated at CT and IT, fish showed more restrained aggression on the final days. This was not expected for CT, at which we had anticipated a stable frequency of restrained aggression between the initial and final observations, due to the absence of temperature manipulation. In fact, an additional effect of social isolation

seems to have affected individual aggressive performance, as it is already known for other teleost fish species (Gómez-Laplaza, Morgan, 2000; Gonçalves-de-Freitas, Mariguela, 2006). This increased aggressive behavior after isolation may be also linked to the previously described emergency life-history stages (Wingfield, 2003), indicating that the individuals are under social stress, which could explain the rise in aggressive interactions seen for *C. paranaense* at CT similarly to IT. Concerning the frequency of overt aggression, we also observed a higher number of aggressive interactions being performed in the final observations for individuals at CT and IT, again revealing a possible effect of social isolation increasing aggressiveness, masking the real effect of temperature in these treatments.

An alternate explanation regarding the change in aggression levels is that social communication was impaired. According to Giaquinto, Volpato (1997) and Gonçalves-de-Freitas *et al.* (2008), chemical and visual signals from contestant fish are required to complete the social rank recognition in the Nile tilapia. Therefore, the mirror image could have offered incomplete information about the “virtual” opponent, which may have led to the continuous overt aggression displayed by isolated fish (Gonçalves-de-Freitas, Mariguela, 2006).

Regarding group-housed fish, there were lower frequencies of restrained and overt aggression at DT, as expected. In terms of aggressive behavior, all three treatments started with the same fighting performance and no differences among their initial observations were seen. Concerning restrained aggression, the final observation for DT revealed a decrease in this behavior compared to the restrained aggression seen in the initial observation. The final observation for DT was also different from the final observations for CT and IT, which was also seen for overt aggression, showing a reduction in the last days compared to the initial ones at DT and when compared to the last observations in the other two treatments, CT and IT. These results corroborated with part of our hypothesis, revealing that a decrease of 6° C in water temperature would lead to reduced aggressive interactions in *C. paranaense*, probably as a consequence of reduced swimming activity at 21° C, showing a possible influence of water temperature on the individual's metabolism (e.g., Claireaux *et al.*, 2006; Pang *et al.*, 2011; Vergauwen *et al.*, 2013; Campos *et al.*, 2017). Nevertheless, this was not the case for 33° C, where initial and final aggressive interactions had no difference, besides the increased swimming activity observed at this temperature. This species continues to respond in the same way to increased water temperature, which leads us to speculate that *C. paranaense* is not such a sensitive species regarding short-term temperature elevation and that the aggressive behavior of those individuals might not be affected over such a short period. Indeed, *C. paranaense* is considered to be a tolerant species, with a high survival rate in impacted environments (Casatti *et al.*, 2009) and possibly able to tolerate increased temperatures. In regard to the CT treatment, we expected that aggressive interactions would

be stable over the days of observation and it occurred as anticipated. Maintaining the water temperature at 27° C was effective in stabilizing fights among *C. paranaense* when the animals were housed in groups. The IT treatment did not show an increase in aggressive interactions among individuals over the days of observation as well. Thus, an increase of 6° C in water temperature did not affect aggressive interactions at IT, contradicting some of our expectations. However, our results are in accordance with studies conducted by O'Mara, Wong (2015), in which increased temperature had no effect on the aggression levels of the Australian bass, *Macquaria novemaculeata* (Steindachner, 1866). This means that the aggressive response depends on the fish species.

When analyzing restrained and overt aggression at the DT treatment, we showed that a decrease in water temperature led to a reduction in the aggressive interactions of *C. paranaense*. These results are similar to those found by Vehanen *et al.* (2000), which showed decreased aggressive interactions among the brown trout, *Salmo trutta* Linnaeus, 1758, in the winter season, when compared to the summer. Thus, the effect of reduced temperatures on aggressive behavior seems to be more generalized among fish species than the effect of increased temperatures.

Our study shows that a decrease in water temperature reduces individuals' swimming activity and aggressive interactions, probably due to a decrease in aggressive encounters between individuals. We also show that, for isolated *C. paranaense* individuals, CTMax is 39° C, being probably inferior for group-housed fish. This investigation revealed how environmental changes, such as temperature, may influence the behavior of social fish, which have to cope with these natural emergencies modulating their aggressive interactions. Despite the findings shown here, long-term effects of temperature and many other factors relating to habitat deterioration on fish's social behavior are crucial and must be intensively investigated, raising questions for future studies. Moreover, the behavior of *C. paranaense* is little known, and information associating physical and environmental alterations with aggressive behavior may support plans to conserve aquatic environments and the fish that inhabit them.

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