



Innate response based on visual cues of sympatric and allopatric predators in Nile tilapia

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ABSTRACT

Predators are an important selective pressure for prey, and responses to visual exposure to non-predators and to allopatric and sympatric predators may assist the understanding of how prey animals recognize and distinguish potential threats. Here, we visually exposed predator-naïve Nile tilapia (*Oreochromis niloticus*) to aquaria with only water (handling-negative control), a non-predator fish (*Cyprinus carpio*), a South American catfish (*Pseudoplatystoma coruscans*, allopatric predator), and an African catfish (*Clarias gariepinus*; sympatric predator). We found that Nile tilapia displayed antipredator responses to both predator catfish but not to non-predator fish or negative control conditions. These antipredator responses, however, were stronger to the sympatric catfish in comparison to the allopatric animal. We concluded that Nile tilapia is able to innately distinguish between a sympatric and an allopatric predator based on visual cues. We assume that the innate recognition of the allopatric predator could be attributed to similarities in the body morphology and movement (key stimuli) of the South American and African catfish. Although this is plausible, the mechanism of the innate allopatric recognition remains unknown and deserves future investigation.

1. Introduction

Predation is one of the major evolutionary forces that drive and shape the life history, morphology and behavior of prey animals (Mikheev and Pasternak, 2006; Sih et al., 1998). Predation also results in the removal of prey individuals from the ecological system, which impacts the population dynamics of the prey (Drossel et al., 2001). As a result of this robust evolutionary force, prey species have developed a range of different predator recognition abilities via sensory cues as well as antipredator responses. For example, Nile tilapia (*Oreochromis niloticus*) exposed to chemical cues (odor) from their sympatric catfish predator decreased activity and increased ventilation frequency (VF), which is a possible way to provide enough oxygen to fish for a defensive response, such as eminent escaping (Miyai et al., 2016).

Vision is also a sense that allows animals to recognize predators. Visual perception provides some evidence of the extent to which animals obtain information from objects in their environment. When fish perceive a threat through visual cues, the animal may also prepare its

body for flight, and consequently, several adjustments occur that indicate the severity of the threat (Barcellos et al., 2007; Barreto et al., 2003). As an example, predator-naïve Nile tilapia increased their ventilatory frequency when visually exposed to an allopatric predator, the spotted catfish (*Pseudoplatystoma coruscans*, a South American catfish, locally known as pintado) (Barreto et al., 2003). Also, in the multi-sensory exposure of predator-naïve Nile tilapia to spotted catfish, tilapia showed avoidance responses (decreased activity and keeping a distance from the predator) and kept their dorsal fin erect, exposing their fin spines as an antipredator response (Freitas and Volpato, 2008). Considering that spotted catfish are indigenous to South America and Nile tilapia originates from Africa, both studies (Barreto et al., 2003; Freitas and Volpato, 2008) speculated that Nile tilapia is innately able to recognize an allopatric predator. Although their conclusion is plausible, these studies did not have the appropriate experimental design to test it. These reports did not consider the predator-naïve Nile tilapia's responses to a sympatric predator, which would be the primordial antipredator defensive response and is consequently important to

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appropriately interpreting the responses to an allopatric predator.

In general, the South American spotted catfish has a dark grey body, with black spots throughout the body, a depressed head and a very long fontanel. They have shorter pectoral fins compared to the African catfish and a forked caudal fin. The African catfish is a more eel-like fish (long dorsal and anal fins with symmetrical, rounded and expanded caudal fin), usually with a dark brown body and a black coloration on the back fading to a white belly. They share similar body movements and habits as they both inhabit deep areas (the bottoms of aquariums) and have nocturnal habits (low daytime activity). Although both are catfish, they share few morphological similarities such as whiskers and a white belly while displaying different colors and slight differences in body shape, especially regarding their fin morphology. Thus, it would be intuitive to suppose that the number of objective key stimuli a prey recognizes as a threat in a sympatric predator could be higher compared to an allopatric predator, and consequently, the prey would display a greater defensive response. The second alternative hypothesis is that a few key stimuli present in both sympatric and allopatric predators (e.g. whiskers and/or body shape and movement) could potentially be enough to trigger a full antipredator response, and therefore, a similar magnitude in antipredator defensive responses would be observed. Hence, in this study, we evaluated predator-naïve Nile tilapia's responses after being visually exposed to a non-predator fish (*Cyprinus carpio*) (Makiguchi et al., 2012) and an allopatric (*P. coruscans*) (Barreto et al., 2003) and sympatric (*Clarias gariepinus*) (DeGraaf et al., 1996) predator. To this end, we measured the ventilatory frequency (VF) (Alvarenga and Volpato, 1995), swimming activity, aggressive interactions (attacks to the visual stimuli) and plasma cortisol and glucose levels as tools to evaluate tilapia's defensive and stress responses to heterospecific fish.

The aggressive interaction was measured because tilapia is a territorial and aggressive fish (Barreto et al., 2011, 2009), thus this response could be an important key to assess how tilapia interprets the threat as a potential predator or territorial invader. The VF indicates an immediate stress response, which enhances the blood oxygenation and prepares the body for an emergency action as 'fight (territorial invader) or flight (threat evasion)' (Barbosa et al., 2012; Barreto et al., 2003). Cortisol is also released during circumstances perceived as stressful by the activation of the hypothalamus-pituitary-inter-renal axis, and its main role is to make energy available, such as plasma glucose, as part of a stress response mechanism (Wendelaar Bonga, 1997). Finally, swimming activity is an important tool for measuring avoidance behavior as part of an antipredator response, since that reduced activity might minimize the likelihood that prey will be detected by a predator (Chelini et al., 2009).

2. Material and methods

2.1. Ethical approval

This study followed the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation - Brazil (CONCEA). The experimental procedures were approved by the Ethical Committee for Animal Research of São Paulo State University (UNESP), Campus Botucatu.

2.2. Animals

The Nile tilapia population used in this study has been bred in laboratory conditions for several generations at the Aquaculture Center of Sao Paulo State University, Jaboticabal, SP, Brazil. The carp and African catfish individuals were purchased from the commercial fish farm, Piscicultura Poletini (Mogi Mirim, SP, Brazil) and the spotted catfish from the fish farm Piscicultura Rio Doce (São João da Boa Vista, SP, Brazil). These stock populations of Nile tilapia (2 tanks, ~ 30 fish per tank, density of ~ 2.2 g l⁻¹), carp (2 tanks, ~ 15 fish per tank, density of ~

1.3 g l⁻¹), African (2 tanks, ~ 10 fish per tank, density of ~ 6 g l⁻¹) and spotted catfish (1 tank, 10 fish per tank, density of ~ 4.2 g l⁻¹) were held in separated fiberglass tanks (300 l) and initially fed with a commercial diet of 2–3% of their body mass twice a day. During this time, the water temperature was ~ 27.5 °C and the tanks were supplied with continuous aeration and a recirculating system through an individual biological filter for each tank to maintain the water quality. Tanks were also supplied with a constant flow of dechlorinated water to avoid loss by evaporation, keeping the water at a constant level in the tanks, and avoiding organic matter accumulation. Oxygen and ammonia levels were monitored daily. The photoperiod was 12 h light: 12 h dark. These fish were never visually exposed to other fish species. The water conditions in fiberglass tanks were monitored daily for oxygen, pH, ammonia and nitrite levels (~6.5 mg l⁻¹; ~7.0; < 0.5 and < 0.05 ppm, respectively).

2.3. Experimental design: the predator exposure

The strategy of this study was to visually expose tilapia to water (control), non-predator fish, and an allopatric or sympatric predator. Tilapia, carps and predators from the stock population described above were acclimated during 1 week in individually-housed 20-l glass aquaria (40 × 20 × 25 cm, width × depth × height). These aquaria were parallel isolated by dividers and paired with an aquarium with one out of four visual stimuli: water (no-fish aquaria, negative control) or non-predator, allopatric, or sympatric heterospecific fish. After the acclimation period, the ventilatory frequency, locomotor activity and aggressive interactions were evaluated for 4 min before exposing the tilapia to the visual cues of water, non-predator fish or predators for the assessment of baseline values (i.e. initial). Thereafter, the divisions were removed, and these parameters were evaluated for 4 min. In addition, 30 min after the onset of the visual exposure, tilapia were anaesthetized, and blood samples were collected to measure plasma cortisol and glucose levels. After the introduction of stressful stimuli, 30 min is the time point corresponding to the peak of the plasma cortisol level in tilapia (Foo and Lam, 1993); therefore, it is suitable to test whether there is or not a difference on this variable.

2.4. Experimental conditions

Fish were netted from the stock tanks described above, anaesthetized (benzocaine, 0.1 g l⁻¹), had their mass and length recorded (see below) and were moved to the experimental aquaria. The fish were then allowed to acclimate for 1 week in the experimental aquaria before the trial and were fed twice a day to apparent satiation. The experimental aquariums were oxygenating with air stone connected through silicone tubing to air pumps. The aquariums were siphoned daily and water replace by ~10%. The experimental aquariums were kept at similar conditions of aeration, temperature and photoperiod compared to fiberglass tanks, with oxygen, pH, ammonia and nitrite levels monitored daily.

The average mass of the tilapia (g; mean ± SEM) and total length (cm; mean ± SEM) were 21.94 ± 1.08 and 8.82 ± 0.19, respectively. There were no significant differences between the mass and total length of tilapia used in this study (one-way ANOVA, P = 0.57 mass and P = 0.64 total length). The average mass and average length (mean ± SE) for the allopatric predators were 127 ± 2 g and 27.3 ± 0.2 cm, 180 ± 10 g and 32.5 ± 0.5 cm for the sympatric predators, and 26.9 ± 0.8 g and 66.5 ± 0.6 cm for the non-predator fish (carp).

2.5. Ventilatory frequency (VF), locomotion activity and aggressive interactions

To assess VF, locomotion and aggressive interactions, the fish were recorded for 4 min both before and after the stimuli exposure. VF was

calculated by visual quantification of the opercular beats for each minute during the 4 min before and 4 min after stimuli imposition. The average of the 4 min for each condition, before and after, were taken as the final VF for each (Alvarenga and Volpato, 1995). To assess locomotion, the rear wall of each aquarium was marked with a grid that contained four quadrants (each quadrant measured 20 cm × 12.5 cm), and the number of times the animal moved into a different quadrant was counted (Barreto et al., 2010). The aggressive interactions were measured as the number of attacks (attempted bites, i.e. open-mouthed contacts with the glass) to the aquaria wall that tilapia addressed directly to the visual stimuli (Arnott and Elwood, 2009).

2.6. Fish sampling and physiological assays

Fish were netted, anaesthetized (benzocaine, 0.1 g l⁻¹) and blood samples were quickly (total time 90 s) drawn from the heart using heparinized syringes. To obtain plasma, whole blood samples were centrifuged for 10 min at 3000 × g. Plasma was stored at -80 °C prior to the analyses. Plasma glucose concentrations were determined by the enzymatic method (Labtest kit, REF: 1012, São Paulo, Brazil) using a spectrophotometer (Model Genesys 10S, Thermo Scientific Inc., Madison, WI, USA), and cortisol levels were measured using a commercial ELISA kit (DRG International, Inc., USA; Cortisol Enzyme-Linked Immunosorbent Assay, EIA 1887) following the manufacturer's instructions.

2.7. Statistical analysis

The level of statistical significance was considered as $P < 0.05$ in all analyses. The data from the number of attacks, VF pre-exposure (basal), delta of VF, swimming activity pre-exposure (basal) and delta of swimming activity failed in normality (Cramer-Von Mises) and/or homoscedasticity tests (Brown-Forsythe) and were compared between groups using a Kruskal-Wallis test, complemented by a Dunn test. Further, a Wilcoxon test within each visual exposure was used to test whether values after exposure were significantly different from the initial values. In addition, the proportions of tilapia that attacked the source of the visual stimuli were compared using a Goodman proportion test (Goodman, 1964), which has been successfully used to compare tilapia behavior (Maia and Volpato, 2018). The cortisol and glucose data were analyzed by one-way ANOVA, because data met the assumption of normality.

3. Results

3.1. Ventilatory frequency

Nile tilapia had a similar VF baseline when compared across the four treatments (Fig. 1A, Kruskal Wallis test; $H = 3.76$; $P = 0.29$). The post-stimuli VF increased when Nile tilapia were exposed to non-predator fish ($Z = 2.16$; $P = 0.031$) and to the allopatric ($Z = 3.52$; $P < 0.0005$) and sympatric predator ($Z = 3.52$; $P < 0.0005$), as revealed by Wilcoxon tests (Fig. 1A). Negative control imposed no changes in VF ($Z = 0.72$; $P = 0.47$). The magnitudes (deviation from the initial) of post-stimuli VFs were dependent on the stimulus and had a graded response (Fig. 1B). The sympatric predator produced the highest increase in VF, while VF responses to the allopatric predator and non-predator fish were similar to each other but higher than the negative control (Fig. 1B, Kruskal Wallis test; $H = 28.18$; $P < 0.0001$).

3.2. Swimming activity

Fish had similar levels of activity before exposure to any stimuli and hence started from a similar baseline level of activity (Fig. 2A, Kruskal Wallis test; $H = 0.29$; $P = 0.97$). Comparing activity before and after visual stimuli presentation (Wilcoxon test), we found that Nile tilapia

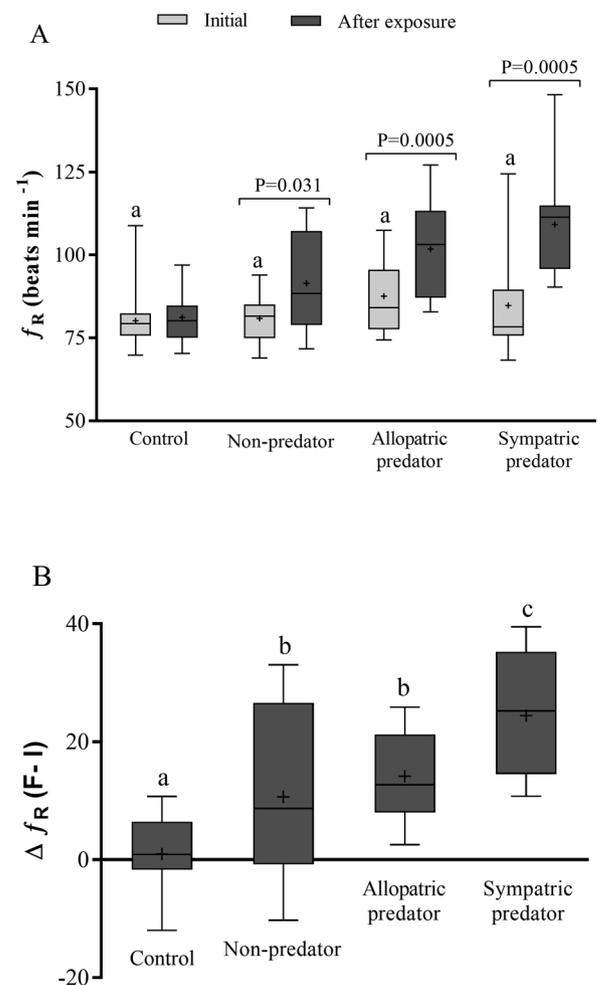


Fig. 1. Ventilatory frequency (A) and delta of VF (B) in the Nile tilapia ($N = 16$), before and after visual exposure to water (control), non-predator fish, and the allopatric or sympatric predators. Values in the figure are median \pm interquartile range. The plus sign indicates the mean.

exposed to non-predator fish showed increased activity ($Z = 2.10$; $P = 0.036$) and showed decreased activity when facing the view of the sympatric catfish predator ($Z = 2.02$; $P = 0.043$). No changes were found for the negative control ($Z = 0.17$; $P = 0.87$) and allopatric catfish predator ($Z = 1.27$; $P = 0.21$). The deviation from initial (baseline value) was higher for Nile tilapia visually exposed to non-predator fish than those exposed to the sympatric predator, while the remaining comparisons were similar to each other (Fig. 2B, Kruskal Wallis test; $H = 9.28$; $P = 0.026$).

3.3. Attack number

Nile tilapia directed more attacks (bite rate) on the tank glass wall when the visual stimulus was the non-predator fish compared to the other conditions that were similar to each other (Fig. 3, Kruskal Wallis test; $H = 38.11$; $P < 0.001$). The proportions of tilapia that attacked the source of the visual stimuli were: negative control, 5/21 (24%); non-predator fish, 10/10 (100%); allopatric catfish, 12/21 (57%); and sympatric predator, 3/21 (14%). Proportion of tilapia that attacked non-predator fish (carp) was higher than the other fish. The proportion obtained for the allopatric catfish was higher than that for the sympatric catfish but similar to the negative control. The proportions observed for the sympatric catfish and negative control were similar.

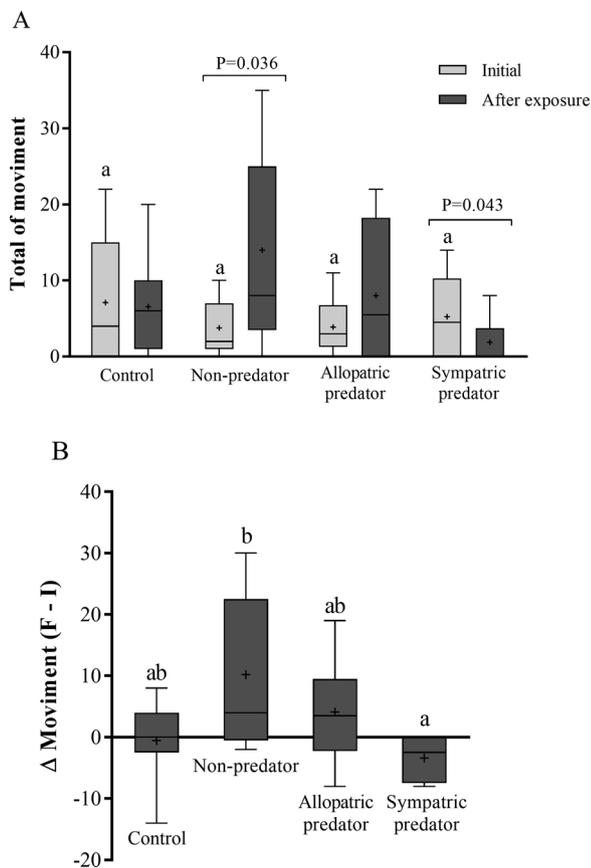


Fig. 2. Swimming activity (A) and delta of swimming activity (B) in the Nile tilapia (N = 8–9), before and after visual exposure to water (control), non-predator fish, and allopatric or sympatric predators. Values in the figure are median ± interquartile range. The plus sign indicates the mean.

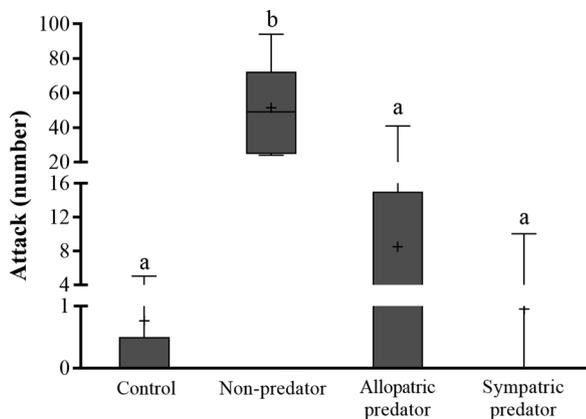


Fig. 3. Number of attacks by the Nile tilapia after visual exposure to water (control), non-predator fish, allopatric or sympatric predators (N = 10–21). Values in the text and figures are median ± interquartile range. The plus sign indicates the mean.

3.4. Plasma cortisol and glucose levels

The visual exposure to aquaria with no fish, non-predator fish, or the sympatric or allopatric predator induced no change (Fig. 4, revealed by one-way ANOVA) in plasma levels of cortisol ($F_{(3;60)} = 0.84$; $P = 0.47$) or glucose ($F_{(3;60)} = 1.43$; $P = 0.24$).

4. Discussion

Tilapia exposed to non-predator fish showed a higher swimming activity and number of attacks as well as a higher proportion of fish that attacked the visual stimuli compared to predator’s stimuli, indicating that tilapia were not intimidated and may have recognized the non-predator fish as a potential invader threat. Consequently, the responses to non-predator fish may be interpreted as territorial defense responses. However, fish exposed to both predators displayed an avoidance behavior with lower swimming activity and a significantly lower number of attacks and proportion of fish that attacked the visual stimuli compared to non-predator fish, suggesting a perception of both catfish as predator threat. Moreover, fish exposed to the sympatric predator had a significantly higher VF and lower proportion of fish that attacked the visual stimuli compared to the allopatric predator which indicates that tilapia had a stronger fear and avoidance responses of the sympatric predator. These results show that Nile tilapia is able to innately recognize both the allopatric and sympatric predator, and visually distinguish between the allopatric vs. sympatric stimuli.

Methodologically, we compared the tilapia’s responses to the predator catfish and to the non-predator fish (carp). Tilapia increased swimming and aggressive activities in response to non-predator fish, a condition that implies increased physical activity, making it necessary to increase oxygen uptake, a response that can be reached by increasing VF (Fernandes and Rantin, 1994), as we observed in this study. In addition, the proportion of Nile tilapia that attacked the non-predator fish was 100%, indicating that tilapia were not intimidated by the non-predator fish. Considering that Nile tilapia is a very territorial fish (Barreto et al., 2011, 2009), the observed behavior can be considered as a typical response to the invasion of a territory. Thus, taken together, we securely conclude that the responses to non-predator fish (carp) were not antipredator in nature.

Ventilatory frequency has been used as a behavioral tool to indicate the visual perception of a predator threat and alarm reaction in fish, such as for Nile tilapia (Barreto et al., 2003) and *Leporinus obtusidens* (Barbosa et al., 2012). In this study, Nile tilapia increased their VF when they were visually exposed to predators. We also observed that tilapia decreased swimming activity after exposure to the sympatric predator, while the tilapia’s exposure to the non-predator fish increased activity, and no significant difference was observed as a result of tilapia’s exposure to the allopatric predator. Moreover, tilapia clearly avoided attacking the sympatric predator and showed decreased swimming activity and increased VF. Thus, this elevation of ventilation in concomitance with decreased activity and fighting avoidance clearly indicates a defensive reaction (contrasting with tilapia’s territorial defense responses to the non-predator fish as we discussed above). In the case of the allopatric predator, the attacks occurred at a lower rate compared to the non-predator fish and in a similar magnitude to the tilapia’s exposure to the negative control (water) and the sympatric predator. The allopatric predator induced no effect on swimming activity but induced an increase in VF, also suggesting a stress response. In other studies, fish exposed to a predator also demonstrated hiding/freezing with a concomitant decrease in swimming activity (Holopainen et al., 1997), avoidance responses (decreased activity, keeping distance from predator) (Cantalupo et al., 1995) and defense responses (keeping the dorsal fin erect and exposing their fin spines) (Freitas and Volpato, 2008). Thus, we confirm that Nile tilapia exhibited a defensive behavior, i.e. an antipredator response (defensive reactions) when exposed to both an allopatric and a sympatric predator.

Predators are considered an important selective pressure for prey animals, leading to characteristic changes through evolution (Ferrari et al., 2010). Depending on the nature and duration of exposure, the sympatric predator acts as a selective force which may modulated prey’s biological traits, such as the ability to respond to a predator threat in a first encounter with a predator (innate recognition of and response to a threat). In this study, the fish exposed to the sympatric predator

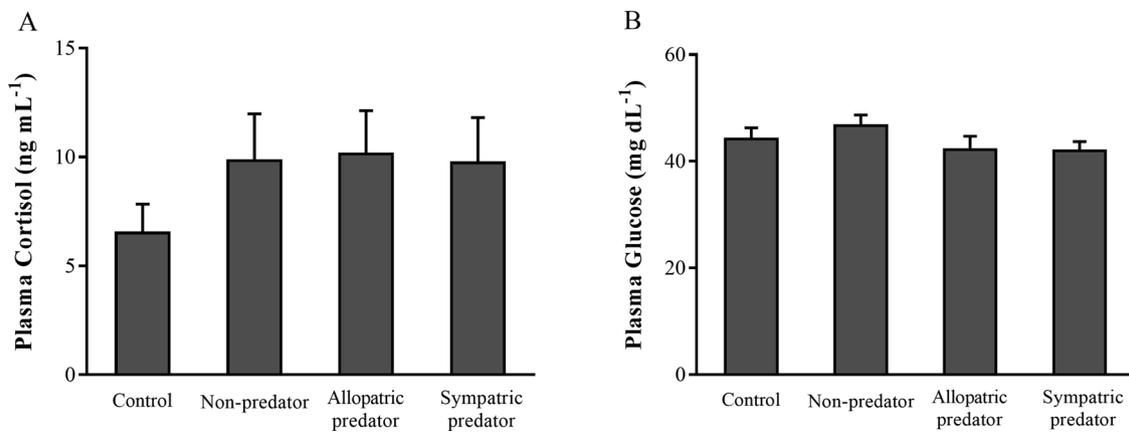


Fig. 4. Cortisol (A) and glucose levels (B) in the Nile tilapia (N = 16) after 30 min. of visual exposure to water (control), non-predator fish, and allopatric or sympatric predators. Values in the text and figures are means \pm 1 standard error of the mean. No significant ($P > 0.05$) differences were observed in plasma cortisol or glucose.

displayed a more intense antipredator defensive response when compared to the allopatric predator as discussed before (higher VF, lower swimming activity and number of attacks, and a significant reduction in the proportion of tilapia that attacked the predator). Different behavioral responses towards a sympatric predator in comparison to an allopatric predator were also observed in paradise fish (*Macropodus opercularis*; Gerlai, 1993) and zebrafish (*Danio rerio*; Bass and Gerlai, 2008), and have been attributed to the number of the key stimuli presents in the both fish that allow prey identify them as predators. In the current study, although in lesser magnitude, tilapia also innately responded to an allopatric predator as well, a factor that did not act as a selective force over tilapia evolution, because the allopatric predator used is a South American catfish and tilapia are an Africa cichlid. Thus, we hypothesized that visual stimuli present in the tilapia's sympatric catfish induce a clear defensive reaction and, because the allopatric catfish bears similar visual traits as native tilapia predators (e.g. whiskers and/or body shape and movement), tilapia is still able to respond innately to this allopatric animal. Considering this, the difference in tilapia's response magnitude is also assumed here as intrinsic to the number of key visual stimuli they can recognize. This is fascinating because tilapia has the ability to identify parts of a situation, making it possible to respond by analogy (i.e. association between key visual stimuli). In addition, tilapia were not intimidated by the non-predator fish and we could also in part attributed this result to the absence of visual cues such as a non-catfish body shape, different activity and body movement, and no whiskers.

Tilapia exposure to both predators increased VF, indicating an immediate stress response mediated by the sympathetic nervous system, typically named a 'fight or flight' reaction that prepares the body for an emergency action (e.g. escape), as we mentioned before. Interestingly, this stressor induced no activation of the hypothalamus-pituitary-interrenal axis because no elevation of plasma cortisol or glucose was observed. However, a neuroendocrine response can be absent in a short-term urgent response to a risky context (Abreu et al., 2016; Ide et al., 2003). Further, Miyai et al. (2016) exposed Nile tilapia to the odor of the same sympatric predator of our study and also observed no differences in plasma cortisol and glucose. Thus, we have no evidence to suggest that this occurred due to the strengthening of the stimuli, which were possibly mild stressors, or if it is the response strategy of Nile tilapia to deal with predators based on visual cues alone. In addition, deal with an elevation in cortisol is energetically costly for fish due to its several impacts in metabolism, osmoregulation, immune and hematological parameters (Wendelaar Bonga, 1997). Thus, cortisol elevation might not have represented an evolutionary advantage in face a visual exposure to predators. However, an increase in plasma glucose was expected in order to prepare and supply the organism with energy

for a potential escape, although it could occur anaerobically. At present, there is no explanation for this result and this question remaining opened for further studies.

The sympatric predators were approx. only 15% larger than the allopatric predators. Recently, Tang et al. (2017) investigated the effect of predator size (up to 2.7-fold for mass) on prey behaviors and found that prey responses to predators were not influenced by their relative size difference. Thus, the small difference between predator sizes could have no or only minor effects and is likely not fully responsible for triggering the different behavioral responses observed in our study between allopatric and sympatric predators. In addition, Bass and Gerlai (2008) demonstrated that a relatively large predatory fish does not represents the stimulus set that elicits a fear response in zebrafish, but rather some specific features unique to the sympatric predator.

In conclusion, we clearly demonstrate that VF can be used as a tool to evaluate fish's visual perception of a predator, and that Nile tilapia is able to visually identify and distinguish an allopatric from a sympatric predator. These findings indicates that exposure to a sympatric predator is important in distinguishing other possible interpretations of predator-naïve prey responses to an allopatric predator. Thus, considering that fear is fundamental to survival and triggers behaviours to protect the animal from threats (Kittilsen, 2013), and that tilapia used in this study were bred for innumerable generations in a hatchery, our results suggest that the recognition of the predators by Nile tilapia has an innate background. Visual recognition of a predator from a non-predator species in naïve fish was also observed by other authors (Barcellos et al., 2007; Gerlai, 1993). Additionally, the innate recognition of the allopatric predator could be attributed to some morphological similarities with the sympatric predator, suggesting an innate background of recognition of these characteristics. However, as the current study was not powered to distinguish which key stimuli elicits an antipredator responses, future studies should be devoted to elucidating how fish respond to key stimuli in the occurrence of defensive behavior that helps them adapt to deal with potential threats. Finally, tilapia is an invasive species and identified as a potential pest in many areas around the world (Linde et al., 2008), and the recognition of the South American catfish as an allopatric predator certainly represents an additional advantage that contributes to their ecological success along South American rivers.

Declarations of interest

None.

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References

- Abreu, M.S., Giacomini, A.C.V.V., Koakoski, G., Piato, A.L., Barcellos, L.J.G., 2016. Evaluating "anxiety" and social behavior in jundia (*Rhamdia quelen*). *Physiol. Behav.* 160, 59–65.
- Alvarenga, C.M.D., Volpato, G.L., 1995. Agonistic profile and metabolism in alevins of the Nile tilapia. *Physiol. Behav.* 57, 75–80.
- Arnott, G., Elwood, R., 2009. Probing aggressive motivation in a cichlid fish. *Biol. Lett.* 5, 762–764.
- Barbosa, A., Alves, F.L., Pereira, A.D.F., Ide, L.M., Hoffmann, A., 2012. Behavioral characterization of the alarm reaction and anxiolytic-like effect of acute treatment with fluoxetine in piauçu fish. *Physiol. Behav.* 105, 784–790.
- Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Quevedo, R.M., da Silva, L.B., Bedin, A.C., Finco, J., Cericato, L., 2007. Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. *Aquaculture* 272, 774–778.
- Barreto, R.E., Luchiar, A.C., Marcondes, A.L., 2003. Ventilatory frequency indicates visual recognition of an allopatric predator in naive Nile tilapia. *Behav. Process.* 60, 235–239.
- Barreto, R.E., Volpato, G.L., Faturi, C.D., Giaquinto, P.C., de Freitas, E.G., de Castilho, M.F., 2009. Aggressive behaviour traits predict physiological stress responses in Nile tilapia (*Oreochromis niloticus*). *Mar. Freshw. Behav. Phys.* 42, 109–118.
- Barreto, R.E., Barbosa, A., Giassi, A.C.C., Hoffmann, A., 2010. The 'club' cell and behavioural and physiological responses to chemical alarm cues in the Nile tilapia. *Mar. Freshw. Behav. Phys.* 43, 75–81.
- Barreto, R.E., Carvalho, G.G., Volpato, G.L., 2011. The aggressive behavior of Nile tilapia introduced into novel environments with variation in enrichment. *Zoology.* 114, 53–57.
- Bass, S.L.S., Gerlai, R., 2008. Zebrafish (*Danio rerio*) responds differentially to stimulus fish: the effects of sympatric and allopatric predators and harmless fish. *Behav. Brain Res.* 186, 107–117.
- Cantalupo, C., Bisazza, A., Vallortigara, G., 1995. Lateralization of predator-evasion response in a teleost fish (*Girardinus falcatus*). *Neuropsychologia* 33, 1637–1646.
- Chelini, M.C., Willemart, R.H., Hebets, E.A., 2009. Costs and benefits of freezing behaviour in the harvestman *Eumesosoma roeweri* (Arachnida, Opiliones). *Behav. Process.* 82, 153–159.
- DeGraaf, G., Galemoni, F., Banzoussi, B., 1996. Recruitment control of Nile tilapia, *Oreochromis niloticus*, by the African catfish, *Clarias gariepinus* (Burchell 1822), and the African snakehead, *Ophiocephalus obscurus*. 1. A biological analysis. *Aquaculture* 146, 85–100.
- Drossel, B., Higgs, P.G., McKane, A.J., 2001. The influence of predator-prey population dynamics on the long-term evolution of food web structure. *J. Theor. Biol.* 208, 91–107.
- Fernandes, M.N., Rantin, F.T., 1994. Relationships between oxygen availability and metabolic cost of breathing in Nile tilapia (*Oreochromis niloticus*) – aquacultural consequences. *Aquaculture* 127, 339–346.
- Ferrari, M.C.O., Wisenden, B.D., Chivers, D.P., 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can. J. Zool.* 88, 698–724.
- Foo, J.T.W., Lam, T.J., 1993. Serum cortisol response to handling stress and the effect of cortisol implantation on testosterone level in the Tilapia, *Oreochromis mossambicus*. *Aquaculture* 115, 145–158.
- Freitas, R.H.A., Volpato, G.L., 2008. Behavioral response of Nile tilapia to an allopatric predator. *Mar. Freshw. Behav. Phys.* 41, 267–272.
- Gerlai, R., 1993. Can paradise fish (*Macropodus opercularis*, Anabantidae) recognize a natural predator – an ethological analysis. *Ethology* 94, 127–136.
- Goodman, L.A., 1964. Simultaneous confidence-intervals for contrasts among multinomial populations. *Ann. Math. Stat.* 35, 716–725.
- Holopainen, I.J., Aho, J., Vornanen, M., Huuskonen, H., 1997. Phenotypic plasticity and predator effects on morphology and physiology of crucian carp in nature and in the laboratory. *J. Fish Biol.* 50, 781–798.
- Ide, L.M., Urbinati, E.C., Hoffmann, A., 2003. The role of olfaction in the behavioural and physiological responses to conspecific skin extract in Brycon cephalus. *J. Fish Biol.* 63, 332–343.
- Kittilsen, S., 2013. Functional aspects of emotions in fish. *Behav. Process.* 100, 153–159.
- Linde, A.R., Izquierdo, J.I., Moreira, J.C., Garcia-Vazquez, E., 2008. Invasive tilapia juveniles are associated with degraded river habitats. *Aquat. Conserv.* 18, 891–895.
- Maia, C.M., Volpato, G.L., 2018. Individuality matters for substrate-size preference in the Nile tilapia juveniles. *J. Appl. Anim. Welf. Sci.* 21, 316–324.
- Makiguchi, Y., Sugie, Y., Kojima, T., Naito, Y., 2012. Detection of feeding behaviour in common carp *Cyprinus carpio* by using an acceleration data logger to identify mandibular movement. *J. Fish Biol.* 80, 2345–2356.
- Mikheev, V.N., Pasternak, A.F., 2006. Defense behavior of fish against predators and parasites. *J. Ichthyol.* 46, S173–S179.
- Miyai, C.A., Sanches, F.H.C., Pinho-Neto, C.F., Barreto, R.E., 2016. Effects of predator odour on antipredator responses of Nile tilapia. *Physiol. Behav.* 165, 22–27.
- Sih, A., Englund, G., Wooster, D., 1998. Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* 13, 350–355.
- Tang, Z.H., Huang, Q., Wu, H., Kuang, L., Fu, S.J., 2017. The behavioral response of prey fish to predators: the role of predator size. *PeerJ.* 5.
- Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.* 77, 591–625.