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Short report

Ventilatory frequency indicates visual recognition of an allopatric predator in naïve Nile tilapia[☆]

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Abstract

Perceiving a possible predator may promote physiological changes to support prey 'fight or flight'. In this case, an increase in ventilatory frequency (VF) may be expected, because this is a way to improve oxygen uptake for escape tasks. Therefore, changes in VF may be used as a behavioral tool to evaluate visual recognition of a predator threat. Thus, we tested the effects of predator visual exposure on VF in the fish Nile tilapia, *Oreochromis niloticus*. For this, we measured tilapia VF before and after the presentation of three stimuli: an aquarium with a harmless fish or a predator or water (control). Nile tilapia VF increased significantly in the group visually exposed to a predator compared with the other two, which were similar to each other. Hence, we conclude that Nile tilapia may recognize an allopatric predator; consequently VF is an effective tool to indicate visual recognition of predator threat in fish.

Keywords: Fish; Nile tilapia; Predator recognition; Ventilatory frequency

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1. Introduction

Visual perceptual faculties provide some evidence of the extent to which animals obtain information from objects in their environment, where an alteration in their behavior may indicate visual awareness of a specific stimulus (Lomas et al., 1998). However, it is not always easy to discern what animals perceive. Hence, establishment of reliable indicators of animal visual recognition is undoubtedly crucial, because they will provide important information for our comprehension about the environmental visual perception in animals.

In predator–prey interactions, early detection of predator presence has an important role, because the animal may prepare its body for flight. In a situation that increases alertness, we may expect adjustment of ventilatory response, since one of the functions of the ventilatory system is to supply

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the body with enough oxygen for all behavioral tasks, such as escape maneuvers. A common ventilatory system adjustment to provide better oxygen uptake is increasing the ventilatory frequency (VF) (Fernandes and Rantin, 1994). For this reason, we suggest the hypothesis that VF will increase when a fish is visually exposed to a heterospecific predator fish. Thus, the aim of the present study was the assessment of fish VF as a behavioral tool to indicate visual recognition of a predator threat. We investigated this possibility measuring changes in VF of the fish Nile tilapia in response to a predator visual stimulus. VF was measured using a simple method of direct observation by counting the frequency of opercular or buccal movements, an easy technique that may provide a rapid and convenient measure of fish visual perception. Finally, VF has been used successfully to detect visual recognition in cephalopods (see, Boal and Ni, 1996), animals that have excellent vision (Hanlon and Messenger, 1996) like fish (Wheeler, 1982), which is one more indication that VF may be an effective tool to evaluate fish visual perception.

2. Material and methods

2.1. Fishes and holding conditions

Three fish species grown in a hatchery were used as our stock population. Juveniles of Nile tilapia, *Oreochromis niloticus*, were housed for about 1 year in 1200-l indoor tank (ca. 1 fish/4 l). Juveniles of pintado (a South American catfish), *Pseudoplatystoma curuscans*, and piauçu (a South American Anostomid), *Leporinus macrocephalus* were held in 20-l aquaria (1 fish per aquarium) for about 1 month. The water temperature averaged 24 °C and was continuously recirculated and aerated through a biological filter. The photoperiod was set from 06:00 to 18:00 h. Commercial dry pellets for tropical fish (Purina® ltda, Campinas, SP, Brazil) of optimal protein content (38%) was offered once per day in excess (over 5% of the fish biomass). There was no previous contact among these three species.

2.2. Experimental design and procedures

Nile tilapia juveniles were randomly chosen and isolated (1 fish per aquarium) in glass aquaria (28.0 × 11.4 × 19.6 cm) for 2 consecutive days. As reported by Höjesjö et al. (1999), food limitation affects heart rate responses, therefore, during these 2 days fish were not fed. Thus, all fish started at the same feeding condition. An aquarium with a heterospecific fish (pintado or piauçu) or only water was set next to each Nile tilapia aquarium and an opaque partition prevented visual contact between them before the tests. The basal values were determined by measuring VF five times every 1 min. After that, the partition was lifted and fish were exposed to a view of the heterospecific fish or to the aquarium with only water. VF was registered immediately after this procedure, every 1 min, up to the 14th min (totaling 15 observations). Three experimental conditions were set up: In the first condition, Nile tilapia was exposed to the view of a predator (pintado catfish). In the second condition, we used a harmless fish (piauçu) to test whether the effect of the view of any fish produced changes in VF. In the last condition, we used only the view of an aquarium with water but without any other fish, controlling handling processes. We set up seven replications of each condition.

Throughout all trials, mean water temperature was 24.75 ± 1.06 °C; and oxygen concentration was near saturation, ranging from 7.26 to 7.49 mg/l. The photoperiod was set up from 06:00 to 18:00 h.

Tilapia standard length and weight were not statistically different among the three conditions. Weight (ANOVA; $F(2; 14) = 1.116$; $P = 0.355$) (g; mean ± S.D.): 21.52 ± 4.58 (Control), 24.69 ± 6.19 (heterospecific non-predator), 25.51 ± 5.75 (heterospecific predator). Length (ANOVA; $F(2; 14) = 0.714$; $P = 0.506$) (cm; mean ± S.D.): 8.74 ± 0.59 (Control), 9.18 ± 0.73 (heterospecific non-predator), 9.42 ± 0.84 (heterospecific predator).

2.3. Ventilatory frequency

VF per minute was calculated by visually enumerating the time necessary for 20 successive opercular or buccal movements (adapted from Alvarenga and Volpato, 1995).

2.4. Statistical analyses

All data of the present study showed normal distribution. VF overtime was assessed by comparing post-stimulus VF of each group, divided in five blocks of three-sample intervals (0–2; 3–5; 6–8; 9–11; 12–14 min), with the pre-stimulus VF (basal level) by using repeated measure ANOVA. Moreover, VFs were studied using the difference between post- and pre-stimulation for each animal. Using the difference we corrected post-stimulus VF level by the basal measurement of each fish; thus each fish acted as its own control. VF difference of each moment was plotted on a graph resulting in an area which best represents an estimation of total beats during the observation period; thus performing this procedure we could demonstrate more clearly the effects of predator visual exposure on Nile tilapia VF. The mean total areas of the three groups were compared by ANOVA (completely randomized design) and followed by Newman–Keuls post hoc test (Zar, 1999).

3. Results

Before stimuli presentation, the mean basal VFs were statistically indistinguishable among groups (ANOVA; $F(2;\ 14) = 1.142$; $P = 0.347$) (beats per min; mean ± S.D.): 122.25 ± 18.17 (Control), 124.83 ± 21.34 (heterospecific non-predator), 117.39 ± 5.65 (heterospecific predator).

Throughout the 14-min period, VF increased over time in the heterospecific predator group when compared with its basal level (repeated measure ANOVA; $F(5;\ 70) = 3.418$; $P = 0.008$), but no difference was obtained for the other two groups (Fig. 1).

The mean total VF areas are expressed in Fig. 2. Considering the total VF area, statistical analysis showed that views of a heterospecific non-predator and control (only water) groups were similar to each other. However, the area of the heterospecific predator group was statistically greater compared with the other two groups (ANOVA; $F(2;\ 14) = 4.559$; $P = 0.029$).

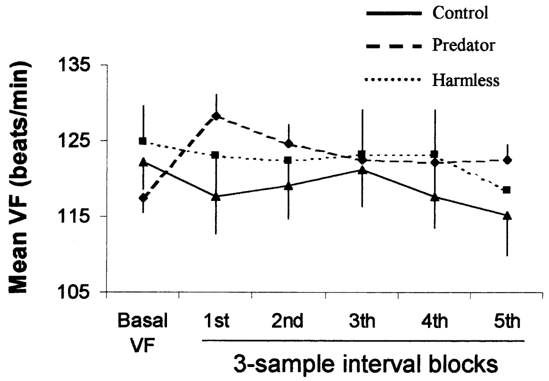

Fig. 1. VF overtime of the fish Nile tilapia, *O. niloticus*, visually exposed to stimuli (mean ± S.E.M.; $n = 7$). Only the heterospecific predator group showed a significant increase in VF when compared with basal VF over time (ANOVA; $P = 0.008$).

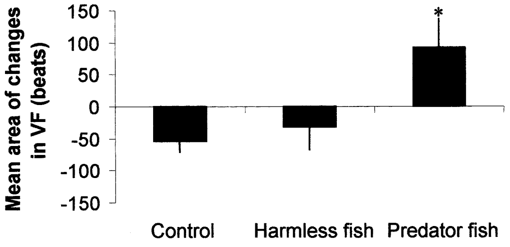

Fig. 2. Area of changes in VF of the fish Nile tilapia, *O. niloticus*, visually exposed to stimuli (mean ± S.E.M.; $n = 7$). *, Denotes statistically difference in relation to the harmless and control group (ANOVA; $P = 0.029$).

4. Discussion

In the present study, we showed that VF may be used as a behavioral tool to indicate fish visual perception of a predator threat. Moreover, we showed that Nile tilapia is able to visually identify and distinguish a predator from a heterospecific non-predator allopatric fish, an evidence of a possible innate basis, because fish used herein were naïve.

A first analysis attempts to evaluate the adequacy of basal VF measurements in this experiment. VF is a parameter inversely proportional to body size (Schmidt-Nielsen, 1996). In the present study, mean basal VF ranged from about 117 to 124 beats per min among groups. Similar results were reported for this species, such as: 76–81 beats per min in slightly longer fish (Volpato et al., 1989); about 50 beats per min in much longer fish (about 3.5 times longer; Fernandes and Rantin, 1989); and about 120 beats per min in fish of the same size as used in this study (Alvarenga and Volpato, 1995). These data support the acceptability of our measurement.

In the present study, Nile tilapia VF increased when they were visually exposed to a predator. However, when fish were visually exposed to an aquarium with only water or to a harmless fish, no elevation of VF was observed. Hence, fish hyperventilation occurred specifically in the presence of a predator. Thus, an increase in VF is closely associated with tilapia visual detection of a predator. Other studies have reported an increase in cardiac (Höjesjö et al., 1999) and ventilatory (Metcalfe et al., 1987) response to visual exposure to artificial modeling of predators in fish. Chemical stimuli also may increase fish VF, occurring when alarming substance is detected by a fish, the initial event of a fright reaction (Pfeiffer, 1962). Moreover, hyperventilation contributes to elevation of oxygen uptake in fish (Fernandes and Rantin, 1994), which is an essential body response to flight from a dangerous situation, such as predation risk. These reports support the association of VF to a predation threat observed in the present study. Thus, we may conclude that VF indicates visual recognition of predator threats in fish, a precise behavioral tool to understand fish visual perception of a predator. Furthermore, VF is a very sensitive parameter because a rapid visual perception of a predator was sufficient to evoke a sudden VF increase.

Another possibility to consider is that fish may avoid a predator not by fleeing but by staying and hiding with a concomitant decrease in activity, along with a decline in ventilation and heart rate, as reported by Holopainen et al. (1997). Thus, decrease in VF would be a response to visual exposure to predator, and, also may be hypothesized as an indicator of predator visual recognition. Another point is that domestication may alter cardiac response to predator exposure (Johnsson et al., 2001) in the Atlantic salmon, *Salmo salar*. Since the tilapia used here have been bred for many generations in a hatchery without wild parents, VF response of Nile tilapia may also be altered. Thus, future experiments focused on the VF response of domesticated- and wild-Nile tilapia may be subject for further researches.

As observed, Nile tilapia certainly recognizes allopatric predators because VF were clearly affected by the view of a pintado (predator) in relation to piauçu (harmless fish) and the aquarium with only water. Nevertheless, this species is not sympatric with pintado or piauçu. Thus, we may not conclude a clear innate recognition. However, the quality of Nile tilapia response evidently showed that it can distinguish between these two species, which may be an indication of a possible innate background. Moreover, Nile tilapia is sympatric with other African predator catfish. Thus, morphological aspects of pintado (primitive features maintained in pintado, e.g. barbells) might have been the key stimuli for Nile tilapia recognition of them as a predator or non-predator, modulating its response. As we proposed, a similar kind of recognition was reported by Csányi (1986), Altbäcker and Csányi (1990). These authors showed that eye-like spots act as a sufficient key stimulus in the recognition and avoidance of a predator by the paradise fish (*Macropodus opercularis*). Furthermore, tilapia used here were naïve (no previous contact with other fish species had been performed); thus it is impossible that the VF of Nile tilapia faced with the predator could be a learned response. Visual recognition of a predator from a non-predator species in naïve fish was obtained by other authors, and associated with genetic factors (Gerlai, 1993; Jordão and Volpato, 2000). As our results show a clear recognition of the predator, we may propose that the Nile tilapia recognition of some allopatric predator have an innate background, suggesting a possible genetic basis.

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