



## Effects of predator odour on antipredator responses of Nile tilapia



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### HIGHLIGHTS

- Predator odour induce anti-predator behaviour.
- Predator odour induce an increase in ventilation rate.
- Increasing of ventilation rate to predator odour prepare Nile tilapia for 'fight or flight'.
- Predator odour did not activate the hypothalamic-pituitary-interrenal axis.

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### ABSTRACT

Several fish species exhibit antipredator responses when exposed to chemicals which indicate risk of predation. One such substance is the scent of a predator (a kairomone) that may induce defensive responses in a potential prey. In the present study, we show that chemical cues (odour) from predator fish induce antipredator and stress responses in Nile tilapia. When exposed to predator odour, Nile tilapia decreased activity and increased ventilation rate (VR), but no increase in plasma levels of cortisol and glucose was found. Although the hypothalamic-pituitary-interrenal axis (HPI axis) was not activated, an increase in ventilation is a fast response which can provide the fish enough oxygen for a 'fight or flight' event when facing a predator. Thus, this respiratory response suggests an anticipated adjustment in order to prepare the body for a defensive response, such as escaping, irrespective of HPI axis activation.

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### 1. Introduction

In predator-prey interactions, the ability to recognize in advance the threat of a potential predator plays a key role in the survival of prey for any animal species [1]. Several fishes exhibit defensive responses when exposed to chemical cues that indicate predation risk [2]. These cues are well-documented as perceived stressors in fish [2–5] and can evoke a coordinated set of behavioural and physiological responses to cope with the threat, enabling the animal to maintain its homeostasis [5]. These cues can be derived from injured conspecifics, (alarm cues [2,6] conspecific blood [7]), dead conspecifics [8], disturbed and non-injured conspecifics [9], injured heterospecific sympatric species [3] or predator odours (kairomones [10,11]). In the case of predator chemical cues, diet may interfere with detection of predators; for instance, prey may detect a predator's odour more easily, as evidenced by most drastic antipredator responses after a predator has fed on conspecific prey [12,13].

To avoid predation, fish have evolved many strategies to deal with such pressures [14]. In these strategies, prey may elicit behavioural

responses, reducing the risk of detection by decreasing locomotor activity [15] or being immobile [16,17]. Further, to escape from a predator after being detected, prey may migrate [18], flee [19] or seek refuge [20–22]. One common antipredator fish behaviour in response to predator chemical cues is a decrease in swimming activity [6,15,21,23]. Minimizing locomotor activity can reduce water vibrations and detection by predators, increasing prey survival chances [3,15].

Although behavioural responses in fish are generally observed when predator odour is present, physiological responses are also required to overcome the threat of predation [24]. These responses have been broadly described as primary, secondary, or tertiary [5]. Primary responses involve some initial neuroendocrine feedbacks after sympathetic autonomic nervous system activation, resulting in catecholamine release from the chromaffin tissue (hypothalamic–sympathetic–chromaffin axis; HSC) [25], and/or the hypothalamic-pituitary-interrenal (HPI) axis stimulation, culminating in discharge of corticosteroid hormones (e.g. cortisol) into the circulation [26,27]. This is accompanied by rapid cardiorespiratory adjustments [5,28] as VR increases [5,6,29]. Secondary responses occur when stressors related to predation risk persist and are characterized by metabolic changes, such as increased plasma glucose levels [5,26,30]. Additionally, tertiary responses

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may occur in chronic stress situations, constricting the animal's well-being, affecting growth, resistance to disease and reproductive capacity [5,31]. In this context, previous studies have reported antipredator behaviour [9,11,19,20], VR increase [29,32,33], cortisol release [27,34,35] and enhanced metabolic response [36–38] in fish exposed to a predator cue. However, none of these provide a link between primary (antipredator behaviour, and increase of VR and plasma cortisol) and secondary responses (increase of plasma glucose).

We selected the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758; -Acanthopterygii; Perciformes; Cichlidae) as our experimental model, a cichlid fish species that visually recognizes and executes antipredator behaviour in the presence of predator fish [39,40] and responds to chemical alarm cues [6,41]. Furthermore, this species also exhibits varied responses to different stressors, such as increasing VR, plasma cortisol and glucose levels [6,42,43]. Thus, we performed three experiments to further investigate the hypothesis that Nile tilapia submitted to the odour of a natural predator which had been fed tilapia, the African catfish *Clarias gariepinus* (Burchell, 1822; Ostariophysi; Siluriformes; Clariidae), could detect and recognize these chemical cues as a predation risk and respond accordingly both behavioural and physiologically.

## 2. Material and methods

### 2.1. Fish and stock conditions

Juvenile Nile tilapias were provided by a commercial fish farmer and were kept in three distinct 310 L tanks. Fish were immature, without sex differentiation, with a (mean  $\pm$  SD) standard length of  $6.37 \pm 0.21$  cm and a mass of  $8.60 \pm 1.06$  g. We used as a natural predator the African catfish, kept in a separate 310 L tank, without sex differentiation. As non-predator control, we used the common carp (*Cyprinus carpio*; Linnaeus, 1758; Ostariophysi; Cypriniformes; Cyprinidae), kept in a separate 310-L tank), also without sex differentiation. Predator and non-predator specimens were obtained from a commercial fish farmer. All fishes were maintained in indoor tanks in appropriate stock density (0.5 g/L) for at least 30 days prior to experimentation and fed commercial fish food to satiation once a day (Guabi - Pira 32% protein). Temperature in all tanks was  $26 \pm 1.5$  °C, in which ammonia (<0.5 ppm) and nitrite (<0.05 ppm) levels were controlled by biological filtration and constant water changes (dechlorinated tap water). The photoperiod was kept constant (12L:12D). None of Nile tilapia had previous contact with either African catfish or common carp.

### 2.2. Experimental design

In experiment 1, we tested the effect of predator odour on prey swimming activity. For such purpose, Nile tilapia were exposed to 50 mL of natural predator odour (African catfish) that had been fed tilapia, 50 mL of non-predator odour (common carp, used as control for the predator odour), or 50 mL of water control. Based on similar studies conducted in other fish species that considered changes in swimming activity as an antipredator response [6,15,21,23], locomotion decrease due to African catfish odour indicated that Nile tilapia recognized this chemical as a predation risk, inducing a typical behavioural response. Thereafter, we conducted a second experiment, to investigate the effect of predator odour on VR as an indicator of physiological responses. We exposed Nile tilapia to either 50 mL of predator odour or 50 mL of water control. A rapid VR response to predator odour indicated the Nile tilapia sympathetic autonomic nervous system had been activated and the primary responses (catecholamine and corticosteroid hormones release) had occurred. Results from experiments 1 and 2 provide evidence to hypothesize that predator odour might have stimulated the Nile tilapia HPI axis, thus causing the release of corticosteroid hormones and increased plasma glucose levels. In light of this, we carried out a third experiment in order to evaluate the effect of predator odour on plasma levels of cortisol and glucose over time, in which Nile tilapia were

exposed to 50 mL of predator odour or 50 mL of water control at 0 min, 15 min, 30 min, 60 min, 120 min and 240 min after initial exposure. An increase in cortisol and glucose plasma levels would indicate the HPI axis activation and metabolic responses, respectively.

### 2.3. Predator and non-predator odour collection

Two African Catfish (standard length: 16.20 cm and 16.60 cm; weight: 35.93 g and 43.75 g, respectively) were individually housed for six days in 42 L aquaria (40  $\times$  30  $\times$  35 cm) with temperature, photoperiod and water conditions similar to those in the stock. African catfish are active, piscivorous and natural predators of Nile tilapia [44–47] and, to ensure that all predators had been fed and subjected to a standardized procedure, fish were fed twice during this period (second and fourth day) with Nile tilapia fresh fillets (with skin, but scaleless; 3% of African catfish weight per feeding). African catfish necessitate 40 h at a temperature of 30 °C to completely evacuate their gut [48], so on the sixth day (i.e. 48 h after the catfish were last fed, to ensure stomachs were empty), fish were rinsed, transferred and individually housed in 22 L aquaria (40  $\times$  24  $\times$  23 cm; stocking density 2 g/L) containing fresh dechlorinated tap water and an air stone, but no filter, and kept for 24 h, without food. After this period, each fish was removed and the water was filtered (to avoid any visible particles), stored into 50 mL aliquots and frozen at  $-20$  °C until required for testing. Non-predator odour was obtained from two common carp (standard length: 15.40 cm and 14.20 cm; weight: 88.43 g and 77.45 g, respectively; stocking density 4 g/L), kept under the same conditions used to collect predator odour. Carp odour was diluted in fresh dechlorinated tap water to attain the catfish odour concentration. Instead of Nile tilapia fillets, carp were fed with the same commercial fish food used in stock conditions (second and fourth day; 3% of carp weight per feeding; Guabi - Pira 32% protein). Procedures were adapted from Ferrari et al. [11].

### 2.4. Experiment 1

We evaluated the effect of predator odour on Nile tilapia behaviour. Fish were exposed to 50 mL of one of the following chemical cues: odour of predator fed on tilapia ( $n = 10$ ); odour of non-predator, used as control for predator odour ( $n = 12$ ); or deionized water ( $n = 9$ ) as a control for presence/absence of chemical cues and lab conditions, totalling 31 individuals. Fish from the stock population were randomly chosen, individually housed and acclimated in glass tanks (40  $\times$  24  $\times$  23 cm; 22 L) for 60 h prior to experimentation. Each aquarium was supplied with constant aeration and water temperature was maintained at  $26 \pm 1.5$  °C. Ammonia (<0.5 ppm) and nitrite (<0.05 ppm) levels were controlled by water changes and the photoperiod was kept constant (12 L:12D). Food was offered once a day, but not on the day of the experiment. After the acclimation period, baseline of behaviour was recorded during 5 min and for another 5 min after inserting one of the stimuli into the experimental aquaria. The assessed behavioural response was locomotion, measured by the number of quadrant changes. Each aquarium had on its back a polystyrene plate divided in nine quadrants (13.3  $\times$  8.0 cm). A quadrant change was considered when at least 75% of the fish's body crossed the line [7]. Locomotion values were considered as the deviation from the initial condition (post-stimulus minus baseline). All movements were quantified using camcorders, set up 1.5 m in front of the experimental aquaria.

### 2.5. Experiment 2

In experiment 1, we found evidence to support the hypothesis that Nile tilapia recognize predator odour as a predation risk by decreasing their swimming activity. Therefore, we conducted a second experiment to investigate whether Nile tilapia VR would increase in response to predator odour. Fish were individually housed and acclimated in the

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