



Brief communication

Ventilation responses to predator odors and conspecific chemical alarm cues in the frillfin goby



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ABSTRACT

The chemical detection of predation risk is direct when based on predator odors, or indirect when an injured conspecific or heterospecific signal it. Physiological adjustments may be necessary in parallel to defensive reactions to cope with an imminent risk. Here, we tested the effects of predator odors and conspecific chemical alarm cues in ventilation response (VR) of frillfin goby, *Bathygobius soporator*, because this response increases oxygen uptake for supporting behavioral tasks. No VR change was detected in response to odors of predators (catfish) that fed on conspecific, heterospecific fish (tilapia), or were deprived of food and to non-predator (tilapia) that fed chow (non-specific odor control) and odor eluent. The goby's VR, however, increased in response to conspecific alarm cues, but not to heterospecific cues or eluent. Clearly, the VR response in fish depends on the nature of the chemical cue. It is in line with 'threat-sensitive hypothesis' as a chemical cue from an injured prey might mean a foraging predator, whilst the mere presence of a predator odor might not. In addition, because VR can increase, decrease or remains unchanged in response to predation risk in other fish species (including other gobies), we reinforces the species-specific characteristic of VR responses in fish, regarding the results obtained here for frillfin gobies.

1. Introduction

The perception of a potential threat imposed by predators is essential for prey survival. The detection of chemical cues that indicates predation risk can be direct, when threat assessment is based on predator odor [10,24]. However, it can also be indirect, when a conspecific or heterospecific prey chemically signals the imminent risk [10,24]. In the case of fish, some species have cells located in the epidermis that when damaged by a predator attack release a substance in water (alarm substance) that induces defensive responses in conspecifics [10]. For instance, detection of alarm substance results in changing the priority of responding to relevant environmental stimuli in fish, such as decreased foraging [15] or territorial defense [4,30].

Overall, the behavioral responses in these contexts have been well documented, but the physiological correlates have not. The predation risk (and other stressful situations) increases alertness, and respiratory adjustments can take place. An increase of ventilation rate (VR) might be a way of preparing the body for escaping tasks since a function of the

ventilatory system is to supply the body with enough oxygen [12]. In fact, increased VR is a response when fish faces a predation risk based on predator odors (direct cues) in Atlantic salmon *Salmo salar* [20–22,29] and Nile tilapia *Oreochromis niloticus* [31] or alarm substances (indirect cues) in Nile tilapia [4,34] and rainbow darters *Etheostoma caeruleum* [19].

Many predators rely on prey's body movements as stimuli to track them [7,8]. Thus, responding to a threat by reducing the VR would increase crypsis, particularly if the prey concomitantly adopts a postural immobility to become less susceptible to detection by predators. In addition, if crypsis fails, a decreased VR would not impair an emergency escape maneuver, because a small escape swimming could be performed anaerobically, requiring no elevation of the VR. In this context, in the presence of a foraging predator, and consequently its odor (direct cue), the strategy of hypoventilation could be a possible action. However, only chemical alarm cues (indirect cues) have been shown to induce hypoventilation in fish, such as in pintado speckled catfish *Pseudoplatystoma coruscans* [5], piauçu fish *Leporinus*

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macrocephalus [3] and the round goby *Neogobius melanostomus* [32].

Fish defensive responses in terms of increase or decrease VR could be supposed as a result of the locomotion strategy adopted: dashing/increased activity or freezing/decreased activity, respectively. However, it is not clear and does not have this positive association between VR and locomotion strategy, because predator odors (Atlantic salmon - [20–22]; Nile tilapia - [31]), as well as alarm substance (Nile tilapia - [4,34]) can lead to freezing or decreased activity with hyperventilation. Thus, no clear prediction can be made regarding how VR is expected to change with perceived threat.

These disparate observations in VR (hyper- or hypoventilation) point toward the need for better comprehending the effects of chemical cues that indicates predation risk in a greater number of fish species. In this context, we wonder if the cue nature (a direct or indirect indicator of risk) could be an important point. Based on this, the present study evaluates the effects of these direct and indirect chemical cues in ventilation rate of frillfin goby (*Bathygobius soporator* - locally known as amborê). The frillfin goby is an avid predator of rocky shores and estuaries that consume small fish and crustaceans [26,37]. However, these fish are also preyed by larger voracious predators, such as catfish [9]. The frillfin goby is also reactive to alarm substance [6]. Accordingly, the gobies exposed to this stimulus exhibited sheltering and decreased swimming activity.

2. Materials and methods

2.1. Fish and holding conditions

Specimens of Frillfin goby were collected from the estuary of the Itanhaem River in Itanhaem city, São Paulo state, Brazil. Funnel traps baited with shrimp were used for capturing fish. The traps were sunk into the estuary water near rocks. After a few minutes, the traps were withdrawn from the water and the captured frillfin gobies were gently placed into plastic lidded buckets filled with seawater. This procedure was repeated several times to have a suitable sample size. Constant aeration was provided to bucket water, from the field to the laboratory, using battery-operated air pumps and air stones connected via plastic tubing (nontoxic silicone). No mortality was observed. After collection, the fish (mean length of ~9 cm, standard length of ~7.5 cm and about 6–7 g of body mass) were acclimated in plastic stock tanks for 15 days before experimental procedures (12 fish/70 L water; stock density = 1.0 g/L of water). The tanks were at constant room temperature (~24 °C) using air conditioning equipment. The water was continuously aerated by air pumps connected to the air stones via nontoxic silicone tubing and with biological and mechanical filtrations. The fish were exposed to natural photoperiod and indirect illumination through lab windows. The fish were fed once a day at 4% of biomass with commercial feed for bottom-dwelling carnivorous fish [36% protein; Poytara carnivoros de fundo® (Bottom-dwelling carnivorous fish chow), Poytara LTDA, Araraquara — SP, Brazil].

2.2. General experimental strategy

The basic strategy was to evaluate the ventilation rate (VR) of the frillfin gobies, *Bathygobius soporator*, (Valenciennes, 1837; Fig. S1) before and after exposure to chemical cues. These cues used here have the potential to indicate, directly (predator odors - Experiment 1) or indirectly (alarm cues - Experiment 2), a presence of a threat imposed by a foraging predator. All subjects in both experiments 1 and 2 were experimentally naive, used only in a single trial.

In experiment 1 (prey direct assessment of predator presence), the frillfin goby (n = 6/each condition) was exposed to predator odor (the madamango sea catfish *Cathorops spixii*). Odor was obtained from predators fed on: 1) conspecific (*B. soporator*); 2) allopatric heterospecific (Nile tilapia *Oreochromis niloticus* – to control the specificity of a potential kairomone); or 3) no food (food deprived catfish). In addition,

for controlling the specificity of response and lab procedures, response to the odor of: 4) Nile tilapia fed on commercial pellets (nonspecific odor – control kairomone) and 5) artificial seawater without any biological scent (eluent - control) were also assessed.

Experiment 2 (prey indirect assessment of predator presence), we exposed the fish (n = 10/each condition) to skin extracts of: 1) conspecific (alarm substance); 2) heterospecific fish (Nile tilapia - control alarm substance); and 3) extract eluent (distilled water). This chemical cue of a heterospecific fish was used to confirm if the response of frillfin goby to conspecific skin extract was specific or a general response to chemical stimuli from any injured fish (based on [25,27]). In a previous study [6], frillfin goby was not behaviorally reactive (in terms of sheltering and locomotion activity) to heterospecific alarm substance (skin extract of platy fish *Xiphophorus maculatus*); however, we decided to include a heterospecific control (Nile tilapia skin extract) herein, because we aimed to assess another response variable, the VR, that is a physiological stress variable linked to sympathetic autonomic nervous system. This heterospecific fish, Nile tilapia (Cichlidae), is a phylogenetically distant species from goby species (Gobiidae) and are therefore unlikely to contain the same chemical alarm cues, hence, a suitable heterospecific control fish.

2.3. Experimental design

For both experiment 1 and 2 the following experimental protocol was conducted. Specimens of frillfin gobies from the stock population were randomly selected and housed in individual glass aquaria (23 L; 40 × 23 × 25 cm; water volume of 20 L; 1 fish/aquarium). The fish were acclimated to the experimental aquaria conditions for five days, where they were fed daily as described above, and the leftover food was removed after 1 h. After this period, fish VR was assessed for 3 min before stimulus presentation (baseline), one time each minute. Next, a chemical stimulus was injected into the experimental aquaria, and fish VR restarted to be assessed 10 s later of that for an additional 3 min (post-stimulus period). The mean baseline and post-cue VRs of each singleton were considered for analysis. A methylene blue dye test demonstrated that the colorant was completely spread throughout the aquarium in ~8 s. Behavior recording occurred from 11 h to 14 h in a random order defined by raffle.

2.4. Chemical cues

The predator (catfish) and non-predator (tilapia) odors were obtained following the procedures that we adapted from Ferrari et al. [13]. Odors from catfish that fed on frillfin goby, tilapia or food deprived ones were obtained from six catfish (mean length of ~22 cm) individually housed for six days in 23-L aquaria (40 × 23 × 25 cm; water volume of 20 L) with temperature, photoperiod and water conditions similar to the experimental aquarium. Fishes were fed twice during this period (second and fourth day) with prey fillets with skin. On the seventh day, catfish were captured, rinsed with artificial sea water and transferred to other 23-L aquaria, where they were kept for 24 h in the same conditions described before, but were not fed. After this period, each fish was removed and water was filtered into 50 mL aliquots and frozen at –20 °C until its use is required for the test trial. The non-predator odor was obtained from two Nile tilapia (mean length of ~18 cm, mean weight of ~170 g) with the same procedures used to collect predator odor, but Nile tilapia were fed (equivalent of 3% of body mass = 5 g) with commercial fish chow (32% protein). Aliquots of artificial sea water were used as procedures control. The 50-mL aliquots were carefully injected, with an aid of syringe attached to a nontoxic silicon tubing, onto the surface of the water from behind a curtain.

The skin extract was prepared as described by Giaquinto and Volpato [18]. The skin donor fish were sacrificed by cephalic contusion without the use of anesthetics to prevent interference from chemical

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