Conspecific and heterospecific alarm substances induce behavioral responses in juvenile catfish *Rhamdia quelen*

Carina Vogel, Paula D. Weber, Carla Lang and Bernardo Baldisserotto

The recognition of chemical information indicating the presence of a predator is very important for prey survival. In this study we tested antipredator behavioral response of juvenile silver catfish (*Rhamdia quelen*) against predator odor released by two different potential predators, *Hoplias malabaricus* and the snake *Helicops infrataeniatus*, and alarm cues and disturbance cues released by conspecifics and by non-predator species, *Megaleporinus obtusidens* and *Astyanax lacustris*. We used juvenile catfish that were naive to predators. The trials consisted of a 10-min prestimulus and a 10-min post-stimulus observation period. The behavioral response displayed by silver catfish exposed to alarm cues comprised a decrease in shelter use and an increase in locomotion, and also a longer latency period before feeding. Our results showed that juvenile silver catfish can perceive chemical cues released by predators, heterospecifics and conspecifics.

Keywords: Alarm cues, Anti-predator behavior, Disturbance cues, Predator odor, Skin extract.

O reconhecimento das informações químicas indicando a presença de predadores é muito importante para a sobrevivência da presa. Neste estudo foi testada a resposta comportamental anti-predação de juvenis de jundiás (*Rhamdia quelen*) a substâncias liberadas por dois predadores potenciais, *Hoplias malabaricus* e a cobra *Helicops infrataeniatus*, e substâncias de alarme liberadas por conspecíficos e pelas espécies não predadoras *Megaleporinus obtusidens* e *Astyanax lacustris*. Foram usados juvenis de jundiá que não possuíam nenhum contato prévio com predadores. Os testes consistiram em observações de períodos de 10 minutos pré estímulo e 10 minutos pós estímulo. As respostas comportamentais apresentadas pelos jundiás expostos às substâncias de alarme consistiram em diminuição no uso do abrigo, aumento na locomoção e também um longo período de latência antes de ingerir o alimento. Os resultados demonstram que juvenis de jundiá percebem substâncias químicas liberadas por predadores, heterospecíficos e conspecíficos.

Palavras-chave: Comportamento anti-predador, Extrato de pele, Odor do predador, Sinais de alarme, Sinais de perturbação.

Introduction

Predation is a strong selective force that shapes behavior, life histories and morphological features in prey (Lima, Dill, 1990; Chivers, Smith, 1998). The ability of prey to discern predators and avoid their threat improves the chance of surviving (Chivers *et al*., 1996). The detection of predator odor by the prey allow them to evade predators from a distance or predators waiting in ambush (Ferrari *et al*., 2010). A second class of pre-attack chemical information comes from chemicals, essentially urinary ammonia (Wisenden, 2015), released by startled or disturbed prey and are referred as conspecific odor. A third class of cues is damaged-released alarm cues.

Predators may damage prey tissue, particularly epidermal tissue, during the attack and subsequent handling of prey prior to ingestion. Damaged epidermal tissues leak chemical compounds that are released in no other context and thus reliably advertise the presence of an actively foraging predator (Wisenden, 2015). All major groups of aquatic organisms, from protists to amphibians, show antipredator responses to alarm cues released from injured conspecifics (Wisenden, 2000).

Chemical alarm signals have been attributed to the superorder Ostariophysi, which includes the Cypriniformes, Characiformes and Siluriformes (Scott *et al*., 2003). The silver catfish *Rhamdia quelen* (Quoy & Gaimard, 1824) is a Siluriformes with Neotropical

Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. (CV) carinavogel@gmail.com, (PDW) paulaweberbio@gmail.com, (CL) carlangbio@gmail.com, (BB) bbaldisserotto@hotmail.com (corresponding author)

distribution, endemic to South America and has high commercial value, being widely employed in aquaculture in southern Brazil (Baldisserotto, 2009). This species responds to alarm cues and predator odor (Kochhann *et al.*, 2009; Weber *et al.*, 2012).

Our objective is to identify if juvenile silver catfish can recognize chemical cues released by two different potential predators, *Hoplias malabaricus* (Block, 1794), a piscivorous fish (Peretti, Andrian, 2008) and *Helicops infrataeniatus* Jan, 1865, a snake with fishes in its diet (Aguiar, Di-Bernardo, 2004). *Helicops infrataeniatus* is a is a potential predator of silver catfish because is commonly found in fishponds in southern Brazil (Giraudo, 2001) and may eventually present a threat to fish farms, bringing losses to production. We also observed silver catfish behavior against disturbance and alarm cues released by conspecifics and the heterospecifics omnivorous and non piscivorous species *Megaleporinus obtusidens* (Valenciennes, 1837) and *Astyanax lacustris* (Lütken, 1875) (Santos, 2000; Vilella *et al.*, 2002). Our hypothesis is that silver catfish will recognize the predator odor of both species and the chemical signals from the conspecifics. We predicted that the fish will present some of the typical antipredator behavior as reduction of inactivity *e.g.* Wisenden *et al.* (2008), increasing use of shelters *e.g.* Wisenden *et al.* (2010) and decreasing foraging *e.g.* Chivers, Smith (1998).

Material and Methods

Fish collection and stimulus preparation. Silver catfish juvenile (3.75 ± 0.52 cm, 0.43 ± 0.16 g) were obtained from two artificial reproductions at the fish culture laboratory of the Universidade Federal de Santa Maria, southern Brazil, and maintained at the Fish Physiology Laboratory of the same institution for 40 days prior to the experiments. The fish were kept in 40-L aquaria and fed three times daily with commercial food (Purina, 45% crude protein). Water was siphoned from the aquaria once a day and all waste was removed with suction.

To obtain the alarm cues, skin extracts for silver catfish, *A. lacustris* and *M. obtusidens* were prepared according to the method of Brown, Smith (1998). Three individuals from each species were selected and immediately killed with a blow to the head. The skin was removed from both sides of each fish (total 5 g of skin), rinsed with distilled water and placed in 50 mL of cold distilled water. The mixture of water and skin was homogenized and filtered through glass wool. The filtrate was diluted to a final volume of 400 mL with distilled water. The skin extract was stored in 25 mL samples at -20°C prior to use. Distilled water was stored in 25 mL samples at the same temperature for use as a control.

To obtain the disturbance cues, three individuals of each species of silver catfish, *M. obtusidens* and *A.*

lacustris were kept separately in a continuously aerated 20-L aquarium for 15 hours. All species were feed with the same commercial food. The water from each aquarium was stored in a refrigerator at 5°C and subsequently used as the conspecific odor cues stimulus.

To obtain predator odor, one specimen of *H. malabaricus* (500 g) and one of *H. infraeniatus* were collected from the wild, quickly transported to the laboratory, kept in an 250-L aquarium for three days at 24°C and fed with five silver catfish juveniles (*ca.* 5.5 cm in length) daily. Each predator was then transferred to a continuously aerated 20-L aquarium and kept in the aquarium for 15 hours. Water from the aquarium was stored in a refrigerator at 5°C and subsequently used as the predator odor stimulus. The *H. malabaricus* and *H. infraeniatus* specimens returned to the wild. Catalog numbers of voucher specimens: *Astyanax lacustris* UFRGS 8426, *Hoplias malabaricus* MCP 37829, *Megaleporinus obtusidens* UFRGS 5176 and *Rhamdia quelen* UFRGS 14114.
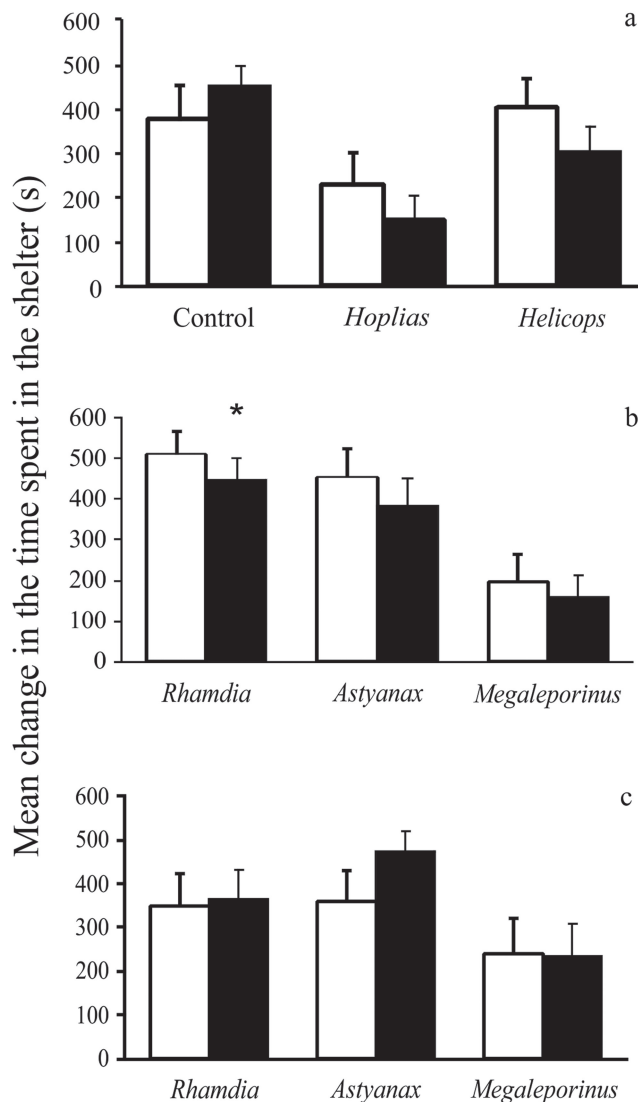
Experiment. Silver catfish were fed and then transferred from the 40-L aquaria to 2-L polyethylene aquaria, where they remained isolated for 24 h before testing began. The experimental aquaria contained a marked midline and a shelter (4x4x4 cm) located at one end of the tank. Each behavioral observation (N = 12 fish for each test, in individual aquaria) lasted 20 min. The observations were conducted using procedures similar to those described by Kochhann *et al.* (2009). The trials consisted of a 10-min pre-stimulus and a 10-min post-stimulus observation period. A 1-mL stimulus sample (distilled water, predator odor, conspecific odor, alarm cues) was added after the pre-stimulus period. The time that the fish spent in the shelter and the number of midline crossings (as an indication of locomotor activity) during the pre- and post-stimulus periods were recorded as described by Scott *et al.* (2003). We also recorded the latency to first feeding attempt.

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This study was approved by the Ethics Committee on Animal Experimentation of UFSM under registration number 25/2007.

Statistical analysis. We calculated the difference between the pre-stimulus and post-stimulus observation periods (post minus pre) for control and experimental trials for time the fish spent in the shelter, the number of midline crossings and time to first feeding attempt, using the non-parametric Wilcoxon Signed-Rank Test. Each analysis was conducted for each chemical cue separately. Data were expressed as mean + SEM. The minimum significance level was set at P < 0.05.
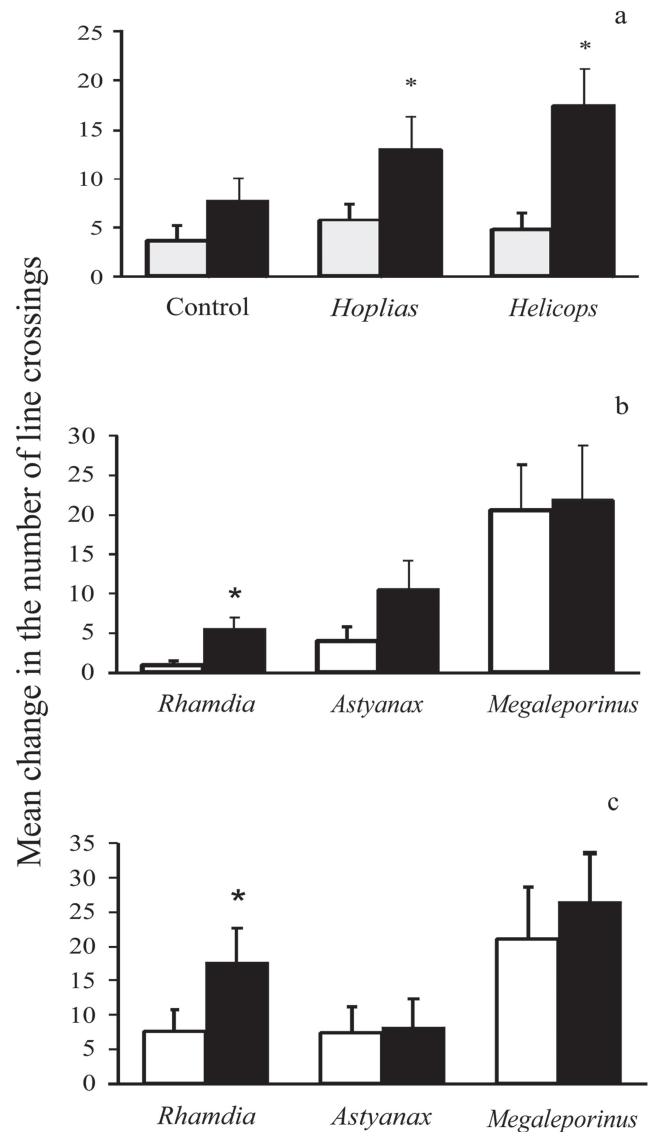
Results

Comparison between post- and pre-stimulus observation periods showed that unexposed juvenile catfish significantly decreased shelter use in response to conspecific odor (V = 66, p = 0.03418). There was no significant difference in shelter use when juvenile were exposed to predators' odor (*Hoplias*: V = 46, p = 0.0665; *Helicops*: V = 50, p = 0.1422), heterospecifics odor (*Astyanax*: V = 49, p = 0.1682; *Megaleporinus*: V = 33, p = 1) or to different alarm cues from conspecifics (V = 25, p = 0.8385) and heterospecifics (*Astyanax*: V = 16, p = 0.2622; *Megaleporinus*: V = 20.5, p = 0.7792) (Fig. 1).

Likewise, there was no significant difference in line crosses when juveniles were exposed to different heterospecifics odor (*Astyanax*: V = 6.5, p = 0.123; *Megaleporinus* V = 39, p = 1) and alarm cues from heterospecifics (*Astyanax*: V = 10, p = 0.5505; *Megaleporinus* V = 10, p = 0.0827). However, there was an increase in swimming activity when fish were exposed to odor from both predators (*Hoplias*: V = 6, p = 0.0322; *Helicops*: V = 4.5, p = 0.0127), heteropecifics odor (V = 0, p = 0.00903) and to alarm cues from conspecifics (V = 6, p = 0.0578) (Fig. 2).
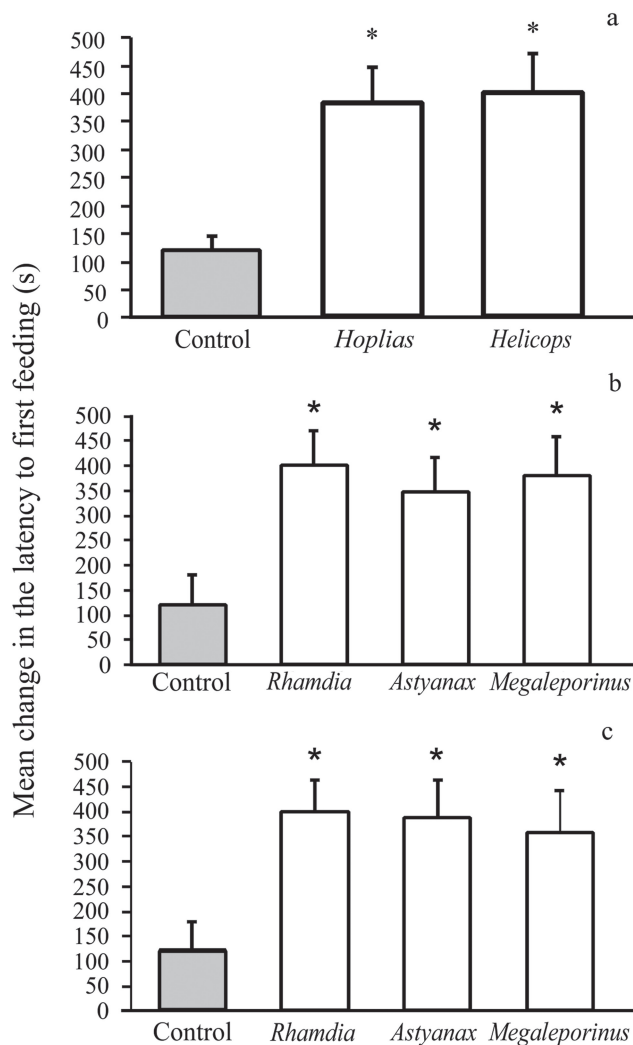

Fig. 1. Mean (± SE) in the time spent in the shelter by juvenile catfish after exposure to **a.** odor of predators *Hoplias malabaricus* and *Helicops infrataeniatus*; **b.** odor and **c.** skin extract of conspecifics and non-predators *Astyanax lacustris* and *Megaleporinus obtusidens*. Open bars = pre stimulus; solid bars = post-stimulus; *indicates a significant difference (p < 0.05).

Fig. 2. Mean (± SE) change in the number on line crossings of juvenile catfish after exposure to **a.** odor of predators *Hoplias malabaricus* and *Helicops infrataeniatus*; **b.** odor and **c.** skin extract of conspecifics and non-predators *Astyanax lacustris* and *Megaleporinus obtusidens*. Open bars = pre stimulus; solid bars = post-stimulus; *indicates a significant difference (p < 0.05).

Silver catfish individuals significantly increased the latency to first feeding in response to predators odor (*Hoplias*: V = 5, p = 0.0048; *Helicops*: V = 7, p = 0.0233;), conspecific (*Rhamdia*: V = 11, p = 0.0309) and heterospecifics odors (*Astyanax*: V = 12, p = 0.0341; *Megaleporinus* V = 6, p = 0.0068) and alarm cues (*Rhamdia*: V = 12, p = 0.0375; *Astyanax*: V = 10, p = 0.021; *Megaleporinus* V = 9, p = 0.0366) when compared do control (Fig. 3).

Fig. 3. Mean (± SE) change in Latency to first feeding (s) of juvenile catfish after exposure to **a.** odor of predators *Hoplias malabaricus* and *Helicops infrataeniatus*; **b.** odor and **c.** skin extract of conspecifics and non-predators *Astyanax lacustris* and *Megaleporinus obtusidens*. Solid bars = control; Open bars = disturbance treatment. *indicates a significant difference when compared to control (p < 0.05).

Discussion

There was some variability in the pre-stimulus response of silver catfish juveniles to time spent in the shelter and in the number of line crossings, mainly those related to the tests with odor and alarm cues from *Megaleporinus*.

Probably this difference is due to the use of a different juvenile population in this analysis, which was done later. All juveniles had the same size range, but those from the experiments with *Megaleporinus* were descendants from a different pair than those from the other experiments. Variations in the response of water controls was also observed in yellow perch (*Perca flavescens*) Harvey, Brown (2004).

The typical Ostariophysans antipredator behavior includes shoal cohesion, seek refuge, decrease movement, decrease foraging, freezing, dashing, avoid alarm cue, change body shape and no response (Lawrence, Smith, 1989; Chivers, Smith, 1998; Wisenden *et al*., 2008; Wisenden *et al*., 2010). Our results demonstrate that juvenile silver catfish are able to sense chemical cues present in the water due to change in their behavior when exposed to conspecifics and heterospecifics odor and alarm cues and predator odor.

The behavioral response displayed by silver catfish exposed to alarm cues comprised no change in shelter use and an increase in locomotion, mainly by using odor stimuli from predators and conspecifics, and a long period of latency before feeding in all experiments. Weber *et al*. (2012) observed a decrease in shelter use in juvenile silver catfish exposed to alarm cue, but no change in locomotion. Kochhann *et al*. (2009) found that silver catfish larvae exposed to conspecific skin extract or to predator odor increased the time spent in the shelter and decreased the number of line crossings, a usual antipredator response (Wisenden *et al*., 2008, 2010). These contrasting results might be a consequence of differences in fish size (larvae 0.027 g, juvenile 1.1 g) used in the two experiments. Apparently, the silver catfish larvae seek concealment because of their small body size, whereas the juvenile increase their amount of movement probably to avoid predator's odor stimulus. However, when adult silver catfish were exposed to conspecifics alarm cues they remained immobile in the first minutes and then increased swimming activity (Souza-Bastos *et al*., 2014), as observed in the present study. Furthermore, *Rivulus hartii* (currently *Anablepsoides hartii*) and yellow perch also demonstrated significant size-dependent trends in response to heterospecific and conspecific alarm cues, respectively, with smaller individuals exhibiting antipredator responses and larger individuals shifting their behavior to increased levels of activity consistent with a foraging or predatory response (Harvey, Brown, 2004; Elvidge *et al*., 2010).

The chemical cues may serve as alarm signals to warn nearby heterospecifics of potential danger and may provide a survival benefit for receivers (Mathis, Smith, 1993; Wisenden *et al*., 1999; Mirza, Chivers, 2003), as described for finescale dace (*Phoxinus neogaeus*) and fathead minnows (*Pimephales promelas*), that learn to respond to heterospecific alarm signals (Chivers, Smith, 1998). This association allows prey to detect predators and execute antipredator responses (Magurran, 1989; Chivers, Smith, 1998; Ferrari *et al*., 2010; Wisenden, 2015). Experience or

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learning may be involved in cross-species responses to alarm signals. For example, when two species occupy similar microhabitats and are exposed to the same predators, they may have the opportunity to associate predation risk with heterospecific alarm cues (Chivers, Smith, 1994). Although the silver catfish used in the present study had never been exposed to heterospecific alarm cues and it seems they can discriminate between predator and no predator using odor, as experiments demonstrate an increase in locomotion. Furthermore, our results showed also the detection of heterospecific alarm cues because, at least, silver catfish increased the latency of first feeding. A similar result was found in juvenile yellow perch, which changed shoaling index, but not shelter use, in response to skin extract of swordtail (*Xiphophorus helleri*), a species phylogenetically distant from perch (Mirza *et al.*, 2003).

Silver catfish increased the number of line crossings and increased time to first feeding when exposed to odor of both predators, a fish (*Hoplias*) and a reptile (*Helicops*) that were fed with silver catfish. Juvenile lemon damselfish (*Pomacentrus moluccensis*) exposed to the odor of the predator moon wrasse (*Thalassoma lunare*) fed fish did not change line crosses, but reduced feeding strikes (Mitchell *et al.*, 2015). On the other hand, sea lamprey (*Petromizon marinus*) was attracted by the odor of the snake *Nerodia sipedon*, and authors considered it a predator inspection behavior (Imre *et al.*, 2014). Thus, an increase in line crossing in this study suggests a recognition of predator and non-predator by the odor; and also, a detection of heterospecific and conspecific alarm cues due to changes on feeding behavior. Altogether, our study shows that silver catfish uses chemical communication in defensive behavior, by responding to chemical cues from different predator species, and by responding to alarm cues.

Acknowledgments

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Submitted April 18, 2016
Accepted March 10, 2017 by Eliane Gonçalves-de-Freitas