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Antipredator responses of *Polypedates maculatus* tadpoles to kairomones from the carnivorous tadpoles of *Hoplobatrachus tigerinus*

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Most anuran amphibians opportunistically breed in temporary ponds and their larvae live in such waters until metamorphosis (SAIDAPUR 2001, MOGALI et al. 2021). Hence, tadpoles of different species that co-occur face threats from desiccation, crowding, and from predators (SKELLY 1997, SAIDAPUR 2001, BENARD 2004, MOGALI et al. 2011, 2016, 2017). Temporary ponds house diverse types of predators, mostly aquatic insects or their larvae, and carnivorous or omnivorous anuran tadpoles (SKELLY 1997, SAIDAPUR et al. 2009, MOGALI et al. 2015). Furthermore, the temporary ponds where tadpole species reside are often turbid or contain aquatic vegetation that obscures visual cues to detect environmental changes. In such conditions, chemical cues are more useful to detect predators (KIESECKER et al. 1996, HICKMAN et al. 2004, SAIDAPUR et al. 2009). Earlier studies have shown that the source of chemical cues emanating from predators and detected by prey tadpoles may differ (SHARMA et al. 2008, MOGALI et al. 2011, 2012, SCHERER & SMEE 2016). The chemical cues may arise from the starved predators, kairomones (SCHOEPPNER & RELYEA 2005), dietary metabolites derived following consumption of conspecific prey and released through feces (WILSON & LEFCORT 1993, LAURILA et al. 1997, MOGALI et al. 2012), alarm pheromones released by injured prey (SCHOEPPNER & RELYEA 2005), or a combination of these factors (SCHOEPPNER & RELYEA 2009 a, b). Earlier studies also revealed that anuran tadpoles showed a variety of antipredatory behaviours to chemical cues of predators. That includes increased activity or high swimming speed in order to escape from predators (MOGALI et al. 2021), reduction of activity (SAIDAPUR et al. 2009, MOGALI et al. 2012), aggregation (SPIELER & LINSENMAIR 1999), or increased use of refuge sites (HOSSIE & MURRAY 2010, MOGALI et al. 2019, 2022).

The Indian tree frog, *Polypedates maculatus* (GRAY, 1834; Anura: Rhacophoridae), is widely distributed in India. It breeds between June–August in South India and females are known to deposit eggs in foam nests attached to vegetation or underneath stones above a water body and bushes over puddles (GIRISH & SAIDAPUR 1999). Development occurs inside the foam nests up to Gosner stage 23 after which tadpoles drop into the water to undergo further development and metamorphosis. The tadpoles of *P. maculatus* are dull green or muddy dark dorsally and ventral surface is whitish. The snout is semicircular, eyes are round and lateral, nares are round and dorsal, and intestine is invisible from the ventral side. Spiracle is single and is found up to Gosner stage 42. The tadpoles attain their maximum size between Gosner stages 37–40. The tadpoles of *P. maculatus* mostly live at the pond bottom and some extent in the water column. They feed on vegetation and detritus matter using serrated jaw sheaths and blunt teeth. The tadpoles are found associated with tadpoles of *Duttaphrynus melanostictus*, *Hoplobatrachus tigerinus*, *Fejervarya limnocharis*, *Euphlyctis cyanophlyctis*, *Microhyla ornata*, and *Sphaerotheca breviceps* (GIRISH & SAIDAPUR 1999, HIRAGOND & SAIDAPUR 2001, HIRAGOND 2002, SAIDAPUR et al. 2009).

Construction of the foam nest for the protection of eggs and early development of tadpoles is a strategy adopted by *P. maculatus* possibly for avoiding predation during early development. During our regular field visits, we noticed that herbivorous tadpoles of *P. maculatus* are preyed by carnivorous tadpoles of *H. tigerinus*. Most of the tadpole prey-predator interactions studies focused mainly on aquatic insects, fishes, or salamanders as predators (CHIVERS & MIRZA 2001, MATHIS 2003, MOGALI et al. 2020, 2022). However, so far there seems to be a paucity of research showing the effect of carnivorous tadpole

predators on the behavioural responses of herbivorous tadpoles. Hence, in the present work, we studied the behavioural responses of *P. maculatus* tadpoles to stimulus solution (kairomones) of the carnivorous predator, tadpoles of *H. tigerinus*. We hypothesized those *P. maculatus* tadpoles, which coexist with their predator in the natural water bodies, should show antipredatory behavioural responses to predator kairomones.

Three foam nests of *P. maculatus* were sighted in temporary ponds during the rainy season (July 2011) on the Karnataka University Campus, Dharwad (latitude 15.440407° N, longitude 74.985246° E), Karnataka State, India. The nests were attached to the vegetation ~20 cm above the water surface. They were immediately brought to the laboratory and each foam nest was placed in a separate plastic container (32 cm in diameter and 14 cm deep) with 1 l of dechlorinated (aged water) tap water along with some substratum collected from the same pond. The tadpoles emerged after four days from foam nests at Gosner stage 23 (GOSNER 1960). Tadpoles from all three nests were then mixed and reared (50 tadpoles from each nest, i.e., 150 tadpoles in total) in a glass aquarium (LWH 90 × 30 × 15 cm) containing 25 l of aged tap water and used for the experiment. The tadpoles of *H. tigerinus* (Gosner stages 29–30; mean total length 32.20 ± 0.55 mm, N = 30) were also collected from the same pond where the foam nests were collected. They were reared individually to avoid cannibalism in plastic containers (19 cm in diameter and 7 cm deep) in 0.5 l of aged tap water. Tadpoles of *P. maculatus* are herbivores and hence were fed with boiled spinach, whereas *H. tigerinus* tadpoles were fed with *P. maculatus* tadpoles during the study.

Preparation of kairomones — The *H. tigerinus* tadpoles were placed individually in separate plastic containers (N = 25 containers; 19 cm in diameter and 7 cm depth) containing 200 ml of aged tap water without food for 96 h to eliminate diet-derived excretory metabolites from the stimulus solution, resulting in a stimulus solution with only kairomones (MOGALI et al. 2012, 2020). After 96 h of starvation, predators were removed from the container and the stimulus solution was filtered (to remove completely, if any, a minute quantity of fecal matter present in the stimulus solution) and used immediately for experimental trials to provide potential kairomones of the predators.

Behavioural responses of *P. maculatus* tadpoles to kairomones of *H. tigerinus* — The behavioural responses of *P. maculatus* tadpoles to kairomones were recorded by placing a single test tadpole (*P. maculatus*; Gosner stage 27–28; mean total length 26.35 ± 0.60 mm) in a rectangular glass tank (LWH 28 × 15 × 15 cm) containing 600 ml of aged tap water. A handycam (Sony, DCR-SR300/E) was fixed above the tank such that it recorded the entire area. The handycam was connected to a computer with the Ethovision Video Tracking System (Noldus Information Technology, The Netherlands) to track the movements of the tadpole before and after the addition of stimulus solution to the test tank. The Ethovision system was used to record maximum

swimming speed (V_{max}), distance traversed by the tadpole, number of swimming spurts and time spent swimming during an entire trial. For each trial, a tadpole of *P. maculatus* was first introduced into the tank and left undisturbed for 5 min. A burette was placed ~1 cm above the water level and 50 ml of aged tap water (chemical blank) was then added at the rate of ~1 ml/s to simulate the disturbance of the later chemical cue would cause. The burette was then removed gently. Movements of the tadpole were then recorded for 5 min using Ethovision to record the baseline activity in the absence of any cues. After tracking baseline activity, 50 ml of stimulus solution containing kairomones of a predator was added as described above. Movements of the tadpole were recorded for another 5 min to determine the activity pattern after exposure to kairomones. Twenty-five trials were conducted. The data on the behavioural responses of *P. maculatus* tadpoles, before and after the addition of the stimulus solution (kairomones) were compared using the Paired-Samples t-test (SPSS ver. 16.0).

Behavioural responses of *P. maculatus* tadpoles to kairomones of *H. tigerinus* — Upon exposure to predators' kairomones, *P. maculatus* tadpoles showed a significant in-

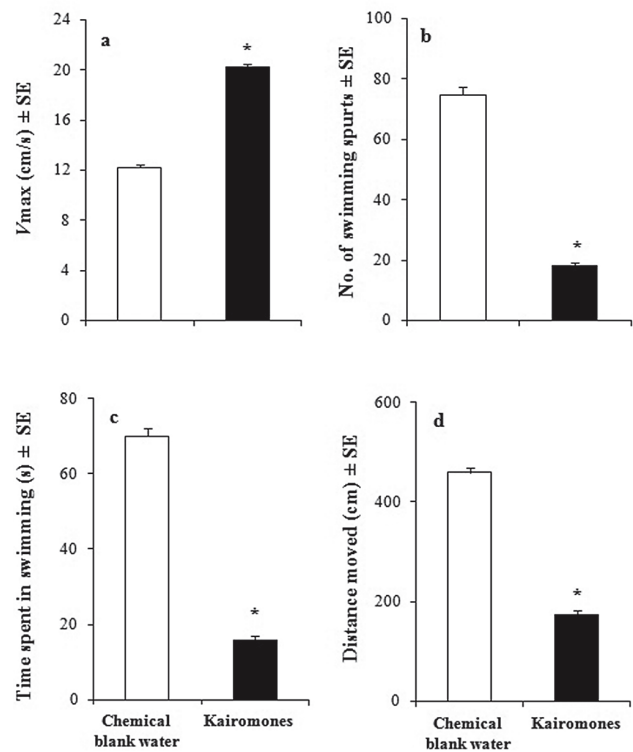


Figure 1. Maximum swimming speed (V_{max}) (a), Swimming spurts (b), Time spent in swimming (c), and Distance moved (d) by tadpoles of *Polypedates maculatus* exposed to chemical blank water (aged tap water) or water conditioned with starved (kairomones) predator, *Hoplobatrachus tigerinus*. Data are represented as mean ± SE; N = 25 trials; data analyzed by Paired-Samples t-test. Asterisks over the bars indicate significant difference between the two groups.

crease in V_{\max} ($T_{24} = -22.648$, $P < 0.01$, Fig. 1a), and a significant decrease in the number of swimming spurts ($T_{24} = 18.660$, $P < 0.01$, Fig. 1b), time spent swimming ($T_{24} = 22.541$, $P < 0.01$, Fig. 1c) and total distance moved ($T_{24} = 20.169$, $P < 0.01$, Fig. 1d) when compared to their baseline activities in stimulus-free water.

In natural aquatic environments, larval anurans are at risk of predation, but the degree of their risk is dependent on their defenses, which have evolved to promote their escape from predators and promote survival (SCHMIDT & AMEZQUITA 2001, RELYEA 2007). In aquatic environments, various types of chemical cues (e.g., kairomones of predators, alarm cues of damaged conspecifics and dietary metabolites of predators) affect the behavioural responses of prey (WILSON & LEFCORT 1993, WISENDEN 2000, SCHOEPPNER & RELYEA 2005, MOGALI et al. 2012). The existence of kairomones (from the body of the starved predator) that elicit antipredator behaviour in prey has been reported in a broad spectrum of animals including anuran tadpoles (KATS & DILL 1998, FERRARI et al. 2010, MOGALI et al. 2011). The results of the present study showed that *P. maculatus* sensed kairomones of a predator (*H. tigerinus*) and quickly decreased their activity levels (i.e., less time spent in swimming, less distance traversed and less number of spurts) during the trial period. Furthermore, it is interesting to note that whenever the *P. maculatus* tadpole moved in the stimulus solution (kairomones), their spurt speed (V_{\max}) was higher than in the stimulus blank solution, indicating their efforts to escape from the perceived kairomones of the predator. Our results are in conformity with earlier studies on *Bufo melanostictus* tadpoles (MOGALI et al. 2011). Thus, *P. maculatus* tadpoles appear to perceive *H. tigerinus* tadpoles as potential predators. This may be because of long ecological co-existence of *P. maculatus* tadpoles with sympatric carnivorous tadpoles such as *H. tigerinus* may have led to the evolution of antipredator defence strategies in response to kairomones of these predators. In summary, tadpoles of *P. maculatus* perceive predation risk by detecting kairomones emanating from the predator, *H. tigerinus* and shown appropriate antipredator behavioural responses.

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