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Antipredator Behavioural Responses of Tadpoles of the Indian Painted Frog *Uperodon taprobanicus* (Parker, 1934) (Microhylidae) to Kairomones from the Predatory Tadpoles of the Indian Bullfrog *Hoplobatrachus tigerinus* (Daudin, 1803) (Dicroglossidae)

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Abstract: In aquatic systems, many prey animals, including anuran tadpoles, predominantly use chemical cues to assess predation threat. In such systems, an array of chemical cues (e.g., kairomones, alarm, dietary) affects the behavioural responses of the prey tadpoles. The study on the behavioural responses of tadpoles belongs to the family Microhylidae to predators are very less hence the outcome of this study will provide a novel information in the field of behavioural ecology of anuran tadpoles with reference to prey-predator interactions. The behavioural responses of tadpoles of Indian painted frog *Uperodon taprobanicus* (Microhylidae) to its naturally co-existing predatory tadpoles of the Indian bullfrog *Hoplobatrachus tigerinus* (Dicroglossidae) were studied in the laboratory. The predator's kairomones (water conditioned by a starved predator) was used to simulate predation threat. The results of the experiment showed that *U. taprobanicus* tadpoles exhibited strong antipredator behavioural responses, i.e. drastically reduced swimming movements and overall time spent in swimming as well as had a higher burst speed in response to kairomones of predators. Thus, *U. taprobanicus* tadpoles consider *H. tigerinus* tadpoles as potential predators. This may be because of long ecological co-existence of *U. taprobanicus* tadpoles with sympatric carnivorous tadpoles, *H. tigerinus* may have led to the evolution of antipredator defence strategies in response to kairomones of these predators. In summary, tadpoles of *U. taprobanicus* perceive predation risk by detecting kairomones emanating from the predator *H. tigerinus* and showed appropriate antipredator behavioural responses.

Key words: Amphibians, burst speed, chemical cues, prey-predator interactions, stimulus solution, swimming activity.

Introduction

The majority of amphibian species opportunistically breed in ephemeral ponds and their larvae live in such waters until metamorphosis (Wilbur 1980, Newman 1992). Hence, all tadpole species reside in such waters face risks from crowding, desiccation as well as from predators (Wilbur 1980, Loman 1999, Benard 2004). Ephemeral ponds are the home of diverse types of predators, mainly aquatic insect larvae and carnivorous anuran tadpoles (Skelly 1994, 1997). In addition, the ephemeral ponds where tad-

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pole species reside are often turbid or contain aquatic vegetation that obscures visual cues to detect environmental changes. In such circumstances, chemical cues are more helpful to detect predators (Kiesecker et al. 1996, Hickman et al. 2004). Earlier studies have shown that the source of chemical cues emanating from predators and detected by prey tadpoles may differ. Chemical cues may arise from kairomones of starved predators (Kiesecker et al. 1996, Schoeppner & Relyea 2005) or dietary metabolites derived from consumption of prey released through faeces (Wilson & Lefcort 1993, Laurila et al. 1997) or alarm cues released by injured prey (Schoeppner & Relyea 2005) or combination of these factors (Schoeppner & Relyea 2009a,b). Earlier studies also revealed that anuran larvae showed a variety of antipredatory behaviours in response to chemical cues of predators. These include increased swimming speed in order to run away from predators (Dayton et al. 2005), overall reduction in activity levels (Lawler et al. 1989, Berec et al. 2016) or increased use of hiding places (Hossie & Murray 2010) and dense aggregation (Spieler & Linsenmair 1999).

In and around the city of Dharwad, Karnataka State (South India), many amphibian species (including the present study species – Indian painted frog *Uperodon taprobanicus* (Parker, 1934) of the family Microhylidae) reproduce in ephemeral ponds formed during the South-West monsoon (Saidapur 2001). The tadpoles *U. taprobanicus* are mainly bottom dwellers and thrive on detritus and algal matter. The visibility is generally low in these waters due to shadows from vegetation, turbid water and the benthic area that is naturally covered by leaf litter and detritus (authors' observations). These water bodies are also home to several groups of invertebrate and vertebrate predators, including the carnivorous tadpoles of the Indian bullfrog *Hoplobatrachus tigerinus* (Daudin, 1802) of the family Dicroglossidae. The tadpoles of *H. tigerinus* are voracious predators that hunt actively and detect their sympatric prey, including anuran tadpoles by means of both visual and chemical senses. During our routine field visits, we noticed that herbivorous tadpoles of *U. taprobanicus* are preyed upon by tadpoles of *H. tigerinus*. Previously, most of the tadpole prey-predator interactions studies have focused mainly on aquatic insects or its larvae, fishes or salamanders as predators (e.g., Skelly 1997, Chivers & Mirza 2001, Mathis 2003). So far, there seems to be paucity of research showing influence of carnivorous tadpoles on the behavioural responses of herbivorous tadpoles.

In the present work, in the laboratory, we systematically studied the behavioural responses of *U. taprobanicus* tadpoles to stimulus solution (kairomones) of the carnivorous predator, i.e. tadpoles of *H. tigerinus*. We hypothesised that those *U. taprobanicus* tadpoles, which coexist with their predator in the natural waters, should show antipredatory behavioural responses to predators' kairomones.

Materials and Methods

Experimental specimens

Tadpoles of *Uperodon taprobanicus* (Gosner stages 27-28, N = 100; see Gosner 1960) and *Hoplobatrachus tigerinus* (Gosner stages 31-32; N = 20) were collected from ephemeral ponds in the Karnatak University Campus, Dharwad (15.440407ºN, 74.985246ºE, altitude 750 m a.s.l.), Karnataka State, India. Tadpoles of both species were immediately transported to the laboratory and each species were reared separately.

Tadpoles of *U. taprobanicus* were reared in a glass aquarium (90 cm L × 30 cm W × 15 cm H) containing 25 L of aged tap water and used as a prey (test) tadpoles. Tadpoles of *H. tigerinus* were reared individually in a plastic tub (14 cm diameter and 7 cm deep) with 500 mL of aged tap water to avoid cannibalism and used as predators. Tadpoles of *U. taprobanicus* are herbivorous in nature, hence they were provided with boiled spinach.

Tadpoles of *H. tigerinus* are carnivorous in nature, hence were provided with tadpoles of *U. taprobanicus*. In all experimental trials, test tadpoles (*U. taprobanicus*) were of comparable body sizes (Total length 18.22 ± 0.72 mm, $\bar{x}$ ± SE; N = 25) and developmental stage (Gosner stages 27-28). The predators (*H. tigerinus*) were comparable in body size (Total length 37.14 ± 0.46 mm, $\bar{x}$ ± SE; N = 10) and developmental stage (Gosner stages 31-32).

Preparation of kairomones

The *H. tigerinus* tadpoles were placed individually in separate plastic containers (N = 10 containers; 19 cm diameter and 7 cm depth) containing 200 mL of aged tap water without food for 96 h to completely eliminate diet-derived excretory metabolites from the stimulus solution, resulting in a stimulus solution with only the kairomones (Mogali et al. 2020). After 96 h of starvation, *H. tigerinus* were removed from the container and the stimulus solution was filtered and used for experimental trials to provide potential kairomones of the predators.

Behavioural responses of *Uperodon taprobanicus* tadpoles to kairomones

The behavioural responses of *U. taprobanicus* tadpoles to kairomones from the *H. tigerinus* were recorded by placing a single test tadpole in a rectangular glass tank (28 cm L × 15 cm W × 15 cm H) containing 600 mL of aged tap water. A handycam (Sony, DCR-SR 300/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 test tadpole before and after addition of stimulus solution (predator's kairomones) to the test tank. The Ethovision System was used to record maximum swimming speed (V_{max}), total distance moved by the tadpoles, number of swimming spurts and total time spent in swimming during an entire trial. For each trial, a test tadpole, *U. taprobanicus* was first introduced into the test tank and left undisturbed for 5 min. A burette was placed ~1 cm above the water level and 50 mL of aged tap water (stimulus-free) 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 test tadpole were then recorded for 5 min using Ethovision to record the baseline activity in the absence of any chemical cues. After tracking baseline activity, 50 mL of stimulus solution (predator's kairomones) was added as described above. Movements of the test tadpoles 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 test tadpoles, *U. taprobanicus* before and after addition of the stimulus solution were compared using the Paired-Samples t-test (SPSS ver. 16.0).

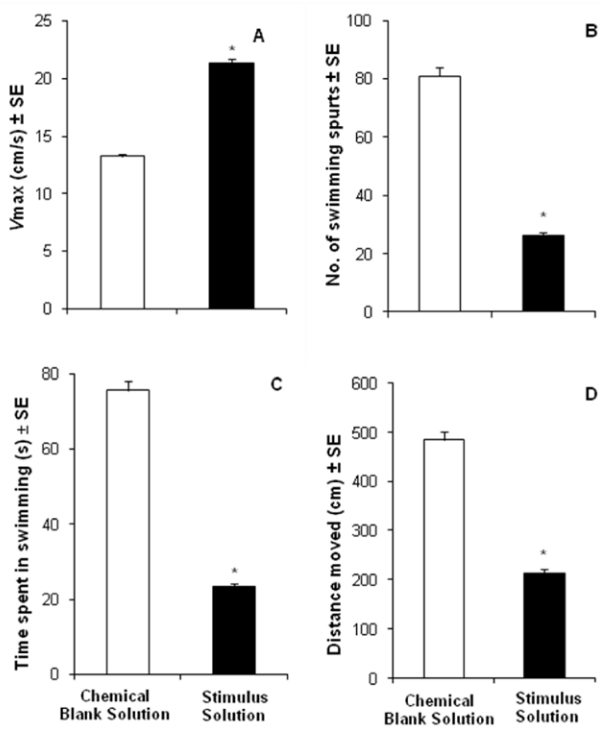

Fig. 1. Maximum swimming speed (V_{max}) (A), Swimming spurts (B), Time spent in swimming (C), and Distance moved (D) by tadpoles of *Uperodon taprobanicus* exposed to chemical blank solution (aged tap water) or stimulus solution (kairomones) of predatory tadpole, *Hoplobatrachus tigerinus*. Data are represented as mean ± SE and analysed by Paired-Samples t-test. N = 25 trials. Asterisks over the bars indicate significant difference between the treatment groups.

Results

Upon exposure to predator kairomones (stimulus solution), *U. taprobanicus* tadpoles showed a significant increase in V_{max} (T_{24} = -24.832; $P < 0.05$; Fig. 1A), and significant decrease in the number of swimming spurts (T_{24} = 17.597; $P < 0.05$; Fig. 1B). There was also a significant decrease in the time spent in swimming (T_{24} = 20.479; $P < 0.05$; Fig. 1C) and total distance travelled (T_{24} = 16.927; $P < 0.05$; Fig. 1D) when compared to their baseline activities in stimulus blank water.

Discussion

In aquatic systems, many prey organisms including the anuran tadpoles are at high risk of predation but the level of their risk is dependent on their defences used, which have evolved to promote their escape from the potential predators hence promote survival (Schmidt & Amezquita 2001, Relyea 2007). In such systems, various types of chemical cues (e.g., kairomones of predators, alarm cues of injured conspecifics and various dietary metabolites of predators) affect the behavioural responses of most prey organisms including anuran tadpoles (Wilson & Lefcort 1993, Wisenden 2000, Schoeppner & Relyea 2005, 2009a, b, Mogali et al. 2011).

Kairomones are generally considered as the chemical signatures of predators. The existence of kairomones (predator odour or smell) that elicit antipredator behaviour in prey has been reported in a wide array of animals including anuran tadpoles (Hazlett & Schoolmaster 1998, Kats & Dill 1998, Gyssels & Stoks 2006, Ferrari et al. 2010, Mogali et al. 2011). The results of the present study showed that *U. taprobanicus* sensed kairomones of a predator (i.e. *H. tigerinus*) and quickly decreased their overall activity levels (i.e., less time spent in swimming, less distance moved and less number of swimming spurts) during the trial period. In addition, it is interesting to note that whenever the *U. taprobanicus* tadpoles moved in the stimulus solution (kairomones), their spurt speed was higher than in the stimulus blank water, indicating their efforts to run away from the perceived kairomones of the predator.

Our results are in conformity with earlier studies on different tadpoles species belongs to different families such as *Bufo melanostictus* (Mogali et al. 2011), *Sphaerotheca breviceps* (Mogali et al. 2022), *Polypedates maculatus* (Mogali et al. 2022) and *Euphlyctis cyanophlyctis* tadpoles (Mogali et al. 2023). Therefore, *U. taprobanicus* tadpoles appear to perceive *H. tigerinus* tadpoles as potential predators. This is because of long ecological co-existence of *U. taprobanicus* tadpoles with sympatric carnivorous tadpoles, *H. tigerinus* may have led to the evolution of antipredator defence strategies in response to kairomones of these predators, as seen in tadpoles of *Rana draytonii* (Anderson & Lawler 2016). In summary, *U. taprobanicus* tadpoles perceive predation threat by detecting kairomones emanating from *H. tigerinus* and shown necessary antipredator behavioural responses.

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References

Anderson R.B. & Lawler S. P. 2016. Behavioural changes in tadpoles after multigenerational exposure to an invasive intraguild predator. Behavioural Ecology 27: 1790–1796.

Benard M. F. 2004. Predator-induced phenotypic plasticity in organisms with complex life cycles. Annual Review of Ecology, Evolution and Systematics 35: 651–673.

Berec M., Klapka V. & Zemek R. 2016. Effect of an alien turtle predator on movement activity of European brown frog tadpoles. Italian Journal of Zoology 83: 68–76.

Chivers D. P. & Mirza R. S. 2001. Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. Journal of Chemical Ecology 27: 45–51.

Dayton, G. H., Saenz D., Baum K. A., Langerhans R. B. & DeWitt T. J. 2005. Body shape, burst speed and escape behaviour of larval anurans. Oikos 111: 582–591.

Ferrari M. C. O., Wisenden B. D. & Chivers D. P. 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology 88: 698–724.

Gosner K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190.

Gyssels F. & Stoks R. 2006. Behavioural responses of fish kairomones and autotomy in a damselfly. Journal of Ethology 24: 79–83.

Hazlett B. A. & Schoolmaster D. R. 1998. Responses of cambarid crayfish to predator odor. Journal of Chemical Ecology 24: 1757–1770.

Hickman C. R., Stone M. D. & Mathis A. 2004. Priority use of chemical over visual cues for detection of predators by graybelly salamanders, *Eurycea multiplicata griseogaster*. Herpetologica 60: 203–210.

Hossie T. J. & Murray D. L. 2010. You can't run but you can hide: refuge use in frog tadpoles elicits density-dependent predation by dragonfly larvae. Oecologia 163: 395–404.

Kats L. B. & Dill L. M. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience 5: 361–394.

Kiesecker J. M., Chivers D. P. & Blaustein A. R. 1996. The use of chemical cues in predator recognition by western toad tadpoles. Animal Behaviour 52: 1237–1245.

Laurila A., Kujasalo J. & Ranta E. 1997. Different antipredator behaviour in two anuran tadpoles: effect of predator diet. Behavioural Ecology and Sociobiology 40: 329–336.

Lawler S. P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. Animal Behaviour 38: 1039–1047.

Loman J. 1999. Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. Amphibia-Reptilia 20: 421–430.

Mathis A. 2003. Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. Chemoecology 13: 193–197.

Mogali S. M., Saidapur S. K. & Shanbhag B. A. 2011. Levels of predation modulate antipredator defense behaviour and metamorphic traits in the toad *Bufo melanostictus*. Journal of Herpetology 45: 428–431.

Mogali S. M., Saidapur S. K. & Shanbhag B. A. 2020. Behavioural responses of tadpoles of *Duttaphrynus melanostictus* (Anura: Bufonidae) to cues of starved and fed dragonfly larvae. Phyllomedusa 19: 93–98.

Mogali S. M., Shanbhag B. A. & Saidapur S. K. 2022. Antipredator responses of tadpoles to kairomones from an omnivorous tadpole. Herpetological Review 53: 404–407.

Mogali S. M., Shanbhag B. A. & Saidapur S. K. 2023. Behavioral responses of omnivorous *Euphlyctis cyanophlyctis* tadpoles to carnivorous *Hoplobatrachus tigerinus* tadpoles. Herpetological Review 54: 195–199.

Newman R. A. 1992. Adaptive plasticity in amphibian metamorphosis. Bioscience 42: 671–678.

Relyea R. A. 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia 152: 389–400.

Saidapur S. K. 2001. Behavioral ecology of anuran tadpoles: the Indian scenario. Proceedings of Indian National Science Academy B67: 311–322.

Schmidt B. R. & Amezquita A. 2001. Predator-induced behavioural responses: tadpoles of the Neotropical frog *Phyllomedusa tarsius* do not respond to all predators. Herpetological Journal 11: 9–15.

Schoeppner N. M. & Relyea R. A. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters 8: 505–512.

Schoeppner N. M. & Relyea R. A. 2009a. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. Functional Ecology 23: 1114–1121.

Schoeppner N. M. & Relyea R. A. 2009b. When should prey respond to consumed heterospecifics? Testing hypothesis of perceived risk. Copeia 2009: 190–194.

Skelly D. K. 1994. Activity level and the susceptibility of anuran larvae to predation. Animal Behaviour 47: 465–468.

Skelly D. K. 1997. Tadpole communities: pond permanence and predation are powerful forces shaping the structure of tadpole communities. American Scientist 85: 36–45.

Spieler M. & Linsenmair K. E. 1999. Aggregation behaviour of *Bufo maculates* tadpoles as an antipredator mechanism. Ethology 105: 665–686.

Wilbur H. M. 1980. Complex life cycles. Annual Review of Ecology and Systematics 11: 67–93.

Wilson D. J. & Lefcort H. 1993. The effect of predator diet on the alarm response of Red-legged Frog *Rana aurora* tadpoles. Animal Behaviour 46: 1017–1019.

Wisenden B. D. 2000. Olfactory assessment of predation risk in the aquatic environment. Philosophical Transactions of the Royal Society London B Biological Sciences 355: 1205–1208.

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