Behavioral responses of tadpoles of *Clinotarsus curtipes* (Anura: Ranidae) to odor cues of dragonfly larvae

Santosh M. Mogali, Bhagyashri A. Shanbhag, and Srinivas K. Saidapur

Karnatak University, Department of Zoology Dharwad-580 003, Karnataka State, India. E-mail: santoshmogali@rediffmail.com.

Abstract

Behavioral responses of tadpoles of *Clinotarsus curtipes* (Anura: Ranidae) to odor cues of dragonfly larvae. In aquatic environments, many prey animals, including anuran larvae, predominantly use chemical cues to assess predation risk. In such systems, a variety of chemical cues (e.g., kairomones, alarm, dietary) affect the behavioral responses of the prey tadpoles. Many anuran tadpoles are able to discriminate different chemical cues and exhibit differential antipredator behavioral responses according to the perceived risk. The behavioral responses of tadpoles of *Clinotarsus curtipes* to predatory larvae of the dragonfly *Pantala flavescens* were studied in the laboratory. The predator's kairomones (water conditioned by a starved predator) or its diet-derived metabolites released in excreta of a predator after consumption of conspecific (*C. curtipes*) or heterogeneric (*Indosylvirana temporalis*) prey tadpoles were used to simulate predation threat. The tadpoles of *C. curtipes* had no behavioral response to predator kairomones. However, the tadpoles showed antipredator behavioral responses i.e., reduced swimming movements and overall time spent swimming, and had a higher burst speed in response to water-borne cues released from the excreta of predators fed both conspecific and heterogeneric prey. The antipredator behavioral responses of tadpoles were most intense in response to cues of predators fed on conspecific prey. The findings of the present study show that tadpoles of *C. curtipes* are capable of assessing levels of predation threat and modulating the intensity of their defense behavior in accordance with the perceived threat.

Keywords: Amphibians, Antipredator behavior, Bicolored frog, Predator, Prey.

Resumo

Respostas comportamentais de girinos de *Clinotarsus curtipes* (Anura: Ranidae) a sinais de odores de larvas de libélulas. Em ambientes aquáticos, muitas presas, inclusive larvas de anuros, usam predominantemente sinais químicos para avaliar o risco de predação. Nesses sistemas, uma variedade de sinais químicos (por exemplo, cairomônios, alarme, dieta) afeta as respostas comportamentais dos girinos. Muitos girinos são capazes de discriminar diferentes sinais químicos e apresentar respostas comportamentais diferenciadas contra predadores de acordo com o risco percebido. As respostas comportamentais dos girinos de *Clinotarsus curtipes* às larvas predadoras da

libélula *Pantala flavescens* foram estudadas em laboratório. Os cairomônios do predador (água condicionada por um predador faminto) ou seus metabólitos derivados da dieta liberados nas excretas de um predador após o consumo de girinos conspecíficos (*C. curtipes*) ou heterogenéricos (*Indosylvirana temporalis*) foram usados para simular o risco de predação. Os girinos de *C. curtipes* não apresentaram resposta comportamental aos cairomônios do predador. No entanto, os girinos apresentaram respostas comportamentais anti-predador, ou seja, redução dos movimentos de natação e do tempo total gasto nadando, e maior velocidade de explosão em resposta a sinais transportados pela água e liberados nas excretas de predadores alimentados com presas conspecíficas e heterogenéricas. As respostas comportamentais antipredador dos girinos foram mais intensas em resposta a sinais de predadores alimentados com presas específicas. Os resultados do presente estudo mostram que os girinos de *C. curtipes* são capazes de avaliar os níveis de ameaça de predação e modular a intensidade de seu comportamento de defesa de acordo com a risco percebido.

Palavras-chave: Anfíbios, Comportamento anti-predador, Predador, Presa, Rã bicolor.

Introduction

The interaction between predator and prey is an evolutionary arms race in which early detection by either party is often the key to their success (Ferrari *et al.* 2010). Predation leads inevitably to the removal of prey individuals from an ecological system which can have a major impact on population dynamics of prey organisms (Lima 1998). Prey organisms must assess predation risk and develop antipredator defense strategies in order to optimize their survival and fitness (Lima and Dill 1990). Sensing the presence of a predator before the actual encounter may offer a selective advantage to prey animals because it would allow them to adjust their behavior and reduce the probability of being detected (Lima and Dill 1990, Smith and Awan 2009, Ferrari *et al.* 2010). Failure to respond to a potential predator can be fatal. At the same time, evoking unnecessary antipredator behavior may have direct energetic costs as well as costs associated with reduced opportunities to feed or grow (Lima and Dill 1990). Sensory information obtained about a predator may assist prey organisms in assessing the potential risk more accurately and therefore reduce these costs (Lima and Dill 1990, Chivers and Smith 1998).

In aquatic environments, many prey animals, including anuran tadpoles, use chemical cues to assess predation risk (Chivers and Smith 1998, Kats and Dill 1998, Hickman *et al.* 2004, Mogali *et al.* 2012, 2020). Chemical cues are useful in such systems because visual information may be obscured in water that is turbid or densely vegetated. Previous 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, [e.g., *Dryophytes versicolor* (LeConte, 1825) (= *Hyla versicolor;* Schoeppner and Relyea 2005), *Polypedates maculatus* (Gray, 1830) (Mogali 2018)], from dietary metabolites derived from consumption of conspecific prey and released through feces [e.g., *Rana aurora* Baird and Girard, 1852 (Wilson and Lefcort 1993), *Duttaphrynus melanostictus* (Schneider, 1799) (Mogali *et al.* 2011), *Indosylvirana temporalis* (Günther, 1864) (Mogali *et al.* 2012)], from alarm pheromones released by injured prey [e.g., *D. versicolor* (= *Hyla versicolor;* Schoeppner and Relyea 2005), *D. melanostictus* (Mogali *et al.* 2011)], or from a combination of these factors [e.g., *D. versicolor, Lithobates pipiens* (Schreber, 1782) (= *Rana pipiens;* Schoeppner and Relyea 2009 a, b)]. Earlier studies revealed that anuran tadpoles showed a variety of antipredatory behaviors in response to chemical cues of predators. These behaviors include increased activity or swimming speed in

order to flee from predators (Mogali *et al.* 2021), reduction in activity (Saidapur *et al.* 2009, Mogali *et al.* 2012, 2020), aggregation (Spieler and Linsenmair 1999), or increased use of refuge sites (Hossie and Murray 2010, Mogali *et al.* 2019, 2022).

The Bicolored frog, *Clinotarsus curtipes* (Jerdon, 1853) usually breeds in the months of August and September along gently flowing streams and isolated pockets of water along sides of the streams in southern Western Ghats of India. The species has a relatively long larval period from six months to a year, occasionally extending to 1.5 years (180–360 days; Saidapur 2001); therefore, their larvae are found throughout the year in these habitats (Hiragond and Saidapur 2001, Hiragond *et al.* 2001, Saidapur 2001). The tadpoles of *C. curtipes* are found in close association with tadpoles of a sympatric species, *Indosylvirana temporalis.* Tadpoles of this species also have a relatively long larval period (90–120 days; Saidapur 2001). Tadpoles of both species are bottom-dwellers and thrive on detritus and algal matter (Hiragond and Saidapur 2001). The visibility is generally low in such water bodies because of shadows cast by dense vegetation and the dark brown color of benthic areas that are typically covered by leaf litter and detritus. These water bodies are also inhabited by several invertebrate predators, including larvae of the dragonfly *Pantala flavescens* (Fabricius, 1798) (Odonata: Libellulidae). The larvae of *P. flavescens* are sit-and-wait predators that move slowly and wait for the prey to approach before attacking. During our regular field visits, we observed larvae of *P. flavescens* actively feeding on early-stage tadpoles of *C. curtipes* (i.e., Gosner stage 25; Gosner 1960) and *I. temporalis.* Previous studies from our laboratory revealed that tadpoles of *I. temporalis* showed antipredatory behavioral responses to larvae of *P. flavescens* (Mogali *et al.* 2012). Whether tadpoles of *C. curtipes* respond to dragonfly larvae is unknown. Thus, tadpoles of *C. curtipes* offer an excellent model to test behavioral responses to larval dragonflies. In this

study, we examined behavioral responses of tadpoles of *C. curtipes* to different odors (e.g., kairomones or dietary cues) of larval *P. flavescens.* We hypothesized that tadpoles of *C. curtipes,* which coexist with this predator in natural water bodies, should show antipredatory behavioral responses to different predator cues. Additionally, we hypothesized that the level of antipredatory responses of tadpoles of *C. curtipes* would vary depending on the type of cue. We expected that tadpoles of *C. curtipes* would show strong antipredator behavioral responses to dietary cues from predators fed with conspecific tadpoles, a moderate response to predators fed with heterospecific tadpoles, and little or no response to starved predators. The outcome of this study will provide novel information in the field of behavioral ecology of anuran larvae with special reference to prey-predator interactions.

Materials and Methods

Tadpoles of *Clinotarsus curtipes* [Gosner stage 25; *N* = 300; 22.30 ± 0.45 mm total length (mean ± SE); Gosner 1960] and *Indosylvirana temporalis* (Gosner stages 27–28; *N* = 300; 22.25 ± 0.39 mm total length) were collected from a stream in the Western Ghats near Anmod village (15.43088° N, 74.37360° E), Karnataka State, India, in September 2011 and brought to the laboratory. Tadpoles of each species were placed separately in glass aquaria (90 L × 30 W × 15 H cm) containing 25 L of aged tap water (dechlorinated) and used as stock. Tadpoles of both species were herbivorous and were fed boiled spinach to sustain growth and development. The last instar larvae of *Pantala flavescens* (*N* = 50; 34.50 ± 1.5 mm total length) were collected from the same location where the tadpoles were obtained and were reared individually in plastic tubs (19 cm diameter and 7 cm depth) with 500 mL of aged tap water to avoid cannibalism. Predators were fed daily with either tadpoles of *C. curtipes* or *I. temporalis.* Behavioral responses of the prey (*C. curtipes*) tadpoles were studied by exposing them to stimulus solution of either

predator kairomones or dietary metabolites of predators fed conspecific (*C. curtipes*) or heterogeneric (*I. temporalis*) tadpoles.

Preparation of Kairomones

Individual dragonfly larvae were placed in separate plastic tubs (*N* = 10 tubs; 19 cm 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 tubs, and the stimulus solutions were filtered to completely remove any fecal matter. The solutions were used immediately (kairomones) for experimental trials.

Preparation of Dietary Cues of Conspecific Origin

Individual dragonfly larvae were placed in separate plastic tubs (*N* = 10 tubs; 19 cm diameter and 7 cm depth) containing 200 mL of aged tap water along with four tadpoles of *C. curtipes* at Gosner stage 25 (at 08:30 h). The dragonfly larvae consumed all tadpoles provided to them by evening (18:30 h). On the following day between 09:30 h and 11:30 h, predators were removed and the water from the tubs was filtered using fine cheese cloth. The filtrate served as the stimulus solution containing the diet-derived excretory metabolites or substances of predators fed conspecific prey and were unlikely to have contained alarm cues of the prey. Prey alarm cues are known to be labile in nature (Ferrari *et al.* 2008, Chivers *et al.* 2013). It is unlikely that prey alarm cues were present in the stimulus solution because all prey were consumed more than 15 h before the solution was collected.

Preparation of Dietary Cues of Heterogeneric Origin

Individual dragonfly larvae were placed in plastic tubs (*N* = 10 tubs; 19 cm diameter and 7 cm depth) containing 200 mL of aged tap water along with four tadpoles of *I. temporalis* (at 08:30 h). The dragonfly larvae consumed all tadpoles provided to them by evening (18:30 h). On the following day, the filtrate was obtained and served as a stimulus solution containing the diet-derived excretory metabolites or substances of predators fed heterogeneric prey.

Behavioral Responses of Tadpoles of C. curtipes to Predator Kairomones

The behavioral responses of tadpoles of *C. curtipes* to kairomones (water conditioned with starved predators) were recorded by placing a single tadpole (Gosner stage 25) in a rectangular glass tank (28 L × 15 W × 15 H cm) containing 600 mL of aged tap water. A handycam (Sony, DCR-SR300/E) was fixed above the tank so 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 movements of the tadpole before and after addition of the stimulus solution to the test tank. The Ethovision system was used to record maximum swimming speed (V_{max}, cm/s), distance traversed by the tadpole (cm), number of swimming spurts (the number of times a test tadpole makes a move after a short stationary period within a given trial of 5 min; Sharma *et al.* 2008) and time spent swimming (s) during an entire trial.

For each trial, a new tadpole (*C. curtipes*) 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 added at the rate of ~1 mL/s to simulate disturbance that the later chemical cue would make. The burette was removed gently. Movement of the tadpole was recorded for 5 min using Ethovision to record its baseline activity in the absence of any cues. After tracking baseline activity, 50 mL of stimulus solution containing kairomones was added as described above. Movement of the

tadpole was recorded for another 5 min to determine the activity pattern after exposure to kairomones.

Behavioral Responses of Tadpoles of C. curtipes *to Dietary Cues of Heterogeneric Origin*

In this experiment, the stimulus solution contained chemical cues from excretory metabolites or substances of predators fed tadpoles of *I. temporalis.* The behavioral responses of tadpoles were recorded as described above, before and after the addition of the stimulus solutions.

Behavioral Responses of Tadpoles of C. curtipes *to Dietary Cues of Conspecific Origin*

In this experiment, the stimulus solution contained chemical cues from excretory metabolites or substances of predators fed tadpoles of *C. curtipes.* The behavioral responses of tadpoles were recorded as described above, before and after the addition of the stimulus solutions.

Twenty-five trials were carried out for each experiment (3 types of stimulus solutions × 25 trials = 75 trials in total). A new tadpole was used for each trial. The test tank was cleaned and replenished with aged tap water between trials.

Statistical Analysis

The data on the behavioral responses of tadpoles of *C. curtipes,* before and after addition of each type of stimulus solution, were compared separately by using the Paired-Samples t-test. The data on the behavioral responses of tadpoles of *C. curtipes* to chemical blank water and various types of stimulus solutions were analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test to determine whether any differences occurred among the various types of stimulus solutions. Statistical tests were performed using SPSS ver. 16.0.

Results

The V_{max} ($t = -0.301$, $df = 24$, $p = 0.766$), frequency of swimming spurts ($t = -0.609$, $df = 24$, $p = 0.548$), time spent swimming ($t = -0.419$, $df = 24$, $p = 0.679$) and total distance traversed ($t = -1.049$, $df = 24$, $p = 0.305$) by tadpoles exposed to chemical blank water were similar to those exposed to kairomones.

Upon exposure to diet-derived metabolites of a predator after it consumed heterogeneric prey, tadpoles showed a significant increase in V_{max} ($t = -27.343$, $df = 24$, $p < 0.001$, Figure 1A), and significant declines in the number of swimming spurts ($t = 23.466$, $df = 24$, $p < 0.001$, Figure 1B), time spent swimming ($t = 21.363$, $df = 24$, $p < 0.001$, Figure 1C), and total distance moved ($t = 17.280$, $df = 24$, $p < 0.001$, Figure 1D) when compared to their baseline activities in stimulus-free water.

Upon exposure to diet-derived metabolites of a predator after it consumed conspecific prey, tadpoles showed a significant increase in V_{max} ($t = -23.269$, $df = 24$, $p < 0.001$, Figure 2A), and significant declines in the number of swimming spurts ($t = 26.575$, $df = 24$, $p < 0.001$, Figure 2B), time spent swimming ($t = 31.881$, $df = 24$, $p < 0.001$, Figure 2C) and total distance moved ($t = 28.741$, $df = 24$, $p < 0.001$, Figure 2D) when compared to their baseline activities in stimulus-free water.

Results of ANOVA showed a significant difference in V_{max} ($F_{3, 146} = 711.0$, $p < 0.001$), number of swimming spurts ($F_{3, 146} = 326.3$, $p < 0.001$), time spent swimming ($F_{3, 146} = 288.0$, $p < 0.001$), and total distance moved ($F_{3, 146} = 353.6$, $p < 0.001$) by prey tadpoles among different treatment groups (Table 1). The intensity of defence behaviors varied significantly with the cue. Those exposed to cues of predators fed with conspecific prey exhibited significantly higher V_{max} ($p < 0.001$) and spent less time swimming ($p < 0.001$) with reduced number of swimming spurts ($p < 0.001$) and moved for shorter distance ($p < 0.001$) compared to any other groups (Table 1). Tadpoles exposed to predators fed with
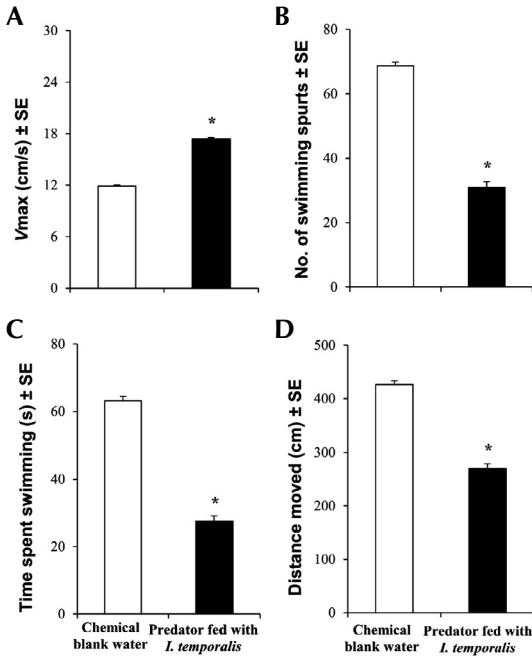
Figure 1. Maximum swimming speed (V_{max}) (**A**), Swimming spurts (**B**), Time spent swimming (**C**), and Distance moved (**D**) by tadpoles of *Clinotarsus curtipes* exposed to chemical blank water (aged tap water) or dissolved diet-derived excretory metabolites of heterogeneric prey (*Indosylvirana temporalis*) fed to larval *P. flavescens*. Data are represented as mean ± SE; *N* = 25 trials; data analyzed by Paired-Samples t-test. Asterisks over the bars indicate a significant difference between the two groups.

Figure 2. Maximum swimming speed (V_{max}) (**A**), Swimming spurts (**B**), Time spent swimming (**C**), and Distance moved (**D**) by tadpoles of *Clinotarsus curtipes* exposed to chemical blank water (aged tap water) or dissolved diet-derived excretory metabolites of conspecific prey (*C. curtipes*) fed to larval *P. flavescens*. Data are represented as mean ± SE; *N* = 25 trials; data analyzed by Paired-Samples t-test. Asterisks over the bars indicate a significant difference between the two groups.

heterogeneric prey also exhibited significantly higher V_{max} ($p < 0.001$) and spent less time swimming ($p < 0.001$) with reduced number of swimming spurts ($p < 0.001$) and moved only short distance ($p < 0.001$) compared to the control (chemical blank water) or kairomone groups (Table 1). There was no significant difference in V_{max} ($p = 0.978$), swimming spurts ($p = 0.991$), time spent swimming ($p = 0.997$) and distance moved ($p = 0.660$) between control (chemical blank water) and kairomone groups (Table 1).
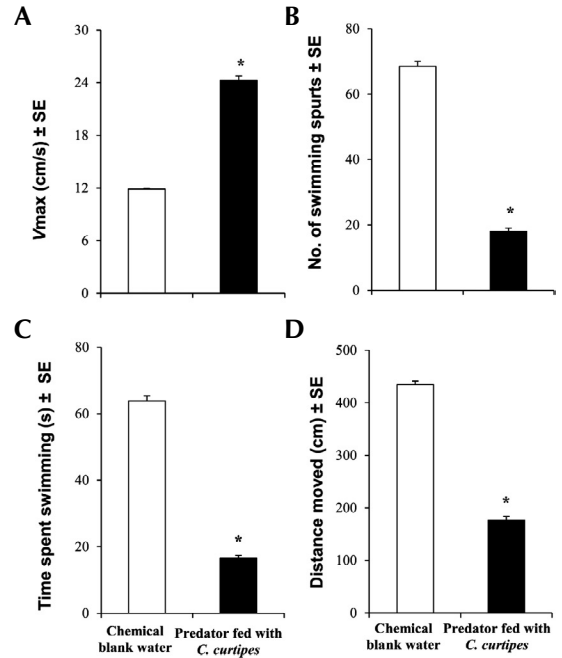
The intensity of defense behaviors are as follows: predator fed on conspecific prey (*C. curtipes*) tadpoles (highest) > predator fed on heterogeneric prey (*I. temporalis*) tadpoles (moderate) > kairomones (starved predators) = chemical blank water (control group, Table 1).

Discussion

In aquatic ecosystems, most prey organisms, including tadpoles, are at risk of predation, but the level of their risk is dependent on defenses

Table 1. Behavioral responses of tadpoles of *Clinotarsus curtipes* to different stimulus solutions (kairomones, dietary cues) of a predator, larval *Pantala flavescens*. Twenty-five trials were conducted in each treatment group. Data are represented as mean ± SE and analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test. Dissimilar letters indicate significant differences between the groups.

Chemical cues	V_{max} (cm/s)	Swimming spurts	Time spent swimming (s)	Distance moved (cm)
Chemical blank	11.91 ± 0.11[a]	67.96 ± 1.01[a]	62.67 ± 1.04[a]	429.17 ± 4.50[a]
Predator's kairomones	12.02 ± 0.12[a]	68.52 ± 1.68[a]	62.28 ± 1.59[a]	439.35 ± 5.96[a]
Predator fed with tadpoles of *I. temporalis*	17.39 ± 0.15[b]	31.00 ± 1.71[b]	27.58 ± 1.53[b]	270.26 ± 8.76[b]
Predator fed with tadpoles of *C. curtipes*	24.25 ± 0.52[c]	18.08 ± 0.96[c]	16.53 ± 0.87[c]	176.12 ± 7.68[c]
$F_{3, 146}$ value	711.0	326.3	288.0	353.6
p value	< 0.001	< 0.001	< 0.001	< 0.001

that they have evolved to escape from predators and sustain survival (Lima and Dill 1990, Kats and Dill 1998, Schmidt and Amezquita 2001). In aquatic environments, a variety of chemical cues (e.g., kairomones, alarm, dietary, and disturbance cues) affect the behavioral responses of tadpoles (Wilson and Lefcort 1993, Schoeppner and Relyea 2005, Mogali *et al.* 2012, 2023, Scherer and Smee 2016, Rivera-Harnández *et al.* 2022). Anuran tadpoles are able to discriminate different chemical cues and exhibit differential antipredator behavioral responses according to perceived levels of risk (Mogali *et al.* 2011, 2023). Results of the present study showed variation in intensity of antipredator defense behavior of tadpoles of *C. curtipes* in response to different chemical cues emanated from predators.

Tadpoles of *Clinotarsus curtipes* did not change their behavior in response to kairomones of dragonfly larvae of *P. flavescens*, thereby suggesting that they did not perceive cues of larval *P. flavescens* as a serious predation risk. Parallel results have been reported for tadpoles of *I. temporalis* (Mogali *et al.* 2012) and *D. melanostictus* (Mogali *et al.* 2020). Larval dragonflies are sit-and-wait predators that move slowly and usually wait for prey to come near before attacking (Miller *et al.* 2014). An earlier study reported intense selection pressure on sit-

and-wait predators to suppress chemical (e.g., kairomones) evidence of their presence because they need the prey to approach closely (Miller *et al.* 2015). It is possible that predation pressure of larval *P. flavescens* on tadpoles of *C. curtipes* is low. If so, tadpoles of *C. curtipes* are better served by preserving their energy by not inducing antipredator defenses in response to kairomones, if any, of larval *P. flavescens*.

In contrast, water-soluble substances in the excreta of *P. flavescens* following consumption of conspecific prey items appear to indicate intense predation risk and elicit strong behavioral changes in tadpoles of *C. curtipes* in the form of reduced levels of swimming activity (i.e., less time spent swimming, less distance traversed, and fewer numbers of spurts). Whenever tadpoles of *C. curtipes* moved in the stimulus solution (dietary cues of conspecifics), their spurt speed (V_{max}) was higher than in the blank stimulus solution, indicating increased efforts to escape from perceived chemical cues of the predator. Our results are in conformity with earlier studies on tadpoles of *D. melanostictus* (Mogali *et al.* 2011, 2020) and *I. temporalis* (Mogali *et al.* 2012). Tadpoles of *D. melanostictus* and *I. temporalis* when exposed to chemical cues of predators reduced their swimming activity (i.e., less time spent swimming, less distance

traversed, and fewer numbers of spurts), but they exhibited a high V_{max} (efforts to escape from the perceived chemical cues of the predator).

Tadpoles of *C. curtipes* displayed antipredator behavioral responses (i.e., reduced swimming activities and high burst speeds) to water-borne chemical cues of larval *P. flavescens* fed on heterogeneric tadpoles. We ruled out the influence of alarm cues released by tadpoles of *C. curtipes* because neither surviving injured individuals nor dead remains of tadpoles were found in the tubs for 15 h prior to obtaining the stimulus solution. Moreover, alarm cues are highly labile in nature (Ferrari *et al.* 2008, Wisenden *et al.* 2009, Chivers *et al.* 2013). Antipredator defense behavior of tadpoles of *C. curtipes* in the present study is therefore specifically in response to diet-derived excretory metabolites or substances released by larval *P. flavescens* that have consumed conspecific or heterogeneric prey.

The results of the present study revealed that tadpoles of *C. curtipes* exhibited a more intense antipredator defense response when challenged by predators fed with conspecific prey items compared to predators fed with heterogeneric prey items. The intensity of defense behaviors of *C. curtipes* are as follows; predators fed on conspecific prey (highest) > predators fed on heterogeneric prey (moderate) > kairomones = chemical blank water. Tadpoles of *C. curtipes* exhibited stronger antipredator defense behavior when conspecific tadpoles become prey to the predator, suggesting a combined additive response toward species-specific alarm pheromones and a predator's chemical cues of dietary origin. Evidently, tadpoles of *C. curtipes* construe injury and death of individuals of their species (conspecifics) as a greater threat than when individuals of other species (heterospecifics) fall prey to predators. This observation suggests that, in the surrounding waters, if the predators selectively prey upon other species, it is unnecessary for unaffected prey species to expend energy evoking and maintaining defense behaviors (Mogali *et al.* 2011). Perception of

high predation risk may lead to decrease in developmental rate and may increase larval duration in tadpoles of *C. curtipes.*

The present study shows that tadpoles of *C. curtipes* exhibit differential assessment of predation risk and accordingly adjust their level of defense strategies in terms of swimming activity. In response to a perceived low predation risk (when predators feed on other species e.g., *I. temporalis*), tadpoles of *C. curtipes* exhibit moderate levels of defenses thereby saving energy for growth and development. These findings on *C. curtipes* lend support to the hypothesis of "threat-sensitive learning" and "threat-sensitive predator avoidance" (Helfman 1989, Mathis and Vincent 2000, Chivers *et al.* 2001).

Acknowledgments

References

Chivers, D. P. and R. J. F. Smith. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Ecoscience 5:* 338–352.

Chivers, D. P., R. S. Mirza, P. J. Bryer, and J. M. Kiesecker. 2001. Threat-sensitive predator avoidance by slimy sculpins: understanding the role of visual versus chemical information. *Canadian Journal of Zoology 79:* 867–873.

Chivers, D. P., D. L. Dixson, J. R. White, M. I. McCormick, and M. C. O. Ferrari. 2013. Degradation of chemical alarm cues and assessment of risk throughout the day. *Ecology and Evolution 3:* 3925–2934.

Ferrari, M. C. O., F. Messier, and D. P. Chivers. 2008. Degradation of chemical alarm cues under natural conditions: risk assessment by larval woodfrogs. *Chemoecology 17:* 263–266.

Ferrari, M. C. O., B. D. Wisenden, and D. P. Chivers. 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.

Helfman, G. S. 1989. Threat-sensitive predator avoidance in damselfish-trumpet fish interactions. *Behavioural Ecology and Sociobiology 24:* 47–58.

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

Hiragond, N. C. and S. K. Saidapur. 2001. Microhabitat choice of tadpoles of seven anuran species. *Current Herpetology 20:* 51–60.

Hiragond, N. C., B. A. Shanbhag, and S. K. Saidapur. 2001. Description of the tadpole of a stream breeding frog, *Rana curtipes*. *Journal of Herpetology 35:* 166–168.

Hossie, T. J. and D. L. Murray. 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. and L. M. Dill. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience 5:* 361–394.

Lima, S. L. 1998. Nonlethal effects of in the ecology of predator-prey interactions. *Bioscience 48:* 25–34.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology 68:* 619–640.

Mathis, A. and F. Vincent. 2000. Differential use of visual and chemical cues in predator recognition and threat-sensitive predator avoidance responses by larval newts (*Notophthalmus viridescens*). *Canadian Journal of Zoology 78:* 1646–1652.

Miller, J. R. B., J. M. Ament, and O. J. Schmitz. 2014. Fear on the move: predator hunting mode predicts variation in prey mortality and plasticity in prey spatial response. *Journal of Animal Ecology 83:* 214–222.

Miller, A. K., B. Maritz, S. Mckay, X. Glaudas, and G. J. Alexander. 2015. An ambusher's arsenal: chemical crypsis in the puff adder (*Bitis arietans*). *Proceedings of the Royal Society B, Biological Sciences 282:* 20152182.

Mogali, S. M. 2018. Predatory cues influence the behavioral responses and metamorphic traits of *Polypedates maculatus* (Anura: Rhacophoridae). *Asian Herpetological Research 9:* 188–194.

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

Mogali, S. M., S. K. Saidapur, and B. A. Shanbhag. 2012. Tadpoles of the bronze frog (*Rana temporalis*) assess predation risk before evoking antipredator defense behavior. *Journal of Ethology 30:* 379–386.

Mogali, S. M., S. K. Saidapur, and B. A. Shanbhag. 2019. Experience of predacious cues and accessibility to refuge minimize mortality of *Hylarana temporalis* tadpoles. *Acta Herpetologica 14:* 15–19.

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

Mogali, S. M., B. A. Shanbhag, and S. K. Saidapur. 2021. Comparative vulnerability of *Indosylvirana temporalis* and *Clinotarsus curtipes* (Anura: Ranidae) tadpoles to water scorpions: importance of refugia and swimming speed in predator avoidance. *Phyllomedusa 20:* 159–164.

Mogali, S. M., B. A. Shanbhag, and S. K. Saidapur. 2022. Knowledge of predators and accessibility to refuge reduces larval mortality of the Bicolored frog, *Clinotarsus curtipes* (Anura: Ranidae). *Salamandra 58:* 157–160.

Mogali, S. M., B. A. Shanbhag, and S. K. Saidapur. 2023. Differential antipredator behavioral responses in *Sphaerotheca breviceps* tadpoles: an experimental approach. *Herpetological Review 54:* 31–35.

Rivera-Harnández, I. A. E., A. L. Crane, M. S. Pollock, and M. C. O. Ferrari. 2022. Disturbance cues function as a background risk cue but not as an associative learning cue in tadpoles. *Animal Cognition 25:* 881–889.

Saidapur, S. K. 2001. Behavioural ecology of anuran tadpoles: Indian scenario. *Proceedings of Indian National Science Academy 67:* 311–322.

Saidapur, S. K., D. K. Veeranagoudar, N. C. Hiragond, and B. A. Shanbhag. 2009. Mechanism of predator-prey detection and behavioral responses in some anuran tadpoles. *Chemoecology 19:* 21–28.

Scherer, A. E. and D. L. Smee. 2016. A review of predation diet effects on prey defensive responses. *Chemoecology 26:* 83–100.

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

Schoeppner, N. M. and R. A. Relyea. 2005. Damage, digestion, and defense: the role of alarm cues and kairomones for inducing prey defenses. *Ecology Letters 8:* 505–512.

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

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

Sharma, S. S., D. K. Veeranagoudar, B. A. Shanbhag, and S. K. Saidapur. 2008. Activity of *Sphaerotheca breviceps* tadpoles in response to chemical cues of predaceous tadpoles *Hoplobatrachus tigerinus*. *Journal of Ethology 26:* 303–307.

Smith, G. R. and A. R. Awan. 2009. The roles predator identity and group size in antipredator responses of American toad (*Bufo americanus*) and bull frog (*Rana catesbeiana*) tadpoles. *Behaviour 146:* 225–243.

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

Wilson, D. J. and H. Lefcort. 1993. The effect of predator diet on the alarm responses of red-legged frog *Rana aurora* tadpoles. *Animal Behaviour 46:* 1017–1019.

Wisenden, B. D., M. L. Rugg, N. L. Korpi, and L. C. Fuselier. 2009. Lab and field estimates of active time of chemical alarm cues of a cyprinid fish and amphipod crustacean. *Behaviour 146:* 1423–1442.

Editor: Fausto Nomura