



Correspondence

Knowledge of predators and accessibility to refuge reduces larval mortality of the Bicolored Frog, *Clinotarsus curtipes* (Anura: Ranidae)

SANTOSH M. MOGALI, BHAGYASHRI A. SHANBHAG & SRINIVAS K. SAIDAPUR

Department of Zoology, Karnatak University, Dharwad 580 003, Karnataka State, India

Corresponding author: SANTOSH M. MOGALI, e-mail: santoshmogali@rediffmail.com

Manuscript received: 1 November 2021

Accepted: 29 January 2022 by ARNE SCHULZE

Interaction between predator and prey is part of an evolutionary arms race in which early detection by either party is often the key to their success (FERRARI et al. 2010). Predation inevitably leads to the removal of prey individuals from an ecological system, which can have a major impact on the population dynamics of prey organisms (LIMA 1998). Most anurans have a complex life cycle that involves an aquatic larval stage (WILBUR 1980, MOGALI et al. 2016). During the aquatic stage, tadpole mortality is frequently due to desiccation or fragmentation or isolation of ponds before the completion of metamorphosis (HIRAGOND & SAIDAPUR 2001, MOGALI et al. 2016), infection with pathogens (BLAUSTEIN et al. 2018), and predation by aquatic predators (MOGALI et al. 2016, 2020). Therefore, the adoption of phenotypic plasticity and other strategies may become important to enhance survivorship.

Under threat from predation, anuran tadpoles are known to alter their behavioural responses in order to reduce detection or capture (LIMA & DILL 1990, SCHMIDT & AMEZQUITA 2001, RELYEA 2007, MOGALI et al. 2012, 2016). Taking shelter under leaf litter, in aquatic vegetation, mud or sand, small pebbles, and other objects that provide refuge and exploiting benthic microhabitats can assist tadpoles in reducing the rate of detection by predators (LIMA & DILL 1990, HOSSIE & MURRAY 2010). In addition, predators may find it hard to capture prey concealed within a shelter; simultaneously, prey animals may have an enhanced chance to assess the severity of threats of predation by the predators living in the vicinity and thus grade the necessity of defensive behaviours (HEMMI & ZEIL 2005). However, hiding and remaining motionless by prey incur trade-offs. These strategies may reduce encounter rates with predators, but also tend to reduce the time available

for foraging. Indeed, habitats that are energetically beneficial will often also be the riskiest, since the commonness of predators tends to parallel prey resource abundance (LIMA 1998). Besides, the refuge selected may not always be optimal for foraging. For example, insufficient time spent feeding is known to lead to the alteration of metamorphic traits (MOGALI et al. 2011).

The breeding period of the Bicolored frog, *Clinotarsus curtipes*, falls into the period from August to September, with reproduction taking place in gently flowing streams and isolated pockets of water along these in the southern Western Ghats of India. This species has a relatively long larval period, i.e., from six months to a year, and as a consequence, their larvae are found throughout the year in such waters (HIRAGOND & SAIDAPUR 2001, HIRAGOND et al. 2001, SAIDAPUR 2001). The tadpoles of *C. curtipes* are bottom-dwellers and feed on detritus and algal matter (HIRAGOND & SAIDAPUR 2001). Visibility in such waters is generally low, due to shadows cast by dense flanking vegetation and the dark brownish colour of the benthic areas that are typically covered with leaf litter and detritus. These water bodies are also inhabited by several invertebrate predators, including water scorpions (*Laccotrephes* sp., Hemiptera: Nepidae). During our regular field visits, we observed *Laccotrephes* sp. actively feeding on *C. curtipes* tadpoles. Therefore, in the present experiment, we assumed that tadpoles of *C. curtipes* profitably use refuge sites (leaf litter) to reduce the predation risk exerted by coexisting predators. An additional assumption was that tadpoles with knowledge of predators from previous encounters (predator-experienced tadpoles) will be more successful in escaping predation by using shelters compared to those facing predators for the first time (predator-naïve tadpoles). To test our hypotheses, tadpoles of

C. curtipes representing either class were exposed to freely hunting *Laccotrephes* sp. in the presence or absence of refuge sites for fixed periods (24 h) each to record their survival/mortality.

Five egg clutches of *Clinotarsus curtipes* were collected from a stream in the southern Western Ghats near Anmod village (15.430888° N, 74.373601° E), Karnataka State, India, in September 2013. They were immediately transported to the laboratory and each clutch was placed separately in a plastic tub (32 cm diameter and 14 cm deep) containing 5 litre of aged tap (dechlorinated) water until hatching (Gosner stage 19; GOSNER 1960). After hatching, the tadpoles of all clutches were mixed to normalize genetic differences throughout the groups. They were then reared in a glass aquarium (LWH 90 × 30 × 15 cm) containing 25 litres of aged tap water. Tadpoles were provided with boiled spinach as food ad libitum. Reference specimens (*C. curtipes*) are deposited in the Museum of the Zoological Survey of India, Calcutta, under catalogue number A9117. Individuals of *Laccotrephes* sp. (predators) were collected from the same site from where the egg clutches of *C. curtipes* were obtained and were reared individually, to avoid cannibalism, in small plastic tubs (14 cm diameter and 7 cm deep) filled with 500 ml of aged tap water; they were fed exclusively with tadpoles of *C. curtipes*.

The tadpoles of *C. curtipes* hatched in the laboratory and not exposed to predators (*Laccotrephes* sp.), or its cues, served as predator-naïve subjects. To obtain predator-experienced tadpoles we exposed groups of 30 tadpoles to a 48-h starved water scorpion for 8 h (09:00–17:00 h). After that period the predator and injured tadpoles were recovered from the tub and returned to separate holding tanks. On average, a predator consumed 4 ± 0.5 and injured 5 ± 0.7 tadpoles ($\bar{x} \pm SE$) in predation attempts during the trial period. Predator-risk experienced but uninjured tadpoles were used in the experiments during the following days, in order to assess their performance against predators in subsequent encounters with them. In the present experiment, leaves of *Aporosa lindleyana* were used as refuge sites; they were collected from the same place as the *C. curtipes* eggs. To normalize their structural properties, they were soaked in water for 2 days (dry mass $15 \text{ g} \pm 1.6$; $\bar{x} \pm SE$), chopped to $\sim 1\text{-cm}^2$ pieces, and then spread out on the bottom of the tubs serving as test arenas.

In all experimental trials, prey (*C. curtipes*) tadpoles (either predator-naïve or predator-experienced) were of comparable body sizes (length 21.12 ± 0.50 mm, width 5.33 ± 0.09 mm, and weight 57.90 ± 1.20 mg; $\bar{x} \pm SE$; N = 30; Fig. 1a) and developmental stage (Gosner stage 25). The *Laccotrephes* sp. used in the various trials were comparable in size, too (length 61.30 ± 0.85 mm, width 10.14 ± 0.12 mm, and weight 615.0 ± 2.30 mg; $\bar{x} \pm SE$; N = 30; Fig. 1b). The body sizes of prey tadpoles and predators were measured with a digital calliper (accuracy 0.01 mm), and their weights were ascertained using an electronic scale (accuracy 0.0001 mg). The experiments used a 2×2 factorial design with the following treatment groups:

Group 1: Predator-naïve tadpoles of *C. curtipes* were exposed to a predator, *Laccotrephes* sp., in the absence of refuge sites

Group 2: Predator-naïve tadpoles of *C. curtipes* were exposed to a predator, *Laccotrephes* sp., in the presence of refuge sites

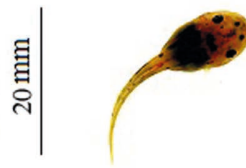
Group 3: Predator-experienced tadpoles of *C. curtipes* were exposed to a predator, *Laccotrephes* sp., in the absence of refuge sites

Group 4: Predator-experienced tadpoles of *C. curtipes* were exposed to a predator, *Laccotrephes* sp., in the presence of refuge sites

Each treatment group was exposed to 20 trials (overall $20 \times 4 = 80$ trials).

For each trial, the test (*C. curtipes*) tadpoles (either predator-naïve or predator-experienced) were released ($n = 30$) into the plastic tub (32 cm diameter and 14 cm deep) containing 3 litres of aged tap water and in the presence or absence of refuge sites and allowed to acclimatize in the tub for 15 min. Then one *Laccotrephes* sp. starved for

a) Tadpole of *Clinotarsus curtipes*



b) Adult *Laccotrephes* sp.

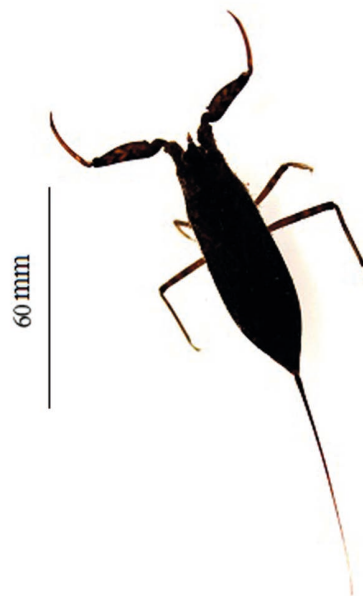


Figure 1. (a) Potential prey tadpole of *Clinotarsus curtipes* and predator, (b) adult *Laccotrephes* sp. used in the experiments.

Table 1. Results of two-way ANOVAs for exposure and refuge accessibility and their interactions. The response variable is the mean number of *Clinotarsus curtipes* tadpoles lost due to predation by *Laccotrephes* sp. * Indicates significant differences.

Source	DF	MS	F	P
Exposure	1	49.613	15.799	<0.01*
Refuge accessibility	1	99.013	31.531	<0.01*
Exposure × refuge accessibility	1	6.612	2.106	0.151

48 h was introduced gently to the tub and left there. Trials were ended after 24 h. The number of surviving tadpoles (either predator-naïve or predator-experienced) in the various trials was recorded to compute the number of tadpoles lost due to predation. All experimental trials were conducted under natural photoperiods and temperatures, with the latter fluctuating in the test room of the laboratory between 27 and 28°C. Prior to an experimental trial, all tadpoles (either predator-naïve or predator-experienced) were fed with boiled spinach. Data were analyzed using a two-way analysis of variance for assessing the overall consequence of past exposure to predator cues and the access to refuges and their effects on tadpole survival. Data on the number of tadpoles consumed between the two treatment groups were analyzed by Independent-Samples t-tests.

The two-way ANOVAs showed significant effects of predator exposure ($P < 0.01$, Table 1) and refuge accessibility ($P < 0.01$, Table 1), but not of their interactions ($P = 0.151$, Table 1). Accessibility to refuges significantly reduced tadpole mortality in predator-naïve tadpoles compared to when these had to do without refuges ($T_{38} = 4.531$, $P < 0.01$, Table 2). A similar trend was evident in the case of predator-experienced tadpoles ($T_{38} = 3.325$, $P < 0.01$, Table 2). Tadpole mortality was significantly lower in predator-experienced tadpoles compared to predator-naïve tadpoles, regardless of the accessibility ($T_{38} = 2.416$, $P < 0.01$, Table 2) or inaccessibility ($T_{38} = 3.181$, $P < 0.01$, Table 2) of refuge sites.

In aquatic environments, a majority of the prey organisms including larval anurans live under immense predatory pressure. This pressure results in the evolution of defence strategies in a quest to escape predation and enhance survivorship (SCHMIDT & AMEZQUITA 2001, RELYEA 2007). The result of the present study shows the significance of accessible refuge sites and of past experience with predators in evoking defensive and improving escape behaviours in *C. curtipes* tadpoles. Both factors independently and not conjointly affect larval survival in encounters with *Laccotrephes* sp. The results reveal that accessibility to refuge sites and past knowledge of predation threats are key determinants of survival in the face of predators in *C. curtipes* tadpoles. Our findings conform with observations reported on other larval anurans (SEMLITSCH & REYER 1992, HETTYEY et al. 2011).

The results of the present study show that predator-experienced tadpoles will learn to escape predation and be-

Table 2. Numbers of predator-naïve and predator-experienced *Clinotarsus curtipes* tadpoles consumed by the predator, *Laccotrephes* sp., in the presence or absence of refuge sites, during a trial period of 24 h. Each trial involved 30 either predator-naïve or predator-experienced tadpoles. Twenty trials were carried out with each treatment group and a total of 80 trials were conducted. *Data analyzed by Independent-Samples t-test, * indicates significant differences.

Treatment group	Tadpoles consumed (mean ± SE)		T* and P values
	Absence of refuge sites	Presence of refuge sites	
Predator-naïve	7.65±0.56	4.85±0.25	$T_{38} = 4.531$ $P < 0.01^*$
Predator-experienced	5.50±0.37	3.84±0.32	$T_{38} = 3.325$ $P < 0.01^*$
T* and P values	$T_{38} = 3.181$ $P < 0.01^*$	$T_{38} = 2.416$ $P < 0.01^*$	

come less vulnerable to predators compared to predator-naïve tadpoles regardless of the accessibility of refuges. This clearly demonstrates that past experience with predators plays a key role in enhancing the survival chances of tadpoles by escaping predation. HEALEY & REINHARDT (1995) made a similar observation on Coho Salmon (*Oncorhynchus kisutch*) vis-à-vis predatory Rainbow Trout (*O. mykiss*). For their part, ÁLVAREZ & NICIEZA (2006), who studied in *Rana temporaria* tadpoles, demonstrated that 48 h of association with the predator were enough to improve their ability to escape predation. The present study on *C. curtipes* shows that even an 8-h exposure to the predator or its cues are enough to evoke stronger effective defence behaviours in subsequent encounters with the predator. Possibly, an even shorter period of exposure may be adequate for tadpoles to evoke such effective defensive behaviour. However, further studies are needed to establish the minimum period of exposure to predators required to condition prey and modify future defensive behaviour. Furthermore, releasing predator cues that remain effective for longer periods may not be in the interest of the predator. Indeed, previous studies have shown that predator cues are transient in nature (PEACOR 2006, SHARMA et al. 2008) and their half-life ranges from 0.2 h to a few days (e.g., VAN BUSKIRK et al. 2014). Thus, it appears that the persistence of predatory cues and their ability to evoke defence behaviour in prey may vary between tadpoles. In the present study, all experimental trials were carried out under natural light, there was not much fluctuation in room temperature, and all test tadpoles were healthy and well-fed before trials. Hence, we are confident that these factors did not affect tadpole movements or mortality.

The present study on *C. curtipes* was conducted at early larval stages (Gosner stage 25). Vulnerability rates to its natural predator and escape mechanisms of *C. curtipes* may not be the same throughout its larval phase, because *C. curtipes* tadpoles have a long larval period, i.e., from six months to

a year, and eventually attain large sizes (SAIDAPUR 2001). Additional studies may shed light on possible differences in predator and predator avoidance during ontogeny.

Acknowledgements

SMM is thankful to UGC's Dr. D. S. Kothari postdoctoral fellowship, New Delhi. BAS and SKS are grateful to the Indian National Science Academy (INSA), New Delhi for the support rendered. This research was conducted according to the ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, under Registration No. 639/02/a/CPCSEA.

References

- ÁLVAREZ, D. & A. G. NICIEZA (2006): Factors determining tadpole vulnerability to predators: Can prior experience compensate for a suboptimal shape? – *Evolutionary Ecology*, **20**: 523–534.
- BLAUSTEIN, A. R., J. URBINA, P. W. SNYDER, E. REYNOLDS, T. DANG, J. T. HOVERMAN, B. HAN, D. H. OLSON, C. SEARLE & N. M. HAMBALÉK (2018): Effects of emerging infectious diseases on amphibians: A review of experimental studies. – *Diversity*, **10**: 81.
- FERRARI, M. C. O., B. D. WISENDEN & 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.
- HEALEY, M. C. & U. REINHARDT (1995): Predator avoidance in naïve and experienced juvenile chinook and coho salmon. – *Canadian Journal Fisheries and Aquatic Sciences*, **52**: 614–622.
- HEMMI, J. M. & J. ZEIL (2005): Animal as prey: Perceptual limitations and behavioural options. – *Marine Ecology Progress Series*, **287**: 274–278.
- HETTYEY, A., K. VINCZE, S. ZSARNÓCZAI, H. HOI & A. LAURILA (2011): Costs and benefits of defenses induced by predators differing in dangerousness. – *Journal of Evolutionary Biology*, **24**: 1007–1019.
- HIRAGOND, N. C. & S. K. SAIDAPUR (2001): Microhabitat choice of tadpoles of seven anuran species. – *Current Herpetology*, **20**: 51–60.
- HIRAGOND, N. C., B. A. SHANBHAG & S. K. SAIDAPUR (2001): Description of the tadpole of a stream breeding frog, *Rana curtipes*. – *Journal of Herpetology*, **35**: 166–168.
- HOSSIE, T. J. & 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.
- LIMA, S. L. (1998): Nonlethal effects in the ecology of predator-prey interactions. – *Bioscience*, **48**: 25–34.
- LIMA, S. L. & L. M. DILL (1990): Behavioural decisions made under the risk of predation: A review and prospectus. – *Canadian Journal of Zoology*, **68**: 619–640.
- MOGALI, S. M., S. K. SAIDAPUR & B. A. SHANBHAG (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., S. K. SAIDAPUR & B. A. SHANBHAG (2012): Tadpoles of the bronze frog (*Rana temporalis*) assess predation risk before evoking antipredator defense behaviour. – *Journal of Ethology*, **30**: 379–386.
- MOGALI, S. M., S. K. SAIDAPUR & B. A. SHANBHAG (2016): Influence of desiccation, predatory cues, and density on metamorphic traits of the bronze frog *Hylarana temporalis*. – *Amphibia-Reptilia*, **37**: 199–205.
- MOGALI, S. M., B. A. SHANBHAG & S. K. SAIDAPUR (2020): Vulnerability of *Hylarana temporalis* tadpoles to predation by water scorpions (*Laccotrephes*, Nepidae). – *Herpetological Review*, **51**: 699–701.
- PEACOR, S. D. (2006): Behavioural response of bullfrog tadpoles to chemical cues of predation risk are affected by cue age and water source. – *Hydrobiologia*, **573**: 39–44.
- RELYEA, R. A. (2007): Getting out alive: How predators affect the decision to metamorphose. – *Oecologia*, **152**: 389–400.
- SAIDAPUR, S. K. (2001): Behavioural ecology of anuran tadpoles: the Indian scenario. – *Proceedings of the Indian National Science Academy*, **B67**: 311–322.
- SCHMIDT, B. R. & A. AMEZQUITA (2001): Predator-induced behavioural responses: tadpoles of the Neotropical frog *Phyllomedusa tarsius* do not respond to all predators. – *Herpetological Journal*, **11**: 9–15.
- SEMLITSCH, R. D. & H. U. REYER (1992): Modification of antipredator behaviour in tadpoles by environmental conditioning. – *Journal of Animal Ecology*, **61**: 353–360.
- SHARMA, S. S., D. K. VEERANAGODAR, B. A. SHANBHAG & S. K. SAIDAPUR (2008): Activity of *Sphaerotheca breviceps* tadpoles in response to chemical cues of the predaceous tadpoles *Hoplobatrachus tigerinus*. – *Journal of Ethology*, **26**: 303–307.
- VAN BUSKIRK, J., A. KRUGEL, J. KUNZ, F. MISS & A. STAMM (2014): The rate of degradation of chemical cues indicating predation risk: an experiment and review. – *Ethology*, **120**: 942–949.
- WILBUR, H. M. (1980): Complex life cycles. – *Annual Review Ecology and Systematics*, **11**: 67–93.