

Experience of predacious cues and accessibility to refuge minimize mortality of *Hylarana temporalis* tadpoles

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Abstract. We explored the effect of a prior experience of predation threat and access to the refuge sites on larval mortality in *Hylarana temporalis* with a 2×2 factorial design. The design included predator-naïve or predator experienced prey subjects, and presence or absence of a natural shelter. Water scorpion adult individuals (*Laccotrephes* species) provided predation threat and also an opportunity to the prey to experience a direct danger of predation. Both previous experience with predators and availability of shelters affected larval survival independently and not conjointly. A prior encounter with predators enabled prey tadpoles to escape predation more effectively with a significant increase in their survival in comparison to the predator-naïve subjects. Similarly, access to refuge sites significantly increased survival of predator-naïve as well as predator experienced tadpoles compared to that in the absence of shelters. Clearly, ability to sense water borne predacious cues in the vicinity and use refuge sites plays a key role in escaping from predation in the bronze frog tadpoles.

Keywords. *Hylarana temporalis*, *Laccotrephes* sp., predator, refuge availability, tadpole mortality.

Predator-prey interactions invariably lead to an evolutionary arms race in which early detection of either party is often the key for success. Progressive elimination of prey individuals from the ecosystem by the coexisting predators can have far reaching impact on their population dynamics. Most anuran amphibians show a biphasic mode of life cycle involving aquatic larval phase. In the aquatic phase of life the larval mortality is often due to desiccation of the ephemeral ponds before completion of the metamorphosis, infection, and to predation by the coexisting aquatic invertebrate and vertebrate predators. Therefore, adoption of phenotypic plasticity and diverse strategies become essential for their survival. Many anuran larvae including *Hylarana temporalis* are known to alter their behavior and the life-history traits under predation risk (Relyea, 2007; Sharma et al., 2008; Saidapur et al., 2009; Mogali et al., 2012, 2015, 2016; Mogali, 2018). For example, *H. temporalis* tadpoles meta-

morphosed early and at a larger body size under predation threat, when provided by a caged dragonfly larva (*Pantala flavescens*), compared to those reared under predation threat-free environment (Mogali et al., 2016). They also decreased swimming activity, by remaining still for longer times, to avoid being detected and increased burst speed on encounter with the predacious cues in the vicinity (Mogali et al., 2012). Taking shelter under the leaf litter, aquatic vegetation and other objects that provide refuge and utilization of benthic habitat can help tadpoles by reducing the risk of being detected by predators (Lima and Dill, 1990; Eklov and Persson, 1996; Hossie and Murray, 2010). Besides, predators may find difficult to capture a prey residing inside a refuge; at the same time, prey may have a better opportunity to assess the actual risk of predation by the predators residing in close vicinity (Hemmi and Zeil, 2005) and, consequently, evoke defensive behaviors. Nevertheless, hiding and stay-

ing still by prey involve trade-offs. These strategies may reduce encounter rates with predators but also tend to reduce time dedicated to foraging activity. Indeed, often the habitats that are energetically very profitable are also the most dangerous, since the distribution of predators tends to match their prey's resource distribution (Lima, 1998). Besides, the refuge may not always be the best place for feeding. For instance, inadequate time spent in feeding leads to alteration in the metamorphic traits in *Bufo melanostictus* (Mogali et al., 2011).

The bronze frog (*Hylarana temporalis*) tadpoles are found along the gently flowing streams and isolated pockets of water along sides of the streams during the post-monsoon season (October-January) in South-Western Ghats of India (Mogali et al., 2012). They are bottom dwellers and thrive on detritus and algal matter (Hiragond and Saidapur, 2001). In these water bodies visibility is low due to shadows of the dense vegetation, brownish-dark color of the benthic area that is typically covered by the leaf litter and detritus matter (Veeranagoudar et al., 2004). The water bodies are also inhabited by several types of invertebrate predators that include aquatic beetles, dragonfly larvae, crabs and water scorpions that prey upon anuran tadpoles (our personal observations). During field visits we have observed water scorpions (*Laccotrephes* sp., Nepidae) actively feeding on bronze frog tadpoles. They detect their prey visually and by mechanical cues. The *H. temporalis* tadpoles have poor vision (Veeranagoudar et al., 2004) as in most anuran tadpoles (Hoff et al., 1999). However, they possess an innate ability to detect predators by chemoreception (Mogali et al., 2012) and evoke antipredator behavior. Further, predator-experienced *H. temporalis* tadpoles show enhanced defensive behaviors on subsequent encounter with predators (Mogali et al., 2012).

The natural habitats in which *H. temporalis* tadpoles live is full of detritus material and leaf litter. Therefore, we hypothesized that tadpoles of the bronze frog profitably use these shelters (leaf litter) to reduce predation risk of the coexisting predators. An additional assumption was that tadpoles having prior experience of predacious cues will be more successful in escaping predation by using shelters compared to the those facing the predators for the first time (predator-naïve). To test our hypotheses, tadpoles of *H. temporalis* were exposed to free hunting water scorpion (*Laccotrephes* sp.). The prey subjects were either predator-naïve or predator experienced. They were exposed to predator in laboratory set ups with or without the refuge sites (leaf litter) for a predetermined period to record survival/mortality of prey.

Eight clutches of *H. temporalis* were collected in November, 2013 from a stream in the Western Ghats near

Anmod village (15.430888°N, 74.373601°E), Karnataka State, India. Each clutch was individually reared in plastic tub (32 cm diameter and 14 cm deep) containing 5 L of aged tap (dechlorinated) water. Hatching time occurred six days later, at Gosner stage 19 (Gosner, 1960). The tadpoles of different clutches were mixed to normalize genetic differences among the groups. After mixing, tadpoles were reared in two separate glass aquaria (90 cm L × 30 cm W × 15 cm H) each containing 25 L of aged tap water. Upon reaching stage 25, tadpoles were supplied with boiled spinach *ad libitum*. The water scorpions (*Laccotrephes* sp.) were collected from the same location from where the eggs of *H. temporalis* were obtained and were reared individually, to avoid cannibalism, in small plastic bowls (14 cm diameter and 7 cm deep) with 500 mL of aged tap water. Prey tadpoles served as food for water scorpions.

The tadpoles born in laboratory, and never exposed to predator, served as the predator-naïve subjects. To obtain predator-experienced tadpoles we exposed groups of 30 tadpoles to a 48 h starved predator for 8 h (09:00-17:00 h). After the 8 h trial period the predator and injured tadpoles were removed from the test bowl. On an average, predator ate 3 ± 0.3 tadpoles and injured 4 ± 0.5 ($x \pm SE$) during the trial period. Predator-threat experienced and uninjured tadpoles obtained from 80 trials were used in the experiments the following days, in order to assess their performance against predators on the subsequent encounter with them.

The leaves of *Aporosa lindleyana* were collected from the same sites from which *H. temporalis* eggs were collected. Structural refuge was made using water soaked (2 days) leaves (dry mass $15 \text{ g} \pm 1.8$; $x \pm SE$) chopped to have $\sim 1 \text{ cm}^2$ pieces. These were spread at the bottom of the testing bowls to serve as shelters/ refuge site).

In all trials, prey tadpoles were of comparable sizes (length 20.48 ± 0.09 mm, width 5.31 ± 0.07 mm, and weight 62.0 ± 0.64 mg; $x \pm SE$; $n = 30$) and developmental stages (stages 28-30). The water scorpions used in various trials were comparable in size (length 31.0 ± 0.08 mm, width 10.11 ± 0.05 mm, and weight 622.0 ± 5.2 mg; $x \pm SE$; $n = 30$). The size of prey and predators was measured using a digital caliper (accuracy 0.01 mm) and weight was recorded using an electronic balance (accuracy 0.001 g).

The experiment involved using a 2×2 factorial design with the following groups.

1. Predator-naïve tadpoles were exposed to predator in the absence of refuge
2. Predator-naïve tadpoles were exposed to predator in the presence of refuge
3. Predator-experienced tadpoles were exposed to predator in the absence of refuge

4. Predator-experienced tadpoles were exposed to predator in the presence of refuge

Each treatment consisted of 30 trials (overall 120 trials).

For each trial the test tadpoles were released ($n = 30$) into the plastic tub (32 cm diameter and 14 cm deep) containing 3 L of aged tap water and with or without the structural refuge and allowed to familiarize themselves to the tub for 15 min. Then one water scorpion starved for 48 h was introduced gently into the tub and left there. The trials were terminated after 24 h. The number of surviving tadpoles in various trials was recorded to compute the number of tadpoles lost due to predation. Data were analyzed using two-way analysis of variance for assessing the overall consequence of a prior exposure to predatory cues and the access to the refuge and their interaction on prey survival. Data on number of tadpoles consumed between the two treatment groups were analyzed by independent-samples t test.

Two-way ANOVA showed significant main effect of predator exposure ($P < 0.001$, Table 1) and refuge availability ($P < 0.001$, Table 1) but not of their interaction ($P = 0.253$, Table 1).

Accessibility to the refuge significantly reduced the larval mortality in predator-naïve tadpoles when compared to that without refuge ($t_{58} = 4.402$, $P < 0.001$, Fig. 1A). A similar trend was also evident in the case of predator-threat experienced tadpoles ($t_{58} = 5.648$, $P < 0.001$, Fig. 1B). The larval mortality was significantly lower in predator-experienced tadpoles compared to predator-naïve tadpoles regardless of the availability ($t_{58} = 4.144$, $P < 0.001$) or unavailability ($t_{58} = 4.353$, $P < 0.001$) of refuge sites (Fig. 1).

The present study shows the importance of available shelters and of a prior experience with predator in evoking defensive behaviors and enhances escape predation in *H. temporalis* tadpoles. Both these factors independently and not conjointly affect the larval survival following their encounter with water scorpion. The results reveal that accessibility to refuge sites and prior experience of predation threat are key determinants of

Table 1. Results of two-way ANOVA for exposure and refuge availability and their interactions. The response variable is mean number of *Hylarana temporalis* tadpoles lost due to predator. Significant values are bolded.

| Source | df | MS | F | P |
|---------------------------------------|----|---------|--------|------------------|
| Exposure | 1 | 108.300 | 35.382 | <0.001 |
| Refuge availability | 1 | 132.300 | 43.222 | <0.001 |
| Exposure \times refuge availability | 1 | 4.033 | 1.318 | 0.253 |

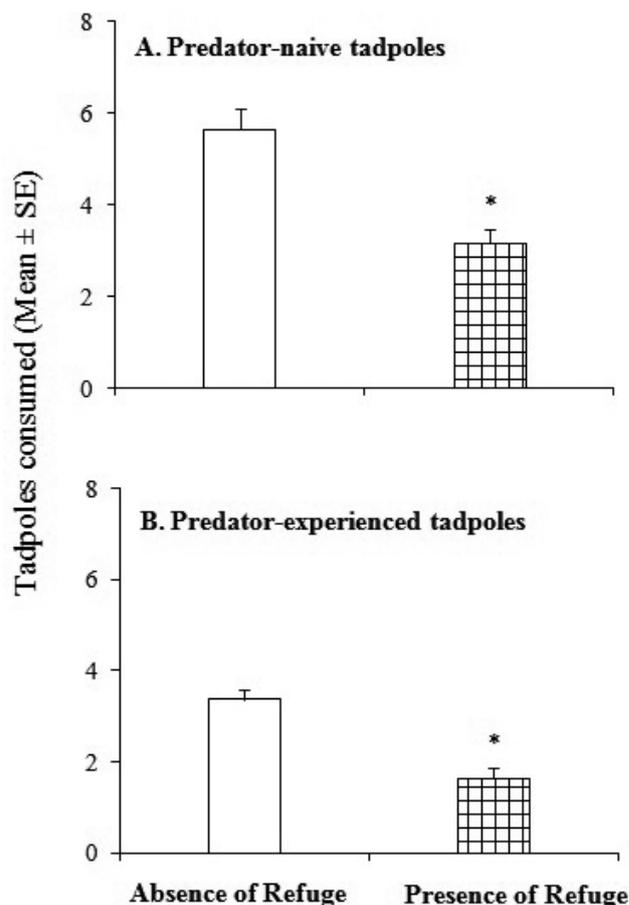


Fig. 1. Number of predator-naïve and predator-experienced *Hylarana temporalis* tadpoles consumed by the predator, *Laccotrephes* sp., in relation to accessibility to refuges in trials of 24 h. An asterisk over the bar indicates a significant difference between two treatments. Data represents $\bar{x} \pm SE$. Each trial consists of 30 tadpoles of either predator-naïve or predator-experienced. 30 trials were carried out for each treatment and total all together 120 trials.

survival from the aquatic insect predators in *H. temporalis* tadpoles. Previous studies have shown that bronze frog tadpoles exhibit antipredator defense behaviors in response to water borne chemical cues of predator by staying away from the predator and reducing their swimming activity (Mogali et al., 2012). Our study revealed a heightened defense response in predator-experienced tadpoles in the subsequent encounters with the predator (Mogali et al., 2012) in conformity with the observations reported on other anuran species (Semlitsch and Reyer, 1992; Hettley et al., 2011). The results of the present study show that predator-experienced tadpoles learn to escape predation and become less vulnerable to predator compared to the predator-naïve individuals regardless of the accessibility to structural refuge. This clearly demonstrates that prior experience with predator plays a key

role in enhancing survival chances of tadpoles by escaping predation. Healey and Reinhardt (1995) made similar observation on Coho salmon against the predacious Rainbow trout. Álvarez and Nicieza (2006), who studied *Rana temporaria* tadpoles, showed that 48 h association with the predator was a sufficient period of time to enhance their ability to escape predation. The present study on *H. temporalis* shows that even mere 8 h exposure to predatory cues is sufficient to evoke a stronger effective defense behavior on subsequent encounters with the predator. Possibly, even a shorter time exposure might be sufficient for prey tadpoles to evoke their defensive behavior on following encounters with predators. However, additional studies are needed to establish the minimum period of exposure to predators that is required to condition the prey and modify their future performance of defensive behaviour. Further, releasing predacious cues that remain effective for longer periods may not be in the interest of the predator. Indeed, several studies showed that predaceous cues are labile in nature (Peacor, 2006; Sharma et al., 2008) and their half life ranges from 0.2 h to a few days (Van Buskirk et al., 2014). Thus, it appears that persistence of predatory cues and their ability to evoke defense behaviors in prey may vary among anuran tadpoles.

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REFERENCES

- Álvarez, D., Nicieza, A.G. (2006): Factors determining tadpole vulnerability to predators: can prior experience compensate for a suboptimal shape? *Evol. Ecol.* **20**: 523-534.
- Eklov, P., Persson, L. (1996): The response of prey to the risk of predation: proximate cues for refusing juvenile fish. *Anim. Behav.* **51**: 105-115.
- Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**: 183-190.
- Healey, M.C., Reinhardt, U. (1995): Predator avoidance in naïve and experienced juvenile chinook and coho salmon. *Can. J. Fish. Aquat. Sci.* **52**: 614-622.
- Hemmi, J.M., Zeil, J. (2005): Animal as prey: perceptual limitations and behavioral options. *Mar. Ecol. Prog. Ser.* **287**: 274-278.
- Hettyey, A., Vincze, K., Zsarnóczai, S., Hoi, H., Laurila, A. (2011): Costs and benefits of defences induced by predators differing in dangerousness. *J. Evol. Biol.* **24**: 1007-1019.
- Hiragond, N.C., Saidapur, S.K. (2001): Microhabitat choice of tadpoles of seven anuran species. *Curr. Herpetol.* **20**: 51-60.
- Hoff, K.V.S., Blaustein, A.R., McDiarmid, R.W., Altig, R. (1999): Behavior: interactions and their consequences. In: *Tadpoles: biology of anuran larvae*, pp. 215-239. McDiarmid, R.W., Altig, R., Eds, University of Chicago Press, Chicago, USA.
- 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.
- Lima, S.L. (1998): Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* **48**: 25-34.
- Lima, S.L., Dill, L.M. (1990): Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**: 619-640.
- Mogali, S. (2018): Predatory cues influence the behavioral responses and metamorphic traits of *Polypedates maculatus* (Anura: Rhacophoridae). *Asian Herpetol. Res.* **9**: 188-194.
- Mogali, S.M., Saidapur, S.K., Shanbhag, B.A. (2011): Levels of predation modulate antipredator defense behavior and metamorphic traits in the toad *Bufo melanostictus*. *J. Herpetol.* **45**: 428-431.
- Mogali, S.M., Saidapur, S.K., Shanbhag, B.A. (2012): Tadpoles of the bronze frog (*Rana temporalis*) assess predation risk before evoking antipredator defense behavior. *J. Ethol.* **30**: 379-386.
- Mogali, S.M., Shanbhag, B.A., Saidapur, S.K. (2015): Strong food odors mask predation risk and affect evocation of defense behaviors in the tadpoles of *Sphaerotheca breviceps*. *J. Ethol.* **33**: 41-46.
- Mogali, S.M., Saidapur, S.K., Shanbhag, B.A. (2016): Influence of desiccation, predatory cues, and density on metamorphic traits of the bronze frog *Hylarana temporalis*. *Amphibia-Reptilia* **37**: 199-205.
- 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., Veeranagoudar, D.K., Hiragond, N.C., Shanbhag, B.A. (2009): Mechanism of predator-prey

- detection and behavioral responses in some anuran tadpoles. *Chemoecology* **19**: 21-28.
- Semlitsch, R.D., Reyer, H.U. (1992): Modification of anti-predator behavior in tadpoles by environmental conditioning. *J. Anim. Ecol.* **61**: 353-360.
- Sharma, S.S., Veeranagoudar, D.K., Shanbhag, B.A., Saidapur, S.K. (2008): Activity of *Sphaerotheca breviceps* tadpoles in response to chemical cues of the predaceous tadpoles *Hoplobatrachus tigerinus*. *J. Ethol.* **26**: 303-307.
- Van Buskirk, J., Krügel, A., Kunz, J., Miss, F., Stamm, A. (2014): The rate of degradation of chemical cues indicating predation risk: An experiment and review. *Ethology* **120**: 942-949.
- Veeranagoudar, D.K., Shanbhag, B.A., Saidapur, S.K. (2004): Mechanism of food detection in the tadpoles of the bronze frog *Rana temporalis*. *Acta Ethol.* **7**: 37-41.