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Adaptive significance of the transparent body in the tadpoles of ornamented pygmy frog, *Microhyla ornata* (Anura, Amphibia)

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

Department of Zoology, Karnatak University, Dharwad-580 003, Karnataka State, India
* *Corresponding author*. Email: santoshmogali@rediffmail.com

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Abstract. In Southern India, during the Southwest monsoon phase, the newly formed ephemeral water bodies harbour several species of tadpoles as well as some of their predators. Tadpoles of *Microhyla ornata* dwell in surface or column zones of water. They face predation threat from aquatic insect predators and carnivorous tadpoles of other anurans though they are invisible due to their transparent body form. We tested whether transparent body form of *M. ornata* tadpoles is a useful attribute against predation by exposing tail fin stained (with the Nile blue) subjects to a naturally occurring predator (*Hoplobatrachus tigerinus* tadpoles that detect prey using both visual and chemical cues). The study shows that susceptibility of stained *M. ornata* tadpoles to predation increased significantly compared to the unstained transparent individuals. We conclude that the transparent body form is of great significance in escaping predation during the larval phase of life in *M. ornata*.

Keywords. *Hoplobatrachus tigerinus*, *Microhyla ornata*, predation, tadpoles, transparent body

Most anuran amphibians are opportunistic breeders; in South India, they often breed communally in rain-filled puddles soon after the onset of southwest monsoon. Therefore, tadpoles of several species coexist during their aquatic larval phase (Saidapur et al., 2009). In such ephemeral ponds, the tadpoles commonly experience desiccation threat, competition for resources from homospecific and heterospecific members (Mogali et al., 2011a, b, 2015, 2017). In addition, predation threat from aquatic insects and their larvae are routinely encountered by the herbivorous tadpoles in such ephemeral ponds (Skelly, 1997). Furthermore, the tadpoles of several species are carnivorous or omnivorous (Saidapur et al., 2009). In response to coexisting predators, the prey tadpoles have evolved defence strategies that often involve utilization of refuge shelters when available, reduced movements, high burst speed when movement is necessary, species-specific habitat choices (Hiragond and Saidapur, 2001; Saidapur et al., 2009; Hossie and Murray, 2010; Mogali, 2018; Mogali et al., 2019) and so on. Within the community of anuran larvae found in the ephemeral water bodies of Southern India, tadpoles of *Hoplobatrachus tigerinus* are notorious predators and hunt actively (Saidapur, 2001; Saidapur et al., 2009).

Of the several species of anuran larvae found in Southern India, the tadpoles of *Microhyla ornata* are unique in many aspects; they are predominantly found in the surface or column zone of the pond, devoid of teeth, feed on micro-plankton, delicate, slow movers, remain still for longer time and more importantly, their body is transparent (Hiragond and Saidapur, 2001) and hence not easily visible. Despite a highly vulnerable nature, *M. ornata* is highly successful and show wide distribution throughout Asia and the Indian subcontinent. Evidently, the success of any anuran species with aquatic larval phase depends on the survival rate of

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their larvae, emergence on the land with optimum size and, subsequent reproduction. We hypothesized that transparent body of *M. ornata* tadpoles is a key feature in ensuring their survival and escape from predation. Therefore, we conducted experiments on *M. ornata* tadpoles whose tail fins were stained with Nile blue or left unstained and then tested for their efficiency to escape from predation using highly predacious sympatric tadpoles of *H. tigerinus*.

Six egg clutches of *M. ornata* were collected from rain-filled puddles/ ponds located on the Karnatak University Campus, Dharwad (latitude 15.440407°N, longitude 74.985246°E) in June 2008. Each egg clutch was placed separately in plastic tubs (42 cm diameter and 16 cm deep) containing 3 L of pond water. Eggs from all clutches hatched almost synchronously at Gosner stage 19 (Gosner, 1960) a day after their collection. Soon after hatching, the tadpoles of different clutches were mixed and reared in 3 separate glass aquariums (75 cm L × 45 cm W × 15 cm H) each with ~ 600 tadpoles in 15 L of pond water to provide plankton material for feeding. The tadpoles in the Gosner stage 25-26 with comparable body size (length 12.65 ± 0.42 mm, Mean ± SE) were used in trials. The tadpoles of *H. tigerinus* (Gosner stage 27-28; ~ n = 70) were also collected from the rain-filled ponds located on the University Campus. They were reared individually to avoid cannibalism in plastic bowls (16 cm diameter and 7 cm deep) in 0.5 L of aged tap water. *Hoplobatrachus tigerinus* tadpoles of Gosner stage 29-30 having comparable body size (length 32.15 ± 0.49 mm Mean ± SE) were used in the trials.

The experiment was made with two sets of tadpoles of *M. ornata* (n = 25 per set) chosen randomly. In the first set of experiment, tadpoles of *M. ornata* (n = 25) with a natural transparent body, were released into a rectangular glass tank (40 cm L × 25 cm W × 30 cm H) with 10 L of aged tap water that created a column height of 10 cm. After acclimatization for 15 min, a single *H. tigerinus* tadpole (starved for 24 h) was introduced into the tank and left there for 24 h to estimate the quantum of predation. After the trial period, the number of surviving prey tadpoles was recorded to compute the number of tadpoles lost due to predation. In the second set of experiment, the tail fins of prey tadpoles were applied Nile blue (1 mg/mL water and filtered by using Whatman filter paper no. 40) using a fine brush before the trials. The Nile blue coloured tadpoles (n = 25) were left with one starved *H. tigerinus* tadpole for 24 h as in the first set of experiment. After completion of the trial duration, the number of tadpoles consumed by the predator was recorded. Twenty-five trials were carried out for both sets of experiments. The data on the number of tadpoles consumed by the predator between the two experiments were analyzed by the Mann-Whitney U test.

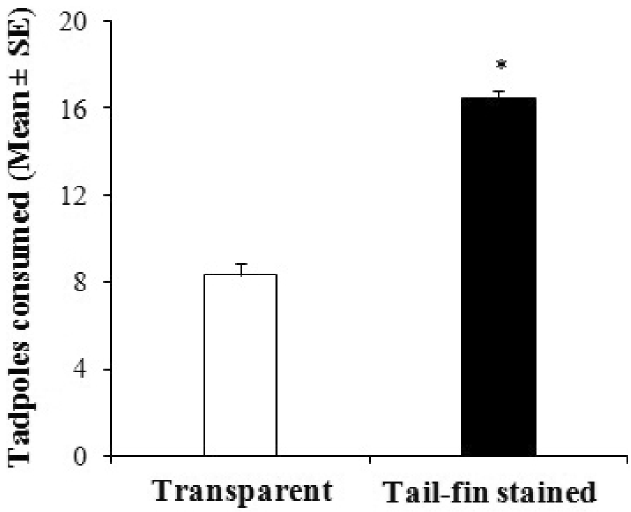

Fig. 1. Number of *Microhyla ornata* (transparent or tail-fin stained) tadpoles consumed by the predator, *Hoplobatrachus tigerinus* tadpoles in trials of 24 h. Data represent Mean ± SE and analyzed by Mann-Whitney U test; n = 25 trials per group with 25 tadpoles per trial. An asterisk indicates a significant difference between the groups ($P < 0.001$).

The results show that the predator consumed a significantly greater number of prey tadpoles that had blue tinge on their tail fins ($U = 15.500$, $P < 0.001$, Fig. 1) compared to those that did not have any colour on the tail fins. Thus, the insatiable predatory tadpoles of *H. tigerinus* that are active chase hunters predated a significantly smaller number of prey subjects that were transparent compared to those whose tail fins showed blue tinge due to Nile blue.

Most previous studies on prey-predator interactions have dealt with the tadpoles residing in the substrate or benthic zones (Relyea, 2001; Saidapur et al., 2009; Jara and Perotti, 2010; Mogali et al., 2011a, 2012). It may be noted that the aquatic insects and their larvae that prey on tadpoles are generally found along with anuran larvae at the substratum and these are mostly ‘sit and wait’ predators; they wait for the prey to come near before attacking them (Luttbeg et al., 2008; Miller et al., 2014). *Microhyla ornata* tadpoles are principally surface/ column zone dwellers (Hiragond and Saidapur, 2001). Therefore, one can expect the least predation of *M. ornata* tadpoles by the aquatic insects and their larvae. On the other hand, predatory *H. tigerinus* tadpoles are generally substrate dwellers but also capable of moving all over. Besides they are active chase hunters which make use of both visual as well as chemical cues of the prey subjects in devouring them (Hiragond and Saidapur, 2001; Saidapur et al., 2009). The predation threat from such predators is enormous even to surface dwellers like the *M. ornata* tadpoles. The present findings clearly show that *M. ornata* tadpoles have a greater capacity to escape predation from the active and chase hunting *H. tigerinus* tadpoles. However, a mere blue tinge of tail fins following application of the vital dye Nile blue made them highly susceptible to predation.

Nile blue used in this study was in micro quantities. Hence, in such large quantity of water, chemical cues arising from the dye, if any, would be presumably very weak. However, the study shows that *H. tigerinus* tadpoles perceive even slightly coloured blue tinged *M. ornata* tadpoles more efficiently than that of the transparent subjects. Thus, it appears that less vulnerability of this tadpole species to predation is largely due to its unique transparent body form. The present study, however, does not convey us about the perception of the type of visual cues arising after application of Nile blue to prey tadpoles by *H. tigerinus* tadpoles. Therefore, further additional and differently designed studies are needed to decipher the impact of different colours/ wavelengths to know what exactly is perceived by the predator. In conclusion, the transparent body form of *M. ornata* tadpoles appears to be a key feature ensuring larval survival and escape from predators.

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