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Comparative vulnerability of *Indosylvirana temporalis* and *Clinotarsus curtipes* (Anura: Ranidae) tadpoles to water scorpions: importance of refugia and swimming speed in predator avoidance

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Abstract

Comparative vulnerability of *Indosylvirana temporalis* and *Clinotarsus curtipes* (Anura: Ranidae) tadpoles to water scorpions: importance of refugia and swimming speed in predator avoidance. The comparative vulnerability of two co-existing tadpole species (*Indosylvirana temporalis* and *Clinotarsus curtipes*) to their common predator, water scorpions (*Laccotrephes* sp.; Hemiptera: Nepidae), and the importance of refugia in predator avoidance were studied in the laboratory. In a total of 60 experimental trials, 10 tadpoles each of *I. temporalis* and *C. curtipes* of comparable body sizes were exposed to water scorpions (starved for 48 h). Thirty trials included refugia while 30 did not. The results of this study showed that in both the absence and the presence of refugia *C. curtipes* tadpoles fell prey to water scorpions more frequently than *I. temporalis* tadpoles. A main difference between the two species is the speed of swimming; V_{max} of *C. curtipes* (24.73 cm/s) tadpoles is lower than that of *I. temporalis* (30.78 cm/s) tadpoles. This is likely to be the reason why more *C. curtipes* tadpoles were preyed upon than were *I. temporalis* tadpoles. Predation risk of tadpoles of both species was affected significantly by the presence of refuge sites. The vulnerability of both tadpole species was lower where refuge sites were available. The present study clearly shows that *I. temporalis* tadpoles avoid predation by water scorpions more effectively than do *C. curtipes* tadpoles.

Keywords: Defensive behavior, Hemiptera, *Laccotrephes* sp., Nepidae, predator-prey relationships, refuge sites, tadpoles.

Resumo

Vulnerabilidade comparativa dos girinos *Indosylvirana temporalis* e *Clinotarsus curtipes* (Anura: Ranidae) aos escorpiões da água: importância de abrigos e da velocidade de natação na evitação de predadores. A vulnerabilidade comparativa de duas espécies de girinos coexistentes (*Hylarana temporalis* e *Clinotarsus curtipes*) ao seu predador comum, o escorpião-d'água (*Laccotrephes* sp.; Hemiptera: Nepidae), e a importância dos abrigos na evitação de predadores foram estudados em laboratório. Em um total de 60 ensaios experimentais, 10 girinos de de cada

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espécie, tamanhos de corpo comparáveis, foram expostos a escorpiões-d'água (mantidos sem alimento por 48 horas). Trinta experimentos incluíram abrigos, enquanto outros 30 não o fizeram. Os resultados deste estudo mostraram que, tanto na ausência como na presença de abrigos, os girinos de *C. curtipes* foram predados mais frequentemente do que os girinos de *I. temporalis*. Uma diferença principal entre as duas espécies foi a velocidade de natação; a V_{max} dos girinos de *C. curtipes* (24,73 cm/s) foi menor que a dos girinos de *I. temporalis* (30,78 cm/s). Provavelmente foi esse o motivo pelo qual mais girinos de *C. curtipes* foram predados em relação *I. temporalis*. O risco de predação de girinos de ambas as espécies foi significativamente influenciado pela presença de locais de refúgio. A vulnerabilidade de ambas as espécies foi menor onde havia locais de abrigo disponíveis. O presente estudo mostra claramente que os girinos de *I. temporalis* evitam mais eficazmente a predação por escorpiões-d'água do que os girinos de *C. curtipes.*

Palavras-chave: comportamento defensivo, girinos, Hemiptera, *Laccotrephes* sp., locais de abrigo, Nepidae, relações predador-presa.

Introduction

In nature, predation is a major selective force acting on prey that forces the evolution of strategies for assessment of predation threat and the development of antipredator defense strategies in order to optimize survival and fitness (Lima and Dill 1990). In aquatic environments, tadpoles of most anurans face varying levels of predation threat and therefore evolve a variety of defense strategies. Antipredator strategies of anuran tadpoles observed in earlier studies include increased activity or high swimming speed in order to run away from predators (Hews 1988, Van Buskirk and McCollum 2000), reduction in activity levels to avoid detection (Kiesecker *et al.* 1996, Schmidt and Amézquita 2001, Saidapur *et al.* 2009, Mogali *et al.* 2011, 2012, 2020a), aggregation (Spieler and Linsenmair 1999) and increased use of refuge sites (Stauffer and Semlitsch 1993, Nystrom and Abjornsson 2000, Hossie and Murray 2010, Mogali *et al.* 2019) depending upon species. Because they exist in aquatic environments, anuran tadpoles mainly use chemical signals to assess predation threats since visual information may be obscured in water that is turbid or densely vegetated (Kiesecker *et al.* 1996, Mogali 2018).

The tadpoles of *Indosylvirana temporalis* (Günther, 1864) and *Clinotarsus curtipes* (Jerdon, 1853) co-exist along gently flowing streams and in isolated pockets of water along sides of streams during the post-monsoon season in the South-Western Ghats of India (Hiragond and Saidapur 2001, Mogali *et al.* 2012, 2016). They are mainly bottom dwellers and thrive on detritus and algal matter (Hiragond and Saidapur 2001). Visibility is low in these water bodies due to shadows from vegetation, and the benthic area that is naturally covered by leaf litter and detritus (Mogali *et al.* 2019). These water bodies are home to several types of predatory invertebrates including water scorpions, *Laccotrephes* sp. (Hemiptera: Nepidae). Water scorpions are considered ambush/sit-and-wait, non-gap-limited predators with excellent vision. In nature, they are well camouflaged in the vegetation or detritus, and ambush unsuspecting prey including tadpoles of *I. temporalis* and *C. curtipes* with a quick grasping action of the forelegs (Mogali *et al.* 2020b).

Earlier studies from our laboratory show that, under predation threat by water scorpions, the availability of refuge sites minimizes the larval mortality of both predator-naïve and predator-experienced *I. temporalis* (Mogali *et al.* 2019) and *C. curtipes* (unpubl. data). They also show that predator-experienced tadpoles of both species use refuge sites more effectively and survive better than predator-naïve tadpoles.

In natural environments, we noticed that

tadpoles of both species co-exist and use similar hiding places when needed, hence it is very important to know about the comparative vulnerability of tadpoles to their common predator, water scorpions. Hence, the present study was designed to determine the comparative vulnerability of wild-caught tadpoles (predator-experienced) of *I. temporalis* and *C. curtipes* of comparable body size at early stages of development (Gosner stages 25–27) to free hunting water scorpions both in the presence and the absence of refuge sites. We hypothesized that there should be a difference in the vulnerability between two anuran tadpole species.

Materials and Methods

Tadpoles of *Hylarana temporalis* (Gosner stages 27–28; N = ~ 800) and *Clinotarsus curtipes* (Gosner stage 25; N = ~ 800) were collected from a stream in the Western Ghats near Anmod village (15.43088° N, 74.37360° E), Karnataka State, India in November and brought to the laboratory. Tadpoles of each species were placed separately in glass aquaria (90 × 30 × 15 cm) containing 25 L of aged tap water and used as a stock. Tadpoles of both species are herbivores and were fed boiled spinach to sustain growth and development. The water scorpions (*Laccotrephes* sp.; predators; N = 70) were collected from the same location that the tadpoles were obtained and were reared individually in plastic tubs (14 cm diameter and 7 cm deep) with 500 mL of aged tap water to avoid cannibalism. Prior to the commencement of the experiment, predators were daily fed equally with both prey species (3 *I. temporalis* + 3 *C. curtipes* tadpoles; Gosner stage 25) for at least four days.

Experiment 1: Comparative Vulnerability of Prey Species

This experiment was designed to determine the comparative vulnerability of *I. temporalis* and *C. curtipes* tadpoles to predatory water scorpions and the importance of refugia in predator avoidance. We carried out a total of sixty experimental trials over a week period. Ten trials were conducted per day, in ten separate experimental tubs each containing one of two treatments. Each trial started at 07:00 AM and ended at 07:00 the next day. In each trial ten tadpoles each of *I. temporalis* (Gosner stages 27–28; 21.12 ± 0.23 mm in total length; 5.37 ± 0.14 mm in width and weight 58.00 ± 3.42 mg; mean ± SD across all trials) and *C. curtipes* (Gosner stage 25; 21.10 ± 0.24 mm in total length; 5.35 ± 0.16 mm in width and weight 57.80 ± 3.50 mg; mean ± SD) of comparable body sizes were released in a plastic tub (32 cm diameter and 14 cm deep) containing 3 L of aged tap water. They were allowed to acclimate for 30 min. Then one water scorpion (61.50 ± 3.49 mm in total length, 10.18 ± 0.24 mm in width and weighing 629.0 ± 13.15 mg; mean ± SD across all trials) starved for 48 h was introduced into the tub. After 24 h the number of surviving *I. temporalis* and *C. curtipes* tadpoles was recorded to compute the number of tadpoles of each species lost due to predation.

In thirty trials (five per day over six days) the tubs containing the tadpoles and predators provided no refugia for the tadpoles. In a second thirty trials, carried out five per day over the same six days, the tubs contained structural refuges made using water soaked (two days) leaves of *Aporosa lindleyana* (dry mass 15 ± 1.6 g; mean ± SD) chopped into ~ 1 cm^2 pieces. These were spread at the bottom of the testing tubs to serve as shelters/ refuge sites. Predation risk was studied as described above. The test tubs in all trials were washed thoroughly before each trial. Both tadpole species were well fed with boiled spinach before trials. However, during the trial hours they were not provided any food. All experimental trials were carried out at room temperature (25°C). Relative vulnerability of *I. temporalis* and *C. curtipes* tadpoles to predation in each experiment was tested using Mann-Whitney *U*-tests (SPSS software ver. 16.0).

Experiment 2: Burst Swimming Speed of Prey Tadpoles

The vulnerability of the prey species (*I. temporalis* and *C. curtipes* tadpoles) to predation by water scorpion differed significantly (Experiment 1). It was thus of interest to know the differences in the swimming speeds between the prey species. To determine V_{max}, a single test tadpole (either *I. temporalis* or *C. curtipes*) of comparable body size (see expt. 1) was placed in a plastic tub (20 cm diameter and 10 cm deep) filled with aged tap water to a depth of 2.5 cm and left undisturbed for 5 min to adjust to new conditions. A handycam (Sony, DCR-SR300/E) was positioned above the tub to record activity in the entire tub. 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. After 5 min of acclimation, the test tadpole (either *I. temporalis* or *C. curtipes*) was chased continuously for 1 min by prodding the tail base with a delicate wire as described by Van Buskirk and McCollum (2000). The movement of the tadpole was tracked to determine the V_{max}. A total of 25 trials were carried out for both tadpole species with a new test tadpole of each species every time. Both tadpole species were well fed with boiled spinach before trials. The V_{max} of two tadpole species was compared by Mann-Whitney *U*-test (SPSS software ver. 16.0).

Results

Experiment 1: Comparative Vulnerability of Prey Species

Either in the absence or presence of refuge sites significantly higher numbers of *C. curtipes* tadpoles fell prey to water scorpions as compared to *I. temporalis* tadpoles (Table 1). Predation risk of tadpoles of both species was affected significantly by the presence of refuge sites. The vulnerability of both tadpole species was lower where refuge sites were available (Table 1).

Experiment 2: Burst Swimming Speed of Prey Tadpoles

There was a significant difference in the swimming speed between tadpoles of the two

Table 1. Number of prey tadpoles (mean ± SE) of *Indosylvirana temporalis* and *Clinotarsus curtipes* consumed by the predator, *Laccotrephes* sp., in a 24 h trial period (N = 30 trials. #Mann-Whitney U-test; *indicates significant difference between two treatments.

Treatment	Tadpoles consumed		U and p values#
	Indosylvirana temporalis	*Clinotarsus curtipes*	
Without refuge sites	2.60 ± 0.23	4.03 ± 0.40	$U = 268.0$, $p < 0.05$*
With refuge sites	1.43 ± 0.21	2.53 ± 0.27	$U = 256.0$, $p < 0.05$*
U and p values#	$U = 229.5$, $p < 0.05$*	$U = 265.5$, $p < 0.05$*	

Table 2. Burst swimming speed (V_{max}; mean ± SE) of *Indosylvirana temporalis* and *Clinotarsus curtipes* tadpoles (N = 25 trials). #Mann-Whitney U test; *indicates significant difference between two species.

Species	V_{max} (cm/s)
Indosylvirana temporalis	30.78 ± 1.24
Clinotarsus curtipes	24.73 ± 1.01
U and p values#	$U = 147.50$, $p < 0.05$*

species. The *I. temporalis* tadpoles exhibited a greater V_{max} (30.78 cm/s) than that of the *C. curtipes* (24.73 cm/s) tadpoles (Table 2).

Discussion

In aquatic environments, most prey organisms including larval anurans live under great predation pressure. This results in the evolution of defense means to escape from predation and promote survival (Schmidt and Amézquita 2001, Relyea 2007). The results of this study showed that in both the absence and the presence of refuge sites, *C. curtipes* tadpoles fell prey to water scorpions more easily than *I. temporalis* tadpoles. A main difference between the two species is the speed of swimming; the V_{max} of *C. curtipes* tadpoles is lower than that of *I. temporalis* tadpoles. Hence, *C. curtipes* tadpoles are more vulnerable to capture by predators than are *I. temporalis* tadpoles. Our results conform to those of earlier studies (Van Buskirk and McCollum 2000, Dayton *et al.* 2005). It is generally believed that refuge sites reduce predation risk (Nystrom and Abjornsson 2000, Mogali *et al.* 2019). In the present study the vulnerability of both tadpole species was lower where refuge sites were available.

The results of the present study show that *C. curtipes* tadpoles are more vulnerable to predators than those of *I. temporalis.* The present study on comparative vulnerability of tadpoles of *I. temporalis* and *C. curtipes* was conducted only at early larval stages of development (Gosner stages 25-27). The vulnerability of the two species may not be the same throughout the larval period because the tadpoles of *I. temporalis* complete its larval period within 3–4 months (Hiragond and Saidapur 1999, Saidapur 2001, Mogali *et al.* 2016) but those of *C. curtipes* grow to larger body size over very long larval periods i.e., from six months to one year (Saidapur 2001). Further studies comparing the species throughout development are therefore needed. The finding of the present study clearly shows that at early stages of development, *I. temporalis* tadpoles have developed better predator avoidance behavior than that of *C. curtipes* tadpoles.

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