

# Predatory influence of dragonfly larvae and water scorpions on eggs and tadpoles of *Indosylvirana temporalis* (Anura: Ranidae)

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## Abstract

**Predatory influence of dragonfly larvae and water scorpions on eggs and tadpoles of *Indosylvirana temporalis* (Anura: Ranidae).** We assessed in the laboratory the vulnerability of Bronzed frog (*Indosylvirana temporalis*) eggs and tadpoles to two potential sit-and-wait insect predators, larvae of a dragonfly (*Pantala flavescens*; Odonata: Libellulidae) and adult water scorpions (*Laccotrephes* sp.; Hemiptera: Nepidae). We exposed a series of different developmental stages of *I. temporalis* (from eggs to metamorphic climax stage) to these two predators. The results of this study showed that larvae of *P. flavescens* preyed on eggs and tadpoles of *I. temporalis* but only to stage 36. *Laccotrephes* sp. did not prey on eggs of *I. temporalis* but on tadpoles of all stages (22 to 42). This difference in predation rate was likely due to the gape size of the predators. The larvae of *P. flavescens* are gape-limited and cannot prey on larger tadpoles (above stage 36). Adults *Laccotrephes* sp. are non-gape-limited predators, using a segmented beak to pierce *I. temporalis* and suck the body fluids. They captured small to large tadpoles by quickly grabbing and immobilizing them using the front pair of raptorial legs. The present study shows that both predatory insects are a threat to *I. temporalis* at early and later stages of larval development.

**Keywords:** Developmental stages, Eggs, Hemiptera, *Laccotrephes* sp., Odonata, *Pantala flavescens*, Predator-prey interactions.

## Resumo

**Influência da predação por larvas de libélulas e escorpiões d'água sobre ovos e girinos de *Indosylvirana temporalis* (Anura: Ranidae).** Avaliamos em laboratório a vulnerabilidade de ovos e girinos de rã-de-bronze (*Indosylvirana temporalis*) a dois potenciais insetos predadores do tipo senta-e-espereira, larvas de uma libélula (*Pantala flavescens*; Odonata: Libellulidae) e escorpiões-d'água adultos (*Laccotrephes* sp.; Hemiptera: Nepidae). Expusemos uma série de diferentes estágios de desenvolvimento de *I. temporalis* (do estágio de ovos ao estágio de clímax metamórfico) a esses dois predadores. Os resultados deste estudo mostraram que as larvas de *P. flavescens* preda ovos e girinos de *I. temporalis*, mas apenas até o estágio 36. *Laccotrephes* sp. não predou ovos de *I.*

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*temporalis*, mas girinos de todas os estágios (22 a 42). Essa diferença na taxa de predação foi provavelmente devida ao tamanho da boca dos predadores. As larvas de *P. flavescens* são limitadas pelo tamanho da boca e não podem apresar girinos maiores (acima do estágio 36). Os adultos de *Laccotrephes* sp. não são limitados pelo tamanho da boca, utilizando um bico segmentado para perfurar *I. temporalis* e sugar os fluidos corporais. Capturaram girinos pequenos a grandes, agarrando-os rapidamente e imobilizando-os com o auxílio do par frontal de apêndices raptorais. O presente estudo mostra que ambos os insetos predadores são uma ameaça para a *I. temporalis* nas fases iniciais e posteriores do desenvolvimento larval.

**Palavras-chave:** Estágios de desenvolvimento, Hemiptera, Interações predador-presa, *Laccotrephes* sp., Odonata, Ovos, *Pantala flavescens*.

## Introduction

Amphibians, especially anurans (frogs), have complex life history strategies consisting of an aquatic and a terrestrial stage (Wilbur 1980). Eggs and larvae of most anurans are vulnerable to a wide range of predators including both invertebrates and vertebrates (Eterovick and Fernandes 2002, Johnson *et al.* 2003, Porthault *et al.* 2007, Burbano-Yandi *et al.* 2018, Mogali 2018, Mogali *et al.* 2020a, b). Anurans usually deposit their eggs either in temporary or permanent ponds or streams with continuous or intermittent flowing water (Hiragond and Saidapur 1999, Eterovick and Barata 2006, Mogali *et al.* 2016, 2017), resulting in early developmental stages being highly vulnerable to predation (Alford, 1999). As a consequence of this predation stress, larval anurans have developed various kinds of defensive mechanisms such as secretion of toxic substances that make eggs or larvae unpalatable or toxic to predators (Gunzburger and Travis 2005), a drastic reduction in activity levels to avoid being detected (Chovanec 1992, Mogali *et al.* 2011, 2012, Gómez 2019), increased use of refuges (Kopp *et al.* 2006, Hossie and Murray 2010, Sanders *et al.* 2015, Mogali *et al.* 2019, 2022), increased activity in order escape predators (Mogali *et al.* 2021), or aggregation and formation of schools (Waldman and Adler 1979, Watt *et al.* 1997, Spieler and Linsenmair 1999), depending upon the species or perceived predation threat.

In Southern India (Western Ghats), Bronze frogs, *Indosylvirana temporalis* (Günther, 1864), breed from September to January (post-monsoon season). They typically breed along the edges of gently flowing streams and/or in pockets of still water along streams; larvae are present in the aquatic habitats until March or April (Saidapur 2001). The larvae are mostly bottom dwellers that thrive on detritus and algal matter (Hiragond and Saidapur 2001). Visibility is low in these habitats because of shadows from vegetation and because the benthic area is naturally covered by leaf litter and detritus (Mogali *et al.* 2019). These habitats harbor both vertebrate and invertebrate predators, although the number of vertebrate predators (e.g., fishes, snakes, and birds) is lower based on personal observations. The streams are home to several types of predatory invertebrates, including beetles (*Dineutus* sp.), crabs (*Barytelphusa* sp.), dragonfly larvae (*Pantala flavescens*, *Bradinopyga geminata*), damselfly larvae (*Ceragrion cerinorubellum*) mayfly larvae (*Baetis* sp.), water striders (*Cylindrostethus* sp.), backswimmers (*Notonecta* sp.), water scorpions (*Laccotrephes* sp., *Ranatra* sp.), caddis fly larvae (*Leptoceris* sp.), and leeches. Among these many predatory invertebrates, water scorpions (*Laccotrephes* sp.; Hemiptera: Nepidae) and dragonfly larvae (*Pantala flavescens*; Odonata: Libellulidae) are the most voracious predators that actively feed on anuran tadpoles including *I. temporalis*. Although we observed that other predatory invertebrates cause damage to anuran eggs or tadpoles, their impact was minimal.

Larvae of dragonflies are considered ambush/sit-and-wait, gape-limited predators. Adult water scorpions are also ambush/sit-and-wait predators, but they are not gape-limited. Both predators have excellent vision. In nature, they are well camouflaged in vegetation or detritus, where they ambush unsuspecting prey including tadpoles of *I. temporalis* (Mogali *et al.* 2020a, b). Although their life histories are remarkably similar to diving beetles that are known to prey on and severely threaten anuran larval populations (Kruse 1983, Cabrera-Guzmán *et al.* 2012, Gould *et al.* 2019, Valdez 2019), the cryptic nature of dragonfly larvae and water scorpions has discouraged the study of predation on tadpoles. Although arthropods are not typically regarded as major vertebrate predators, they can have a large impact on vertebrate populations and communities (McCormick and Polis 1982, Valdez 2020, Nyffeler and Altig 2020). Our study investigates whether one or if both of these predators negatively affects larval populations of *I. temporalis*.

In this experiment, we exposed a series of developmental stages of *I. temporalis* (i.e., from egg to metamorphic climax) to freely hunting predators, *P. flavescens* (gape-limited) and *Laccotrephes* sp. (not gape-limited). Hence, we hypothesized that there should be a difference in the predation rate between the two predatory insects.

## Materials and Methods

Developmental stages are according to Gosner (1960). Eggs (stages < 10,  $N \sim 8000$ ) from eight clutches and tadpoles (stages 25–28,  $N \sim 500$ ) of *Indosylvirana temporalis* were collected from a stream in the Western Ghats near Anmod village (15.43088° N, 74.37360° E), Karnataka State, India, in November 2015 and immediately brought to the laboratory. Eggs were placed in a glass aquarium (90 × 30 × 15 cm) containing 25 L of aged (dechlorinated) tap water and used as stock for the experiment. Hatching occurred five days later at stage 19.

The tadpoles were reared in another glass aquarium (90 × 30 × 15 cm) containing 25 L of aged tap water and mainly used as food for predators. Upon reaching the feeding stage (stage 25), the tadpoles were fed boiled spinach to sustain growth and development.

Larvae of *P. flavescens* ( $N = 60$ ), and adult *Laccotrephes* sp. ( $N = 60$ ) were collected from the same location as the eggs and tadpoles. Because both insects are highly cannibalistic in nature (Mogali *et al.* 2020a, 2021), they were reared individually in plastic tubs (14 cm diameter and 7 cm depth) with 500 mL of aged tap water to avoid cannibalism. All predators were fed each day with 5–8 tadpoles of *I. temporalis*.

## Predatory Experiments with Dragonfly Larvae and Water Scorpions

In these experiments, a series of trials was conducted. In each trial, 20 individuals in the same developmental stage (egg stages 10–12 and tadpole stages 17–42) of *I. temporalis* and of comparable body sizes were released into a tub (32 cm diameter and 14 cm depth) containing 3 L of aged tap water and allowed to familiarize themselves to the tub for 15 min. One *P. flavescens* (late instar, 35 mm long) or one adult *Laccotrephes* sp. (35 mm long excluding the siphon length) that had been starved for 48 hours was introduced gently into a tub and left there for a period of 24 hours. After the trial period, the number of prey that survived or had been predated was noted. Five trials were conducted for each developmental stage using each predator. For each trial, a new set of eggs or tadpoles ( $N = 20$ ) was used. The test tub was washed thoroughly before each trial.

Including both experiments, we carried out 270 experimental trials over a 90-day period. Each trial started at 07:00 h and ended at 07:00 h the next day. A minimum of five trials (of a particular developmental stage of prey) was conducted each day. An average of 2–3 days between experiments allowed the eggs or

tadpoles to attain the next developmental stage. All experimental prey were healthy, and tadpoles were well fed with boiled spinach before trials, but they were not provided food during the trials. For each trial, a new set of eggs or tadpoles were used. Predators (*P. flavescens* or *Laccotrephes* sp.) used in the experiments were healthy, and all individuals were fed daily with a sufficient number of tadpoles, except that prior to use in experimental trials predators were starved for 48 hours. Individual predators (either *P. flavescens* or *Laccotrephes* sp.) were used in up to three trials each and were re-used only after a gap of at least five days. Over the course of our experiments, both predator species grew in size. For all experimental trials, we used only last instars of *P. flavescens* of comparable body size; similarly, we used only adult *Laccotrephes* sp. of comparable body size. During the course of our experiments, only the larvae of 10 *P. flavescens* metamorphosed into adult dragonflies. All experimental trials were carried out at room temperature (25°C). The data on the number of eggs/tadpoles consumed by the two predators at each developmental stage were analyzed by the Mann-Whitney U test.

## Results

The number of eggs (stages 10–12) or tadpoles (stages 17–42) of *I. temporalis* consumed by the predators is given as mean  $\pm$  SE (Table 1). Larvae of *P. flavescens* consumed both eggs and tadpoles of *I. temporalis* but consumed tadpoles only up to stage 36. They did not kill or consume any tadpoles in stages 37–42. Adults of *Laccotrephes* sp. did not consume eggs of *I. temporalis*, but they consumed tadpoles from stage 22 until metamorphic climax.

The results also show that *P. flavescens* consumed a significantly greater number of eggs/tadpoles of early developmental stages (stages 10–12 and 17–23) compared to those that of *Laccotrephes* sp. (Table 1). However, there was no significant difference in the number of

tadpoles consumed by both predators at stage 24 (Table 1). From stage 25 to 42, *Laccotrephes* sp. consumed significantly greater number of tadpoles compared to those that of *P. flavescens* (Table 1).

## Discussion

In aquatic environments, amphibian eggs and larvae are vulnerable to various types of predators (McCormick and Polis 1982, Kruse 1983, Gould *et al.* 2019, Valdez 2019) but differences exist in the risk and intensity of predation among predator species (Portheault *et al.* 2007, Cabrera-Guzmán *et al.* 2012). In the present study, we observed differences in the number of tadpoles or eggs of *I. temporalis* consumed by two species of predatory insects (*P. flavescens* and *Laccotrephes* sp.). Larvae of *P. flavescens* preyed upon eggs and tadpoles to stage 36 of *I. temporalis*. They were apparently not able to kill or consume tadpoles from stages 37–42. *Laccotrephes* sp. did not prey on eggs of *I. temporalis* but consumed tadpoles of all stages, i.e., from 22–42.

We believe these differences in patterns of predation risk are related to gape size of the predators. The larvae of *P. flavescens* are gape-limited predators (Mogali *et al.* 2016), and cannot prey on larger tadpoles (above stage 36). Other mechanisms may counteract this limitation. For example, like adult diving beetles, they may time their offspring to hatch at the same time as tadpoles (Valdez 2019, Gould *et al.* 2019). Adult *Laccotrephes* sp. are non-gape-limited and use a segmented beak to pierce *I. temporalis* and suck their body fluids. They are able to capture small to large tadpoles by quickly grabbing and immobilizing the tadpoles by using the front pair of raptorial legs (Mogali *et al.* 2020b). Even though starved for 48 h, the water scorpions did not eat eggs of *I. temporalis*. Perhaps the small, non-mobile eggs of *I. temporalis* may not catch the attention of adult water scorpions or they may not feed on small prey. An earlier study revealed that water scorpions mostly feed on moving prey (Ohba and Swart 2009).

**Table 1.** Number of eggs/tadpoles of different developmental stages of *Indosylvirana temporalis* (prey) consumed by the predators, *Pantala flavescens* (sit-and-wait, gape-limited; total length 35.05 ± 0.62 mm) and *Laccotrephes* sp. (sit-and-wait, non-gape-limited; 35.30 ± 0.85 mm; excluding the siphon length) in 24-hour trial periods. Data represent mean ± SE and analyzed by Mann-Whitney U test. N = 5 trials for each stage.

Stages	Prey total length (mm)	Number of eggs/tadpoles consumed by the predator		
		<i>Pantala flavescens</i>	<i>Laccotrephes</i> sp.	U and p values
10–12 (eggs)	1.84 ± 0.06 (diameter)	0.60 ± 0.24	0.00 ± 0.00	U = 5.000, p = 0.050
17	2.56 ± 0.02	0.80 ± 0.20	0.00 ± 0.00	U = 2.500, p = 0.014
18	2.84 ± 0.03	1.20 ± 0.20	0.00 ± 0.00	U = 0.000, p = 0.004
19	3.02 ± 0.07	1.40 ± 0.24	0.00 ± 0.00	U = 0.000, p = 0.005
20	3.92 ± 0.04	1.60 ± 0.24	0.00 ± 0.00	U = 0.000, p = 0.005
21	4.80 ± 0.05	2.20 ± 0.20	0.00 ± 0.00	U = 0.000, p = 0.003
22	5.58 ± 0.07	2.60 ± 0.24	1.00 ± 0.44	U = 2.000, p = 0.021
23	6.78 ± 0.04	3.20 ± 0.37	2.00 ± 0.00	U = 2.500, p = 0.018
24	7.64 ± 0.06	4.00 ± 0.31	3.40 ± 0.40	U = 8.000, p = 0.288
25	9.42 ± 0.04	7.80 ± 0.66	13.60 ± 0.67	U = 0.000, p = 0.009
26	17.16 ± 0.05	5.80 ± 0.58	11.40 ± 0.50	U = 0.000, p = 0.009
27	20.13 ± 0.01	5.20 ± 0.37	8.40 ± 0.25	U = 0.000, p = 0.008
28	24.32 ± 0.01	4.20 ± 0.20	6.20 ± 0.20	U = 0.000, p = 0.005
29	24.72 ± 0.02	3.40 ± 0.25	5.80 ± 0.20	U = 0.000, p = 0.006
30	28.35 ± 0.02	2.60 ± 0.25	5.60 ± 0.25	U = 0.000, p = 0.007
31	28.90 ± 0.03	2.20 ± 0.20	5.40 ± 0.25	U = 0.000, p = 0.006
32	29.02 ± 0.05	2.00 ± 0.00	4.80 ± 0.20	U = 0.000, p = 0.004
33	29.33 ± 0.01	1.60 ± 0.25	4.60 ± 0.25	U = 0.000, p = 0.007
34	30.41 ± 0.03	1.00 ± 0.00	4.40 ± 0.25	U = 0.000, p = 0.005
35	30.65 ± 0.02	0.60 ± 0.25	4.20 ± 0.20	U = 0.000, p = 0.006
36	31.70 ± 0.02	0.20 ± 0.20	4.00 ± 0.00	U = 0.000, p = 0.004
37	32.29 ± 0.03	0.00 ± 0.00	3.60 ± 0.25	U = 0.000, p = 0.005
38	32.46 ± 0.02	0.00 ± 0.00	3.20 ± 0.32	U = 0.000, p = 0.005
39	32.65 ± 0.02	0.00 ± 0.00	2.80 ± 0.37	U = 0.000, p = 0.005
40	33.24 ± 0.02	0.00 ± 0.00	2.60 ± 0.25	U = 0.000, p = 0.005
41	33.43 ± 0.01	0.00 ± 0.00	2.40 ± 0.25	U = 0.000, p = 0.005
42	33.80 ± 0.02	0.00 ± 0.00	2.60 ± 0.25	U = 0.000, p = 0.005

*Pantala flavescens* are relatively ineffective egg predators but they actively feed on small tadpoles. The immobile eggs or less mobile early stages of tadpoles of *I. temporalis* (stages < 23) may not readily catch the attention of sit-and-wait predators. In nature, other aquatic predators, such as caddis fly larvae, leeches, water beetles, water boatman, and other small insects or their larvae mainly feed on eggs of *I. temporalis* (e.g., Henrikson 1990, Cabrera-Guzmán *et al.* 2012). To counteract such predator pressure, tadpoles of *I. temporalis* have been shown to have higher survivorship by reducing their activity levels (Mogali *et al.* 2012). They also seek refuge sites in the presence of predators (Mogali *et al.* 2019, 2021) and complete their larval period early with a larger body size (Mogali *et al.* 2016). It is unknown what effect these predators have on natural populations of tadpoles of *I. temporalis*. The present study shows that the two predatory insects studied can be threats to *I. temporalis* at most stages of larval development.

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