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Predatory activity of *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* on mosquito larvae in Darjeeling, India

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Abstract

Background & objectives: Predation potential of the dytiscid beetle, *Rhantus sikkimensis* Regimbart 1899 and the larvae of *Toxorhynchites splendens* Wiedemann 1819 occurring along with the larval stages of the mosquitoes in the annual lentic water bodies of Darjeeling was evaluated using the larvae of *Culex quinquefasciatus* Say 1823 as preys, in the laboratory under simulated natural conditions.

Methods: Field collected *R. sikkimensis* and larvae of *Tx. splendens* were offered IV instar larvae of *Cx. quinquefasciatus* to observe the rate of predation, at varying prey and predator densities. Based on the data obtained on the predation for a period of three consecutive days, two indices of predation, predatory impact (PI) and clearance rate (CR) values were estimated, and compared between the predator species.

Results: The rate of predation of IV instar *Cx. quinquefasciatus* larvae by *R. sikkimensis* ranged between 21.56 and 86.89 larvae per day, depending on the prey and predator densities. The PI value remained between 18.67 and 35.33 larvae/day depending on prey densities, while the CR ranged between 2.21 and 2.23 larvae litres/day/predator. Compared to these, the *Tx. splendens* larvae consumed the prey larvae at the rate of 0.67 to 34.22 larvae per day, depending on the prey and predator densities. The PI value ranged between 7.67 and 11.33 larvae/day, and the CR value ranged between 1.41 and 1.76 larvae litres/day/predator. The rate of predation, CR values and PI values of *R. sikkimensis* and *Tx. splendens* varied significantly.

Interpretation & conclusion: Both the predators *R. sikkimensis* and larvae of *Tx. splendens* can consume a good number of mosquito larvae, though the rate of consumption between the two predators vary owing to the difference in the life history traits and features. It can be assumed that these predators play an important role in larval population regulation of mosquitoes and thereby impart an effect on species composition and interactions in the aquatic insect communities of Darjeeling Hills, India.

Key words Beetles – *Culex quinquefasciatus* – Darjeeling – larvae – predation – *Rhantus sikkimensis* – *Toxorhynchites splendens*

Introduction

The annual lentic water bodies in Darjeeling hills, India provide a congenial habitat for different species of mosquito immatures to thrive along with other aquatic organisms. The dytiscid beetle, *Rhantus sikkimensis* Regimbart 1899 (Coleoptera: Dytiscidae) and the larval stages of the nonbiting mosquito species *Toxorhynchites splendens* Wiedemann 1819 (Diptera: Culicidae) were observed to predate on mosquito larvae in some of these habitats. In view of this, an attempt was made to evaluate the predation potential of *R. sikkimensis* and the larvae of *Tx. splendens* using the larvae of *Culex quinquefasciatus* Say 1823 (Diptera: Culicidae) as prey. As such, little is known about the larval ecology and aquatic predators that regulate larval populations of mosquitoes recorded from this place[1-5]. In this respect this is the first record of predation of mosquito immatures by the co-inhabiting predators—the larvae of *Tx. splendens* and *R. sikkimensis.* Also, considering eastern Himalayas in general and Darjeeling in particular, biological control of mosquito immatures using natural enemies is a viable alternative compared to the traditional control methods using pesticides that have an adverse effect on the rich biodiversity of this region. Evaluation of predatory efficiency of *R. sikkimensis* and the larvae of *Tx. splendens* on mosquito larvae would reflect upon the possible species interactions in the aquatic insect communities of the lentic water bodies besides their control potential against mosquitoes in Darjeeling.

Material & Methods

The adult morphs of *R. sikkimensis* and the larvae of the mosquitoes *Tx. splendens* and *Cx. quinquefasciatus* were collected time-to-time as per the requirement, from the cemented pools and temporary water bodies in and around Darjeeling Government College campus, Darjeeling, and were kept separately in glass aquaria 12´´×6´´×6´´ capacity containing harvested rain-water (pH 8.1–8.9). The IV instar larvae of *Cx. quinquefasciatus* were separated from the heterogeneous population and were used in the experiments. The small instar larvae were kept in an enamel tray of 12´´×8´´×4´´ capacity for growth to IV instar stage using yeast grains as food.

The early instars (II & III) of *Tx. splendens* were isolated and cultured in the presence of equivalent instars of *Cx. quinquefasciatus* larvae following Jones and Schreiber[6], to obtain 0-day old IV instar of *Tx. splendens* used in the experiments.

The *R. sikkimensis* beetles were provided with larvae of *Cx. quinquefasciatus* as food during a brief period of five days of acclimatisation in the laboratory. Prior to using any predator individual in the experiments, they were fed to satiation followed by a period of 10–12 h of starvation. Estimation of predation by *R. sikkimensis* and the IV instar larvae of *Tx. splendens* was made through the following experiments in the laboratory at a room temperature of 19–22°C.

Experiment 1: To a single predator individual, 50 or 100 IV instar *Cx. quinquefasciatus* larvae were offered as preys in a 500 ml breaker for 24 h and the number of prey killed was noted. Nine replicates were made for each prey density, with predators or without predators (control), separately, for *R. sikkimensis* and larvae of *Tx. splendens*. Using the same predator individuals, the rate of predation was noted for three consecutive days, the prey density being set to same value after every 24 h. The data obtained on predation were put to the following equation to calculate the predatory impact (PI) following Hampton *et al*[7] with necessary modifications:

$$PI = \frac{\sum_{n=1}^{3} P_E}{T}$$

Where, PI = Predatory impact (nos. of prey larvae/day);
PE = Nos. of prey eaten/killed; and T = Time in days, here T = 3.

Experiment 2: To three predator individuals, 100 IV instar *Cx. quinquefasciatus* larvae were offered as prey in an enamel tray 8´´×5´´×3´´ capacity with 1.5 litre of harvested rainwater, and the number of preys killed after 24 h was noted. Nine replicates for experiment and control were set in respect to the predators—*R. sikkimensis* and the larvae of *Tx. splendens*, separately. For each predator, observations were made for three consecutive days using same individuals (predators) and resetting the prey density every 24 h. The data obtained on the number of prey killed were applied to the following equation to estimate the clearance rate (CR) following Gilbert and Burns[8] with necessary modifications:

$$CR ? \frac{V(InP)}{TN}$$

Where, CR= Clearance rate of predators (nos. of prey killed litres/day/predator); V = Volume of water (l); P =Nos. of prey killed; T= Time (in day); and N = Nos. of predators.

The data obtained on the numbers of prey killed were subjected to t-test following Zar[9], to judge the difference in predation between the days as well as between the predator species. PI and CR values were also compared through t–test.

Results

Experiment 1: A single *R. sikkimensis* consumed on an average 21.56 to 31.44 larvae of *Cx. quinquefasciatus* per day depending on the prey density. When the prey density was 50, a total of 65.78 ± 3.15 prey larvae were consumed in three days. At a prey density of 100 the value was 92.89 ± 2.58. The rate of predation did not vary between the days significantly (for prey density 50, between day 1 and 2, $t = 0.243$, not significant (N.S.); between Day 1 and 3, $t = 0.3646$, N.S.; between Day 2 and 3, $t = 0.098$, N.S. for density 100; between Day 1 and 2, $t = 1.828$, N.S.; between Day 1 and 3, $t = 1.270$, N.S.; and between Day 2 and 3, $t = 0.119$, N. S.; for all t-values, df = 8).

A single IV instar *Tx. splendens* larva consumed on an average 0.67 to 14.44 IV instar larvae of *Cx. quinquefasciatus* per day. The predation rate varied between the days and prey density significantly (for prey density 50, between Day 1 and 2, $t = 3.744$, $p < 0.005$; between Day 1 and 3, $t = 7.056$, $p < 0.0005$; between Day 2 and 3, $t = 12.623$, $p < 0.0005$; for prey density 100, between Day 1 and 2, $t = 5.055$, $p < 0.0005$; between Day 1 and 3, $t = 20.225$, $p < 0.0005$; and between Day 2 and 3, $t = 14.67$, $p < 0.0005$, for all t-values, df = 8). In the three day period, a IV instar *Tx. splendens* larva consumed on an average 23.44 ± 0.34 and 28.11 ± 1.37 prey larvae at the prey density 50 and 100 respectively.

The numbers of prey larvae consumed by the predators–*R. sikkimensis* and IV instar of *Tx. splendens* in respect to prey densities and days are shown in Fig. 1.

The PI values for *R. sikkimensis* ranged between 18.33 and 28.33 larvae/day at a prey density of 50, and for prey density 100, the values ranged between 26.33 and 35.33 larvae/day. Compared to these, the PI values for *Tx. splendens* ranged between 7.33 and 8 larvae/day for prey density 50, and between 7.33 and 11.33 larvae/day for prey density 100. The PI values of the predators differed significantly (Fig. 2).

Experiment 2: R. sikkimensis were found to predate 79 to 92 IV instar *Cx. quinquefasciatus* larvae/day, while the IV instar of *Tx. splendens* consumed on an average 13 to 34 numbers of the same prey per day. The numbers of prey consumed by the predators are shown in Fig. 3.

For *R. sikkimensis*, the predation rate did not vary between the days significantly (between Day 1 and 2, $t = 1.648$, N.S.; between Day 1 and 3, $t = 1.298$, N.S.; between Day 2 and 3, $t = 0.118$, N.S.; for all

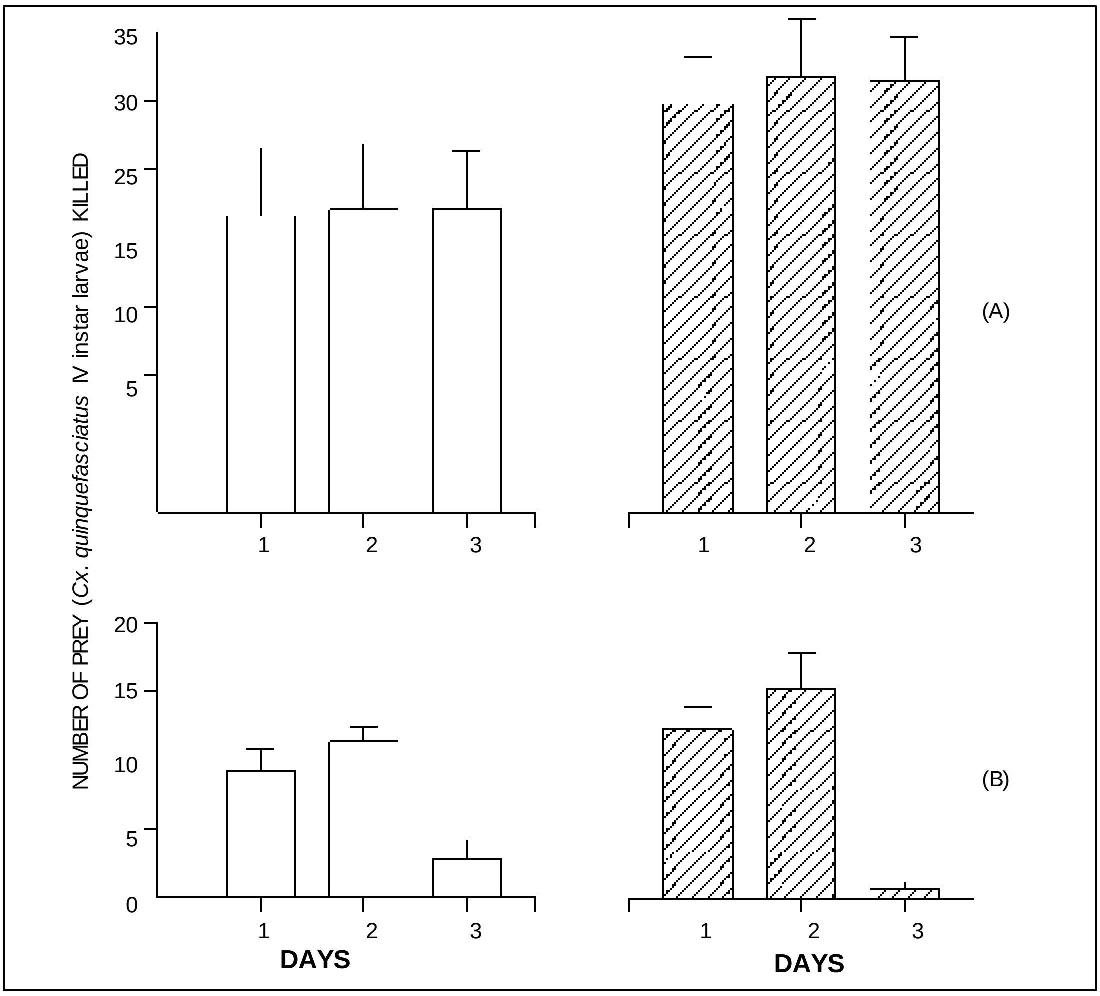

Fig. 1: Number (±S.E.) of IV instar *Cx. quinquefasciatus* larvae killed by an individual of *R. sikkimensis* (A); and a IV instar *Tx. splendens* larvae (B), per day (for three consecutive days). The prey densities were 50 (open bar) and 100 (hatched bar)

t-values, df = 8). The predation rate of *Tx. splendens* larvae varied significantly between the days (between Day 1 and 2, t = 8.621, $p < 0.0005$; between Day 1 and 3, t = 0.766, N.S.; between Day 2 and 3, t = 9.159, $p < 0.0005$, for all t-values, df = 8).

The clearance rate (CR) for *R. sikkimensis* ranged between 2.21 and 2.23 prey larvae litres/day/predator, and for *Tx. splendens* the values ranged between 1.41 and 1.76 prey larvae litres/day/predator. The CR values between the predators varied significantly with respect to the days (Table 1). Not a single larva of *Cx. quinquefasciatus* (prey) died in any of the control sets, with respect to both the experiments.

Table 1. Comparative accounts of clearance rates of *R. sikkimensis* and *Tx. splendens* (n = 9 sets) against *Cx. quinquefasciatus* IV instar larvae as prey

Species	Day 1	Day 2	Day 3
R. sikkimensis	2.22–2.25 2.23±0.01	2.17–2.26 2.21±0.03	2.17–2.26 2.22±0.03
Tx. splendens	1.28–1.65 1.46±0.15	1.65–1.80 1.76±0.05	1.32–1.55 1.41±0.1
t-values	15.242 $p < 0.0005$	20.206 $p < 0.0005$	22.772 $p < 0.0005$

Values are in Range, mean ±S.E. in larvae litres/day/predator (t-values and level of significance given above between the predators on a particular day).

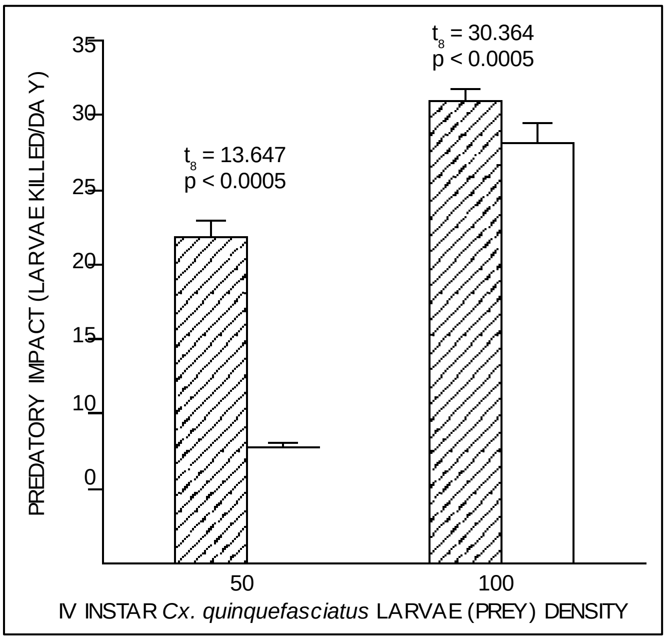

Fig. 2: Comparative account of predatory impact (PI) of *R. sikkimensis* (hatched bar) and IV instar *Tx. splendens* (open bar) larvae, at two prey densities for a period three days (n = 9 sets). t-values between the predators are shown in the graph, and between prey densities for *R. sikkimensis* (t = 7. 179, $p < 0.001$, df = 8) and for *Tx. spelndens* (t = 3.648, $p < 0.05$, df = 8)

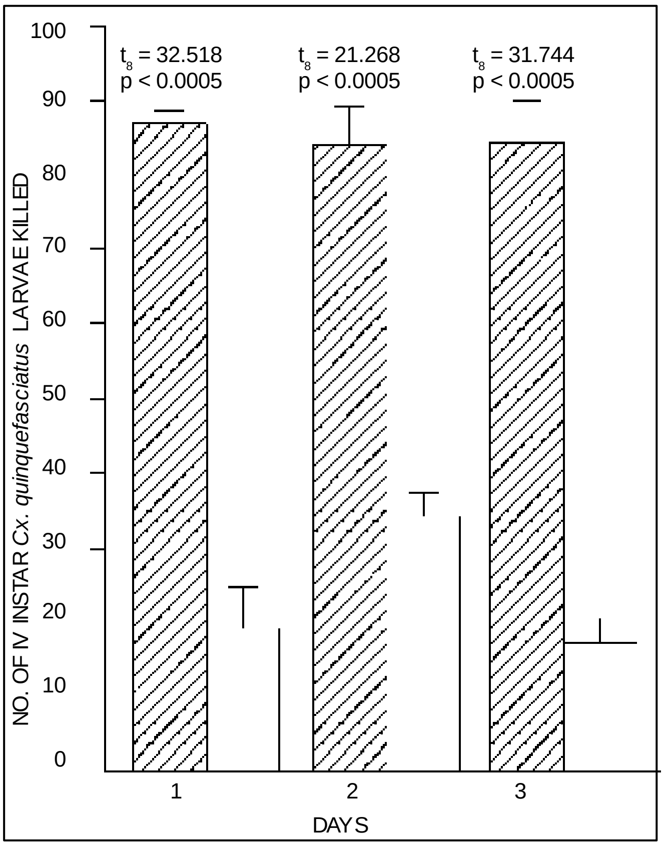

Fig. 3: Number (±S.E.) of IV instar *Cx. quinquefasciatus* larvae killed by three individuals of *R. sikkimensis* (hatched bar) and IV instar larvae of *Tx. splendens* (open bar) per day, for three consecutive days, at a prey density of 100/ 1.5 l; (t-values were calculated to show difference in predation rate between the predators)

Discussion

From the results it is evident that both the predator species *R. sikkimensis* and the larvae of *Tx. splendens* can consume a good number of larvae of *Cx. quinquefasciatus*, though considerable difference in the rate of predation between the two species exists. The predatory impact, reflecting the prey killing capability of *R. sikkimensis* is expectedly more compared to the larvae of *Tx. splendens*, owing to its size and energy requirements. Also, variation in consumption rate by the larvae of *Tx. splendens* between the days can be attributed to its developmental characteristics. As a IV instar larvae of *Tx. splendens* proceeds pupation, the predation rate drops. A sort of parabolic curve results if the predation of a IV instar larva of *Tx. splendens* is observed over its age (i.e. from 0-day old IV instar till pupation, lasting approximately 72–96 h)[10-13].

When the predation rate of the two predators are considered in respect to the prey densities, the rate of consumption rate varied in *R. sikkimensis* reflecting its ability to kill more preys. This proportionate killing was absent in *Tx. splendens*, revealing its lower ability to kill target prey compared to *R. sikkimensis*. Also, the body-sizes of the predators are different and thus, *R. sikkimensis* had a greater predatory impact compared to the larvae of *Tx. splendens* (Fig. 2) to sustain its feeding requirements. However, certain general rules guide the pattern of arthropod predation related to body size, prey density and other factors pertaining to the biology of predators[14-16]. The rate of prey consumption by both the predators followed this

(Figs. 1 and 2). Field evaluation on the predation in respect to varied prey types, space availability and habitat variation would prove the efficiency of *R. sikkimensis* and *Tx. splendens* as predators of mosquito larvae, more appropriately.

The number of prey killed remained higher when three predators were present. The CR, reflects the combined effect of search ability, killing and consumption by the predator and the prey evasion, in unit time and space. Keeping the prey consumption ability apart, both the predators *R. sikkimensis* and the *Tx. splendens* larvae were efficient in searching preys, however, in case of the latter, the proportionate increase in prey consumption is noticed on Day 2 (Figs. 1 and 3). Though the present study reveals that in comparison to *Tx. splendens* the beetle *R. sikkimensis* is much more efficient as predator, the predation rate as well as clearance rate for both the predators, need to be judged in situations, where the habitat is structurally complex. In case two aquatic predators, *Buoena* and *Notonecta* the predation and clearance rate differed in respect to simple and complex habitat structure[7,8].

Larval stages of *Tx. splendens* are known predators of the larvae of other mosquitoes. They have been reported from several diverse habitats[17] including sewage drains[13,18] and rice fields[19]. Their presence in the larval habitats in Darjeeling, obviously provides an opportunity to exploit them as natural predators of mosquitoes in this region. On the other hand, predation of mosquito larvae by the aquatic beetle *R. sikkimensis* is being reported here for the first time. In recent years, predation and population regulation of *Culex* larvae by the dytiscid beetles *Hydroporus* sp, *Colymbetes paykulli*, *Ilybus ater* and *Rhantus* sp have been noted in a pond in Sweden[20]. The rate of predation of *R. sikkimensis* is high compared to these beetles, though in a different habitat.

The species composition, structural complexity and environmental features of a habitat influence the abundance and function of aquatic predators of mosquito immatures[21-23]. From the viewpoint of biological control, the aquatic predators should have a wide range of adaptability in the habitats apart from the predation of target mosquito larvae. Though the larvae of *Tx. splendens* have been found to be quite efficient in this respect[24], largely due to similarity in biology with that of other mosquitoes, it needs to be judged for *R. sikkimensis.*

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