PREDATORY EFFICIENCY OF THE SEWAGE DRAIN INHABITING LARVAE OF *TOXORHYNCHITES SPLENDENS* WIEDEMANN ON *CULEX QUINQUEFASCIATUS* SAY AND *ARMIGERES SUBALBATUS* (COQUILLETT) LARVAE

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Abstract. The rate of predation by stage IV instar *Toxorhynchites splendens* larvae on the equivalent instar stage larvae of *Culex quinquefasciatus* and *Armigeres subalbatus*, co-occurring in sewage drains, were noted for a period of three consecutive days in the laboratory using different prey densities and combinations. The rate of predation varied by age of the predator, density of prey, and prey type. The number of *Ar. subalbatus* larvae consumed by a single *Tx. splendens* larva ranged between 0.50 ± 0.71 and 16.40 ± 2.01; while for *Cx. quinquefasciatus* larvae, the number consumed ranged from 0.20 ± 0.42 to 20.40 ± 1.43 per day. The pupation rates of the prey species varied in respect to control, with a minimum of 0.20 ± 0.42 pupa/day to a maximum of 12.20 ± 2.30 pupa/day in the presence of *Tx. splendens*. The values for the controls were 1.00 ± 0.87 and 14.44 ± 2.83 pupa/day, respectively. Irrespective of prey densities and combinations, a single *Tx. splendens* fourth instar larvae was found to consume on average 10.07 larvae on the first day 16.57 larvae on the second day and 4.38 larvae on the third day, killing a total of 17.70 to 45.10 larvae, in three days. In the presence of *Tx. splendens*, the cumulative pupation, irrespective of prey, remained between 12.20 and 45.10, and differed significantly from control where the values were between 13.90 and 54.70. The results indicate that *Tx. splendens* can significantly reduce immature numbers and lower the rate of pupation of *Cx. quinquefasciatus* and *Ar. subalbatus*. *Tx. splendens* may be a potential biological resource in the control of mosquitoes inhabiting sewage drains.

INTRODUCTION

Reduction of the mosquito population through predation of immature stages by natural enemies can be an effective alternative to pesticides (Bay, 1967; Bay *et al*, 1976; Huffaker *et al*, 1976; Service, 1976; Collins and Blackwell, 2000; Marquardt *et al*, 2000; Becker *et al*, 2003). Habitat similarity and natural occurrence of larval predators of mosquitoes are helpful in terms of biological control of vector mosquito species (Bay *et al*, 1976; Su and Mulla, 2002; Becker *et al*, 2003; Chansang *et al*, 2004; Kumar and Hwang, 2006). The predatory larvae of *Toxorhynchites* mosquitoes have the potential to combat pest and vector mosquitoes (Steffan and Evenhuis, 1981; Collins and Blackwell, 2000). Since the adult stages of *Toxorhynchites* mosquitoes are non-biting, they do not fall under the category

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of a pest or vector species. Various species of *Toxorhynchites* mosquitoes have been found in both temperate and tropical countries (Steffan and Evenhuis, 1981; Collins and Blackwell, 2000). Generally, the larvae of *Toxorhynchites* mosquitoes are found in containers, tree-holes (Focks, 1982; Chuah and Yap, 1984; Yap and Foo, 1984; Tikasingh and Eustace, 1992; Toma and Miagi, 1992; Schreiber and Jones, 1994), temporary pools (Carlson *et al*, 2004) and rice fields (Pramanik and Raut, 2005). In the sewage drains of Kolkata, India, the larvae of *Toxorhynchites splendens* Wiedemann, 1819, exist as predators, along with other mosquito species (Pramanik and Raut, 2003a, 2004). This provides the possibility for their use in controlling mosquito larvae in this and similar habitats. Few natural enemies of mosquito larvae are known to inhabit sewage systems, apart from the fish *Poecilia reticulata* and *Gambusia affinis,* which prey upon mosquito larvae (Becker *et al*, 2003; Kumar and Hwang, 2006) and may be beneficail like the larvae of *Tx. splendens* (Collins and Blackwell, 2000). Apart from being a preferred habitat for several invertebrate species, the sewage drains are also a major source of different species of pest and vector mosquitoes, in urban settlements (Pramanik and Raut, 2002, 2003a, 2004). The use of *Tx. splendens* can be a successful measure to control these mosquitoes.

In Kolkata, India, the two dominant vector mosquitoes growing in the sewage drains are *Armigeres subalbatus* (Coquillett, 1898) (Diptera: Culicidae) and *Culex quinquefasciatus*, Say, 1823 (Diptera: Culicidae) (Hati, 2001; Pramanik and Raut, 2000, 2002). The relative abundance of these vector mosquitoes along with the predator, *Toxorhynchites splendens*, varies by season (Pramanik and Raut, 2003a, 2004). An assessment of the predation potential of *Tx. splendens* larvae was made under laboratory conditions, against the larvae of *Ar. subalbatus* and *Cx. quinquefasciatus*. As an indicator of the effect of predation, the pupation rates of the prey mosquitoes were evaluated. In Indian context, the larvae of *Tx. splendens* have shown potential for the control of vector mosquitoes that breeds generally in the smaller aquatic habitats (Amalraj and Das, 1994a,b, 1998). The results of the present study will further substantiate their use as biological resource against the pest and vector mosquitoes in the sewage drains and similar habitats.

MATERIALS AND METHODS

Collection of mosquito larvae

The second and third instar larvae of *Tx. splendens* were collected from sewage drains and cesspits in and around the Ballygunge Science College campus, Kolkata, India. They were reared in a mixture of sewage and tap water (1:1, v/v) in an enamel tray with equivalent instars of *Cx. quinquefasciatus* larvae for 2 - 4 days following the methods of Ent Guide #3 (www.pherec.org). This allowed successful development of *Tx. splendens* to the 0-day old fourth instar larvae (<12 hours old). From the same spots, the larval stages of *Ar. subalbatus* and *Cx. quinquefasciatus* were collected and fourth instar larvae were separated and used for the initial experiments. The rest of the heterogeneous larval population was reared in enamel trays separately for the two species with Tokyu® fish food *ad libitum* to obtain fourht instar larvae for later experiments.

Method

To monitor the predation rate, one fourth instar *Tx. splendens* larva was provided with 30, 35 and 40 individuals of the two prey species *Ar. subalbatus* and *Cx. quinquefasciatus* (fourth instar larvae) separately or together. The ratios of predater to prey were 5:25, 25:5, 10:25, 25:10, 15:25, and 25:15. Observations were made for three consecutive days (after which the predator larva pupated). For each ratio, 10 sets were studied. Control sets without a predator, were used for comparison. The rate of predation (number of prey killed by a predator per day) and the number of prey larvae pupating per day were noted for each set. The number of larvae pupating in the control sets were also recorded, following which prey were added to both the experimental and control sets, while the predators remained the same. The water in each set was renewed every 24 hours. For both the experimental and control sets finely grained Tokyu® fish food was added (10 mg per replicate) to serve as food for the *Ar. subalbatus* and *Cx. quinquefasciatus* larvae.

Data obtained on the rate of predation and pupation were evaluated by *t*-test and ANOVA following Zar (1999) to discover significant differences between the days of predation in the experimental sets and between the controls and experimental sets in respect to pupation.

The water used for the experimental and control sets was a mixture of sewage and tap water (1:1, v/v) at a pH of 8.1-8.6 with a temperature of 25-28.5ºC. The experiments were carried out in plastic containers with 200 ml of this water.

RESULTS

The number of *Ar. subalbatus* larvae consumed by a single *Tx. splendens* larva ranged from 0.50 ± 0.71 to 16.40 ± 2.01; for *Cx. quinquefasciatus* larvae, the number consumed ranged from 0.20 ± 0.42 to 20.40 ± 1.43 per day depending upon the age of the predator, the type and number of prey (Table 1). The Student's *t*-test conducted on the rate of predation per day among the prey consumed, showed significant differences (day 1, $t = 2.43$, df = 8, $p < 0.025$; day 2, $t = 1.94$, df = 8, $p < 0.05$; and day 3, $t = 2.37$, df = 8, $p < 0.025$).

Two-way ANOVA carried out on the rate of predation by *Tx. splendens*, with respect to a particular prey type, showed significant difference between days, when prey species were present separately (for *Ar. subalbatus*: between days, df = 2, $F_{2,4} = 33.42$, $p<0.001$, between densities, df = 2, $F_{2,4} = 1.25$ NS; for *Cx. quinquefasciatus*: between days, df = 2, $F_{2,4} = 421.40$, $p < 0.001$, between densities, df = 2, $F_{2,4} = 2.45$ NS). When the prey species were present together in different numbers and ratios, the predation rate varied by age of the predator (between days, df = 2, $F_{2,10} = 9.42$, $p < 0.01$; between prey ratios, df = 5, $F_{5,10} = 1.97$ NS).

The rate of pupation in the subjects and controls differed significantly in both prey species for the first two days, but by the third day, it differed for *Cx. quinquefasciatus* only (for *Ar. subalbatus*, day 1, df = 8, $t = 4.04$, $p < 0.0025$; day 2, df = 8, $t = 3.77$, $p < 0.05$; day 3, $t = 0.62$, NS; for *Cx. quinquefasciatus*, day 1, df = 8, $t = 4.47$, $p < 0.0025$; day 2, df = 8, $t = 4.23$, $p < 0.0025$, day 3, df = 8, $t = 2.15$, $p < 0.05$). The rates of pupation for the subject and control sets are shown in Fig 1.

The rate of predation by fourth instar *Tx. splendens* larva was also positively correlated with the number of prey present on a particular day (Table 1). The cumulative consumption of prey larvae ranged between 17.70 and 45.10 depending on the prey type and ratio (Fig 2). Irrespective of prey type, cumulative pupation, in the presence of *Tx. splendens,* ranged from 12.20 to 46.50, while in the controls, it ranged from 19.30 to 54.70 which is significantly different ($t = 22.54$, df = 11, $p < 0.001$).

DISCUSSION

It is evident from the result that the IV instar larvae of *Tx. splendens* can kill, by way of predation, a good number of equivalent instar larvae of both *Ar. subalbatus* and *Cx. quinquefasciatus*, when the preys were present,

Table 1
Number [range, (mean ± SE)] of fourth instar larvae of *Ar. subalbatus* and *Cx. quinquefasciatus* consumed by a single *Tx. splendens* fourth instar larva, at different prey ratios (n = 10 replicates/prey ratio).

Prey ratio	Day 1		Day 2		Day3	
Ar.:Cx.	*Armigeres*	*Culex*	*Armigeres*	*Culex*	*Armigeres*	*Culex*
40:0	2-8 (4.20±1.81)		13-19 (16.40±2.01)		1-3 (2.20±0.79)	
35:0	2-9 (8.00±3.30)		3-18 (14.20±2.62)		0-2 (1.40±0.84)	
30:0	2-12 (5.70±3.43)		3-18 (11.10±5.53)		0-2 (0.90±0.88)	
25:5	5-12 (7.50±2.95)	1-5 (2.10±1.73)	8-17 (11.40±2.75)	2-3 (2.30±0.48)	0-2 (0.70±0.67)	0-2 (1.00±1.05)
25:10	5-10 (8.00±1.63)	3-5 (4.10±0.88)	9-20 (13.20±3.16)	2-8 (5.20±1.87)	0-2 (0.50±0.71)	0-1 (0.20±0.42)
25:15	4-10 (7.00±2.31)	4-11 (6.70±3.80)	8-17 (13.20±3.62)	2-12 (4.00±3.02)	0-2 (0.90±0.88)	0-2 (0.60±0.84)
15:25	1-7 (4.40±1.65)	10-14 (12.80±1.93)	3-6 (4.70±1.06)	10-14 (12.60±1.65)	2-5 (2.90±1.10)	3-12 (7.70±3.06)
10:25	2-8 (5.30±2.63)	4-16 (9.90±3.21)	2-6 (3.60±1.43)	5-18 (13.70±4.14)	0-3 (1.30±1.06)	5-16 (9.00±4.00)
5:25	1-3 (2.00±0.67)	5-10 (7.50±2.27)	2-4 (2.70±0.82)	10-13 (11.70±0.95)	0-3 (0.80±0.92)	8-12 (9.30±1.49)
0:30		3-10 (8.20±2.62)		17-21 (19.20±1.40)		2-6 (3.10±1.19)
0:35		6-11 (8.60±1.58)		19-21 (19.20±0.92)		3-8 (5.40±1.51)
0:40		3-11 (8.80±2.20)		19-21 (20.40±1.43)		2-7 (4.70±1.70)
r	0.51 NS	0.71 NS	0.95 $p<0.001$	0.96 $p<0.001$	0.09 NS	0.55 NS
χ^2 (total)	15.90 NS		4.94 NS		44.10 $p<0.001$	

separately or together. The rate of predation depended on factors such as age of the predator, and density of the prey and prey species. Although consumption increased proportionately with relative numbers of prey, the larvae of *Cx. quinquefasciatus* were more vulnerable than *Ar. subalbatus*, at all ratios. The biomass of the prey was a factor in the predation pattern observed. The larvae of *Ar. subalbatus* (~ 5.20 - 6.50 mg, wet weight) are larger than *Cx. quinquefasciatus* (~1.90 - 2.70 mg wet weight), thus the predator needed more effort to subdue it. However, if captured it provided more return in terms of energy to the predator. The rate of predation decreased as a function of age. During the second day, the predation rate was noted to be maximal, followed by the first and the third days of fourth instar larvae of *Tx. splendens*. This attribute has been observed to be independent of the

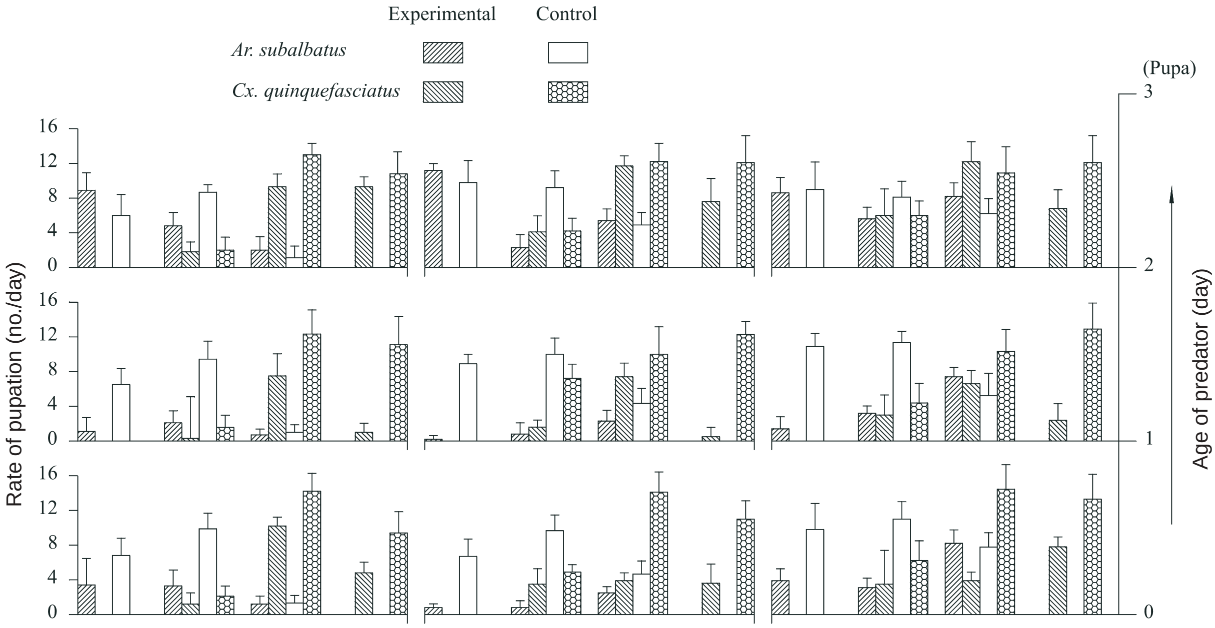

Fig 1–Comparative account of pupation of *Ar. subalbatus* and *Cx. quinquefasciatus* fourth instar larvae in the presence and absence of *Tx. splendens* larva fourth instar, when present at different prey ratios, for a period of three consecutive days (n= 10 sets/prey – predator ratio).

prey mosquitoes (Pramanik and Raut, 2003a). Therefore, the efficiency of *Tx. splendens* may be expected to vary by age, density and species of prey. The ratio of prey to *Tx. splendens* mosquitoes observed in nature (Pramanik and Raut, 2004) follows these predatory attributes.

At all densities, the pupation rates of the prey mosquito species were low in the presence of *Tx. splendens* larvae compared to the controls. This indicates that predation lowered the number of prey which reached the pupal stage, even when the population was asynchronous. However, the size of the adults was not considered, which could have reflected the fitness of the prey spared by *Tx. splendens*. In nature, the rate of pupation can be a parameter to evaluate the efficacy of a biocontrol agent in situations when larvae coexist with predators. The effect of *Tx. splendens* larvae on prey are similar to those of aquatic insects such as *Sphaerodema (=Diplonychus) annulatum*, *S. rusticum* (Pramanik and Raut, 2003b; Aditya *et al*, 2004, 2005; Saha *et al*, 2007a, b) and *Enithares indica* (Wattal *et al*, 1996). These insects and dragonfly nymphs (Mathavan, 1976; Miura and Takahashi, 1988), the crustaceans *Mesocyclops thermocyclopoides* (Mittal *et al*, 1997; Schaper, 1999; Kumar and Rao, 2003; Chansang *et al*, 2004) and *Triops newberryi* (Su and Mulla, 2002) are effective in habitats other than sewage drains. In general, arthropod predation follows several ecological aspects of prey-predator interactions (Beddington *et al*, 1976; Hassell *et al*, 1976; Cock, 1978), which, in the present study with *Tx. splendens* have been dealt to a limited extent, though yielded convincing results.

Some studies of *Tx. splendens* reveal prey selection to be frequency dependent (Amalraj

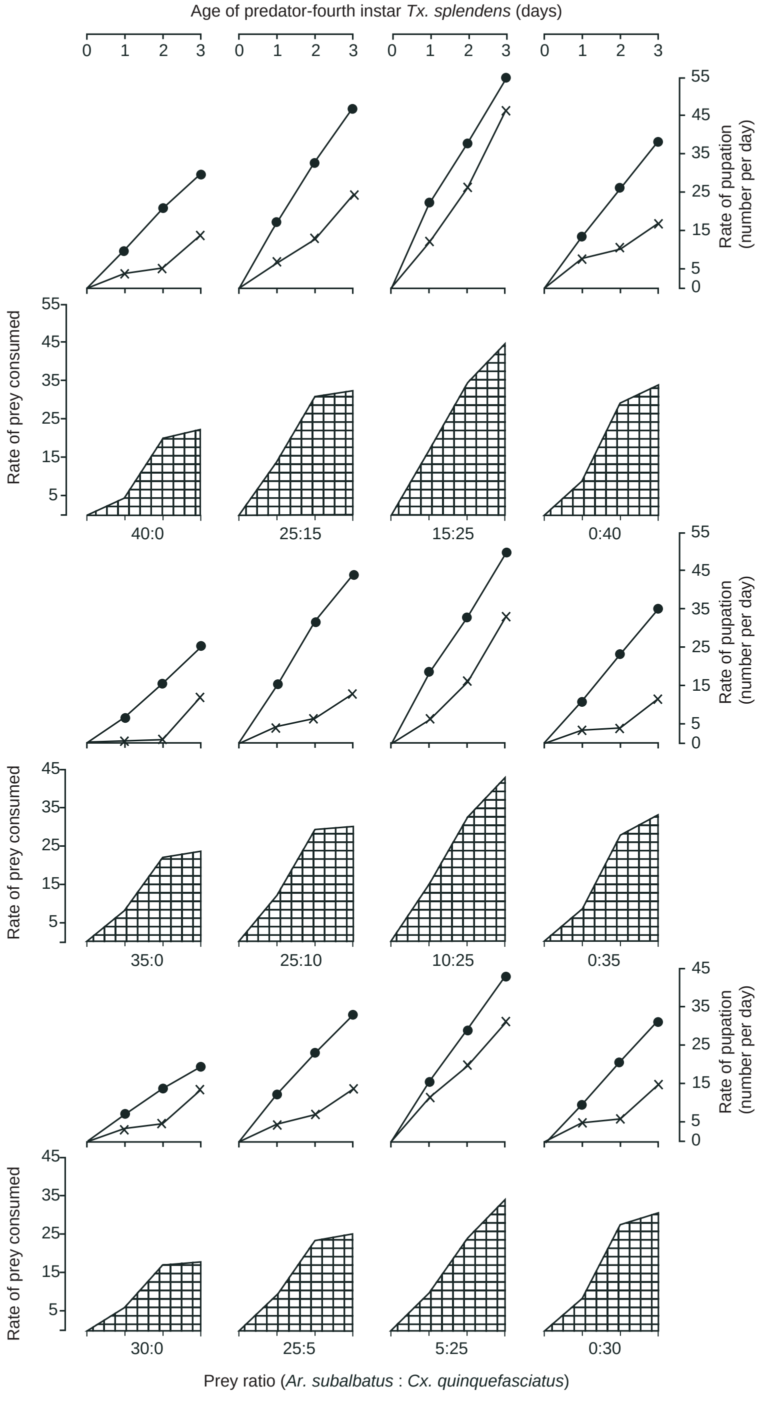

Fig 2–Comparative predation and prey pupation rates (Predator – *Tx. splendens* fourth instar larvae and prey – *Ar. subalbatus* and *Cx. quinquefasciatus* fourth instar larvae) for a period of 3 consecutive days (n= 10 sets/prey:predators ratio). Three sets of graphs represent three prey densities. Cumulative pupation is represented irrespective of species. (Crossed symbols are for experimental and dot symbols are for control sets).

and Das, 1996). Our observations, are simlar to those as well as to the frequency dependent selection shown by *S. annulatum* and *S. rusticum* (Aditya *et al*, 2005), although the numbers consumed depended on the prey species. The pupation rate remained low compared to the controls, reflecting the effect of predation by *Tx. splendens* larvae. Similar findings were found with the water bug *S. annulatum* against the larvae of *Cx. quinquefasciatus* (Aditya *et al*, 2004). The similarity between *Tx. splendens* and other mosquito species reveals its advantage as a biological control agent. Generalist predators, such as the dytiscid beetle *Rhantus sikkimensis* (Aditya *et al*, 2006b; Aditya and Saha, 2006), and water bugs (Wattal *et al*, 1996; Aditya *et al*, 2004; Saha *et al*, 2007a,b), exhibit a high preference for mosquito larvae as prey (Collins and Blackwell, 2000). The Crustacean, *Mesocyclops thermocyclopoides* (Mittal *et al*, 1997; Kumar and Rao, 2003; Chansang *et al,* 2004) is effective against smaller instars, whearas larval *Tx. splendens* can prey upon any instar stage of the target mosquito. This has been noted in studies of the larvae of *Tx. splendens* and other species of the genus *Toxorhynchites* throughout the globe (Focks, 1982; Chuah and Yap, 1984; Tikasingh and Eustace, 1992; Toma and Miagi, 1992; Schreiber and Jones, 1994; Amalraj and Das, 1994a,b, 1998). These attributes of *Toxorhynchites* mosquitoes place them ahead of other biocontrol agents to fight pest and vector mosquitoes and thereby mosquito-borne diseases.

The occurrence of *Tx. splendens* in sewage drains and cesspits (Pramanik and Raut, 2003a, 2004), rice fields (Pramanik and Raut, 2005) and cement water tanks (Aditya *et al*, 2006a,b) offers a new avenue for their use in controlling mosquitoes in larger habitats. The results of the present study indicate the prey ratio, relative density, and age of the predator are important determinants of a control program using *Tx. splendens*. Habitat size (Sunahara *et al*, 2002) and complexity are important factors that influence predator-prey interaction under natural conditions. The predatory efficiency of *Tx. splendens* needs to be evaluated under these conditions, where multiple prey are available in structurally complex habitats. Observations of *Tx. splendens* larvae as natural predators in sewage drains against other mosquitoes (Pramanik and Raut, 2003a, 2004) are promising. Further studies of the colonization potential and bioecology of *Tx. splendens* need to be carried out to evaluate it as a potential biological resource for mosquito control.

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