Asian Pac J Trop Med
2009;2(3):29-34

Original article

Immatures of *Lutzia fuscanus* (Wiedemann, 1820) (Diptera: Culicidae) in ricefields: implications for biological control of vector mosquitoes

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Abstract

Objective: Rice fields are dynamic mosquito larval habitats with assemblage of different predator taxa, including the larva of the mosquitoes *Lutzia*. Entomological surveillance in the ricefields is essential to evaluate the potential of these predators as biological resource to regulate vector mosquito population. In view of this, a survey of ricefields for immatures of different mosquito species including *Lutzia* was conducted. **Methods:** Survey of selected ricefields was carried out to evaluate the species composition of mosquitoes. Laboratory evaluation of the immatures of *Lutzia* mosquitoes was carried out to assess its predation potential using mosquitoes and chironomid as preys. **Results:** The survey revealed the presence of five mosquito species belonging to the genera *Anopheles* and *Culex* and the predatory immatures of the mosquito *Lutzia fuscana* (Wiedemann, 1820). The ratio of prey and predatory larva ranged between 1.46 and 4.78 during the study period, with a significant correlation on the relative abundance of the larval stages of *Lt. fuscanus* and *Anopheles* and *Culex* larvae. Under laboratory conditions, a single IV instar larvae of *Lt. fuscanus* was found to consume on an average 5 to 15 equivalent instars of *Anopheles* sp. and *Culex* sp. larvae per day depending on its age. The prey consumption reduced with the larval stage approaching pupation. When provided with equal numbers of chironomid and Anopheles or Culex larvae, larva of *Lt. fuscanus* consumed mosquito larvae significantly more compared to chironomids. **Conclusion:** The survey results and the preliminary study on predation are suggestive of the role of *Lt. fuscanus* in the regulation of vector mosquito populations naturally in the ricefields. Since *Lt. fuscanus* is common in many Asian countries, further studies on bioecology will be helpful to justify their use in mosquito control programme.

Keywords: Mosquito larvae; *Lutzia fuscanus*, *Anopheles*; *Culex*; Chironomid larvae; Ricefield; Biological control

INTRODUCTION

The rice fields, temporary pools and wetlands are congenial habitats for the immatures of different mosquito species. These habitats hosts a range of the predators that feed on the mosquito larvae like the aquatic bugs, dytiscid beetles, odonate nymphs, planaria, copepods and fishes[1-6]. The presence of these natural predators provides an opportunity for regulation of vector and pest mosquitoes thriving in the ricefields and wetlands[7-9]. However, information on the bióecology of many of these predators are still fragmentary and requires to be given priority keeping in view the resurgence and geographical range expansion of several mosquito borne diseases. In a recent survey on the ricefields in a rural area of Howrah, West Bengal, the larvae of the mosquitoes

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Lutzia fuscanus (Wiedemann, 1820) (Diptera: Culicidae)[10-14] were found in abundant throughout the cultivation period. Following this, a survey of this habitat was continued to record the presence of the predatory larvae of *Lt. fuscanus* mosquitoes in the ricefields of West Bengal and possibility of exploring them as biological resource against vector mosquitoes.

The larvae of the mosquitoes *Lutzia* are predators of the larval stages of different mosquitoes and are thus bear potential as biocontrol agent. Adult *Lutzia* have been recorded to feed on avian blood and do not attack human[13]. Previous studies from India and China have revealed that the larvae of *Lt. raptor* and *Lt. fuscanus* can consume a good number of *Culex* mosquitoes under laboratory conditions[15-20]. However, the abundance of this mosquito in the rice field provides a way to regulate vector and pest mosquitoes and thus reduction of mosquito borne diseases. In the present study besides observations on the relative abundance of the *Lt. fuscanus* larvae, a preliminary experiment on the predation potential was also carried out. Earlier reports indicate that these larvae feed on the chironomid larvae too[20]. So, the relative preference of mosquito and chironomid larvae by these predatory larvae was assessed. The results are expected to highlight the predatory ecology of *Lt. fuscanus* and help to evaluate its suitability in the use against vector and pest mosquitoes.

MATERIALS AND METHODS

Field survey

The survey on the immatures of the mosquito *Lt. fuscanus* was made in the *boro* ricefields in Amta, a rural area in West Bengal, India. During the period between February and April 2006, in three different plots of the ricefields (after a preliminary study on the presence of these mosquitoes in a sample of 30 ricefields, chosen at random), sampling of immature of mosquitoes was conducted. The sampling of the rice-plots were carried out using a plankton net (200nm mesh size) attached to a long wooden handle following Robert et al[21]. The mosquito immatures collected were emptied in a plastic tray and the larvae of *Lutzia* were separated using a suction pipette. These larvae were cultured further to adult stage using the laboratory-reared larvae of *Culex quinquefasciatus* larvae as food. A portion from the rest of the mosquito immatures was reared in separate plastic trays with Tokyu® fish food ad libitum to adult stage. The adults were identified following appropriate keys[10-14, 22]. The plant height and the water depth of the sampling site of the rice field were also recorded. A correlation between the predatory and prey larval species as well with the plant height and water depth of the field was analysed following Zar[23].

Predation experiments

In the laboratory, preliminary experiment on the predation of the IV instar larvae of *Lt. fuscanus* was carried out using the IV instar larvae of *Lt. fuscanus* as predator and equivalent instars of *Anopheles* and *Culex* larvae and chironomid larvae as preys. From the field collected heterogeneous populations, the smaller instars of the predator and prey larvae were separated and placed in plastic trays (25 × 16 × 5 cm) containing aged tap water. For chironomid larvae 3mg of dry sediment was added per 500 mL of water. From the trays, each of the smaller (II and III instars) *Lt. fuscanus* were separately placed in a 100 mL beaker and fed with equivalent instars of *Culex* and *Anopheles* larvae to obtain 0-day old IV instar stages. For the prey larvae including chironomids, few grains of fish food (Tokyu®, Japan) was added in the plastic containers to rear them to IV instars stages (for chironomid larvae ≥ 14 mm length; for *Anopheles* = 7-9 mm; for Culex = 7.5-9 mm length from head to anal segment). In the first set of experiments 50 preys (25 each of *Anopheles* and *Culex*) was provided as prey to 1, 2 and 3 0-day old IV instar larvae of *Lt. fuscanus* separately in plastic trays containing 700 mL of aged tap water. Twelve replicates were set per predator density and the data on the prey consumed was recorded every 24 hours. After counting, in each replicate the prey density were reset for observation of the next 24 hours. The experiment was carried out for three consecutive days. The data obtained on predation was subjected to three-way factorial ANOVA[23] using predator density prey types and days as variables.

In another set-up 20 prey larvae was provided to a single IV instar larvae of *Lt. fuscanus*. Two combinations of prey types were used. The chironomid larvae (10 nos), was common in both along with equal proportion *Anopheles* larvae in one and in the other *Culex* larvae was present. Nine replicates were made for each combination and the observations on the prey consumption were made for 24 hours period. The data was subjected to paired *t*-test[21] to justify the vulnerability of the prey types if any.

In all cases, the predator larvae - IV instar *Lt. fuscanus* - were subjected to a period of 12 hours starvation before using them in the experiments. The experiments were carried out during March - April 2006 in plastic containers stated above using 500 mL of aged tap water (pH 7.8; water temperature 27-29°C).

RESULTS

Field survey

Among the different rice field studied all the 30 plots (a sampling unit) were positive for the Lutzia larvae at one or the other point of time. However, the study on three adjacent plots was continually sampled at an interval of three days, for 45 days. In 9 sampling days with a gap of 10 days immature of the mosquito *Lt. fuscanus* were not observed, but the larvae of the prey mosquitoes were found throughout the sampling period. Apart from the mosquitoes, the belostomatid bugs, diving beetles, odonate nymphs, crustaceans and planktons were present, but not considered in the present study. The number of *L. fuscanus* larvae and pupae ranged between 0 and 11 and 0 and 4 respectively. The anopheline larvae ranged between 0 and 205 and 0 and 48 respectively per sample. The prey *Culex* larvae ranged between 0 and 59 and 0 and 15 respectively. The ratio of prey and predators remained 14.85 ± 4.38 S.E. during the sampling period. All the prey mosquito immatures could not be reared to the adult stage. However, a total of 280 anopheline and 59 Culex larvae were reared to adult stage. The Anopheline species recorded from the sample of total 280 pupae, 78 were *An. vagus* (28%), 69 were *An. culicifacies* (25%), 52 were *Anopheles annularis* (19%), 52 were *An. barbirostris* (19%) and 29 were *An. subpictus* (10%). In a sample of 59 Culicine pupae 19 were *Culex tritaeniorhynchus* (32%) 30 were *Cx vishnui* group (51%) and 10 *Cx. quinquefasciatus* (17%). A negative correlation on the relative abundance of mosquito immatures with plant height/water depth indicator against the prey and predator mosquito larvae was noted (Table1). The relative abundance of the immatures of the predator and prey mosquito along with the ratio of plant height and water depth is shown in Figure 1. The regression equation on the abundance of *Lt. fuscanus* (dependent variable, y) with the prey larvae was:

With *Anopheles* (x): $y = 2.38 + 0.02x$; $r^2 = 0.19$; $F_{(1,21)} = 5.04, P < 0.03$

With *Culex* (x): $y = 2.06 + 0.11x$; $r^2 = 0.23$; $F_{(1,21)} = 6.12, P < 0.02$

The regression equation for the overall prey/predator ratio (y) and the plant height/ water depth (x) was: $y = 2.05 + 0.23x$; $r^2 = 0.09$; $F_{(1,21)} = 1.62$, not significant.

The equations reveal positive interactions between the growth of the plant and water height in rice fields with the prey predator ratio.

Predation Experiments

The predatory larvae of *Lt. fuscanus* were observed to consume both *Anopheles* and *Culex* larvae (Figure 2). The rate of consumption varied with the predator density and the age of the predators. In day two, three *Lt. fuscanus* larvae consumed a total of 30 larvae. The vulnerability of *Culex* larvae was higher than *Anopheles* larvae and the numbers of prey consumed was low in the third day, following which in all the experimental trays, the predators pupated. The results of three-way ANOVA reveal significant differences in the prey consumption by *Lt. fuscanus* larvae with respect to the age (days) and density of the predators and the prey species. Significant differences in the interactions between variables confirm that the difference in prey consumption is dependent on the interaction of these factors (Table 2).

Table 1 Correlation matrix for the mosquito immatures and the paddy plant height and water depth of rice field (N = 23 sampling day).

	Paddy plant height	*Lutzia fuscanus*	*Anopheles*	*Culex*
Water depth	-0.201	-0.022	0.169	0.258
Paddy plant height		-0.139	-0.490	-0.487 *
Lutzia fuscanus			0.439 *	0.475 *
Anopheles				0.896 * *

* Correlation is significant at $P < 0.05$ level (2-tailed). * * Correlation is significant at $P < 0.01$ level (2-tailed).

Table 2 The results of three way factorial ANOVA on the prey consumption by *Lt. fuscanus* larvae using age of the predator (day), predator density and prey type as variables.

Source of variation	Sum of Squares	df	Mean Square	F-value
Predator density (PD)	1 254.23	2	627.12	146.70
Days (D)	2 530.34	2	1 265.17	295.96
Prey species (PS)	2 096.89	1	2 096.89	490.52
PD × D	298.10	4	74.53	17.43
PD × PS	295.12	2	147.56	34.52
PS × D	251.23	2	125.62	29.39
PD × PS × D	139.21	4	34.80	8.14
Error	846.42	198	4.28	
Total	7 711.55	215		

Compared to chironomid larvae *Lt. fuscanus* larvae seemed to prefer mosquito larvae as revealed from the prey consumption pattern when both chironomid and *Anopheles* or *Culex* larvae present together. A significant difference was noted in the t-values when the relative consumption of chironomid and mosquito larvae was compared (Figure 3). However, the total prey consumption by *Lt. fuscanus* did not vary significantly ($t = 0.921$; df = 8) when the combinations of mosquito preys were different (when Anopheles & chironomid larvae mean consumption remained 9.11 ± 0.78 SD and with *Culex* and chironomid larvae combinations the value was 9.67 ± 1.22 SD).

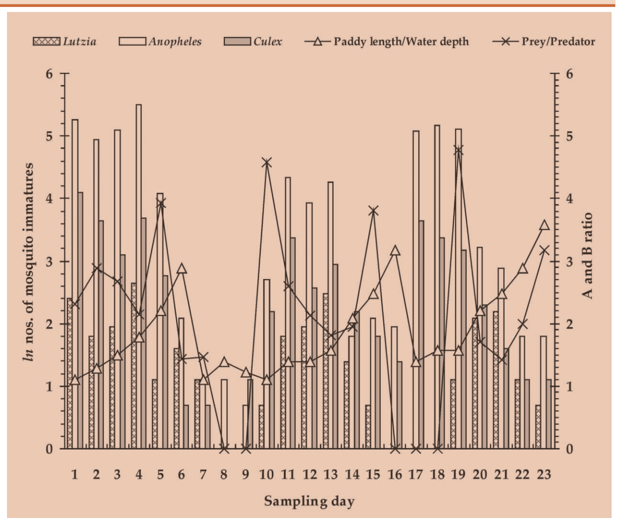

Figure 1 The total number of different mosquito immatures sampled between February and April 2006 from the rice fields in Amta, Howrah, along with the ratio of plant height and water depth (A) and the prey and predators (B) in the samples.

DISCUSSION

The sampling of the rice field for a period of 45 days confirms that the predator and prey mosquito populations are abundant throughout the period of cultivation with periodic oscillation in the prey predator ratio. The density of the mosquito larvae varied with the patches in the sampling area with the higher density of predator mosquitoes between the plantations or open habitats. Besides mosquitoes, the species assemblage consisting of different taxonomic groups including the belostomatid bugs, notonectid bugs, odonate and coleopteran larvae and adults among the predators and the chironomid larvae and planktonic rotifers and cladocerans among the prey classes. The abundance of the *Lt. fuscanus* larvae in presence of other mosquito predators indicates a possibility of intraguild predation. In such system, the higher-level predators like the odonate naiads[7], coleopteran larvae[8] and belostomatid bugs[24] might regulate the population of these mosquitoes. Perhaps, the observed relative abundance are inclusive of such effects and the ratio of the prey mosquito larvae and the *Lt. fuscanus* larvae are in equilibrium, along with other mosquito larval predators in the rice fields.

It appears from the results on prey consumption, that the larvae of the mosquitoes *Lt. fuscanus* can consume both chironomid and mosquito larvae with a preference for the latter. Between Anopheles and *Culex* larvae, the vulnerability of *Culex* larvae was

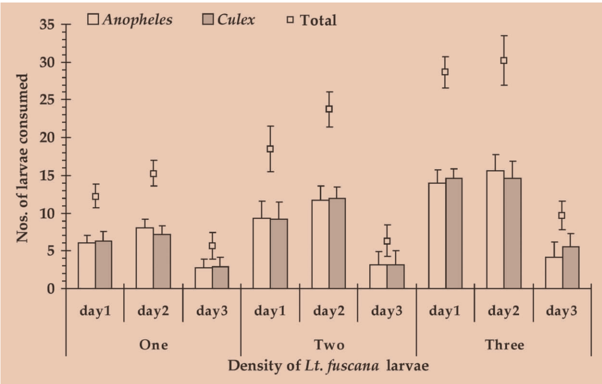

Figure 2 The numbers of prey consumed by IV instar *Lt. fuscanus* larvae when equal proportions of Anopheles and Culex larvae were present. (n = 12 per predator density; increase in day refers to increase in age of the predator)

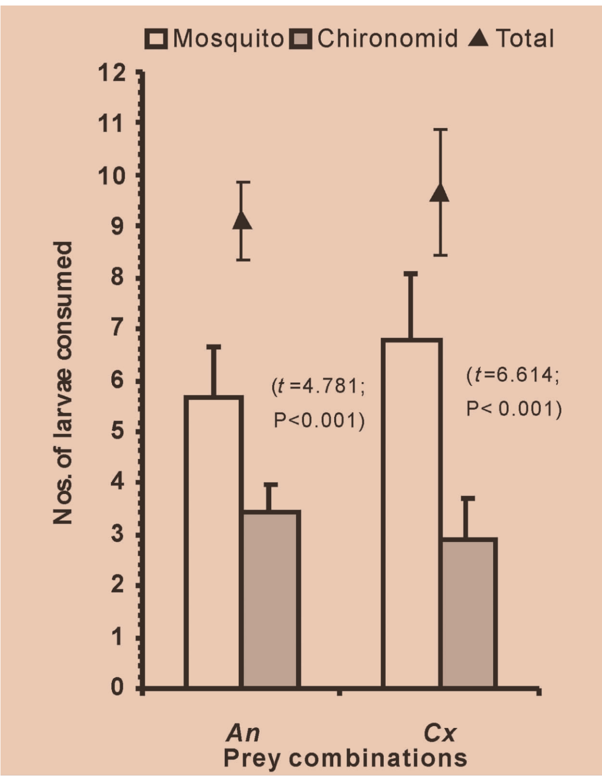

Figure 3 The relative numbers of chironomid and mosquito larvae killed when equal proportion of both the preys were presented to the IV instar Lt. fuscaus larva. (= 9 trials per prey combinations)

higher, possibly due to the presence of siphon or the orientation in water the floating pattern of *Culex* larvae were oblique to perpendicular to the surface compared to Anopheles larvae that remained parallel. The larvae of *Lt. fuscanus* remained parallel and thus the orientation could be a possible constrain in capturing the Anopheles larvae as prey. Besides, the predator in many cases attacked the siphon of *Culex* larvae. Possibly these factors added to the higher vulnerability of *Culex* larvae. The rate of predation decreased with the age of the predators, similar to the larval stages of *Tx. splendens*[23-25]. Although the number of prey consumed by larvae of *Lt. fuscanus* was lower to many species of odonate naiads[7] and the larvae of the dytiscid beetles *Acilius*[8], the specificity for mosquito immatures contrast to chironomid was higher. Nonetheless, considering the similarity of habitats and basic biology with the mosquitoes[10-14], the larvae of *Lt. fuscanus* have high potential to serve as biological resource in regulating mosquito immatures, alike the larvae of the mosquito *Toxorhynchites*[25-27].

The field evidence suggest that larval stages of predatory mosquitoes *Toxorhynchites* and *Lutzia* suits not only the containers and phytotelmata[11-13, 25], but also larger mosquito larval habitats like rice fields[11,23] and sewage drains[24]. Under field conditions, the habitats are complex owing to the presence of physical structures and alternative preys like chironomids. It is known that for belostomatid bugs[22] the effect of habitat complexity influence the predation of *Culex* larvae. Therefore, this needs to be evaluated for *Lutzia* larvae considering the complexity of aquatic habitats in rice fields. The use of *Tx. splendens* mosquitoes in biological control has been proposed earlier particularly in mosquito larval habitats like containers and treeholes[25]. The larvae of *Lt. fuscanus* bear such potential too, particularly when the phytotelmata like bamboo stumps are known to inhabit mosquitoes[28]. In the context of India and other Asian countries, different species of *Lutzia* have been reported. Evaluation of these species as biological resource needs to be evaluated for their use in biological control of mosquitoes. As an initiative, the present study provides satisfactory results in substantiating the rice fields as larval habitats of Lutzia and its predation potential against mosquito immatures. Further studies in this regards will help to include these mosquitoes in vector mosquito control programmes.

ACKNOWLEDGEMENTS

The authors are grateful to the respective Heads, Department of Zoology, University of Calcutta and The University of Burdwan, Burdwan for the facilities provided.

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