Observations on the predatory potential of *Lutzia fuscana* on *Aedes aegypti* larvae: implications for biological control (Diptera: Culicidae)

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Abstract
Among the natural predators, larval stages of the mosquito *Lutzia fuscana* (Wiedemann, 1820) (Diptera: Culicidae) bear potential as a biological control agent of mosquitoes. An estimation of the predatory potential of the larva of *L. fuscana* against the larva of the dengue vector *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) was made to highlight its use in vector management. Laboratory experiments revealed that the larva of *L. fuscana* consumes 19 to 24 *A. aegypti* larvae per day, during its tenure as IV instar larva. The consumption of *A. aegypti* larvae was proportionate to the body length (BL) and body weight (BW) of the predatory larva *L. fuscana* as depicted through the logistic regressions: $y = 1 / (1 + \exp(-(-2.09 + 0.35*BL)))$ and $y = 1 / (1 + \exp(-(0.4 + 0.06*BW)))$. While the prey consumption remained comparable among the days, the net weight gained by the *L. fuscana* larva showed a decreasing trend with the age. On the basis of the results, it is apparent that the larva of the mosquito *L. fuscana* can be used in the regulation of the mosquito *A. aegypti* through augmentative release, particularly, in the smaller mosquito larval habitats.

Key words: *Aedes aegypti*; *Lutzia fuscana*; biological control; smaller mosquito larval habitats; larval predation.

Introduction

Biological control of mosquitoes using natural predators is promoted owing to least effect on the ecosystem functions and species assemblages. Interaction between the natural predators and the mosquitoes is a part of relationship among the community members at large. Predators of mosquito are found in diverse numbers in larger mosquito larval habitats (Banerjee et al. 2010) in contrast to the smaller larval habitats. Among the few predators (Aditya et al. 2006, 2007) that are common in the smaller mosquito larval habitats, larval stages of the mosquito *Lutzia fuscana* (Widemann, 1820) (Diptera: Culicidae) (Pramanik & Aditya 2009; Singh et al. 2013, 2014) are common to abundant in different mosquito larval habitats in India, specifically in Kolkata (Calcutta). Recent taxonomic studies (Tanaka 2003) have lead to the elevation of subgenus *Lutzia* to the genus level, hence the erstwhile name of *Culex* (*Lutzia*) *fuscanus* (Wiedmann, 1920) is presently replaced as *Lutzia* (*Metalutzia*) *fuscana* (Wiedmann, 1820), which is further substantiated through further studies in recent years (Kitching et al. 2014).

The larval stages of *Lutzia* are predatory and feed on larvae of other mosquito species (Geetha Bai et al. 1982; Panikcer et al. 1982; Pramanik & Aditya 2009) as well as on the chironomid larvae and tubificid worms (Jin et al. 2006). Different species of *Lutzia* are recorded from India and other tropical countries (Ikeshoji 1966; Tanaka 2003; Kitching et al. 2014; Ohba et al. 2014), with their potential as biological control agent of different mosquito species (Prakash & Ponniah 1978; Thangam & Kathiresan 1996; Kirti & Kaur 2004; Singh et al. 2013, 2014) being highlighted. However, little effort (Singh et al. 2014) has been made to evaluate their predatory potential to promote them as biological control agent against the vector mosquitoes thriving in smaller mosquito larval habitats. In view of these, an attempt was made to evaluate the growth and predatory potential of the larval stages of *L. fuscana* against the dengue vector *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) under laboratory conditions. The results are expected to highlight the possible use of these mosquitoes in the biological regulation of the vector mosquitoes, especially thriving in the smaller larval habitats.

Materials and methods

The larval stages of the mosquito *L. fuscana* were encountered in different densities in the temporary containers in and around the campus of University of Calcutta, Kolkata, India, during the monsoon and post-monsoon (September to November) period of 2014 and 2015. The collection of the predatory larvae was made in course of entomological survey of the vector mosquitoes in the concerned area. However, the collections were irregular (1 out of 120 containers sampled) and the relative density remained low (predatory larvae 1: 25 vector mosquito larvae on average, as discussed below), and thus the experiments were carried out as and when the larvae of *L. fuscana* were encountered and collected. Of the total of 1800 containers (prospective larval habitats) surveyed following the protocol of the assessment of *Aedes* larval habitats in Kolkata, India (Banerjee et al. 2013, 2015), 16 containers (11 earthen pots and deep bowls, of 1 to 2l volume; 5 plastic containers; broken bucket, bowls and boxes 500ml to 1l volume) were found positive for the *L. fuscana* larvae (mean 5.06 ± 0.54 S.E.; range 2-10) along with the *Aedes* larvae (mean 120.56± 10.44 S.E.; range 40-247), resulting in a ratio of predator to prey larvae of ca. 1: 25 (mean 1: 25.03 ± 1.89 S.E.; range 1: 16 – 48.6).

The collected larvae of *L. fuscana* were brought to the laboratory and placed in enamel trays with 3L of tap water and adequate number of mosquito larvae (IV instar stages of *Culex quinquefasciatus* or *A. aegypti*) was provided as prey. The prey mosquito larvae were present in the collections or obtained from the laboratory culture. The collected predatory larvae were mostly in the late IV instar larvae, evident from the pupation of 54 larvae within 24 h period.

The immature of *Aedes* mosquitoes were collected from different smaller earthen and porcelain containers from the same area. The collected immature (P-generation) were placed in the enamel trays and the pupa were segregated, and placed in separate vials. Following emergence of the adult, the species-specific identification as *A. aegypti* was made and reared (F_1- and subsequent generations) in the laboratory for steady supply of the IV instar prey larva (Banerjee et al. 2013). Owing to the continuous culture of *A, aegypti*, this species was considered as prey in the experiments, though in field collections of the concerned geographical area, both *A. aegypti* and *A. albopictus* co-occur (Banerjee et al. 2010, 2013). The body length and the body weight of the larvae of *L. fuscana* followed a power regression as: body weight = 0.751* body length$^{1.291}$; $r^2 = 0.848$ (mean length 9.32 ± 0.23 mm, range 8.1 – 10.9 mm and mean body weight 13.46 ± 0.49 mg, range 11.2 – 17.4 mg) while the larvae of *A. aegypti* conform to the power regression as: body weight = 0.032* body length$^{2.227}$; $r^2 = 0.748$ (mean length 6.57 ± 0.13 mm, range 5 – 7.6mm; mean body weight as 2.19 ± 0.11 mg, range 1.14 – 3.25 mg).

The experiment constituted assessment of the predation of the instar IV *A. aegypti* larva along with the measurement of the length and weight of *L. fuscana* larva. In a plastic container containing 250ml of tap water (aged tap water kept in storage tanks, pH 7.2), 25 instar IV larvae of *A. aegypti* were placed along with a single *L. fuscana* larva of known length and weight. The length of the larva was measured under binocular fitted with ocular micrometer (Erma®, Japan) and the wet weight was measured in a pan balance (Afcoset®, India) to the nearest 0.1mg.

At the end of a 24 h period, the total numbers of prey consumed by the predator were noted, along with the changes in the length and weight of the predator. Following record of the numbers of *A. aegypti* consumed, the experimental containers were reset with the same number of prey and the observations were made using the same predator larva. The record of predation and the growth of the predator larva were noted for three consecutive days until the larvae metamorphosed to pupal stage. Although the collections of the predatory *L. fuscana* larvae were more, data of only 27 larvae that completed the whole phase of three days predation was considered. Consumption of the prey larva by the predators on each day was subjected to logistic regression analysis (Addinsoft 2010) to deduce the relationship between the length of the predator and the number of prey consumed. Further the gain in weight in each day was considered as a predictor with the number of prey consumed by the *L. fuscana* larva. The logistic regression complied with the propositions of the binomial generalized linear model with the proportion of the prey larva (instar IV *A. aegypti*) consumed as the response variable against the different explanatory variables, like days, body weight and the body length of the predator larva. In this assumption, the prey consumed (response variable) follows a binomial distribution (n, p) with n replicates (samples) of observations, while the linear combination of the explanatory variables (day, body length and body weight) was presented through the probability parameter, p. A weighted binary function with logit link was employed and with the equation being, prey consumed (y) = $1/(1+ \exp(- (a + b_1 x_1)))$. The parameters (intercept and the explanatory variable) were estimated through maximum likelihood with the significant contribution being measured through the Wald's χ^2 (Addinsoft 2010). An indicator of the efficacy of the predatory larva was assessed against the body weight, using linear regression (Zar 1999). Here, the weight gained per unit prey larva consumed was the response variable against the body weight of the predator larva.

Results

The instar IV larva of the mosquito *L. fuscana* consumed 17 to 24 larvae of equivalent instar larva of *A. aegypti* with significant variations among the days of the experi-

ment. As a consequence of the prey larva consumption, the weight gained by the predatory decreased with time, and followed a power function (Fig 1). A logistic regression of consumption of *A. aegypti* larva by the larva of *L. fuscana* could be represented as: consumption, $y = 1/(1+ \exp(1 / (1 + \exp(-(0.81 + 0.34*Day))))$, with the model parameter (day) being significant (intercept = 0.81 ± 0.2; Wald's χ^2 = 15.344; day = 0.34 ± 0.1; Wald's χ^2 = 10.778). The proportion of the prey consumed by the *L. fuscana* as a function of the body length (Fig 2a) could be presented as: prey consumed, $y = 1 / (1 + \exp(-(-2.09+0.35*BL)))$ and the model parameter (BL, body length of the predator) were significant (intercept = -2.09 ± 0.88; Wald's χ^2 = 5.67; P < 0.017; Body length = 0.352 ± 0.087; Wald's χ^2 = 16.197; P<0.0001). Similarly, the proportion of the prey consumed as a function of the body weight (Fig 2b) of *L. fuscana* can be represented as: $y = 1 / (1 + \exp(-(0.4 + 0.06*BW)))$, with the parameter (BW, body weight) being significant (intercept = 0.4 ± 0.137; Wald's χ^2 = 1.72; P= 0.188; Body weight =0.06 ± 0.016; Wald's χ^2 = 12.107; P<0.0001). It was apparent that the prey consumption was non-linear in terms of the days of predation event considered and thus the relationship could be explained in terms of the logistic regression. At the end of the experiment the increment of length and the weight of the larvae of *L. fuscana* followed a power regression as: body weight = 0.034* body length$^{2.727}$; $r^2 = 0.796$ (mean length 10.19 ± 0.15 mm, range 8.1 – 12.3 mm and mean body weight 19.73 ± 0.85 mg, range 11.2 – 29.1 mg). Further assessment of efficacy of the predatory larva of *L. fuscana* could be represented in terms of weight gained per unit larvae consumed against the body weight (Fig. 3). A decreasing trend in the weight gained per unit larvae consumed was observed with reference to the body weight of the *L. fuscana* larva. At the end of the feeding period of three days, the body length of the larva of *L. fuscana* was 10.88 ± 0.13mm (range 10.3 to 12.3mm) and corresponding body weight was 28.71 ± 0.78 mg (range 23.3 – 33.1 mg).

Discussion

From the results it is apparent that the larva of *L. fuscana* can consume considerable number of *A. aegypti* larvae while exhibiting its growth to the pupal stages. The prey consumption varied with the days in correspondence to the growth of the larva of *L. fuscana* indicating that the satiation level varies with the age of the predator. In course of the consumption of the prey, the energy acquired by the predatory larva of *L. fuscana,* is assimilated in the growth of the somatic and reproductive tissue. With the increase in the biomass, the energy required for the maintenance increases, which is reflected through the efficacy indicator. In the initial day, the weight gained per unit larvae killed by early IV instar larva was quite high contrast to the late IV instar larva. A decline in the number of the IV instar

A. aegypti larvae killed by IV instar *L. fuscana* larva was observed as it reached pupation. The results are similar to the observations made on the predation on *A. aegypti* by *L. fuscana* in Rajasthan, India (Singh et al. 2014). Considering the larval habitats of *A. aegypti*, particularly the waste containers and bamboo stumps, it is highly probable that the augmentative release of *L. fuscana* can be a possible mode of intervention. In India (Geetha Bai et al. 1982; Panikcer et al. 1982; Pramanik & Aditya 2009; Singh et al. 2013, 2014) and several Asian countries (Jin et al. 2006; Ohba et al. 2014), the availability of *L. fuscana* calls for their use in the regulation of the dengue vectors, particularly, when the larval habitats and the life cycle patterns are similar for the target and the predator mosquitoes. Earlier studies on the taxonomy (Tanaka 2003; Kitching et al. 2014) and the occurrence of *Lutzia* spp. in China (Jin et al. 2006), India (Geetha Bai et al. 1982; Panicker et al. 1982; Prakash & Ponniah 1978; Thangam & Kathiresan 1996; Pramanik & Aditya 2009; Singh et al. 2013, 2014), Venezuela (Berti Moser et al. 2009) and Vietnam (Ohba et al. 2014) support their co-occurrence in the mosquito larval habitats known to be exploited by *A. aegypti*. As revealed through the gut analysis, the larva of *L. fuscana* consumes chironomid larvae also (Jin et al. 2006), which may be a possible concern for the regulation of the target mosquito. The selection of mosquito larvae by the predatory larva of *L. fuscana* will vary in situations where the mosquito larva coexists with the chironomid larva. Therefore further studies are necessary to evaluate the prey preference of *L. fuscana* in situations where multiple preys exists in the larval habitats, as observed in many container habitats in Kolkata, India (Mohan et al. 2014). Predatory efficacy of *L. fuscana* may also be limited in situations where the prey availability is low, since larval *Lutzia* spp. switches to cannibalism in absence of adequate prey (Yashuno 1965). Nonetheless, the present study demonstrated that the predation of the *L. fuscana* larva is dependent on size and gains biomass through the predation process. As a result of increase in the biomass of the larva, subsequent fitness of the pupa and adults are assured for *L. fuscana,* though the availability of the prey and the size of the larval habitats may be important factors. Keeping in view the low relative density with respect to the prey larvae in the positive *Aedes* larval habitats, it appears that the smaller habitats may be a constraint for the mosquito *L. fuscana.*

As oviposition sites, *Lutzia* spp. is known to explore a wide range of habitats, ranging from the ground water habitats and phytotelmata and small containers (Kitching et al. 2014). Earlier studies substantiate rice fields (Pramanik & Aditya 2009), varied types of cemented tanks (Singh et al. 2013, 2014) and slow water drains (Jin et al. 2006) as larval habitats of *L. fuscana*, and cemented tanks and sewage pools (Prakash & Ponniah 1978; Thangam & Kathiresan 1996) for other species of *Lutzia*. In the present instance, the larvae of *L. fuscana* were encountered in larger among the known sizes of the *Aedes* larval habitats (500ml
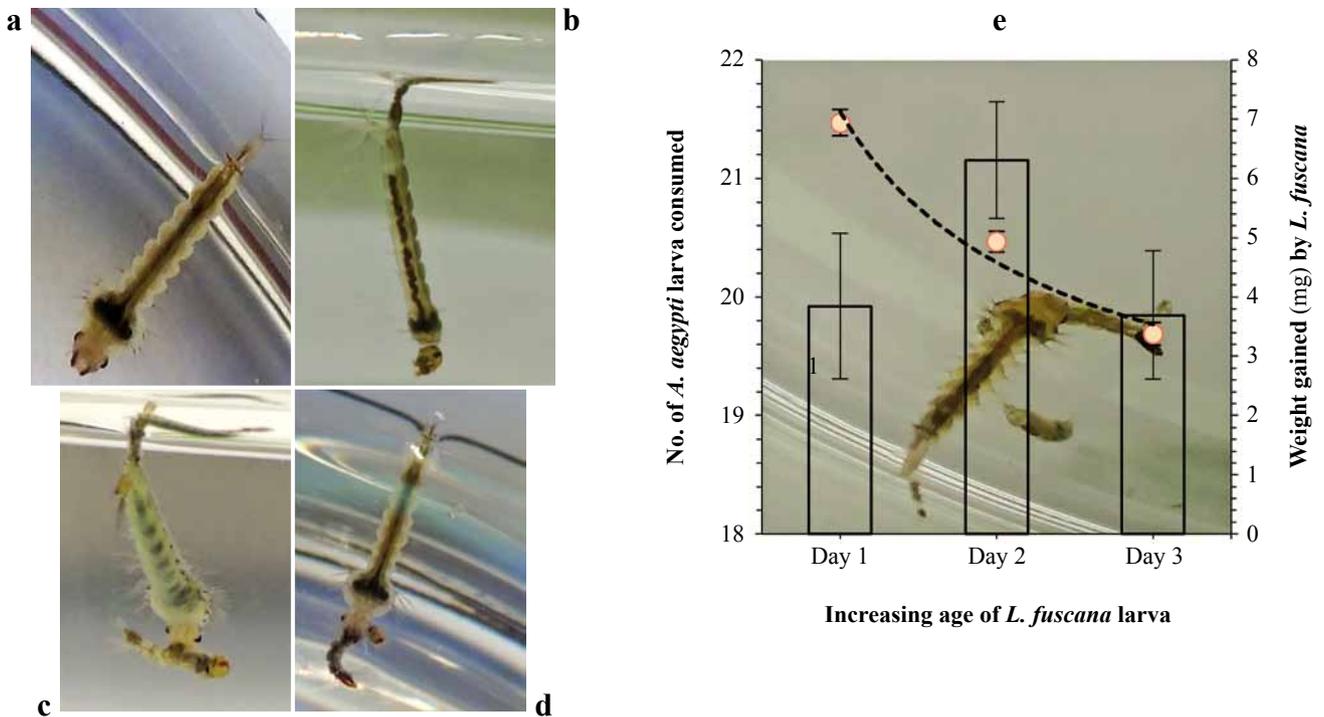
Fig. 1 – **a**, an instar IV larva of *L. fuscana*, the predator; **b**, an instar IV larva of *A. aegypti*, the prey; **c**, larva of *L. fuscana* predating on the larva of *A. aegypti* (ventral view); **d**, same as c, dorsal view; **e**, the number of *A. aegypti* larvae killed by IV instar larva of *L. fuscana* during three consecutive days under laboratory conditions. (n = 39 replicates).
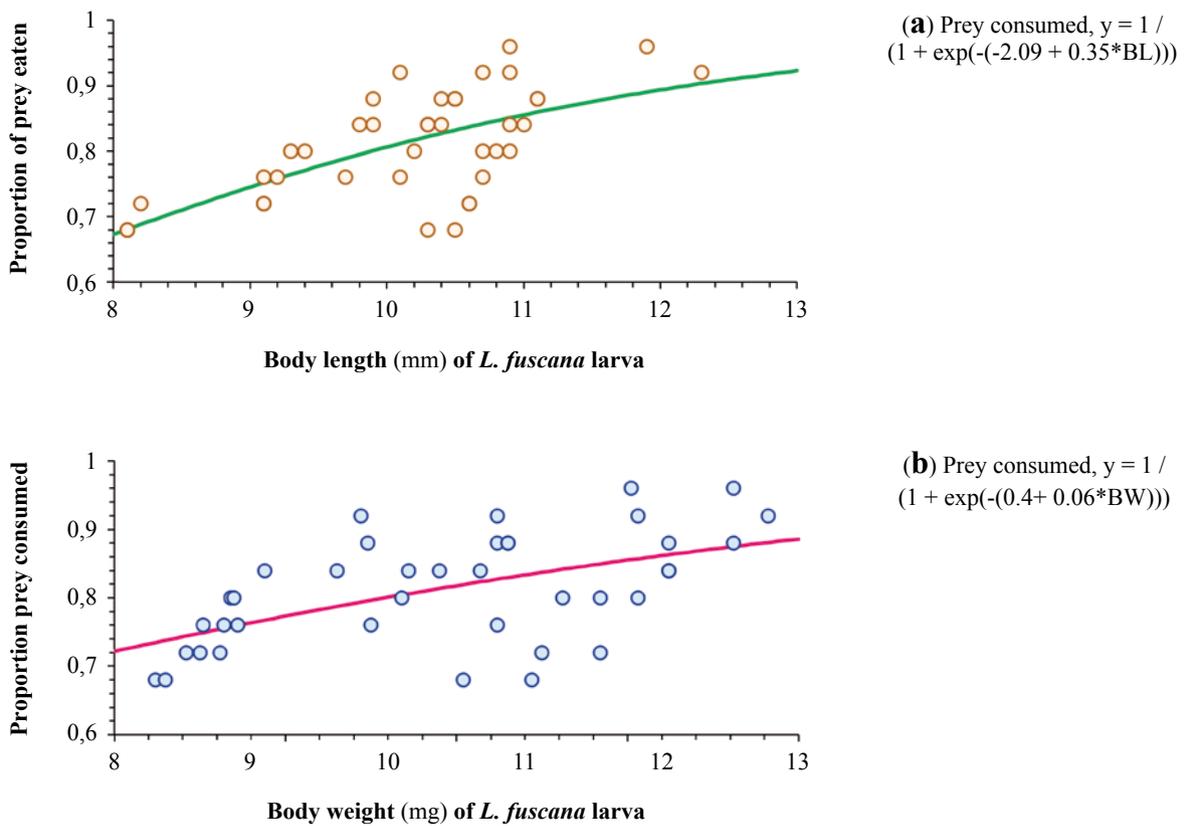

(**a**) Prey consumed, y = 1 / (1 + exp(-(-2.09 + 0.35*BL)))

(**b**) Prey consumed, y = 1 / (1 + exp(-(0.4+ 0.06*BW)))

Fig. 2 – **a**, the proportion of the *A. aegypti* larvae consumed in a day by the predatory larvae of *L. fuscana* presented as a function of the body length (BL, in mm) of individual *L. fuscana* larva, irrespective of day; **b**, the same, as a function of the body weight (BW, in mg).

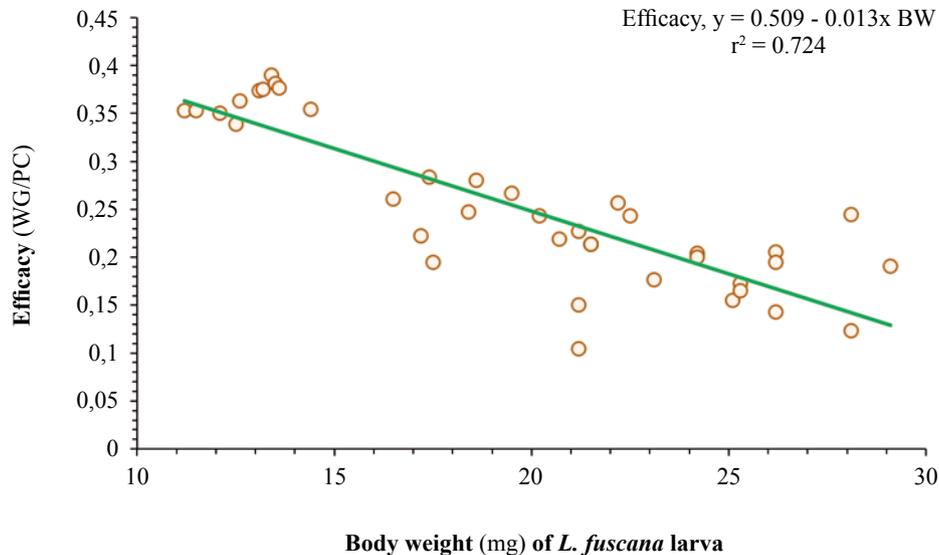Efficacy, y = 0.509 - 0.013x BW
$r^2 = 0.724$

Fig. 3 – The relationship between the predatory efficiency against the initial weight of instar IV *L. fuscana* larva consuming instar IV *A. aegypti* larva. Here efficacy was estimated as weight gained, (WG) per unit prey consumed (PC). BW– Body weight (in mg) of *L. fuscana* larva.

to 2l size plastic buckets or earthen pots and bowls) (Banerjee et al. 2013, 2015; Mohan et al. 2014), and comparable to the oviposition sites observed in Rajasthan, India (Singh et al. 2014). Larval habitat size is linked with higher prey availability (Banerjee et al. 2010) and therefore may be preferred by the predatory mosquito *L. fuscana* as oviposition sites. Further studies should be carried out to establish the range of habitats exploited by the mosquito *L. fuscana,* along with its prey preferences to understand the prospective natural colonization and successful regulation of target mosquitoes. Laboratory colonization for mass release and further establishment of the predatory mosquito (Panicker et al. 1982) need to be carried out with caution, since female *Lutzia* require blood meal (Kitching et al. 2014) and involved in disease transmission in birds (Ejiri et al. 2009). Considering the observations of the present instance and earlier studies (Singh et al. 2014), use of *L. fuscana* in classical or augmentative release method of biological control against *A. aegypti* seems to be feasible. However, further studies should be initiated to explore the life history and ecology of *L. fuscana* to determine its efficacy against vector mosquitoes, including *A. aegypti* in smaller larval habitats like containers and tree-holes.

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