



Distribution of mosquito larvae in rice field habitats: a spatial scale analysis in semi-field condition

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ABSTRACT

The distribution of the mosquito larvae in the breeding habitats varies at the spatial scale depending on the availability of the resources and the predators. This proposition was assessed through the observation of the spatial distribution of *Culex* larvae (*Culex tritaeniorhynchus*) in artificially constructed rice field habitats. Using a binomial generalized linear model with logit link, the disparity in the abundance of the larvae was evaluated to justify the effects of light (light vs shade), vertical (surface vs bottom), and horizontal (wall vs center) distribution as explanatory variables. Under light availability, the spatial occupancy of the mosquito larvae was higher in the center than in the walls of the mesocosms. However, the larval orientation was higher on the surface than at the bottom of the mesocosms in all instances. In comparison to open spaces, the larval aggregation was higher in the presence of the floating vegetations like *Azolla* and *Lemna*, indicating that the habitat heterogeneity of the mesocosms influenced the distribution of the mosquito larvae in the available spaces. A reduction in the larval aggregation pattern in the spaces was observed in the presence of the predator (*Anisops* sp.) reflecting the possible evasion tactics of the mosquito larvae. The observations suggest that the mosquito larvae may utilize the vegetation in the rice field habitats quite effectively and occupy empty spaces of predators. The results may be considered as a prototype of the prospective localization of the mosquito larvae in the rice fields and help to frame the strategies of spraying the biopesticides to achieve optimal efficacy in mosquito regulation.

KEYWORDS

mosquito larva, *Culex* sp., rice fields, spatial distribution, vegetation, *Anisops* sp.

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INTRODUCTION

Rice fields are exploited as breeding sites by a variety of mosquito species linked with the transmission of diseases affecting human and domestic animals (Pramanik et al., 2006; Sunish et al., 2006; Dale and Knight, 2008). Empirical studies around the world indicate that various species of *Anopheles* and *Culex* dominate the rice field habitats with the pupal productivity varying with the paddy rice cultivation pattern (Sunish and Reuben, 2002; Muturi et al., 2008; Mwangangi et al., 2008; Ohba et al., 2013; Lytra and Emmanouel, 2014). Application of the natural enemies alone, or in combination with the biopesticides, is considered as a feasible way of mosquito management. Successful mosquito control is however related to the understanding of larval dispersion patterns in the rice fields. Although the relative abundance of the mosquito larvae in rice fields varies with the time period of the rice cultivation, the dispersion of the mosquito immature is a crucial factor in successful regulation (Ohba et al., 2013, 2014; Watanabe et al., 2013).

The efficacy of the predators and biopesticides depends on the contact with the mosquito larvae, and thus the dispersion of the mosquito larvae in the rice fields is a critical factor. Different studies have substantiated the asymmetric distribution of the larvae in the periphery and the center part of the rice field habitats (Mogi and Miyagi, 1990; Mogi et al., 1995; Sunish and Reuben, 2002; Das et al., 2006; Watanabe et al., 2013). The presence of the mosquito larvae in the central and the peripheral parts of the rice fields may relate to the oviposition habitat selection of the mosquitoes, perhaps in relation with the presence of the natural predators, like *Anisops* sp., dytiscid beetle, odonate larvae, etc, that may induce mortality to the larvae.

The habitat in the rice fields is complex owing to the presence of the paddy plants and physical structures including the sediment and the detritus. Often the mosquito larvae and co-occurring organisms utilize the physical structures as a refuge thereby altering the possible interactions within and between organisms (Saha et al., 2008, 2009). In order to evade

the predation pressure or utilize food resources in the available space, mosquito larvae can be found associated with the refuges, thereby denying the predators access (Saha et al., 2009; Ohba et al., 2013; Watanabe et al., 2013). Empirical evidence suggests that the vulnerability of the mosquito larvae to the insect and fish predators is affected by the heterogeneity in the habitat conditions arising from the presence of the plants, twigs, and detritus (Saha et al., 2008, 2009; Aditya et al., 2012). Habitat complexity of the rice fields is, therefore, a factor that can affect the interactions of the predators and the mosquito prey and determine the success of the regulation of the target mosquito. Further, the surrounding landscape with trees and shrubs may provide ample shades, particularly along the banks and edges of the rice field proper. In effect, even during the daytime, the heterogeneity of the light and shade in the rice fields is available that can in turn influence the distribution of the larvae in the habitat spaces. In the milieu of the habitat heterogeneity, the distribution of the mosquito immature may vary spatially. Therefore, the aggregation of mosquitoes may change with the interactions of shade and light and the habitat structures (Pitcairn et al., 1994; Overgaard et al., 2002; Overgaard, 2007). With time, the growth of the paddy plants augments the degree of heterogeneity thereby affecting the dispersion and the interactions among the predators and prey, including mosquito larvae. Based on this proposition, an evaluation of the distribution and dispersion of the mosquito larvae amidst the physical structures in the rice fields was made through mesocosm study in semi-field conditions. The mesocosms were built to mimic the rice field conditions such that the distribution of the mosquito larvae can be assessed at the microhabitat conditions. The spatial distribution of mosquito larvae in larval habitats have been evaluated using different species of *Aedes* (Walker and Merritt, 1991; Juliano and Reminger, 1992; Juliano et al., 1993; Awasthi et al., 2015), *Culex* (Workman and Walton, 2003), and *Anopheles* (Walker and Merritt, 1993; Wallace and Merritt, 2004) mosquitoes. The present study was undertaken with the same principle of localization of mosquito larvae in the context of the complexity of the rice plants and associated vegetation. The impact of the botanical and microbial formulations against mosquito with paddy plants as a key element of habitat conditions can be appraised through the distribution of the mosquito larvae in the mesocosms. The results are also expected to highlight the density-dependent and water height-dependent effects on the mosquito larvae. Thus, the focus of the study was to emphasize the spatial occupancy of mosquito larvae in the rice fields in relation to (1) paddy plants and associated habitat conditions, (2) in the presence of floating weeds and (3) in the presence of insect predators.

1. MATERIALS AND METHODS

1.1. Collection of the Mosquito Larvae and the Paddy Plants

The University farmhouse in Tarabag, Burdwan, West Bengal, India, includes an area of 10 ha for paddy rice cultivation throughout the year. The paddy plants were procured from the farmhouse at the time of transplantation, while the mosquito larvae were collected from the rice fields from the same area using insect net fitted with a long handle. In the course of the collection of the larvae, the floating macrophytes were also collected in accordance with the requirements of the experiment.

1.2. Preparation of Mesocosms

In order to assess the mosquito immature distribution in the rice fields, multiple numbers of mesocosm were constructed using porcelain basins (41×21×6 cm) as the substrates. The required amount of mud and water was used to fill the space to mimic the conditions available in paddy rice cultivation in field conditions. Following this setup, six paddy plant bunches were planted and allowed to grow in the mesocosms. The mud and water were brought from the rice fields of University Farm House, Tarabag, Burdwan, India (Fig. 1a). The water from the rice fields was brought and mixed with tap water (1:1::v:v) and added to the mesocosms at regular intervals to maintain the water height at par with the rice field conditions. The addition of the water to the mesocosms was continued to maintain the desired water level of around 10 cm (Fig. 1b and c). The growth of the paddy plant continued in the mesocosms and the sediment architecture shaped accordingly creating a crest and trough between the adjacent paddy plants (Fig. 1d). After the end of 2-weeks period, the mesocosms were considered ready for initiation of the experiment. The mesocosms were set in open-air conditions, with equal numbers of replicates. The sidewalls of the porcelain mesocosm had two slits to get rid of excess water during an event of rain. The observations were repeatedly taken and the average of at least three counts per unit space (each surface and wall in a mesocosm) was considered for the number of mosquito larva at that instance. Counting of larvae in the mesocosms was made at a time gap for each surface, wall or bottom, to avoid the randomly moving larvae in and out of the visible counting space. Although in course of the experiments, data on the mortality of the larvae in the mesocosms were counted, the rate of pupation was evident (pupa per day/mesocosm; range 1 and 7.33; mean 3.41±0.64 SE) and the replaced pupa from the mesocosms remained between 12 and 88 (mean 42.17±7.63 SE). In the initial day, the experimental mesocosm was filled with an average of 76.3±5.6 larvae, and subsequently, in the following days, an average of 68±9.65 larvae was added in the mesocosms. However, for the rest of the experimental setup, for experiments with vegetation and insects, additional larvae were added such that the abundance

remained different. In the experiments using vegetation in the mesocosms, the larval abundance remained between 100 and 177 ($133.35 \pm 5.43SE$) with an average of 72.3 ± 4.9 pupa emerging from the mesocosms in course of the experiment. The reasons for such variations are due to the differences in the collections from the rice fields on the particular day. The productivity of the larvae in the rice fields differed in the course of the experimental period and thus the collections (Fig. 1e).

1.3. Experimental Method

The experiment was initiated by introducing the mosquito larvae, collected from the rice fields, into the mesocosms. Prior to introduction, the collected mosquito larvae were segregated as larva and pupa as well as other insects and macroinvertebrates were excluded for entry into the mesocosms. However, any propagule or the benthic macroinvertebrate that remained associated with the paddy saplings and the mud remained inside the mesocosm. Even if some detritivore were found in the mesocosms, these were screened out to make the mesocosm free of any aquatic insects. The experimental observations were made on the larvae (pupae that were formed from larvae subsequent to the introduction in the mesocosms were removed) under two settings – light (natural sunlight) and shaded conditions.

The observations were made on the position of the larvae in the mesocosms where two space types were available: (1) areas around the paddy plants and (2) open spaces. Water depth varied with the two spaces by a gap of some 2–3 cm such that the microhabitat conditions were different. The aim of the study was to analyze the spatial distribution and dispersion of the mosquito immature in the mesocosms. In each mesocosm, the observations were made for a fixed time period of 30 min and the average number of mosquito immature observed during the period was recorded. Although the larvae could not be identified to species level, following the continuous collection of the pupa, and subsequent rearing to the adult stages, the species of mosquito was determined.

In order to observe the position of the mosquito immature, six different positions were considered as shown in Fig. 1. The numbers of larvae present in the spaces were counted through visual observations each day following the introduction to the mesocosm. The observations were made between 10 am and 4 pm each day on the 14 mesocosms; 10 of which were placed under direct light conditions, while four were placed in the shade. The position of the mosquito larvae was considered in two settings: (1) surface against wall (including the base of paddy plants) and (2) surface against bottom in accordance with earlier works (Juliano and Reminger, 1992) with modifications.

In the second experiment, the position of the mosquito larvae was analyzed following the introduction of the floating vegetation, *Azolla pinnata* and *Lemna minor*, in the proportions normally observed in the rice fields. Following collections of the vegetation, the individual units of the same were rinsed to prevent the entry of any macroinvertebrates

associated with the plants. After the vegetation settled in the mesocosms, the mosquito larvae were introduced (range 150–275; mean 170 ± 2.5 not more than 200 depending on the collections) and the counting was carried out using visual inspection as well as by surfing small net for the collection of the larvae. The numbers of vegetation units and the mosquito larvae in the center and the side were counted and placed back for the second counting in the following days. Eight replicates were considered with the vegetation and six replicates were considered without the vegetation (control) for the purpose.

In the third experiment, the mosquito predators were introduced in the mesocosms, and the position of the mosquito larvae was checked. Two backswimmers, *Anisops bouvieri*, were added in each mesocosm and the count of the mosquito larvae was conducted as before to evaluate the effects of the predators. The mesocosms without predator were considered as control, and six such replicates were used against the eight replicates containing the backswimmers. In all instances, the mesocosms used in the experiments were separate such that each mesocosm qualifies as a replicate (Hurlbert, 1984). Apart from the counting of the mosquito immature in each quadrat (mesocosm), the luminosity (lux meter – Kusam Meco LUX-99 Digital Lux Meter, India) of the conditions along with the water quality parameters like temperature, pH, salinity, total dissolved solids, conductivity (Water multiparameter Testr 35 series, EUTECH, USA), water depth (dipping a stick and measuring the water level, in cm), and plant heights (from the surface to the tip of the extended leaf, in cm) were measured. For measuring the plant heights, at least three data were taken from separate bunches in the mesocosms and the mean value was recorded.

1.4. Data Analysis

For both conditions, the mesocosms were placed: in the shade and in the sunlight; the position of the mosquitoes in the mesocosms was attributed to two different categories: (1) center against sidewalls and (2) surface against the bottom. The application of contingency χ^2 was made to justify the differences in the position at the spatial scale. The data obtained on the position of the mosquito larvae in the spaces within the mesocosms at two scales horizontal (center and sidewalls) and vertical (surface and bottom) were used in logistic regression following binomial generalized linear model (GLM) with logit links. The space (surface versus bottom, for instance) and the availability of light (light vs shade) were considered as explanatory factors for the observed position of the mosquito larvae in the mesocosms. In the logistic regression, the position at a particular dimension is assumed to follow the binomial distribution (n, p) with n replicates (mesocosms) for each level of the explanatory variables (light and space). The probability parameter p represents the linear combination of the explanatory variables. A weighted binary function with logit link was employed, and the parameters were estimated through maximum likelihood using the software XLSTAT (Addinsoft, 2010). The equation of the logistic regression is represented as: relative

abundance of mosquito ($y=1/(1+\exp(-(a+b_1x_1+b_2x_2)))$), where, x_1 is the space and x_2 represents light. A χ^2 value (Wald's χ^2) was used to deduce the significant contribution of the explanatory variables to the distribution of the larvae.

The factors, light (sunlight and shade) and the spatial scales (horizontal and vertical) were used as the explanatory variables for the observed differences in the mosquito larval position. Similarly for the experiment with vegetation, the light and the degree of vegetations (none, *Azolla* dominant, and *Lemna* dominant) were considered as the explanatory variables against the number of the mosquito larvae at the horizontal scales as dependent variables. In the experiment with the addition of the backswimmers, the horizontal scale position of the mosquito larvae was considered as the response variable. Thus, the density of the larvae in the presence and absence of the predators was considered. The data were further subjected to the generalized linear mixed models analysis of variance (ANOVA) (Legendre and Legendre, 1998; Zar, 1999) for identifying the significant effect of the response variables on the distribution of the larvae in the mesocosms. Assuming that the habitat conditions remained even throughout the mesocosms, the distribution throughout the space would remain equal for the mosquito immature and irrespective of the genera. The data recorded on the mosquito larval distribution in the mesocosms were noted for a period of 2 weeks coinciding with the first round of waterlogged conditions of the paddy rice cultivation in the field conditions. Observations were continued after a brief gap of 1 week following the second round of flooding of the rice fields. Thus, a total of 4-week observations were made in terms of the spatial distribution of the mosquito larvae in the mesocosms. The experimental design and analysis are provided in Fig. 1. The data on the water quality, water depth, and the plant height were used to correlate (Zar, 1999) (taking in all 138 random samples from different mesocosms) with the abundance of the mosquito during the experimental period, to highlight the influence of the environmental condition on the larval abundance in the mesocosms.

2. RESULTS

The number of *Culex* larvae present in the mesocosms varied in relative numbers owing to the differences in the time of collection and the relative density in the sampling sites. In the course of the experiment, the adults of either *Culex tritaeniorhynchus* or emerged from the mesocosms. Few individuals of the *Culex*

vishnui complex, *Anopheles subpictus*, and *Anopheles vagus* emerged at the end of the experiment, which were not considered in the data for analysis. Thus, the analysis was restricted to the distribution of the *Culex* mosquitoes with *C. tritaeniorhynchus* being the dominant species, identified following suitable key (Rattanarithikul et al., 2005).

The relative abundance of the mosquito larvae varied with the quadrats and the light available in the mesocosms (Fig. 2). The relative number of *Culex* larva present in unit space in the sunlight (mean 8.51±1.17 SE) differed with the shaded (mean 10.39±1.84 SE) regions significantly (78 paired counting unit from sunlight and shaded mesocosms $t_{(2),77}=10.109$; $P<0.001$). However, for the mesocosms in the sunlight area, the variations in the relative abundance of *Culex* larva remained significantly different, while for the shaded mesocosms no such differences were observed (Fig. 2). Following a comparison in the distribution of the *Culex* larvae in the center and sidewalls of the mesocosms, significant differences were observed both at the level of the sites and availability of light (Fig. 3). Using the position of the larvae in center or wall, the logistic regression yielded: larval abundance, $y=1/(1+\exp(-(-6.64+2.65*\text{site}-1.72*\text{position})))$, while the logistic regression for the surface or deep position yielded the equation: larval abundance, $y=1/(1+\exp(-(-0.0001+0.16*\text{site}+0.90*\text{position})))$, with the parameters being significant at $P<0.001$ level (here site – sunlight and shade; position – wall or center, or position – surface or depth) (Table 1). The GLM and the mixed model ANOVA supported the significant differences in the distribution of the larvae in the concerned spaces (Table 2).

In the presence of vegetation, the distribution of the larva varied significantly with impacts in the plots of the vegetation. The logistic regression yielded an equation, larval abundance, $y=1/(1+\exp(-(-1.02-0.07*\text{vegetation}-0.13*\text{site})))$, with the parameters being significant at $P<0.001$ level (intercept=-1.02±0.04 Wald's $\chi^2=537.64$; vegetation=-0.07±0.02; Wald's $\chi^2=20.29$; sites=-0.132±0.01; Wald's $\chi^2=283.08$). The Kruskal-Wallis ANOVA indicated significant differences in the larval abundance in the three microhabitat conditions, with vegetations (high *Azolla*, high *Lemna*, and without vegetation) (Fig. 4a). The distribution of the *Culex* larvae varied with the presence or absence of the backswimmers significantly (Fig. 4b) revealed through the Kruskal-Wallis ANOVA. Larval abundance as shaped by the presence of the predators could be represented through logistic regression as: larval abundance, $y=1/(1+\exp(-(-3.34-2.5*\text{Predator}+0.95*\text{sites})))$, with the pa-

Table 1. The parameters site (x_1) and position (x_2) for the observed variations in abundance of *Culex* sp. in compliance with the equation: relative abundance (y)= $1/(1+\exp(-(a+b_1x_1-b_2x_2)))$

| Spatial features | Intercept | Site | Position |
|---------------------|---------------------|-------------------|--------------------|
| Sidewall and center | -6.639±0.109 | 2.646±0.08 | 1.722±0.07 |
| Surface and bottom | 0.0001±0.16 | 0.155±0.02 | -0.899±0.04 |

The values in bold indicate significance at $P<0.05$ level based on Wald's χ^2 following the logistic regression model.

Figure 1. The outline of the experimental design and the pattern of introduction of *Culex* larvae in the mesocosm

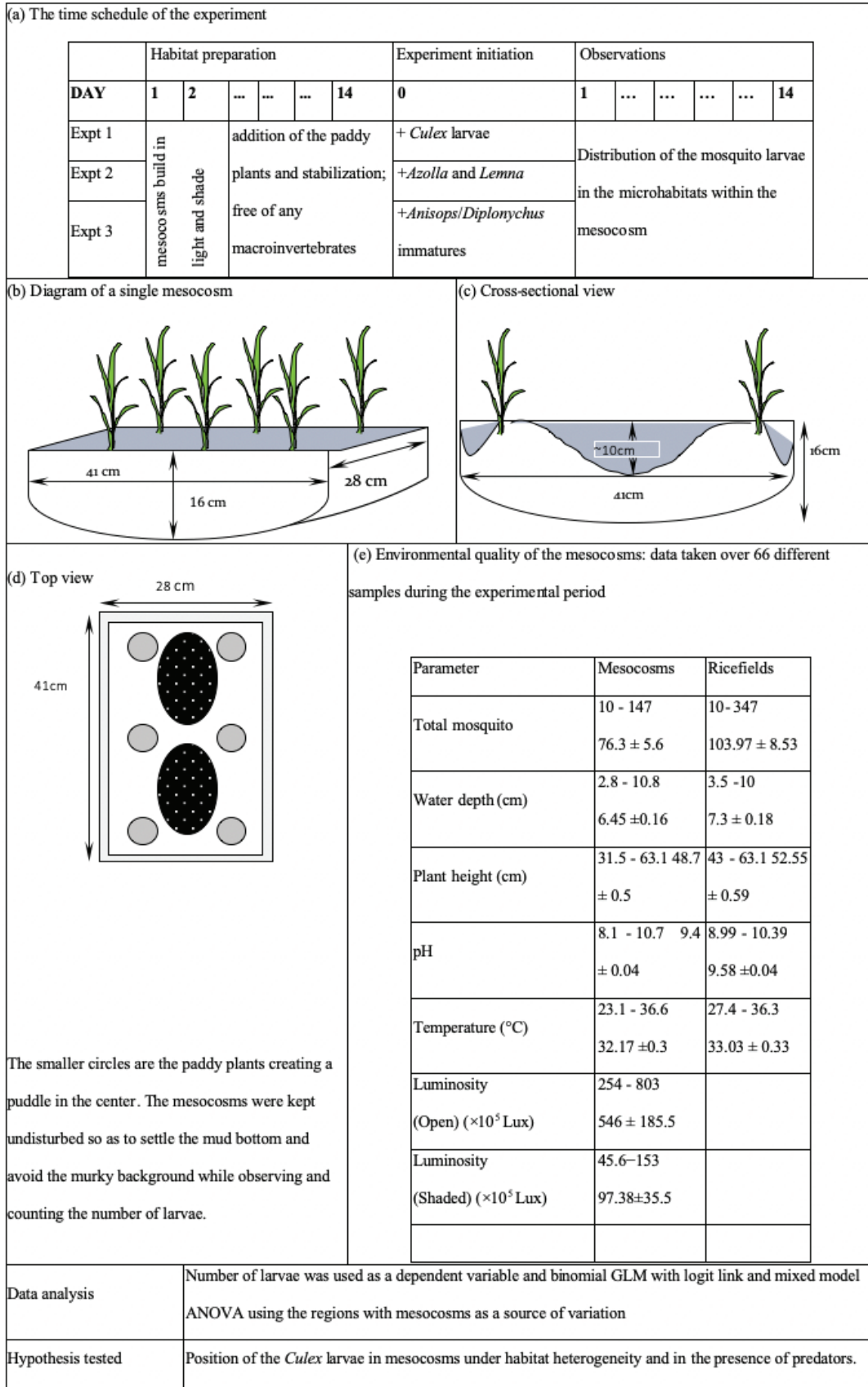
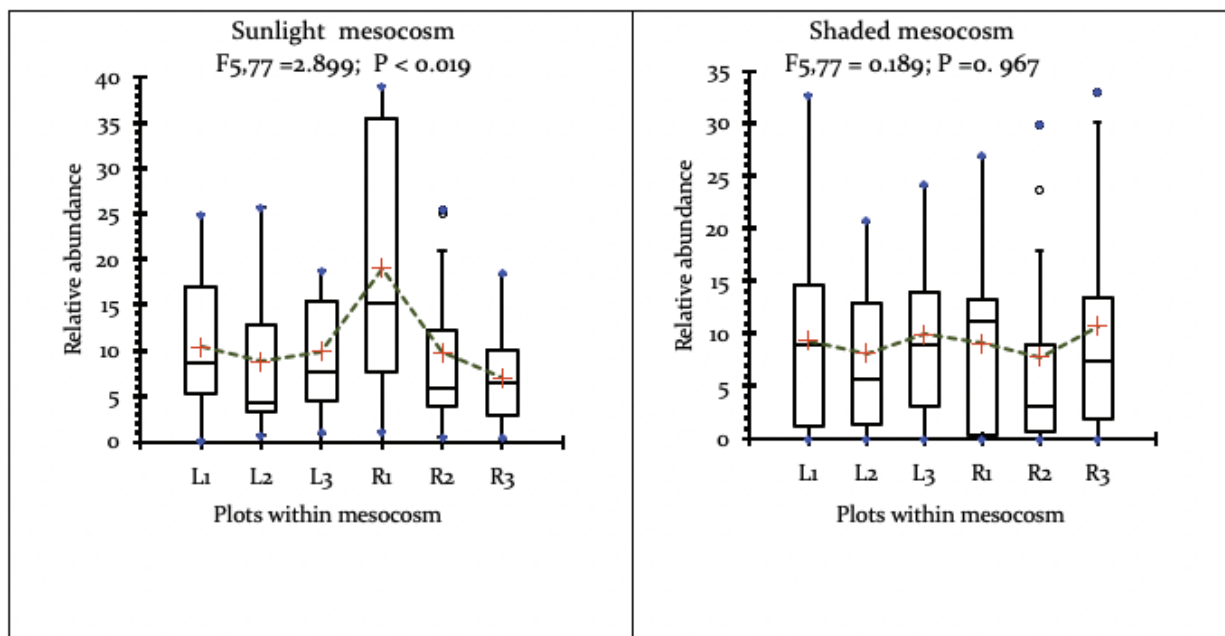


Figure 2. The overall differences in the relative abundance of *Culex* larvae in the mesocosms under sunlight and shaded areas along with the results of the analysis of variance (ANOVA), justifying the differences among the plots within the mesocosms. L: left side and R: right side of the mesocosm



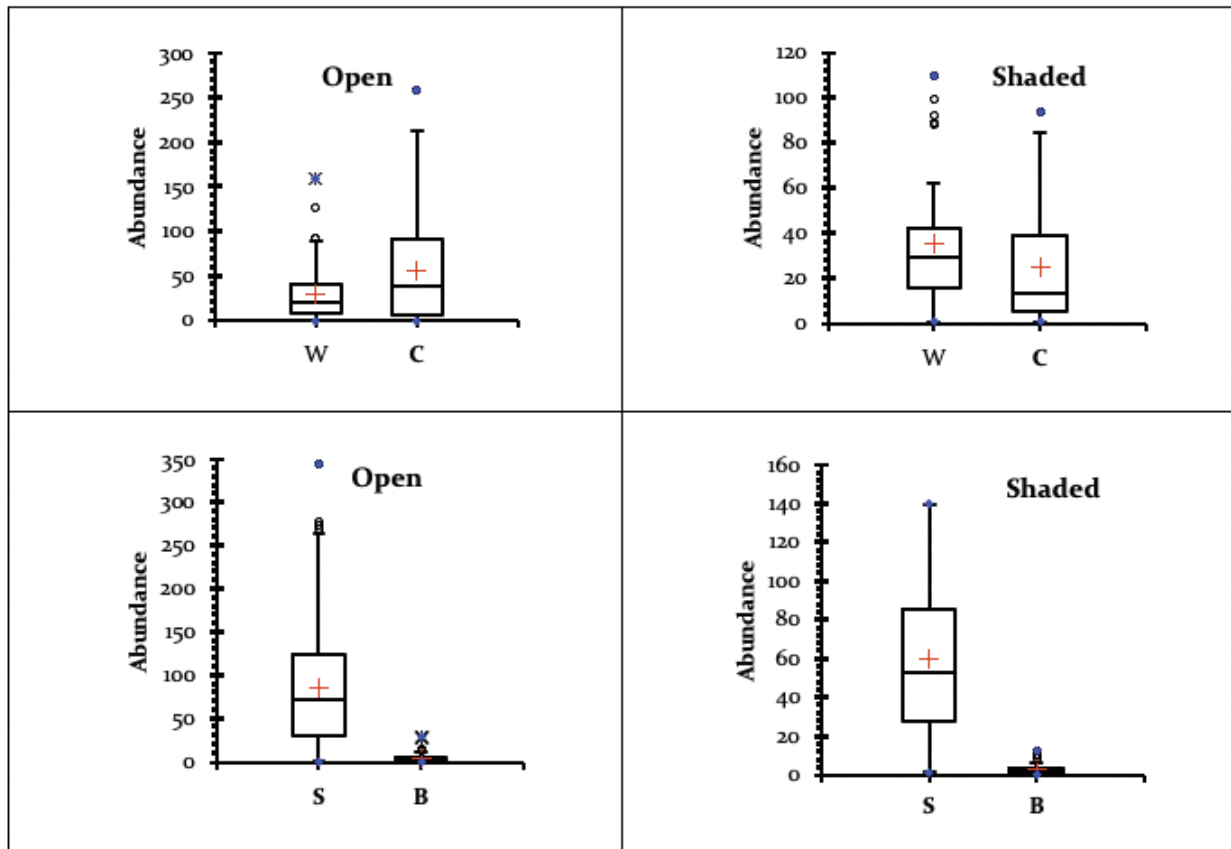
rameters being significant at $P < 0.001$ (intercept = -3.34 ± 0.08 ; Wald's $\chi^2 = 1570.268$; Predator = -2.5 ± 0.07 ; Wald's $\chi^2 = 1175.29$; sites = 0.948 ± 0.05 ; Wald's $\chi^2 = 359.85$). Thus, in the presence of vegetation and the predator in the microhabitats within the mesocosms, the distribution of the *Culex* larvae varied significantly. The correlation matrix (Pearson's product moment correlation coefficients (r)) between the water quality parameters including water depth and the paddy plant height with the larval abundance is shown in Table 3. The paddy plant height and the water depth showed different levels of correlation on the larval abundance.

3. DISCUSSION

The habitat elements like the paddy plants and the soil surface create heterogeneity of the habitat conditions in the rice fields. As a consequence, the activities of the species inhabiting the rice fields are affected, which includes foraging and movement. Species interactions are also influenced under such conditions, where the paddy plants pose a physical obstruction for movements in the available space. Effectively, the reduced space may increase the competition among the constituent species through the enhanced density of the organisms. Interaction between predators and prey are also influenced by the habitat heterogeneity, due to the limitation in the available space. While the paddy plants and floating detritus of varied origins are utilized by the macroinvertebrates like mosquito larvae, the predators face the difficulty in chasing the prey in refuge. Empirical estimates suggest that the habitat complexity and availability of light influence the efficacy of the insect

predators against mosquito immature (Saha *et al.*, 2008, 2009). The observations on the numbers of mosquito larvae in the unit spaces reflect the effects of the light and vegetation as factors influencing the spatial distribution. The predators of the mosquito larvae must face an obstacle in the rice field habitats due to the vegetation, and thus the mosquito larvae gain an advantage from habitat heterogeneity. As such, the presence of vegetation like the *Myriophyllum aquaticum* cover reduces the abundance through alteration of the larval microhabitats (Orr and Resh, 1992). Spaces in the habitats with vegetation are preferred for oviposition by *Anopheles minimus* (Overgaard, 2007), and the aggregation of the larva is high in spaces where the cover of riparian ferns is high (Overgaard *et al.*, 2002). In general, the distribution of the mosquito larvae in the rice fields are highly aggregated is evident from the observations on *Anopheles freeborni* and *Culex tarsalis* in California, USA (Pitcairn *et al.*, 1994). However, the feeding conditions are preferentially in the open water spaces than in the vegetated regions of the habitats as observed for the mosquitoes *Anopheles quadrimaculatus* (Walker and Merritt, 1993; Wallace and Merritt, 2004). In the present instance, the larvae of the *C. tritaeniorhynchus* exhibited an aggregated pattern of spatial distribution similar to the observations made on the anopheline mosquitoes that aggregate at the air–water interface near the plants and algal mats (Walker and Merritt, 1993; Wallace and Merritt, 2004). The shaded regions were more preferred by the *C. tritaeniorhynchus* larvae, complying with the same principle of aggregation exhibited by the larvae of *A. quadrimaculatus* possibly as a result of lower temperature than the open regions. The response to the light by the larvae of different species of *Culex*

Figure 3. Box plot representation of the distribution of the mosquito larvae at two different scales: wall–centre center (W–C) and surface–bottom (S–B) under open and shaded habitat conditions



varies considerably. In comparison to *Culex quinquefasciatus*, *C. tarsalis*, and *Culex stigmatosoma*, the response of *Culex erythrothorax* larvae shows greater sensitivity to the changes in the light conditions (Workman and Walton, 2003). Although in the present instance the light and shade conditions were available in different experimental containers, the spatial distribution of the larvae of *C. tritaeniorhynchus* complied with the findings on *C. erythrothorax* and other mosquitoes. The observations indicate that the open water vegetations like *Lemna* and *Azolla* provided refuge for the mosquito larvae, which is why more larvae were aggregated near the vegetated conditions than in open water conditions. This result is in parity with the distribution of *C. erythrothorax* in constructed wetlands, *A. quadrimaculatus* in ponds, and *Aedes triseriatus* under laboratory mesocosms. In all instances, the physical structures like detritus and leaves and vegetations were used as a refuge by the mosquito larvae (Walker and Merritt, 1991; Workman and Walton, 2003; Skiff and Yee, 2014).

Spatial distribution of mosquito larvae were observed on a continuous basis for fixed time periods in the laboratory (Walker and Merritt, 1991; Juliano and Reminger, 1992; Merritt et al., 1992; Juliano et al., 1993; Workman and Walton, 2003; Skiff and Yee, 2014), which enabled estimating the behavioural manifestations with higher precision. In these observations, the

role of the habitat structures has been little emphasized, which was a redundant feature in the present instance. The habitat complexity, attributable to the paddy plants and the bottom sediments, appeared as constrain to observe and record the activities of the individual *C. tritaeniorhynchus* larva in the present study. However, the mesocosms remained comparable to the rice field conditions and, therefore, enabled assessment of the larval distribution in the presence of the weeds and the predators with higher precision. In the laboratory experiments, deciphering the behavioral pattern and the distribution of the *Aedes* larvae, the role of the detritus remained similar to that of the vegetation in the mesocosms of the present study. Variations in the behavioral pattern and distribution are also observed in the presence of the predator like copepods (Awasthi et al., 2015). Distribution of the *C. tritaeniorhynchus* larvae varied with the presence of the predators in the mesocosms in agreement with the observations on the distribution of the *Aedes aegypti* in the presence of the predator *Mesocyclops* (Awasthi et al., 2015). The findings of the present instance indicate that the movement and distribution of the mosquito larvae is reduced in the presence of predators. The orientation was more toward the walls instead of the center of the available microhabitats, thereby evading the attack of the insect predator *Anisops* sp. Empirical studies have shown that light availability

Figure 4. (a) Box plot representation of the relative abundance of the *Culex* larvae in the microhabitats with and without vegetations (AH – Azolla high; LH – Lemna high; OS – open surface; without vegetation) in the mesocosms. The results of the Kruskal–Wallis analysis of variance (ANOVA) with Steel–Dwass–Critchlow–Fligner multiple comparisons are being shown in the figure. Box plots with similar letters represent significant difference at $P < 0.01$ level. (b) Box plot representation of the relative abundance of the *Culex* larvae in the microhabitats with and without predator (*Anisops* sp.) in the mesocosms. The results of the Kruskal–Wallis ANOVA are being shown in the figure. The circles represent the extreme values and the blue dots the outliers, while the median value are shown as a black line in the box and the cross signs the mean values

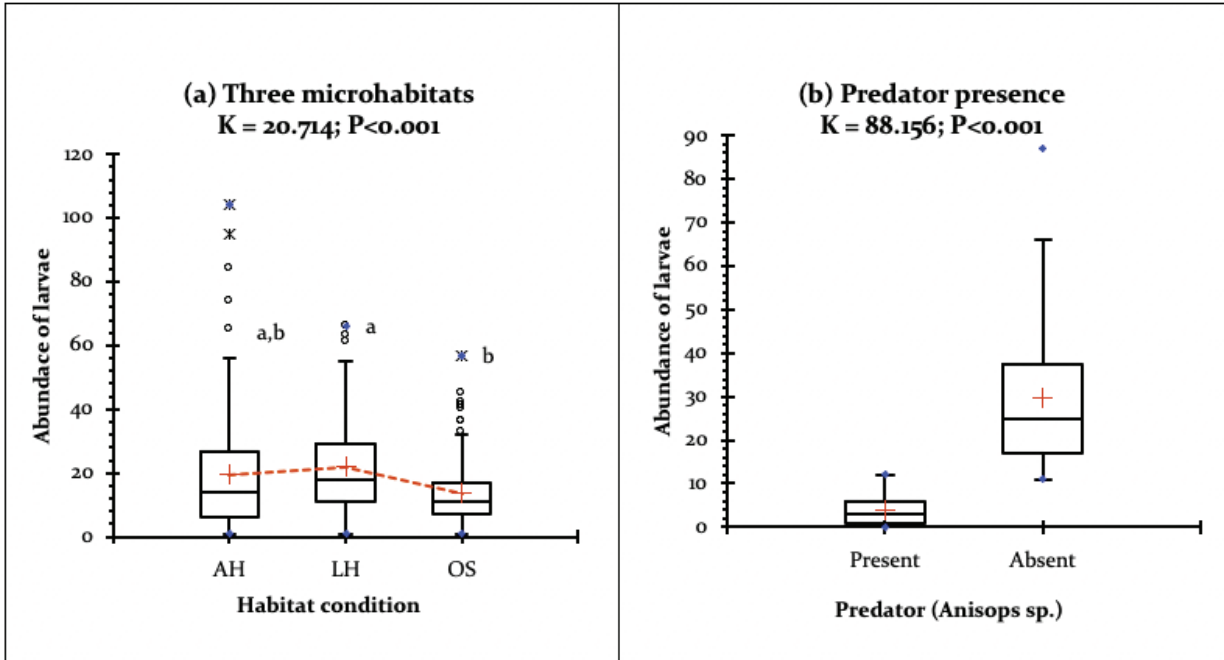


Table 2. The results of the mixed model analysis of variance to explain the differences in the distribution of the *Culex* mosquito larvae in two different conditions: open or shaded under artificially created rice field habitats

(a) Wall or center under open or shaded conditions (site)

| Effects | Num df | Den df | F |
|-------------------|---------|--------|---------------|
| Site | 1 | 250 | 4.998 |
| Wall–center (WC) | 1 | 250 | 8.099 |
| Model parameters: | | | |
| Source | Value | SE | t |
| Intercept | 33.265 | 10.985 | 3.028 |
| Site | -12.545 | 5.630 | -2.228 |
| W/C | 14.690 | 5.162 | 2.846 |

(b) Surface or bottom under open or shaded conditions (site)

| Effects | Num df | Den df | F |
|---------------------|---------|----------------|----------------|
| Site | 1 | 235 | 2.809 |
| Surface–bottom (SB) | 1 | 235 | 146.294 |
| Model parameters: | | | |
| Source | Value | Standard error | t |
| Intercept | 168.568 | 13.086 | 12.881 |
| Site | -13.082 | 6.769 | -1.933 |
| Surface–bottom (SB) | -73.995 | 6.118 | -12.095 |

Table 3. Correlation matrix for the environmental variables and the mosquito productivity in the experimental mesocosms maintained throughout the experimental period (n=138 readings spanning over a 3-weeks period; WD – water depth; PH – paddy plant height; TDS – total dissolved solids)

| Variables | Immature abundance | WD (cm) | pH (cm) | Conductivity (μ S) | TDS (ppm) | Salinity (ppm) |
|-------------------------|--------------------|---------------|--------------|-------------------------|--------------|----------------|
| WD (cm) | 0.611 | | | | | |
| pH (cm) | 0.423 | 0.385 | | | | |
| Conductivity (μ S) | -0.281 | -0.565 | 0.116 | | | |
| TDS (ppm) | -0.277 | -0.573 | 0.109 | 0.994 | | |
| Salinity (ppm) | -0.187 | -0.425 | 0.085 | 0.648 | 0.651 | |
| Temp ($^{\circ}$ C) | 0.183 | 0.218 | 0.469 | 0.171 | 0.169 | 0.043 |

(Saha et al., 2008) and the habitat complexity (Saha et al., 2009) impose a barrier to predation of mosquito larva by the aquatic insect predators. Perhaps, the distribution of the mosquito larvae enables evasion of the predatory attack and therefore the reasons for the predators being less efficient. In addition, the wet and dry conditions of the paddy rice cultivation provide a fair chance of variations in the load of the predators (Watanabe et al., 2013), thereby resulting in the reduced vulnerability of the mosquito larva. Although the predators and prey may coexist, the vulnerability of the prey is reduced, and therefore the effective regulation of the prey mosquito is reduced. Perhaps, the use of alternative forms of biological regulation will be more effective for reducing the population of *C. tritaeniorhynchus* and similar mosquitoes in rice field conditions.

Information on the larval dispersion and distribution in the larval habitats is required for successful reduction in the mosquito population using the predators or microbes. The effective regulation of the mosquito larvae will require the successful interaction between the predator and the concerned mosquito larvae as prey, which is greatly influenced by the habitat heterogeneity of the space. As observed in other mosquitoes (Workman and Walton, 2003; Wallace and Merritt, 2004) and in *C. tritaeniorhynchus* of the present instance, the distribution of the mosquito larvae in the space concerned is highly influenced by the habitat complexity arising out of the physical structures and the paddy plants. Apparently, the mosquito larvae utilize the vegetation as a refuge and thereby the resultant distribution in the space is skewed. The presence of the predators in the habitat also induced similar changes in the spatial distribution of the mosquito larvae. Mosquito larvae avoid the spaces where the predators are present and orient in the spaces where the predators are absent. The distribution of the mosquito larvae in the space appears to comply with the evasion tactics against the predators using the floating vegetation as a refuge. Thus, from the present observation, it may be assumed that the predators and the mosquito prey may coexist in the rice fields with little reduction in the effective population size. Also, the use of microbes or botanicals specifically targeting the mosquitoes may be helpful in this instance. From the observations on the distribution of the *C. tritaeniorhynchus* larvae in the rice fields, it may be assumed that the microbial application will be more appropriate, though the contact with

the granular forms of biopesticides may vary since mosquitoes orient more on the surface while the granules tend to reach the sediment. Nonetheless, the results support the view that the habitat complexity of the rice fields provides ample microhabitat conditions, for refuge of the mosquito larvae and evasion from the predators, while orienting in favorable conditions. Further studies may provide insights about the feeding of the larvae on the surfaces required to substantiate this proposition using rice field habitats as mosquito breeding sites.

4. CONCLUSIONS

In mosquito breeding habitats, the spatial distribution of the larvae determines the interaction with the predators and thus effective regulation. The availability of the floating vegetations in the habitats may further provide refuge to the larvae to evade predation or effective contact with the botanicals or biopesticides. As observed in the present study, the mosquito larvae, represented by *C. tritaeniorhynchus*, exhibited greater abundance in the center than in wall in areas where the light are available, but reverse under shaded condition. Irrespective of the light or shade condition, the larvae were abundant on the surface than on the bottom of the paddy plant mesocosms. The abundance of the mosquito larvae was higher in floating vegetation than in open areas. In the absence of predators, the mosquito larvae exhibited higher abundance. The spatial orientation of the *C. tritaeniorhynchus* larvae in the rice field habitat condition appeared to be similar to *Aedes* and *Anopheles* mosquitoes in the respective breeding habitats. Such orientation of the mosquito larvae in the rice field provides an advantage for reducing vulnerability to the predators and avoids the direct sunlight, ultimately enhancing the survival probability.

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