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Observations on the pupal productivity of *Culex tritaeniorhynchus* in rice fields of West Bengal, India: implications for vector management (Diptera: Culicidae)

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Abstract
The mosquito *Culex tritaeniorhychus* is an established vector of Japanese encephalitis in India and many Asian countries. Entomological monitoring enables estimation of the abundance of the vector mosquitoes and therefore aids in vector management and disease control. In compliance with this proposition, an assessment of the pupal productivity of the mosquito *C. tritaeniorhynchus* was made from selected rice fields of West Bengal, India. The results are expected to provide the present status of mosquitoes in an endemic region of Japanese encephalitis. In course of sampling of the rice fields, the mosquito *C. tritaeniorhyhchus* was found in abundance with other mosquitoes like *Culex bitaeniorhynchus, Anopheles subpictus, Anopheles vagus, Anopheles barbirostris*. Application of multivariate analysis indicated that the plant height, water depth and temperature could explain the variations in the pupal productivity of *C. tritaeniorhynchus* in rice fields. The correspondence of the immature abundance of *C. tritaeniorhynchus* with plant height suggests persistence of the population throughout the paddy rice cultivation period. Using the plant height as an indicator, the abundance of the mosquito *C. tritarniorhynchus* can be predicted enabling application of appropriate strategies for population intervention and thus the possibility of the disease.

Key words: *Culex tritaeniorhynchus,* rice fields, mosquito vector, Japanese Encephalitis

Introduction

Entomological surveillance is a pre-requisite for estimating the variations in the mosquito immatures in the rice fields and allied habitats (Chase and Knight 2003; Derraik 2004; Keiser et al. 2005; Dale & Knight 2008; Hassan et al. 2010; Li et al. 2011; Ohba et al. 2013, 2015; Amarasinghe and Weerakkodi 2014; Amarasinghe & Dalpadado 2014; Lytra & Emmanouel 2014). Random sampling of the selected sites in the rice fields are carried out to infer about the relative abundance of different mosquito species in space and time (Aditya et al. 2006; Ohba et al. 2013, 2015; Lytra & Emmanouel 2014). Rice fields are heterogeneous habitats with changing community composition with time coinciding with the cultivation period and the harvest periods (Kant et al. 1992; Sunish et al. 2006; Yasuoka & Levins 2007; Muturi et al. 2008; Watanabe et al. 2013; Ohba et al. 2013, 2015; Lytra & Emmanouel 2014). During the wet conditions, the rice fields and allied habitats provide congenial conditions for the breeding of different species of vector and pest mosquito species (Aditya et al. 2006; Sunish et al. 2006; Yasuoka & Levins 2007). Systematic study employing different sampling techniques enables estimation of the abundance of different species of the mosquito during the wet phase (Aditya et al. 2006; Pramanik et al. 2006, 2007). Empirical studies on the rice field mosquitoes from different parts of the world highlights the significance of entomological monitoring of the rice fields for facilitating vector management.

The resurgence of Japanese encephalitis in Indian subcontinent (Kanojia 2007; Kanojia & Geevarghese 2004) calls for monitoring of the rice field habitats for evaluation of the population abundance of the vector mosquitoes *C. tritaeniorhynchus* and *C. vishnui* species. Earlier studies reveal the importance of the mosquito *C. tritaeniorhynchus* as a vector of Japanese encephalitis in India and adjoining regions (Hati 1976; Banerjee & Chandra 2004). Estimation of abundance of the vector mosquitoes facilitates population regulation as well as enables the prediction of the possibilities of the disease with higher precision. Thus entomological surveillance is considered as an essential part of the vector management to monitor the abundance of the target mosquito group, and adopt appropriate intervention strategies. The results of the present study will be useful in predicting the state of the vector mosquito, *C. tritaeniorhynchus* and assess the possibility of Japanese encephalitis in the concerned geographical area.

Materials and Methods

Study area

The sampling of the mosquito immature were carried out in the rice fields and allied habitats of the University Farm House, Tarabag (23°15'7" N, 87°50'35" E), The University of Burdwan, Burdwan, India during the paddy rice cultivation periods in the rainy and the winter seasons between 2010, 2012 and 2013. Selected sites of the rice fields were considered as sampling sites during the wet conditions of the paddy rice cultivation period.

Sampling method

Using an insect net (200µm mesh size) fitted to a rectangular frame (20cm X15 cm) and attached with a handle was used for collection of the mosquito immature (larva and pupa) (Robert et al. 2002; W.H.O. 2003). The net was moved through the column of the water in between the paddy plants for trapping the mosquito larvae. The study site constituted discrete paddy cultivation plots spanning a radius of 20 km with the University Farm House, Tarabag, Burdwan, India, at the centre. In a particular paddy rice cultivation plot, the collections were made from three sites and the total collection constituted a single unit. For a particular sampling day, at least three rice plots were selected spanning around the entire paddy rice cultivation area in the Farm House. During each of the cultivation season, the sampling was carried out only during the wet conditions, when the water depth remained between 10cm and 25 cm. The collected mosquito immature in the net were emptied in a plastic packet filled with sufficient water collected from the rice field and brought to the laboratory. In the laboratory, the contents of the plastic bag were emptied in an enamel tray (46cm X 32cm X 6cm) for segregation and identification of the immature up to the genus level (Rattanarithikul et al. 2005). Following segregation, each of the pupa was placed in individual glass vial (25mm X 10 mm) for emergence as adult for identification up to species level. The rest of the larvae were counted and subsequently reared to the adult stage for identification up to the species level (Sirivanakaran 1970, 1975; Reuben et al. 1994). From each spot of the paddy rice cultivation plots, the water depth and the paddy plant height were recorded along with the water pH and temperature as descriptor of the environmental conditions of the sampling space. The record of the total number of the immature and the species specific relative abundance were made for each sampling effort from the rice fields.

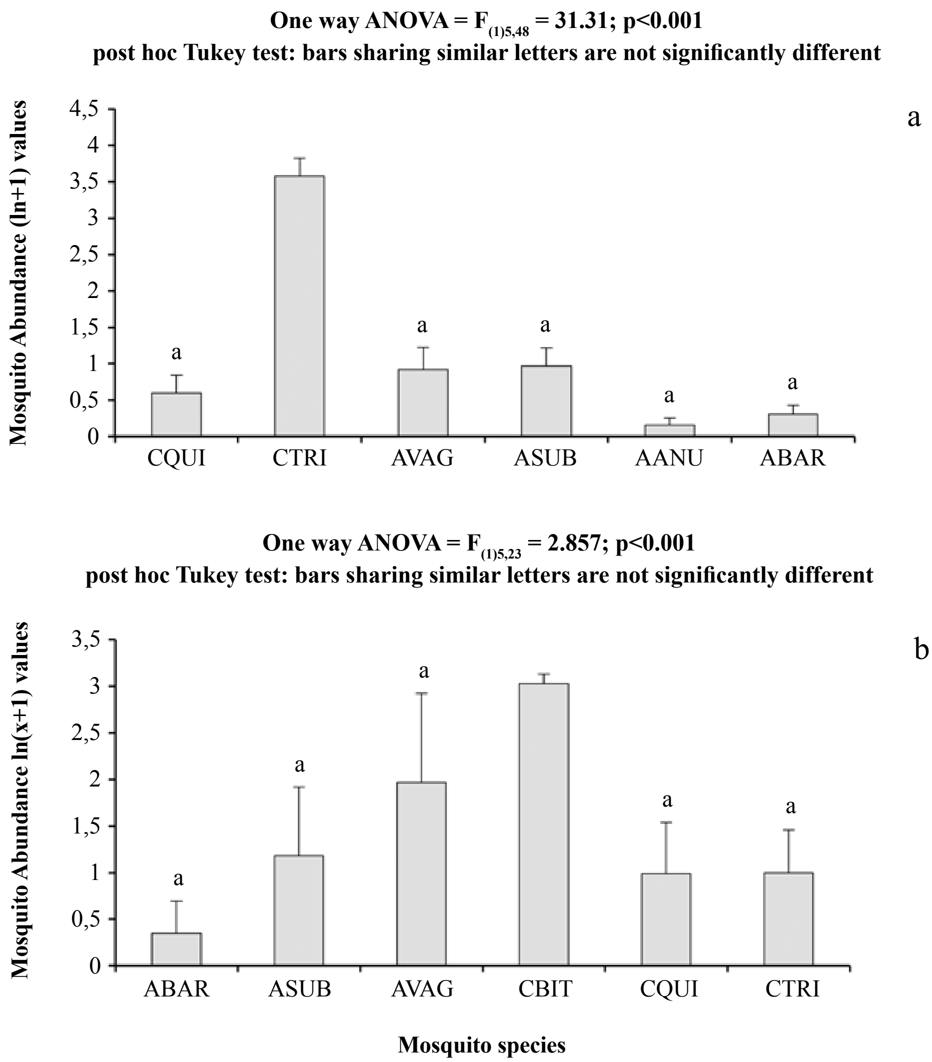
Species specific difference in the productivity (relative abundance) was assessed using the one way ANOVA (Zar 1999). A GLM was also applied following bionomial regression with logit link to define the differences in the productivity in the winter and rainy period of paddy rice cultivation in the rice fields. The paddy plant height and the water depth were used as explanatory variables against the immature abundance to judge the efficacy of these habitat parameters to serve as the indicators for monitoring the mosquito immature abundance in the rice fields. A Principal component analysis was applied to portray the relation among the different environmental variables and the larval abundance in the rice fields.

Results

Mosquito species found in rice field

A total of 4512 mosquitoes were obtained from the field collections during the years 2010 and 2013, separately. In the rice fields, 3 different species of *Culex* namely, *Culex tritaeniorhynchus* Giles, 1901, *Culex quinquefasciatus* Say, 1823, and *Culex whitmorei* Giles, 1904 and four species of *Anopheles* mosquitoes, namely, *Anopheles vagus* Doenitz 1902, *Anopheles subpictus* Grassi, 1899, *Anopheles annularis* Van der Wulp, 1884, *Anopheles barbirostris* Van der Wulp, 1884 and one species of *Lutzia, Lutzia fuscana* (Wiedemann, 1820) were observed in different numbers in each of the samples and between the rice cultivation periods. Among the total collection 2229 *Culex* larvae and 1421 *Anopheles* larvae were recorded. In rainy season 1316 *Culex l*arvae and 1201 *Anopheles* larvae were collected, whereas in dry season the number of *Anopheles* larvae was much lesser than rainy season. A total 1403 *Culex* and 376 *Anopheles* larvae were collected in dry season. Among them only 826 mosquitoes were identified up to species level. Rest were identified up to genus level and the data were recorded for statistical analysis. We observed that *Culex tritaeniorhynchus* (384 individuals) was the most abundant species followed by *Anopheles vagus* (23 individuals) and *Anopheles subpictus* (22 individuals) during the boro cultivation period (winter season). A very small number of *Culex quinquefasciatus* (12 individuals), *Anopheles annularis* (02) and *Anopheles barbirostris* (03) were found during the winter season. But during *aman* cultivation (rainy season) *Culex tritaeniorhynchus* (80 individuals) showed highest abundance followed by *Anopheles vagus* (76 individuals) and *Anopheles subpictus* (29 individuals). *Anopheles barbirostris*, *Culex bitaeniorhynchus* and *Culex quinquefasciatus* were very low in number (5, 11 and 14 respectively). The mean values of the species represented in each sample are shown in Figs 1 a-b, for winter and rainy seasons respectively.

Using GLM with logit link (logistic regression) on the data of abundance of the mosquito species observed in the samples and their relative contribution was evaluated. For winter season, the equation was - Abundance = 1/(1+exp(-(-0.29 – 0.653*species))); species = -0.653 ± 0.04; Wald's Chi square = 260.876; P < 0.0001; While for the rainy season, the equation was - Abundance = 1/(1+exp(-(-0.56 – 0.23* species))); species =-0.233 ± 0.04; Wald's Chi square = 27.65; P<0.00001. The results indicate that the relative abundance of the species varied significantly during both the paddy rice cultivation periods.



Figs 1 a-b – The mosquito species assemblages observed in the winter (**a**) and rainy (**b**) seasons in the rice fields. (**CQUI** *Culex quinquefasciatus*, **CBIT** *Culex bitaeniorhynchus*, **CTRI** *Culex tritaeniorhynchus*, **AVAG** *Anopheles vagus*, **ASUB** *Anopheles subpictus*, **AANU** *Anopheles annularis*, **ABAR** *Anopheles barbirostris*). The results of the one way ANOVA are shown in the graph.

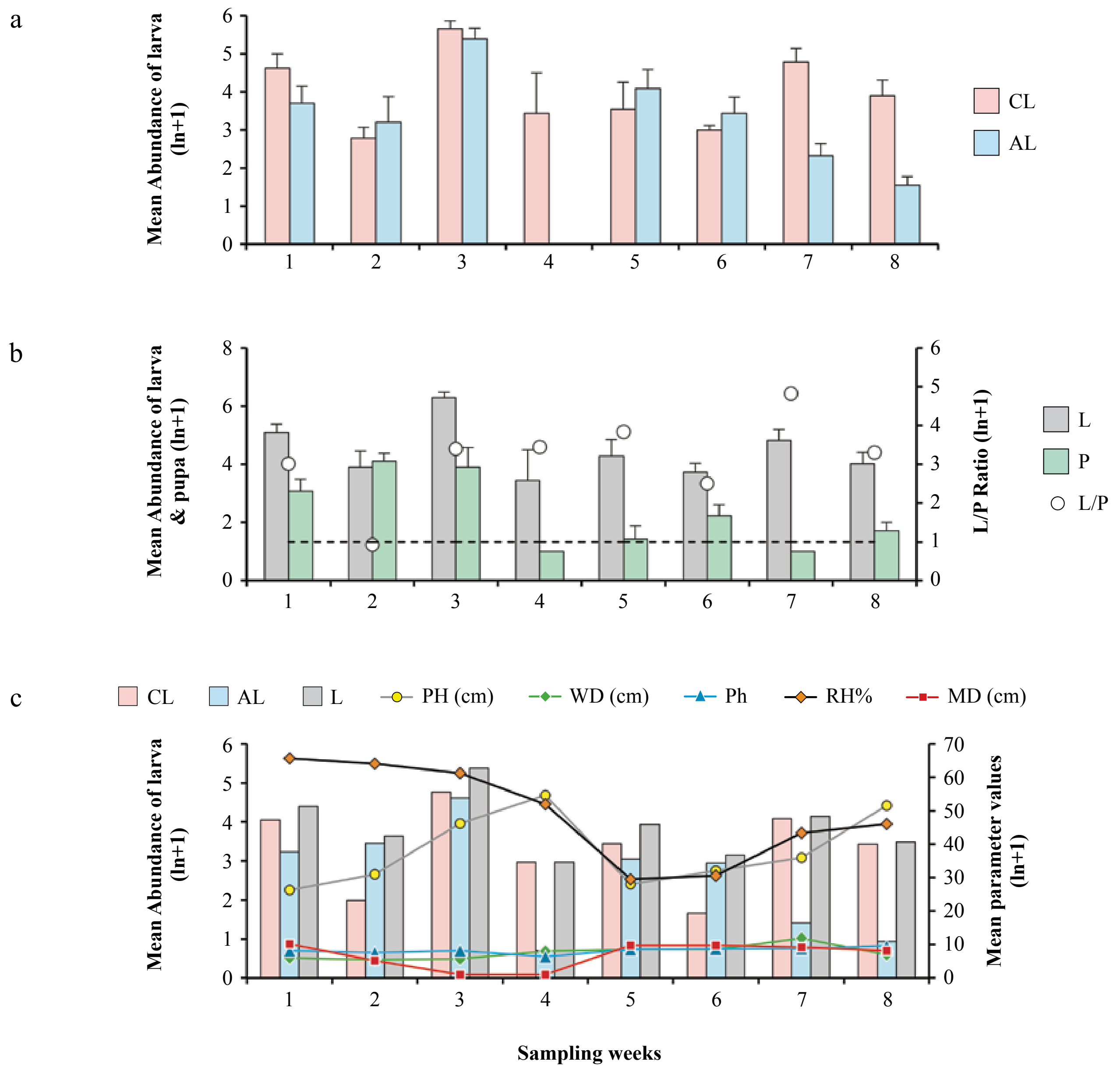
The application of the one way ANOVA on the mosquito abundance in the different time periods indicated significant differences in case of the winter season ($F_{(1)5,48}$ = 31.31; p<0.001) but no differences were observed for the rainy season ($F_{(1)5,23}$ = 2.857; p<0.001). Although the species remained dominant for both the seasons, the relative abundance varied with the time and the period of sampling from the rice fields.

Ratio of C. tritaeniorhynchus with other mosquitoes

The ratio of *C. tritaeniorhynchus* larvae with the other mosquito larvae of dry season was significantly higher than the rainy season (Figs 1 a-b). The ratio of *C. tritaeniorhynchus* larvae with the total larvae found in rice field were 0.86 and 0.0258 in winter and rainy season respectively. Whereas the ratio of *C. tritaeniorhynchus* larvae with the total *Culex* larvae were 0.968 and 0.105 in winter and rainy season respectively. The relative abundance of the mosquitoes were compared between the rainy and the winter seasons of paddy rice cultivation, where it was observed that the winter season hosted significantly higher number of mosquitoes than in rainy season (Figs 1 a-b). The variations in the mosquitoes were also observed to be critical with reference to the species composition with complete absence of certain mosquitoes in one or the other season.

Larva pupa ratio of rice fields

The instantaneous larva to pupa ratio in the collected samples ranged between 3.31- 203.71. A positive correlation was observed between the mosquito immature with the paddy plant height and the water depth in the samples. The larva to pupa ratio provided an impression about the instantaneous productivity of the pupa and the dynamics of the larval development and prospective adult production (Figs 2 a-c). The ratio of the larva and pupa remained dif-



Figs 2 a-c – The abundance of the culicine and anopheline mosquitoes in the consecutive weeks of sampling during the paddy rice cultivation period, along with the ratio of the larva/pupa in the corresponding period. (**a**) *Culex* and *Anopheles* larva in different collection weeks; (**b**) Total larva/pupa ratio; (**c**) Environmental variables and mosquito larvae.

ferent within the same paddy rice cultivation period. Using the data of the winter paddy rice cultivation period of eight consecutive weeks revealed that the ratio of larva to pupa differed significantly suggesting the differences in the propensity of the development process and thus the emergence of the adult mosquitoes (Figs 2 a-c).

Environmental variables and the mosquito abundance in the rice field

The data on the environmental variables and the mosquito larval abundance over the period of the collections was subjected to PCA (Figs 3 a-c). As indicated by the Bartlett's sphericity test the application of the PCA was justified by the significant value of almost infinity. The results indicate that the first two extracted factors could explain more than 53.09% variations of the data on the mosquito abundance and the environmental period for the total sampling period. The first three factors represented the eigen values of more than 1 with representation of more than one variable for the factors (Figs 3 a-c). The biplot indicates the relative differences in the contribution of the variables towards the variations observed in the individual samples over the period of collection. The correlation matrix showed that few variables were highly significant while rest were low or poor though the colinearity of the data was maintained all through.

a

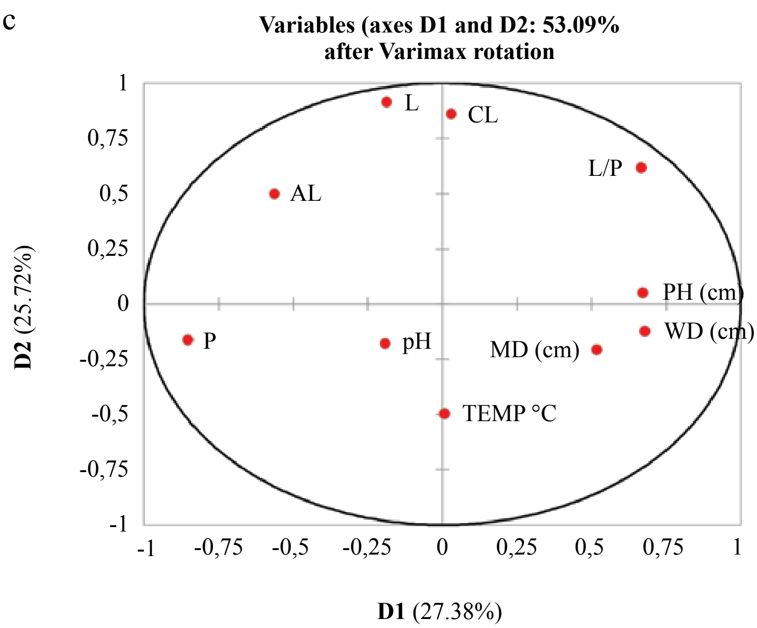
	F1	**F2**	**F3**	**F4**
Eigen value	2.738	2.572	1.331	1.146
Variability (%)	27.376	25.719	13.307	11.465
Cumulative (%)	27.376	53.095	66.402	77.867

b

	F1	**F2**
CL	0.029	**0.861**
AL	**-0.564**	0.499
L	-0.187	**0.914**
P	**-0.852**	-0.163
L/P	**0.666**	0.618
PH (cm)	**0.671**	0.051
WD (cm)	**0.678**	-0.123
MD (cm)	**0.517**	-0.205
pH	**-0.192**	-0.178
TEMP°C	0.006	**-0.497**

Variables	**CL**	**AL**	**P**	**PH**	**WD**	**MD**	**pH**
AL	**0.342**						
P	0.126	**0.328**					
PH	0.002	-0.129	**-0.300**				
WD	-0.170	**-0.280**	-0.224	**0.302**			
MD	-0.244	**-0.392**	-0.247	0.178	**0.413**		
pH	-0.069	-0.091	-0.052	**-0.450**	-0.093	0.064	
TEMP°C	-0.222	-0.085	-0.063	-0.098	0.209	**0.398**	0.222

c



Figs 3 a-c – The results of the PCA representing the redundancy of the data and the variations explained by the extracted factors. The correlation between the plant height and the pupal productivity remained significant at P<0.05 level. **CL** *Culex* larva, **AL** *Anopheles* larva, **L** Larva, **P** pupa, **L/P** Larva/pupa ratio, **PH** Paddy plant height, **WD** water depth, **MD** mud depth, **Temp** Temperature.

Discussion

The results of the study indicate that the rice fields of the study area hosted considerable number of mosquito species with the abundance of the *C. tritaeniorhynchus* being comparatively higher during the winter rice cultivation than in the rainy season. Empirical observations on the rice field mosquitoes in India and countries where paddy rice cultivation is a normal practice, the abundance of Anopheline and Culicine mosquitoes are guided by the environmental factors and the habitat permanence of the cultivable lands (Kant et al. 1992, 1998; Sunish & Reuben 2002; Banerjee & Chandra 2004; Keiser & al. 2005; Sunish et al. 2006; Ohba et al. 2013, 2015; Lytra & Emmanouel 2014). The time period of water logged conditions in paddy rice cultivation vary with the cultivation method employed as well as with the seasons. In flooded conditions of the rice fields the colonization pattern of the mosquitoes are crucial determinant for the future abundance and dominance. Earlier studies have noted that the colonization of different species of mosquitoes occurs in sequence and the dominance pattern changes as a function of time (Chandlar & Highton 1975; Bang & Pant 1984; Begum et al. 1986). Spatial occupancy of the rice field habitats vary with the event of colonization by different macro invertebrates, fish and tadpoles with increasing complexity of interactions (Kant et al. 1998; Watanabe et al. 2013; Zuharah & Lester 2010). The colonization, establishment and abundance of the species in the rice fields are interrupted by the dry and wet cycle of the fields. As a result, the species composition and the stability of the rice field habitat changes which may be reflected through the relative abundance of different species occupying the habitats (Wada et al. 1967; Chandlar & Highton 1975; Mogi & Miyagi 1990; Mogi et al. 1995; Watanabe 2013). Thus hydro period and habitat permanence of the rice fields are the two crucial factors that guide the diversity of the species available in the concerned spaces. As observed in the present study, prominent relationship among the variables are lacking except for the plant height and the water depth with the mosquito immature abundance. Although the water levels change in a cyclic manner in course of the paddy rice cultivation, paddy plant height exhibits a sequential increase with time and thus becomes a key parameter to corroborate with the changes in the species composition inclusive of different mosquito species (Banerjee & Chandra 2004). The results of the repeated fluctuations of the water depth and dry conditions may induce differential level of development of the mosquito immature leading to the differences in the relative abundance of the mosquito within a short time scale. A possible reason, apart from the sampling error, might be that the mosquito abundance remained fluctuating randomly without a specific pattern of change in abundance.

As a result of random fluctuation, the mosquito immature abundance exhibited non-linear relationship against the different environmental parameters considered in the study with low levels of correlations. While the strength of correlation could have increased with the sample size, but the fluctuations in relative abundance could not be directly related to the environmental factors. Other ecological parameters (e.g. physico-chemical, biological such as predators or microbes) could play a role in abundance and species composition prediction. Water availability in the rice field remains a single crucial factor that changes the habitat conditions from the dry terrestrial to amphibious and wet aquatic ecosystems. Mosquito life cycle has to be synchronized with the changing habitat quality to dominate the community assemblages. As revealed through the relative density of the mosquito immature in the different weeks the stability in the relative abundance is lacking. This has been observed in rice fields in Japan and Kenya though the practice of paddy cultivation differs tremendously in these areas (Wada et al. 1967; Chandlar & Highton 1975; Muturi et al. 2008; Ohba et al. 2013, 2015).

During the paddy rice cultivation period, the species composition varies with the alternating dry and wet conditions. Under such scenario, the associated trap ponds in the landscapes play a crucial role acting as a source for the propagules. In dry conditions, dispersal of the predator and prey species between the rice fields and associated trap ponds and canals has been observed in many instances (Watanabe et al. 2013; Ohba 2013, 2015). The habitat heterogeneity and the prey and predator refuge provided by the trap ponds and canals are considerably higher since no de-weeding activity is undertaken alike the rice fields proper. The movements of the species between the rice fields and the associated water bodies can occur either way depending on the dispersal ability of the individual species. In such respect, the movements are more pronounced for the species capable of active flight and swimming, which for the mosquito immature is constrained. In absence of any connection between the trap ponds and the rice field proper, the movements of the mosquitoes may remain restricted to the occupied spaces. It is relevant to mention here that mosquitoes in rice field habitats are encountered in puddles and micro ditches where even with the restricted water, the mosquitoes are able to thrive. However, with the drying of the puddles, the chances of emergence as adult mosquitoes may dwindle. Water retention period in the rice fields is therefore crucial for the abundance of the mosquito species, which is why the discrete but random sampling of the rice fields in the present instance did not yield a linear correspondence with the environmental factors.

The mosquitoes observed in the present instance exhibit a similarity with the findings of earlier studies in India (Kant et al. 1998; Sunish & Reuben 2002; Sunish et al. 2006) or elsewhere (Muturi et al. 2008; Stoops et al. 2008; Lytra & Emmanouel 2014; Ohba et al. 2013, 2015) though the species richness remained low. This may possibly due to the sampling error or due to the restricted space included in the sampling strategy. This study was carried out using Burdwan district as the geographical area, and may represent a specific region where these species were available. While in the studies Muturi et al. 2008; Stoops et al. 2008; Lytra & Emmanouel 2014; Ohba et al. 2013, 2015) being compared with the present one included a larger sampling area and time, the present study was accomplished within a short spatial range. However, when compared with the findings with the similar spatial range of habitat, the mosquito species richness remained comparable (Pramanik et al. 2006). Thus the species observed in the rural areas of West Bengal, India, remained comparable with the present finding, with *C. tritaeniorhynchus* being the dominant species in many of the samples of winter paddy rice cultivation period. In essence the rice fields of the southern districts of West Bengal hosts the vector of Japanese encephalitis consistently posing a risk for the event of an epidemic (Hati 1976; Banerjee et al. 1979; Banerjee & Chandra 2004) in the concerned geographical area.

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