



Productivity of *Culex tritaeniorhynchus* in Rice Fields of West Bengal, India: Correlates of Immature and Adult Features

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Abstract

Background: Surveillance of larval habitats is a part of entomological monitoring to determine the abundance and facilitate vector mosquito management.

Objective: Survey of the rice field habitats was carried out to characterize the productivity of *Culex tritaeniorhynchus* Giles, 1901 (Diptera: Culicidae), in West Bengal, India. Apart from the numerical abundance, the life history traits of the mosquito were assessed to enhance the information on the fitness of the individuals.

Methods: Mosquito immature were collected following repeated sampling of rice fields during the paddy rice cultivation. The pupal weight (PW), adult body weight (AW) and wing length (WL) of *Cx. tritaeniorhynchus* and co-occurring mosquitoes were subjected to multivariate statistical analysis and ANOVA. Correlates of environmental factors, abundance and life history traits were validated as predictors of *Cx. tritaeniorhynchus* productivity.

Results: Four different mosquitoes namely *Culex quinquefasciatus*, *Anopheles annularis*, *An. subpictus* and *An. vagus* were encountered in varied number along with *Cx. tritaeniorhynchus*, which could be discriminated significantly using the PW, AW and WL as variables. Water depth ($r = +0.349$) and plant height ($r = -0.423$) appeared to be a significant ($P < 0.05$) predictor of the productivity of the mosquito *Cx. tritaeniorhynchus*. Variations in the correlated traits PW, AW and WL of *Cx. tritaeniorhynchus* corresponded to the time period of paddy rice cultivation substantiated through the ANOVA.

Conclusion: The pupal productivity as well as the correlated traits PW, AW and WL of *Cx. tritaeniorhynchus* varied with the time period of paddy rice cultivations, paddy plant height and water depth. The correlated variations with productivity calls for inclusion of the life history traits in entomological monitoring of *Cx. tritaeniorhynchus* to appraise the fitness and the population abundance with higher precision.

Keywords

Culex tritaeniorhynchus; Japanese encephalitis; Pupal weight; Body weight; Wing length; Rice fields

Introduction

Rice fields are breeding ground of numerous species of mosquitoes that are associated with disease transmission affecting health of human and domestic animals [1,2]. The vector of Japanese encephalitis, *Culex tritaeniorhynchus* Giles, 1901 (Diptera: Culicidae) prefers to breed in rice field habitats, evident from the studies around the world [3,4]. Entomological monitoring of the mosquito species is being employed to provide information about the population abundance and prediction of the prospect of the disease at a spatio-temporal scale. Thus, entomological surveillance on the mosquito *Cx. tritaeniorhynchus* is carried out in areas where Japanese encephalitis is common [5,6]. The abundance of *Cx. tritaeniorhynchus* in the rice fields corresponds to the prevalence of Japanese encephalitis, both in northern [1,7] and southern [1] India. Observation in rice fields of West Bengal, India, indicates the presence of *Cx. tritaeniorhynchus* in the rice fields along with other vector mosquitoes [8]. In Indian context, however, information on the productivity and the life history traits of *Cx. tritaeniorhynchus* from the breeding sites are little known, in comparison to the adult population [6] and the Japanese encephalitis virus they transmit [9,10]. Thus the present study was aimed at assessment of the productivity as well as the life history traits of *Cx. tritaeniorhynchus* from rice fields was made using Burdwan, West Bengal, India as a focal geographical area.

Entomological monitoring of the rice fields for *Cx. tritaeniorhynchus* in different parts of the world [11-16] emphasize the numerical abundance of the mosquito, though the fitness aspects of the mosquitoes remain undermined. Life history traits like pupal weight and the adult body size serve as good indicators of the efficacy of transmission of disease by mosquitoes [17-19]. In insects including mosquitoes the appraisal of the pupal weight and the adult features are essential to predict the fitness of the individuals constituting the population. Thus inclusion of the life history traits in mosquito assessment programs provides better information to characterize the prevalence and the fitness of the concerned mosquito species, evident from the studies on the dengue vectors in Thailand [20]. Therefore to predict the fitness of the individuals thriving in the rice fields, the pupal weight and the adult dry weight and wing length of the *Cx. tritaeniorhynchus* was carried out. The pupal weight is an indicator of the larval effort towards the development of a competent adult with increased weight as a positive contributor to the fitness. The resource acquisition by the larval stages through feeding is crucial for the development into a successful pupa and subsequent adult stages [17-23]. Since the pupa is a non-feeding stage the resource accumulated by the larva is reflected through the pupal weight. Thus pupal weight can be used as a surrogate of the resources acquired during the larval development that enables emergence of a fit adult [17-19]. Using the correlates of the pupal weight, adult body weight and wing length, as a measure of the larval development in the rice fields, the fitness of *Cx. tritaeniorhynchus* can be deduced. The results of the present study will substantiate this proposition and its significance in the entomological surveillance program. Comparative assessment using the parameters will enable understanding the life history strategies and variations in the mosquitoes breeding in the rice field habitats.

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Materials and Methods

Study area

The mosquito immature were sampled from 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, and few rice fields in adjacent district of Hooghly, (Khanyan, 23°03'6"N, 88°30'8" E) West Bengal, India. During the paddy rice cultivation periods in the rainy and the winter seasons between 2010, 2012 and 2013 sampling was done. Selected sites of the rice fields were considered as sampling sites during the wet conditions of the rice paddy cultivation period.

Sampling method

The collections of mosquitoes were made [24,25] at regular interval from the rice fields during the wet conditions, following transplantation of paddy plants. Collections were restricted to wet conditions since in the dry conditions the rice field proper lacked water and thus the mosquito immature and other aquatic macro invertebrates. The sampling was conducted first week onward following transplantation of the paddy plants in the rice fields. Random sampling of selected rice plots of the University Farm House at Tarabag, Burdwan, and Khanyan, Hooghly was continued for the *boro* cultivation (February –March) of 2012 and 2013. Random stratified sampling was employed following standard protocols [24,26], to collect mosquito immature from selected rice fields. An insect net (200µm mesh size) fitted to an iron frame (20cm X 15 cm) with long handle was dredged in the inundated rice fields covering 1m² area, chosen randomly. At least nine samples were considered for a rice field of 1 ha area. The net trapped organisms and debris were emptied in a plastic bag and brought to the laboratory for identification and counting. In the laboratory, the plastic bags were emptied in enamel tray (46 X 32 X 6cm) containing tap water and the mosquito larvae and pupae were counted and separated and further placed in separate vials to emerge as adults. The larval stages were identified according to proper keys [27]. Prior to placement into the glass vials, individual pupa were weighed to the nearest 0.1 mg in a pan balance and the vials were marked with the date and the weight. Following emergence the adults were killed through starvation and the dead adults were allowed to dry naturally. The adults were further weighed and recorded. The wing length of individual adult was measured underneath a bionocular fitted with ocular micrometer (Erma, Japan) and the data were recorded to the nearest 0.1mm. In each sampling day irrespective of the mosquito species the data on the pupal weight (PW, in mg), adult weight (AW, in mg) and the wing length (WL, in mm) were recorded. The emerged adults were identified up to the species level following suitable key [13,28-31].

Data analysis

A Discriminant Function Analysis (DA) [32,33] was performed to classify the different mosquito species based on the pupal weight, wing length and the adult weight as explanatory variables. The purpose of the discriminant function analysis was to portray the differences in the life history traits of the mosquito occurring in the same habitat conditions. The Fisher's distance was used as an estimate to denote the differences among the species and sex of the mosquitoes. Using the time (period of paddy rice cultivation) and the sex as the source of variations, the differences in the pupal weight, adult body weight and wing length of *Cx. tritaeniorhynchus* were judged through 2-way ANOVA. Correlations among the immature

productivity of *Cx. tritaeniorhynchus* and the selected environmental parameters including paddy plant height and water depth were carried out. The data on the pupal weight, wing length and the adult weight of the mosquito *Cx. tritaeniorhynchus* were subjected regression analysis [34], to justify the correspondence among the life history traits, for both the sexes separately. For each trait, a degree of sexual dimorphism [18] was estimated and subjected to one-tailed t-test to justify the differences in the sexes with reference to that trait. Since the collections were made at different time interval and the individuals were considered at random, each of the data represented a true replicate following the norms of interspersion and randomization [35].

Results

The five mosquito species namely *Culex quinquefasciatus* Say, 1823, *Cx. tritaeniorhynchus*, *Anopheles annularis* van der Wulp, 1884, *An. subpictus* Grassi, 1899 and *An. vagus* Donitz, 1902 were observed in varied number from the samples of the rice fields. A total 67 samples were taken from 9 different weeks including Golapbag, Burdwan (42 samples) and Khanyan, Hooghly, (25 samples) West Bengal, India. The concerned rice fields remained inundated for different time period, which limited continuous sampling of the mosquito larvae from the rice fields. However, the time span of the sampling included nine different sampling weeks in parity with the paddy rice cultivation in these areas. In course of sampling the plant height (5.5 – 63.1cm, mean 42.88 ± 19.15 cm SE) and the water depth (3.5 – 41cm, mean 16.96 ± 18.9 cm SE), varied considerably as were the total mosquito immature in each sample (2 – 347 individuals, mean 103.97 ± 69.3 SE). The water quality were characterized through, conductivity (921 - 2400 µs, mean 1412.318 ± 260.14 µs SE), salinity (453 – 1220ppm, mean 700 ± 134.21 ppm SE), Total dissolved solids (TDS; 653 – 1680ppm, mean 1000.83 ± 182.38 ppm SE), pH (8.8 – 10.5, mean 9.58 ± 0.34SE) and the temperature (27.4 – 36.3°C, mean 33.03 ± 2.7 °C SE). The luminosity of the rice fields during the sampling period remained on average 546×10⁵ ± 185.54 lux (range 254×10⁵ to 803×10⁵ lux.). A total of 436 pupa collected from the samples successfully emerged as adults that could be identified to the species level [13,28-31]. Corresponding variations in the pupal weight, adult weight and wing length were observed for the male and female mosquitoes (Figure 1), which are further reflected through the discriminant function analysis (DA). Using the pupal weight, adult weight and wing length as explanatory variables, the mosquitoes could be separated on the basis of species and sex (Figure 2). Significant differences between the mosquito *Cx. tritaeniorhynchus* with other species were observed reflected through the Fisher's distance. Comparison among other mosquito species was restricted due to the low sample size for the respective species (Figure 2), though the dominance of *Cx. tritaeniorhynchus* was observed in the samples. The correlations matrix of the environmental variables and the total larval count is shown in Table 1. The plant height was significantly negatively correlated while the water height was positively correlated for the total count of immature. However, the data were discrete in term of the various rice fields sampled thereby restricting the assessment of the effects of the environmental variables as a regulator of the productivity of the mosquitoes.

The time scale dependent variation in the pupal weight, adult weight and the wing length was observed for *Cx. tritaeniorhynchus* (Figure 3). The results of ANOVA indicated significant differences in the life history traits corresponding to the time of sampling

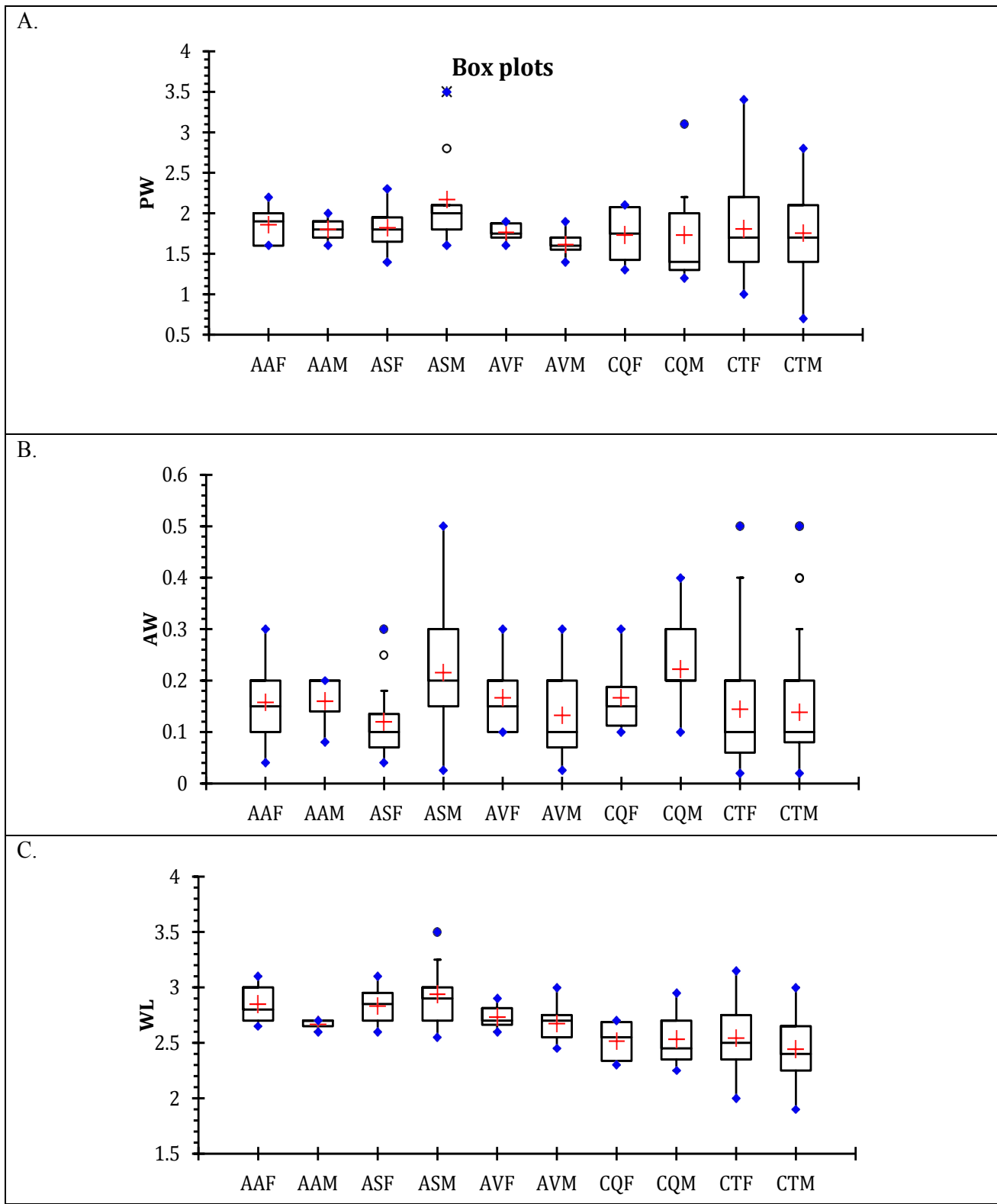
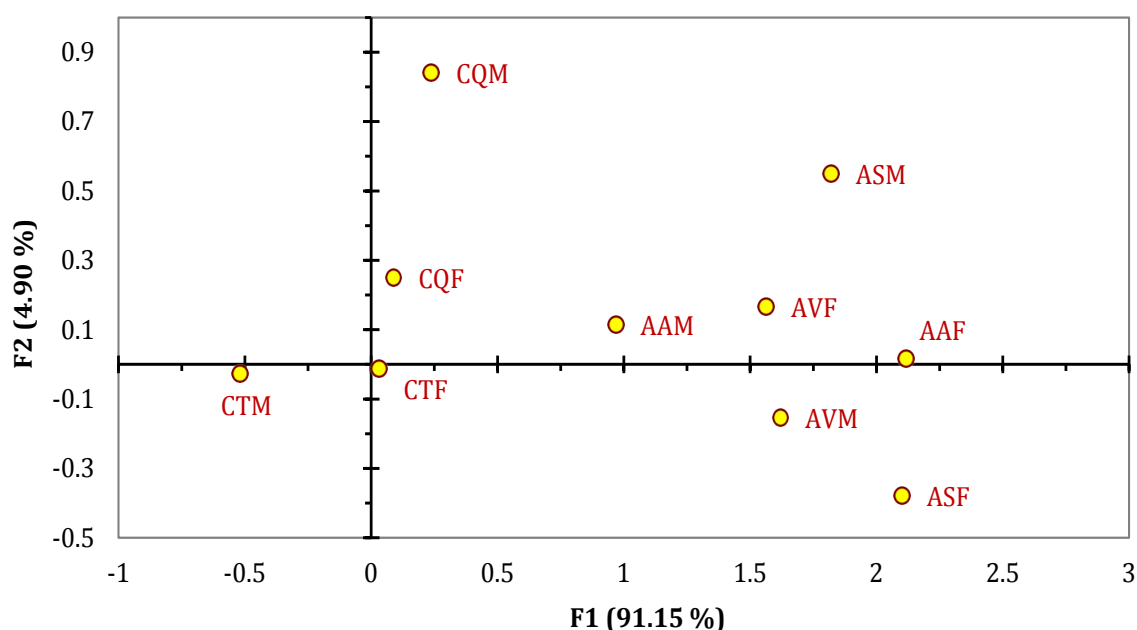


Figure 1: Box plot representing –[A] the pupal weight (PW), [B] adult dry weight (AW) and [C] wing length (WL) of the mosquito species encountered in the rice fields in Burdwan, India. Among the mosquitoes, *Cx. tritaeniorhynchus* (CT; n= 373), remained dominant with relative low representation of *Cx. quinquefasciatus* (CQ; n= 15), *An. vagus* (AV; n= 21), *An. annularis* (AA; n= 18), and *An. subpictus* (AS; n=24). F- Female and M- male.

| | | | | | | | |
|------------------------|--------|--------|---------|-----|--------|--------|--------|
| | F1 | F2 | F3 | | F1 | F2 | F3 |
| Eigenvalue | 0.525 | 0.028 | 0.023 | P W | -1.375 | -0.068 | 1.180 |
| Discrimination (%) | 91.154 | 4.900 | 3.945 | A W | 0.050 | 1.050 | -0.359 |
| Cumulative % | 91.154 | 96.055 | 100.000 | W L | 1.736 | -0.085 | -0.096 |
| Canonical correlations | 0.587 | 0.166 | 0.149 | | | | |



| | AAF | AAM | ASF | ASM | AVF | AVM | CQF | CQM | CTF |
|-----|---------------|-------|---------------|---------------|--------------|---------------|-------|--------------|--------------|
| AAM | 0.830 | | | | | | | | |
| ASF | 0.198 | 1.281 | | | | | | | |
| ASM | 0.767 | 1.036 | 2.330 | | | | | | |
| AVF | 0.347 | 0.251 | 0.943 | 1.075 | | | | | |
| AVM | 0.665 | 0.553 | 1.445 | 3.257 | 0.255 | | | | |
| CQF | 3.821 | 0.540 | 6.436 | 4.519 | 2.176 | 3.669 | | | |
| CQM | 4.701 | 0.888 | 9.706 | 5.488 | 2.698 | 5.418 | 0.498 | | |
| CTF | 7.021 | 0.894 | 20.327 | 10.889 | 4.739 | 13.062 | 0.286 | 2.871 | |
| CTM | 11.252 | 2.196 | 32.422 | 17.768 | 8.543 | 22.264 | 0.937 | 4.268 | 9.773 |

Figure 2: The results of the discriminant function analysis to segregate the mosquitoes on the basis of species and sex using pupal weight (PW) adult weight (AW) and wing length (WL) as explanatory variables. Among the mosquitoes, *Cx. tritaeniorhynchus* (CT), remained dominant with relative low representation of *Cx. quinquefasciatus*(CQ), *An. vagus*(AV), *An. annularis*(AA), and *An. subpictus* (AS). F- Female and M- Male. The Wilk's λ value was 0.624; $F_{27, 1274} = 8.281$; $P < 0.0001$.

Table 1: Correlation matrix for the environmental variables and the mosquito productivity in the rice field samples from West Bengal, India. (Water depth in cm, WD; Paddy plant height in cm, PH; Total dissolved solids TDS)

| Variables | Immature Abundance | WH (cm) | PH (cm) | Conductivity (µS) | TDS (ppm) | Salinity (ppm) |
|------------------|--------------------|---------|---------|-------------------|-----------|----------------|
| WD(cm) | 0.349 | | | | | |
| PH (cm) | -0.423 | -0.897 | | | | |
| Conductivity(µS) | -0.251 | -0.075 | 0.115 | | | |
| TDS (ppm) | -0.251 | -0.077 | 0.117 | | | |
| Salinity(ppm) | -0.237 | -0.057 | 0.095 | 0.929 | 0.929 | |
| Temp(°C) | 0.120 | 0.118 | -0.072 | 0.290 | 0.289 | 0.245 |

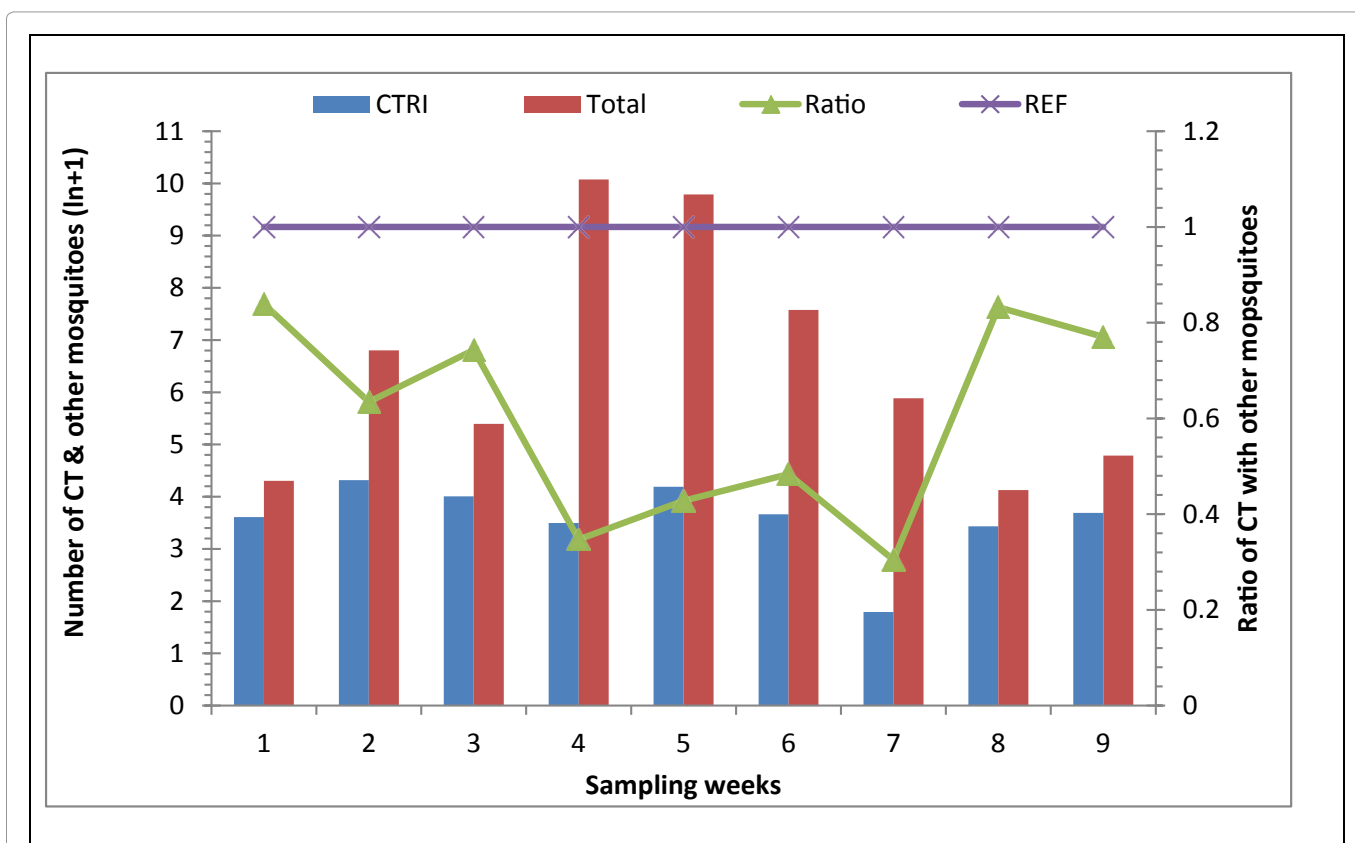


Figure 3: The proportion of *Cx. tritaeniorhynchus* against the total number of mosquito emerging from the collected immature in the sampling weeks from West Bengal, India. The reference line (dashed line) indicates the ratio of abundance of *Cx. tritaeniorhynchus* and other mosquitoes, if they are equal to unity.

(weeks) and the sex (Table 2). Positive correlations among the life history traits viz., pupal weight, adult weight and wing length were observed reflected in linear regressions (Figure 4), for both sexes of *Cx. tritaeniorhynchus*. For all the three life history traits, the degree of sexual dimorphism was significantly inclined towards the female of *Cx. tritaeniorhynchus* (Figure 5).

Discussion

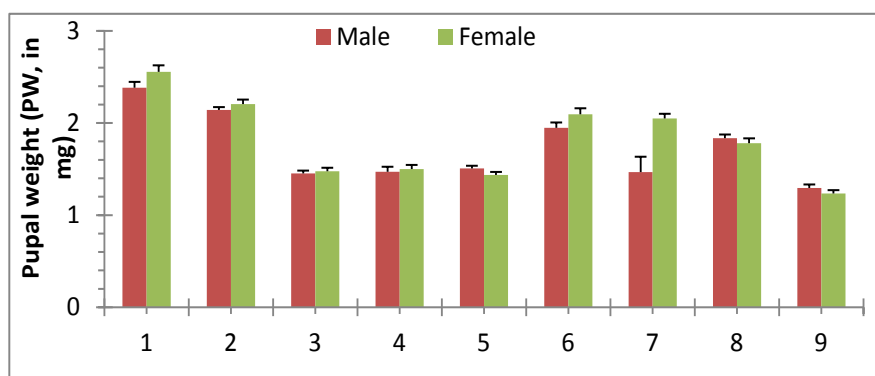
Rice fields are congenial breeding sites for vector mosquitoes [12,14-16]. Empirical evidences suggest that different Anopheline and Culicine species are abundant in the rice fields of Sri Lanka [36,37], Korea [38-40], Indonesia [41,42], Philippines [43], Japan [14,44], China [45], Malaysia [46], Greece [16], Vietnam [15], Mali [47] and Kenya [12,48], and to a lesser extent in the rice fields of Australia [49] and Portugal [50]. Among the Culicine mosquitoes, *Cx. tritaeniorhynchus*, the vector of Japanese encephalitis, breed in

the rice fields, as evident from the JE endemic countries like Japan [14,44], India [51,52] and several other tropical countries [12,14-16,36-38,40-44,46-48]. In Indian context, the relative abundance of *Cx. tritaeniorhynchus* is observed in the rice fields, in both Southern and Northern regions [51-54]. As observed in the present study, the mosquito *Cx. tritaeniorhynchus* remained a dominant species in the rice field habitats. Apart from the numerical abundance the pupal weight, wing length and adult weight of *Cx. tritaeniorhynchus* and the co-occurring mosquitoes remained sufficiently different to segregate and discriminate the mosquito population in terms of species and sex (Figure 2). However, the species diversity of the mosquitoes were low compared to other places [15,16]. The pattern of diversity of mosquitoes in rice fields varies with a suite of conditions including geographic, environmental and paddy rice cultivation pattern. Perhaps a combination of different factors including sampling error and the dry and wet regime of the rice fields may have contributed to the low species diversity of mosquitoes.

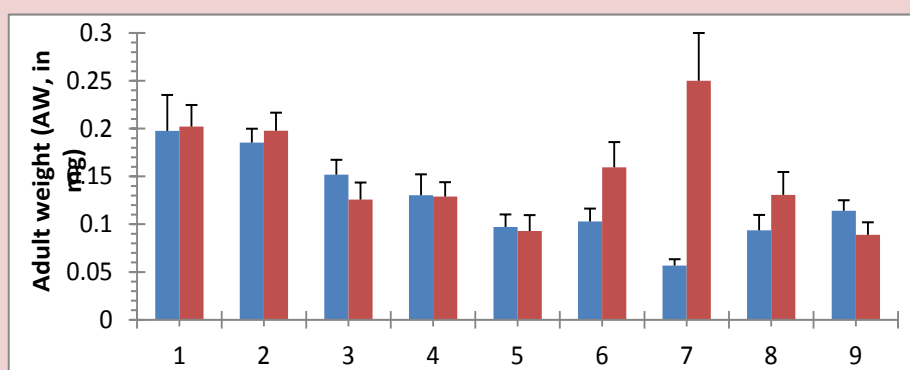
Table 2: The results of the univariate ANOVA using the time and sex as explanatory variables for the observations on pupal weight, adult weight and wing length of *Cx. tritaeniorhynchus*.

| Source of variation | Pupal weight | | Adult weight | | Wing length | |
|---------------------|--------------|---------|--------------|-------|-------------|--------|
| | df | F | df | F | df | F |
| Time | 8 | 150.403 | 8 | 8.644 | 8 | 16.884 |
| Sex | 1 | 9.311 | 1 | 4.698 | 1 | 8.343 |
| Time * Sex | 8 | 2.581 | 8 | 1.458 | 8 | 0.462 |
| Error | 359 | | 358 | | 359 | |
| Total | 376 | | 375 | | 376 | |

A.



B.



C.

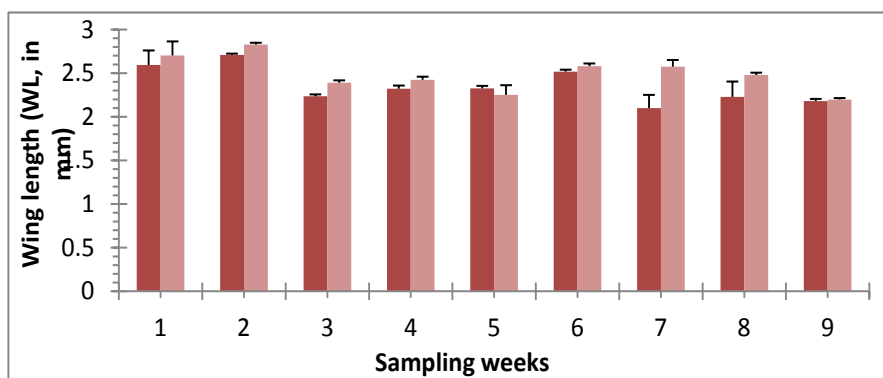


Figure 4: The life history traits (pupal weight, PW in mg, in A; adult dry weight, AW in mg, in B; and wing length, WL in mm, in C) of *Cx. tritaeniorhynchus* sampled from rice fields for nine consecutive weeks post transplantation. The univariate ANOVA applied on the data of pupal weight (PW), adult weight (AW) and wing length (WL) using the time period and sex as explanatory variables. (n= 373 individuals).

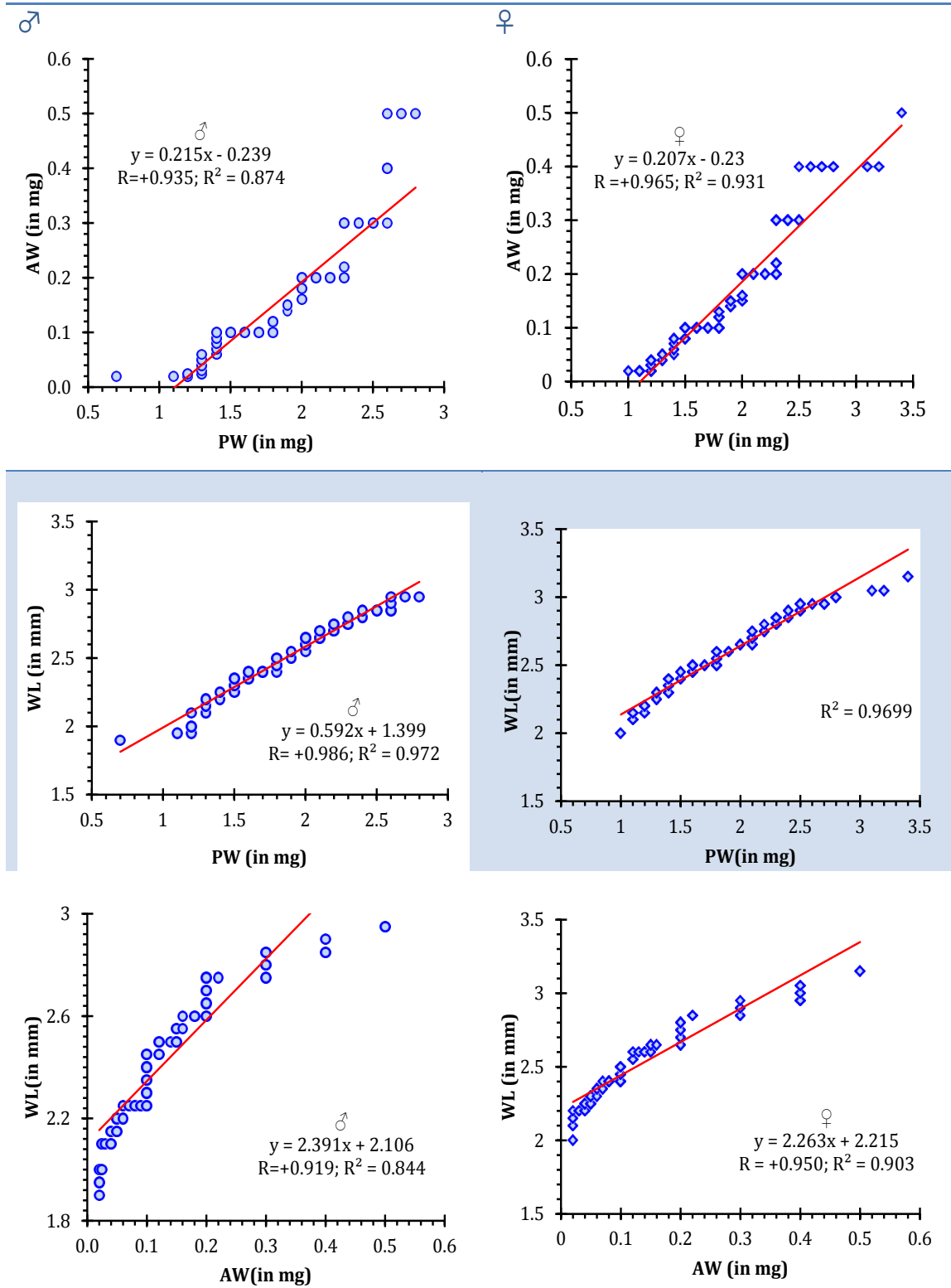


Figure 5: The regression equations depicting the relationship among the three life history traits, pupal weight (PW), adult dry weight (AW) and wing length (WL) in male (♂) and female (♀) *Cx. tritaeniorhynchus* (♂ = 177; ♀ = 194).

The habitat conditions in the rice fields change temporally, that is reflected in the water depth and the paddy plant height. Whereas the paddy plants may increase in height with the time period of the cultivation, the water logged conditions and thus the water depth may vary with time. Owing to the alternative dry and wet conditions the rice fields vary in habitat permanence which may influence the colonization pattern and development pattern of the mosquitoes. As a result the productivity of the mosquitoes may vary in the time scale of paddy rice cultivation, but show a positive relation with the water depth and a negative relationship with the paddy plant height [1,7,14,15,51,53]. Similarly, a positive correlation between number of immature and water depth and a negative correlation between plant height and the immature was observed in the present instance. A negative correlation results between the paddy plant height and the water depth in the rice fields over the time period of cultivation. Thus the paddy plant height and the water depth may serve as indicators to predict the productivity of *Cx. tritaeniorhynchus*. Similar observations have been made for other mosquito species of the rice fields where the productivity varied with the time period of the paddy cultivation [46,54].

The numerical dominance of adult *Cx. tritaeniorhynchus* among other vectors has been attributed to the sampling design and differences in biting activity, host preference and host-seeking strategy [55,56]. For the present observations, the prospective reasons for the dominance can be attributed to the phenology of the co-occurring mosquitoes and the conditions of the oviposition habitat sites [46-48]. Owing to the intermittent water logged condition, the rice fields vary in terms of tenure and quality as mosquito larval habitat. As a consequence, the larval resource acquisition may have varied resulting in corresponding variations in the pupal weight, adult body weight and wing length of the mosquito *Cx. tritaeniorhynchus*. Thus, in the rice field habitats, the productivity of *Cx. tritaeniorhynchus* is not limited to the variations in the numerical abundance, as observed in earlier studies [1,4,7,42,53], but also varies in terms of the life history traits over the period of paddy rice cultivation. The correspondence of the pupal weight with the adult body weight and wing length indicate that the pupal size can be used as an indicator to predict the prospective adult size, and consequently the efficacy of disease transmission. The relation of adult body size and disease transmission (wing length and body weight) is established for the dengue vectors [19,20] (Figure 6). Although, similar direct observations are lacking for Japanese encephalitis, the estimate of the life history traits coupled with the productivity may enhance the information retrieved through monitoring of *Cx. tritaeniorhynchus* in rice field habitats. While the adult emergence pattern of the mosquito may be relevant to understand the prospective population abundance, the body weight and the wing length features would indicate the variation in the transmission efficiency of Japanese encephalitis virus. However, this opinion needs to be substantiated through empirical studies on the virus and *Cx. tritaeniorhynchus* interaction using the morphometric features as the explanatory variables.

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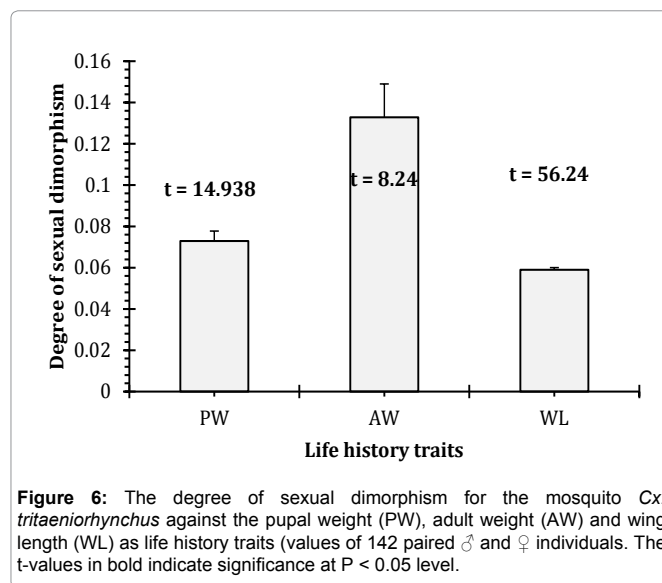


Figure 6: The degree of sexual dimorphism for the mosquito *Cx. tritaeniorhynchus* against the pupal weight (PW), adult weight (AW) and wing length (WL) as life history traits (values of 142 paired ♂ and ♀ individuals). The t-values in bold indicate significance at $P < 0.05$ level.

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