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Pupal productivity of dengue vectors in Kolkata, India: implications for vector management

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***Background & objectives*: Entomological surveillance of the dengue vectors using pupal productivity as indicators can be helpful in effective management. On this basis, an assessment was made on the relative importance of the larval habitats of *Aedes* mosquitoes in Kolkata, an endemic zone for dengue in West Bengal, India.**

***Methods*: Monthly collection of larvae and pupae of *Aedes* from larval habitats categorized as earthen, plastic and porcelain containers and tyres, was carried out from selected sites. Pupal weight was recorded and degree of sexual dimorphism was calculated. The data on pupal weight, sexual dimorphism and immature density were used for regression analysis.**

***Results*: The number of positive sites for each type of larval habitats varied with months and mosquito species. Based on mean density per month, the plastic containers were the most productive habitats and the tyres were least productive for both *Aedes* species. The pupal weight of both *Ae. aegypti* and *Ae. albopictus* varied with the relative density and type of larval habitats. Significant differences in pupal productivity, positive sites and the proportion of pupae were observed in the habitats. Species-specific differences in the degree of dimorphism were noted with the females being larger in size than males, irrespective of the habitats.**

***Interpretation & conclusions*: Pupal productivity of *Aedes* mosquitoes in Kolkata differed in terms of the type of the larval habitats with the immature density affecting the body size of the adults. This habitat-based study is a pioneer effort considering Kolkata and calls for a management plan for source reduction of these habitats to minimize *Aedes* mosquitoes and thus potential risk of dengue.**

Key words *Aedes aegypti* - *Ae. albopictus* - Kolkata - larval habitats - vector management

Dengue is an arboviral disease prevalent in the tropical and subtropical regions of the world[1] transmitted to human by the bites of vectors *Aedes aegypti* (Linneaus, 1774) and *Aedes albopictus* (Skuse, 1893) (Diptera: Culicidae). The incidence of dengue is largely dependent on vector populations and the frequency of contact between the vectors and susceptible human hosts, reflected by a positive correlation of *Aedes* abundance and prevalence with dengue[2-4]. This supports the necessity of entomological surveillance to assess and predict the abundance of vectors and possibilities of occurrence of dengue[5-7]. Conventionally, assessment and prediction of the vector population levels are made using the productive larval habitats and data on human demography as components of different indices recommended by the WHO[6]. The information provided by these indices is useful supplements for the management strategies against the dengue vectors, in different parts of the world[4,6,7]. Estimates of *Aedes* abundance in different dengue endemic regions of India are available that help vector management and enhance necessary precautionary measures. However, uninterrupted monitoring of the vector population is necessary to make successful assessment of the vector population as well as to note the deviation in the population loads, if any. In view of these and considering unique ecological environment of Kolkata, West Bengal, India, being dengue endemic, an appraisal of the dengue vector abundance based on pupal productivity was made to supplement vector management and to provide a platform for comparison with other areas in India and elsewhere in the globe.

Several methods of entomological surveillance have been proposed with higher resolutions of forecasting abundance of mosquitoes. These include sequential stratified sampling methods incorporating pupal density as one of the predictor variable of abundance. In other methods, immature density, habitat types and location are given priority. However, the adult population and its ability to transmit disease depend on the features of the individual mosquitoes that constitute the population. Biological features such as longevity and reproductive success of individual mosquito define the persistence of the population. Longevity and reproductive success are positively correlated with the adult body weight, which is linked to the pupal weight. Thus, pupal weight can be used as an indicator to infer about the adult longevity and reproductive success and the consequences at the population level. Incorporation of pupal weight in the surveillance in addition to the density will help enhance prediction about the population abundance and its possible variations, with higher precision. This proposition has been tested in Thailand[8], where adult wing length was found to vary with the *Aedes* immature density in the habitats and with months. In the present study, pupal weight was used as an indicator of the prospective adult body size, which as a trait contributes to the fitness of the individual and the population as a whole. Thus, evaluation of the pupal weight of *Aedes* mosquitoes from different larval habitats will help to correlate the density effect on the pupal weight and indirectly the adult features.

Material & Methods

Monthly sampling of selected sites from Kolkata was carried out between January and December 2008. In each site the *Aedes* mosquito larval habitats were chosen randomly, based on four broad types, earthen pots, plastic and porcelain containers, and tyres. A total of 75 numbers of each habitat were considered per sampling site per month. The features of the habitats surveyed are presented in Table I. From each of the positive larval habitats, the pupae and larvae were sampled and placed in sample containers (100 ml sample container Tarsons®, India) and brought to the laboratory for counting and recording the number of immature collected. This was followed by recording the pupal weight (wet weight up to nearest 0.1 mg using METTLAR-TOLEDO ® Al-104 balance, India), each pupa was placed in vials individually, and allowed to emerge to adult stage. The sex and species of the adults were identified based on appropriate keys[9]. The numbers of individuals of *Ae. aegypti* and *Ae. albopictus* were considered only for analysis. The data on the pupal weight of males and females were used to construct the degree of sexual dimorphism based on the formula[10]:

Degree of sexual dimorphism (DD) = $PW_f - PW_m / [(PW_f + PW_m)/2]$

Where, PW_m = pupal weight of ith male, and

PW_f = pupal weight of ith female (i= 600)

Table I. Features of the *Aedes* mosquito larval habitats surveyed

Habitat	Water volume (ml)	Water height (cm)	Width diameter (cm)	*p*H	Sampled with
Earthen	<500	11-14	7-12	7.4-7.8	Pipette (100 ml) or whole content was emptied in glass beaker (500 ml)
Plastic, Porcelain, Tyres	<100	02-10	8-20	7.2-7.5	Pipette (100 ml) or whole content was emptied in glass beaker (500 ml)

The data on the pupal weight of the females and males were arranged in ascending order in each density class, and for each pair, the DD was calculated using the above formula. To comment on the species specific variation in the abundance of *Aedes* mosquito, the data on the pupal weight, proportion of positive larval habitats (container index) were subjected to two-way factorial ANOVA analysis, using habitats, species and sex as variables. The data on pupal weight, sexual dimorphism and immature density were considered for regression analysis. The statistical analyses were performed following Zar[11] using the SPSS[12] and *XL*STAT[13] software.

Results

The observations on the *Aedes* larval habitats revealed that the number of habitats positive for *Aedes* immature exhibited significant rise and fall with the months and the *Aedes* species. On an average out of 75 habitats per month, the habitats positive exclusively for *Ae. aegypti* varied between 0 and 24 (~35%) for earthen, 0 and 23 (~30%) for plastic and porcelain, 0 and 5.5 (~07%) for tyres, depending on the months. The exclusive habitats positive for *Ae. albopictus* varied between 0 and 23 for earthen (~31%), 0 and 25 for plastic (~35%), 0 and 24 for porcelain (~35%), 0 and 6 for tyres (~10%). The habitats common for both mosquitoes differed between 0 and 19 for earthen (~ 25%), 1 and 14 for plastic (~19%), 0 and 19 for porcelain (~26%), 0 and 6 (~10%) for tyres (Fig. 1). During the months of March no habitat was found to be positive for *Ae. aegypti* alone, although a few tyre habitats were positive for both *Ae. aegypti* and *Ae. albopictus*. The mean number of pupae per positive habitats was observed to vary significantly with the months during the survey period (Fig. 2). During March, no *Ae, aegypti* or *Ae. albopictus* was recorded alone in any of the habitats, however, plastic containers recorded both the species in small numbers. The post-monsoon months were found to be most productive in terms of nurturing both the species individually as well as in groups. To carry out ANOVA, the data on pupal productivity were log transformed to ascertain homogeneity of variances among the groups (habitat type). The Levene's test (W) for homogeneity of variance[11,12] was applied that yielded a value of 46.103 (df = 3,188, P<0.001), for pupal productivity per habitat indicating the unequal variance among the groups (habitat type) across the months, though their mean values were unequal (indicated by: Welch's robust test of equality of means[11,12], which was 30.485; df = 3, 78.54; P < 0.001; F distributed asymptotically). Transformation of the values by log (x+1) yielded homogeneity of variance among the groups [Levene's test (W) 2.100; df = 3,188, P=0.102] and Welch's robust test of equality of means was 46.623 (df = 3, 102.63; P<0.001). Similar transformation on the data on the positive habitats did not reduce the heterogeneity of variances among the groups (untransformed data, Levene W=80.465; df=3,188, P<0.001; transformed data, Levene W=113.573; df=3,188, P<0.001) and, therefore, the original values were used for ANOVA. In all of the positive habitats, the pupal productivity varied with the species and habitat types considerably as reflected by the two-way ANOVA. Tukey test revealed significant differences between and among the variables (Table II). Significant correlations between the productivity and the sampling period were observed for both the species irrespective of the habitat types (Table III). The data on pupal productivity was further applied to a 3-way multivariate ANOVA that revealed significant differences in the productivity between the months, habitat types and species concerned (Table IV). The significant differences in the interactions between the month, species and habitat indicate that the pattern of productivity in the habitats varied with the species and months indicating differences in the population dynamics of *Ae. aegypti* and *Ae. albopictus* as well as choice of habitats for breeding. The value of Wilk's is indicative of significant variations in the pupal productivity with reference to the habitat type, months and the two species of *Aedes*.

Using the data on the pupal weight as indicator of adult fitness, regression equations revealed pupal weight as a function of density of the habitats (Table V). A significant difference in the relation could be observed with the correlation coefficient values for the density pupal weight relationship varying with the habitat type. In case of both the species, male pupal weight did not vary with the density of the habitats, while for the females in all the habitats, the density affected the pupal weight, *i.e.* a negative correlation coefficient value was observed. A deviation to this was observed in case of *Ae. aegypti* from the plastic and *Ae. albopictus* from the earthen habitats. However, for the males the density effect was seen for the tyre habitats, for *Ae. albopictus* only.

The degree of dimorphism was prominent for both *Ae. aegypti* and *Ae. albopictus* based on the pupal weight.

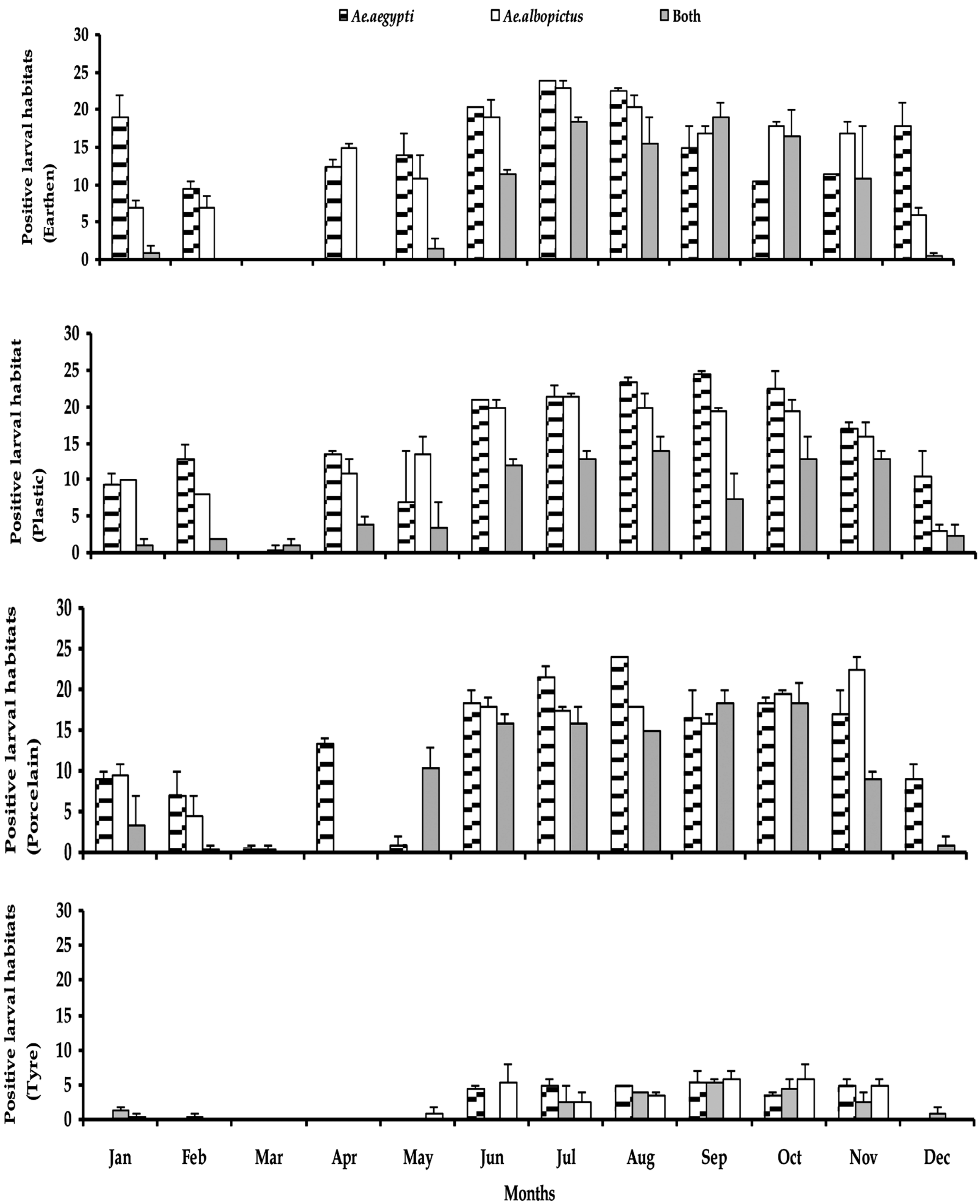

Fig. 1. The number (mean ± SE) of habitats positive with only *Ae. aegypti*, only *Ae. albopictus* and both during the survey period (n=75 habitats/habitat type/month). This is equivalent to container index (CI).

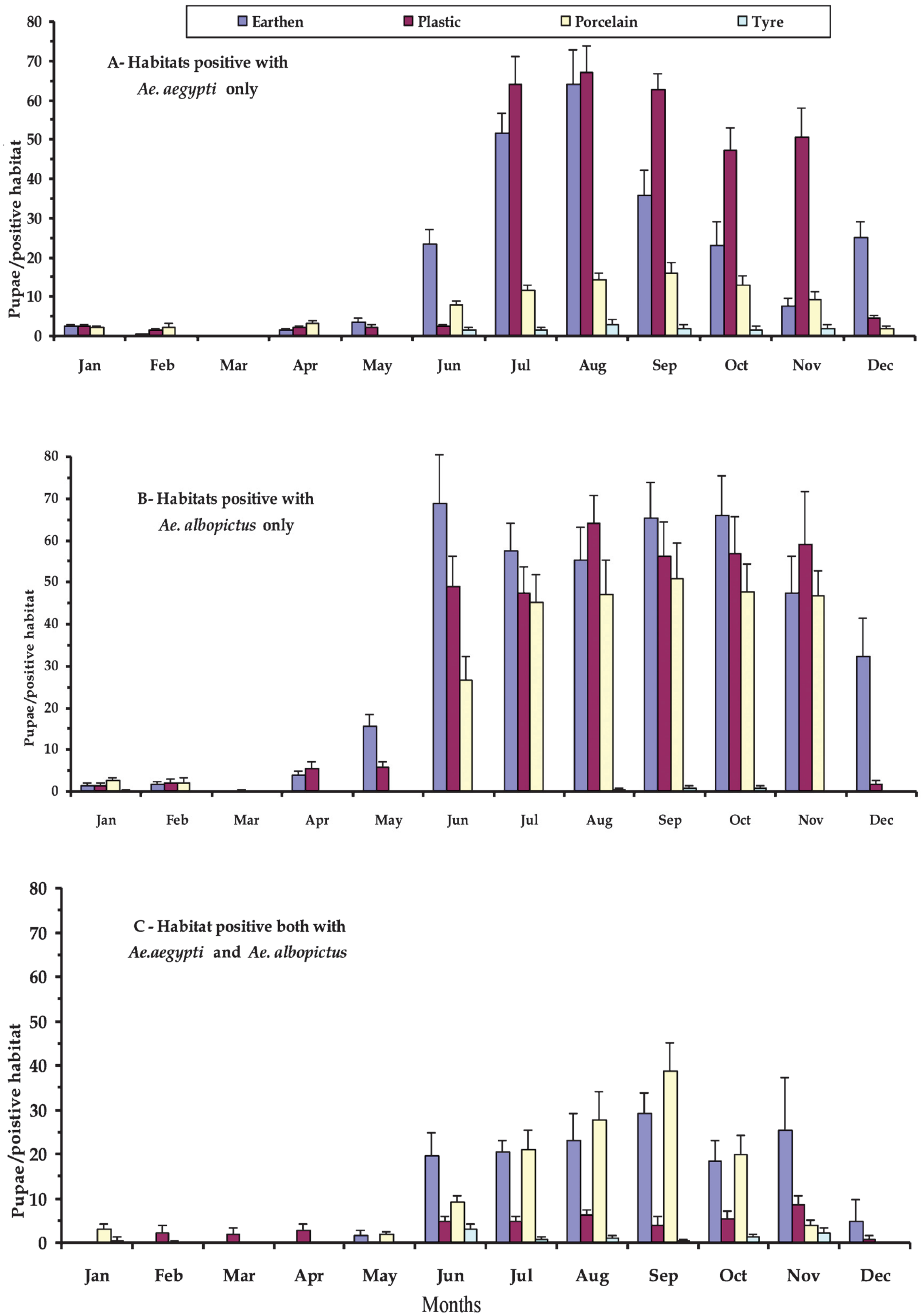

Fig. 2. The number (mean ± S.E.) of pupae per positive habitats sampled for only *Ae. aegypti,* only *Ae. albopictus* and both, in different months during the survey period.

Table II. Results of 2-way factorial ANOVA on (a) the variation in positive habitat and (b) the variation in immature *Aedes* mosquitoes using larval habitats and species as variables.

(a) Positive habitat

Source	Sum of squares	df	Mean square	*F*
Species (S)	4794.88	1	4794.882	23.865
Habitat (H)	14369.42	3	4789.808	23.839
SXH	14146.57	3	4715.524	23.469
Error	36968.97	184	200.918	
Total	70279.85	191		

Post hoc Tukey test (S.E. = 2.893) Studentized range q = [|(I-J)|/S.E.]; df = 191, 3

Habitat (I)	Habitat(J)	q
earthen	plastic	19.823
earthen	porcelain	19.930
earthen	tyre	20.177
plastic	porcelain	0.107
plastic	tyre	0.354
porcelain	tyre	0.247

(b) Pupal productivity

Source	Sum of squares	df	Mean square	*F*
Species (S)	0.017	1	0.017	0.025
Habitat (H)	64.94	3	21.65	**31.77**
SXH	1.253	3	0.418	0.613
Error	125.343	184	0.681	
Total	191.553	191		

Post hoc Tukey test (S.E. = 0.168) Studentized range q = [|(I-J)|/S.E.]; df = 191, 3

Habitat (I)	Habitat (J)	q
earthen	plastic	0.009
earthen	porcelain	0.420
earthen	tyre	1.427
plastic	porcelain	0.411
plastic	tyre	1.418
porcelain	tyre	1.006

(Habitats positive for both the species were excluded. Observed data on immature productivity were transformed as log (x+1) to ascertain homogeneity of variance). All F values significant at $P<0.05$ level are marked in bold (N=192 habitats).

However, a variation in the degree of dimorphism was noted depending on the density of the immature in the concerned habitats. The regression equations and the correlation coefficients suggested that with increasing density the degree of dimorphism decreased (Table VI), except for *Ae. albopictus* in plastic habitats.

Discussion

Entomological surveillance of dengue vectors have been a key aid in understanding the population persistence and dynamics inclusive of relative abundance at a spatiotemporal scale[6]. The information on these aspects has been useful in predicting the population state of the vector mosquitoes and the possibility of the diseases. Appropriate intervention of the population could be outlined based on the data on the population fluctuations. This is substantiated through the studies in Trinidad[4,14], Thailand[8], Brazil[15,16], Vietnam[17,18] and tropical region of Australia[19,20]. A comprehensive account of the different survey methods and its utility in the vector management suggest a shift from the classical indices for entomological monitoring of the dengue vectors, which has been employed in the studies carried out in recent years. Considering India and Kolkata in particular, such information was fragmentary[21], with information on the seasonal prevalence only[21,22]. In the present study, an attempt was made to emphasize on the importance of the pupal productivity for the entomological monitoring, thereby adding to the possible population and life historical consequences of the vector mosquitoes. The pupal weight was used as an indicator so that the possible fecundity and population growth could be assessed. Our study reveals that the containers with higher density of immature yielded smaller pupae, thereby the prospective adults having lower fitness in terms of longevity and fecundity. This is deduced from the fact that in case of insects and mosquito in particular, the adult life history features are correlated with the pupal weight, which in turn is dependent on the larval development. Both *Ae. aegypti* and *Ae. albopictus* exhibited this pattern, for the number of containers surveyed during this year long period. The impact of density effects on the pupal

Table III. Correlations between the months in terms of pupal productivity of *Ae. aegypti* and *Ae. albopictus* irrespective of the habitats

Ae. aegypti

	Jan	Feb	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Feb	**0.213**									
Apr	0.096	0.054								
May	**0.255**	-0.074	0.056							
Jun	**0.149**	-0.048	0.066	0.016						
Jul	**0.278**	0.042	-0.054	**0.361**	**0.299**					
Aug	**0.294**	0.021	**0.200**	**0.447**	**0.195**	**0.543**				
Sep	**0.277**	0.109	0.119	**0.356**	0.070	**0.609**	**0.494**			
Oct	**0.214**	0.104	0.120	**0.202**	**0.181**	**0.539**	**0.396**	**0.449**		
Nov	0.055	0.018	0.079	**0.170**	**-0.223**	**0.455**	**0.347**	**0.515**	**0.467**	
Dec	0.089	-0.116	-0.085	**0.328**	**0.439**	**0.309**	**0.313**	**0.189**	**0.149**	-0.083

Ae. albopictus

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Feb	**0.225**										
Mar	0.140	-0.032									
Apr	0.179	0.005	-0.048								
May	0.041	0.020	0.049	**0.231**							
Jun	-0.022	0.085	0.098	**0.185**	**0.336**						
Jul	0.033	0.039	-0.016	**0.188**	0.193	**0.270**					
Aug	0.188	0.138	-0.104	**0.263**	**0.261**	**0.301**	0.392				
Sep	**0.236**	0.115	0.049	**0.284**	0.072	**0.246**	**0.461**	**0.442**			
Oct	0.001	0.066	-0.027	0.159	**0.289**	**0.368**	**0.382**	**0.427**	**0.388**		
Nov	0.025	0.141	-0.087	0.058	0.094	**0.239**	**0.363**	**0.420**	**0.233**	**0.505**	
Dec	-0.108	-0.102	-0.032	0.102	**0.350**	0.099	**0.275**	0.097	**0.226**	**0.297**	0.106

For *Ae. aegypti* data for March were nil (a total of 100 habitats that were positive for the *Ae. aegpti* and *Ae. albopictus* separately). The values in bold are significant at P<0.05 level, reference value $r_{(2),\ 0.05,\ 98} = 0.197$)

weight could be extended to the sexual dimorphism, which as a biological factor is important in mate choice and successful mating. The changes in the degree of sexual dimorphism with increasing larval density is a possible indication that the vector mosquitoes are able to make a trade-off with the size fecundity relationship such that in situations with lower density they are able to produce larger adults that can live long and serve as continuity and dispersal of the population for the next favourable season. It would be appropriate to judge this proposition through further studies incorporating the life history analysis under variable environmental conditions. The important finding is that higher density of the immature of *Ae. aegypti* and *Ae. albopictus* in the larval habitats produces smaller pupae and thus affects the individual fitness of the adults.

The variations in the immature density were prominent with the months, type of containers (larval habitat) and the mosquito species. The trend in the variation was similar for both the species, though the preference of the larval habitats was different. While *Ae. albopictus* utilized plastic and tyres, *Ae. aegypti* utilized earthen and porcelain habitats to a higher extent. When both species were found, *Ae. albopictus* was competitively superior with higher immature density than *Ae. aegypti*. The competitive effects substantiate

Table IV. The results of the three-way multivariate ANOVA using the species (*Ae. aegypti*, *Ae. albopictus* alone and both), habitats (earthen, plastic, porcelain, tyre) and months as explanatory variables for the observed variations in the pupal density of *Aedes* mosquitoes in the positive habitats η2 = (1 - Wilk's Λ)

Source	Sum of squares	df	Mean square	F-value	η2
Tests of between-subject effects					
Species (S)	205556.41	2	102778.206	**129.01**	0.473
Habitat (H)	376548.49	3	125516.163	**157.55**	0.621
(S) X (H)	147965.28	6	24660.88	**30.954**	0.392
Error	229450.24	288	796.702		
Total	*959520.42*	299			
Tests of within-subjects effects					
Months (M)	857786.38	11	77980.58	**134.98**	0.319
(M) X (S)	201825.56	22	9173.889	**15.88**	0.099
(M) X (H)	336374.66	33	10193.172	**17.644**	0.155
(M) X (S) X (H)	253875.85	66	3846.604	**6.658**	0.122
Error (MONTHS)	1830206.72	3168	577.717		
Total	4439589.59	3599			
Multivariate tests Wilks' Λ					
Effect	Value	F	Hypothesis df	Error df	**η^2**
Months (M)	0.167	125.62	11	278	0.833
(M) X (S)	0.4	14.697	22	556	0.368
(M) X (H)	0.222	16.557	33	819.74	0.394
(M) X (S) X (H)	0.286	5.97	66	1493	0.189

All F values and Wilk's Λ-values are significant at $P<0.05$ level

the findings of a study[23], where the mosquito *Ae. albopictus* competitively displaced *Ae. aegypti* under experimental and natural conditions.

In urban areas the landscape pattern may contain barriers for dispersal of *Aedes* mosquitoes[19]. Therefore, it is more probable that within restricted areas where habitats are available, the density of the immature will be high, if dispersal is limited. This is supported by the observations of Williams *et al*[24] where water storage in containers facilitated *Aedes* abundance. However, the differences in pupal productivity in residential and non-residential areas provide further evidence to this proposition. It has been noted that certain species of *Aedes* like *Ae. oceanicus* utilizes domestic containers in the similar geographical area[25]. In the present study, the pupal productivity and its variations in different months followed similar pattern. Theoretically, in rainy season the container habitats had higher chances to be filled with water and thus adult dispersal can be limited[26]. This further enhances the chance of high density of larvae in the habitats. It has been observed that variation in water storage containers leads to a corresponding variation in pupal productivity[17]. A positive correlation between the numbers of household water storage containers and abundance of *Ae. aegypti* immature was found, in rural areas in Vietnam[17]. Pupae were abundant in large concrete water storage jars or concrete water storage tanks. The relation between the positive containers and the pupal productivity in Kolkata followed a similar pattern. Thus it appears that a common pattern of pupal productivity exists possibly due to the similar socio-economic and geographical settings of the cities in the tropics.

Table V. The regression equations showing pupal weight as a function of density (log value) in the different larval habitats surveyed

Habitat type	Species	Regression equation	$r_{(2),598}$	r^2	$F_{1,598}$
Earthen	*Aedes aegypti*	male: y = 1.857 - 0.341 * log x	0.116	0.014	**2.728**
		female: y = 3.534 - 0.415 * log x	-0.258	0.066	**42.566**
	Ae. albopictus	male: y = 1.191 + 0.176 * log x	**0.104**	0.01	0.471
		female: y = 2.667 - 0.069 * log x	0.028	0.011	**6.505**
Plastic	*Ae. aegypti*	male: y = 1.237 + 0.131 * log x	**0.132**	0.017	**10.59**
		female: y = 2.265 + 0.263 * log x	**0.182**	0.033	**20.474**
	Ae. albopictus	male: y = 1.281 + 0.062 * log x	**0.098**	0.01	**5.83**
		female: y = 2.738 - 0.187 * log x	**-0.185**	0.034	**21.142**
Porcelain	*Ae. aegypti*	male: y = 1.517 + 0.031 * log x	0.023	0.01	0.328
		female: y = 3.51 - 0.097 * log x	-0.038	0.01	0.864
	Ae. albopictus	male: y = 1.218 + 0.067 * log x	**0.093**	0.01	**5.199**
		female: y = 3.407 - 0.3 * log x	**-0.243**	0.059	**37.37**
Tyre	*Ae. aegypti*	male: y = 0.654 + 0.669 * log x	0.195	0.038	1.497
		female: y = 5.351 - 1.652 * log x	**-0.319**	0.102	**4.318**
	Ae. albopictus	male: y = 1.144 + 0.219 * log x	-0.052	0.003	0.104
		female: y = 4.899 - 1.709 * log x	-0.185	0.034	1.353

N=600 for each species each habitat type. Here y represents pupal weight and x represents density of immature in the habitat. Values in bold are significant at P<0.05 level

Table VI. The regression equations of sexual dimorphism as a function of immature density (log value) in the different larval habitats surveyed

Habitat	Species	Regression equation	r	r^2	F-value
Earthen	*Aedes aegypti*	y = 2.47 - 0.29 * log x	**-0.157**	0.025	**15.17**
	Ae. albopictus	y = 0.04 - 0.03 * log x	-0.022	0.0004	0.298
Plastic	*Ae. aegypti*	y = 2.38 - 0.12 * log x	-0.052	0.003	1.652
	Ae. albopictus	y = 0.33 + 0.15 * log x	**0.205**	0.042	**26.35**
Porcelain	*Ae. aegypti*	y = 3.25 - 0.05 * log x	-0.022	0.0004	0.297
	Ae. albopictus	y = 0.35 - 0.08 * log x	**0.197**	0.039	**24.25**
Tyre	*Ae. aegypti*	y = 13.61 - 8.36 * log x	-0.252	0.064	2.587
	Ae. albopictus	y = 4.88 - 1.96 * log x	-0.242	0.058	2.354

N=600 for each species each habitat type, except tyres, N = 40. The r-values are two tailed

Pupal productivity differed in the larval habitats depending on the size and material of the container type. This was observed in rural areas of American Samoa[26]. Outdoor, non-potable water storage containers posed significant breeding risk contrast to potable water storage containers. Plastic drums and small plastic containers were the key habitats of *Ae. aegypti* breeding. Discarded plastic pots were identified as the most productive containers. Similar observations were made from the present study with the plastic containers being more productive than other types. Considering Kolkata and the type and amount of solid waste generated there, it is more probable that the plastic, glass and rubber waste materials generated from household or otherwise augment the availability of possible larval habitats and their utility as oviposition sites by *Aedes*. This has been observed in urban areas of Dibrugarh and Duliajan, Assam[27]. Our study reflects that the pupal productivity can be used as an indicator of the abundance of the *Aedes* mosquitoes, serving as a supplement to assess the possibilities of the dengue in Kolkata. A choice of the larval habitats by *Ae. aegypti* and *Ae. albopictus* was observed, though both the species exploited all the container types considered in the study. The individual and population level fitness of the principal vectors of dengue can be evaluated from the densities in the respective container habitats. As an intervention and management strategy, monitoring of the discarded containers and tyres should also be included keeping in view the amount of solid waste generated in the city[28,29]. This applies to other similar areas of India, so that the possible numbers of larval habitats are checked, thereby reducing the population of *Aedes* mosquitoes. Further studies should be carried out to note the population dynamics of *Aedes* in relation to the changing numbers of plastic, porcelain and tyre waste available to substantiate the contribution of solid waste in the maintenance of *Aedes* population.

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