ASSESSMENT OF PUPAL PRODUCTIVITY OF *AEDES* AND CO-OCCURRING MOSQUITOES IN KOLKATA, INDIA

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Abstract. Monitoring of dengue vectors provide baseline information about the abundance and subsequent management strategy. An appraisal of mosquito abundance using dengue vectors as focal species was made in respect to Kolkata, India as geographical area. The data on immature abundance in the container larval habitats *viz*, earthen, porcelain and plastic materials were subjected to three-way factorial ANOVA, using months, habitats and species as variables. Similar tests were done on pupal weight and wing length. It was observed that *Anopheles subpictus*, *Culex quinquefasciatus* coexist with *Ae. aegypti* and *Ae. albopictus* in different container habitats, that varied with months and habitats. *Ae. aegypti* and *Ae. albopictus* were found in higher proportions in porcelain and plastic containers. In earthen containers a stable ratio of three mosquitoes was observed. Sex specific variations in pupal weight and wing length were noted in both species of *Ae. aegypti* and *Ae. albopictus.* The wing length of adult *Aedes* mosquitoes showed correspondence with pupal weight suggesting the use of pupal weight can be used as indicator of prospective adult body size. Although less known as container breeding, presence of *An. subpictus* and *Cx. quinquefascistus* along with *Aedes* indicates that availability of waste containers in environment increases risks of dengue and other mosquito borne diseases. The present habitat-based study calls for a strict vector management strategy to reduce the sources of ovipostion in various container habitats to minimize the mosquito vectors and thus potential risk of dengue and other mosquito borne diseases.

Keywords: *Aedes aegypti*, *Ae. albopictus,* larval habitats, vector management, India

INTRODUCTION

Entomological surveillance of vector mosquitoes is a prerequisite for predicting possibilities of disease outbreak and framing management strategies (Focks, 2003; Focks and Alexander, 2006; WHO-SEARO, 2011). For dengue vectors, entomological monitoring has been emphasized for long as evident from studies from various parts of the globe (Kalra *et al,* 1997; Katyal *et al,* 1998; Medronho *et al*, 2009; Vezzani and Albicócco, 2009). Survey methods employed for monitoring dengue vec-

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tors may accentuate larval stages or adult stages or both of a particular geographical area. Often the intensity of abundance of dengue vectors are expressed as container index, house index and alike, where the probability of encounter a particular form of *Aedes*, is expressed as a function of putative containers or positive human settlement (Tun-Lin *et al,* 1995, 1996; Ray and Tandon, 1999; Sharma *et al,* 2005a,b; Chadee, 2009; Arunachalam *et al,* 2010). Although widely used in monitoring dengue abundance, these indices fail to indicate the possibility of an adult to successfully emerge from larva or pupa. The prospective risk from a dengue vector remains obscured since the abundance of mosquitoes are being appraised without characterizing the pupa or larva or immature. Characteristic features like mosquito body size and wing length are good indicators of the traits like longevity and fecundity (Agnew *et al,* 2000, 2002; Ellis, 2008; Padmanabha *et al,* 2011a,b; Muturi *et al,* 2012). The body weight of adult mosquito in turn depends on the resource acquisition by larval stages. Correlates of different life history traits suggest that population of adult mosquitoes and its fitness depends heavily upon the larval foraging success and characteristic resource acquisition in terms of energy reserves. Inclusion of one or more of the larval adult indicators of body mass would enable to assess the prospective longevity and reproduction. Few studies till date have highlighted the significance of life history indicators (Focks *et al,* 2000; Alto *et al,* 2003; Strickman and Kittayapong, 2003; Alto *et al,* 2008; Banerjee *et al,* 2013a). The purpose of the present study is to include features like pupal weight and wing length, so as to predict the possible features of the adult with higher precision. As a consequence, the prospect of larval control strategies can be framed to restrict the epidemic cycle of dengue and DHF.

Considering Kolkata, India as a dengue endemic zone and the significance of pupal productivity based assessment of dengue vectors, the present study aimed at bridging the gap between pupal productivity and prospective characteristics of the dengue larval abundance. Spatio-temporal variations in abundance of dengue vectors and characteristic energy reserve would enable appraisal of the population characteristics and prospective dengue transmission potential. Alike earlier studies, the assessment of dengue vector abundance will supplement necessary information for dengue vector management (Pramanik *et al,* 2007; Silver, 2008; Arunachalam *et al,* 2010; Becker *et al,* 2010). In the present study assessment of dengue vector abundance included appraisal of pupal weight and wing length of individual adult mosquitoes, apart from immature density in the habitats. As a consequence predictions can be made on the prospective fitness of dengue vectors and its chances to disease transmission. The survey included various larval habitats of *Aedes* thereby enabling assessment of variations in adult features emerging from diverse habitats. Inclusion of pupal weight and adult wing length as indicators of adult fitness, would enable prediction of habitat based variations in life history traits with higher precision. The link between habitat type and possible variations in the *Aedes* population can be evaluated, which would enhance habitat based vector management program.

MATERIALS AND METHODS

Study area

The inspection of various containers, for *Aedes* larval habitats, generated due to multipurpose usage, was carried out at selected sites of Kolkata and its adjacent locality. The sampling sites included four sampling spots located in the north, east, west direction centring from Ballygunge campus of University of Calcutta, Kolkata, India. The spots chosen for the present survey were: Dunlop (North), Ballygunge (Central), Chetla (East), and Jadavpur (South).

Methods

Immature of mosquitoes were collected from discarded earthen pots, porcelain and plastic containers and sewage drains, in and around the study area, time to time as per the requirement of the study. Water-filled earthen, porcelain and plastic containers, if found positive with immature (larvae/pupae) of mosquitoes, were considered as positive larval habitats. At each site the mosquito larval habitats were chosen randomly, based on three container types: earthen pots, plastic and porcelain containers. In course of the study, objects like coconut shells, discarded tires were also observed to be positive with the mosquito immature, but these were not considered for analysis. Random sampling of selected sites from Kolkata was carried out on a monthly basis, between January 2007 and December 2010, using WHO methods (WHO, 1999; WHO SEARO, 2012) and following Krebs (1999) and Focks and Alexander (2006) according to the suitability of the habitats with required modifications. A total of 20 numbers of each habitat was considered per sampling site per month. The features of the habitats surveyed and the sampling scheme followed are presented in Table 1. The container type units (plastic containers and earthen pots) were sampled with the help of pipette (if > 100 ml approximately (Aditya *et al*, 2008, 2009) or the whole content was emptied in (if < 100 ml) in a separate specimen containers (Tarson® specimen container, 100 ml capacity; Banerjee *et al*, 2010). The sewage drains were sampled using short plankton net (7 or 9 cm diameter, 200 μm mesh size). For all containers excess water was flushed and sieved with the help of plankton net (200 μm mesh size, rectangular in shape 30 cm x 15 cm) or using a circular plastic net (10 mm mesh size) to collect remaining immature, if any. The specimens collected in each sample were poured into plastic bags (2 liter volume) or Tarsons® specimen container (100 ml) and brought to the laboratory. In the laboratory the specimens were placed in enamel trays for identification and recording the data. The mosquito larvae were reared separately, after initial identification into genera, to the adult stages for identification of the sex and species following appropriate key (Christophers, 1933; Barraud, 1934; Nagpal *et al*, 2005; Reinert *et al*, 2009; Banerjee *et al*, 2010). However for further analysis, the number of individuals of *Ae. aegypti* and *Ae. albopictus* were only considered. The *Aedes* spp pupa collected were weighed to record the pupal weight (wet weight up to nearest 0.1mg using Mettlar-Toledo® Al-104 balance), each pupa were placed in vials individually, and allowed to emerge to adult stage. Following natural death of *Aedes* spp adults, the wing length (WL) of adults were measured to the nearest 0.1mm using a dissecting stereo microscope (Olympus® SZX, Olympus, Tokyo, Japan) fitted with a graduated eyepiece (Erma®, Tokyo, Japan) (appropriate magnification, scaling and conversion of eyepiece to mm was followed). The data on the wing length of males and females were used to construct the degree of sexual dimorphism based on the formula explained in Sharmila Bharathi *et al* (2004):

Degree of sexual dimorphism (DD_{WL}) $= WL_f - WL_m / [(WL_f + WL_m)/2]$

where, WL_m = wing length of *i*th male, and WL_f = wing length of *i*th female (*i*=360):

The data on the wing length of the females and males were arranged in ascending order in each density class, and for each pair, the DD was calculated using the formula above.

To comment on the variations in the abundance of mosquitoes based on habitat type and month, the data on abundance of mosquito immature in different habitat types were considered for three-way factorial ANOVA using month, species, and habitat as variables. Further the data on pupal weight were subjected to four way factorial ANOVA using habitat types months, species and sex as variables whereas the data on wing length were considered for three way ANOVA taking types of habitat, sampling months and sex as sources of variation. Paired *t*-test was done to find out the differences, if any, between the pupal weights and wing lengths of both sexes of each species. The statistical analyses were performed following Zar (1999) using the SPSS version 10 (Kinnear and Gray, 2000) and *XL*STAT software version 10.0 (Addinsoft SARL, Paris, France).

RESULTS

The ratio of individual of each mosquito genus of either *Culex quinquefasciatus*, *Ae. aegypti*, *Ae. albopictus* and *Anopheles subpictus* varied seasonally between the different containers. However *An. subpictus* was observed only in the earthen containers and was not encountered in either porcelain or plastic habitats. Irrespective of the sites, in earthen containers, *Culex quinquefasciatus*, *Aedes* spp and *An. subpictus* maintained a constant ratio all throughout the study period. No specific peak or dip was observed, however the ratio of *Ae. aegypti* and *Ae. albopictus* was noted to be highest during May to September while that of *Aedes aegypti* and *Ae. albopictus* and *An. subpictus* showed the maximum during October to December. In porcelain container habitats, *Culex quinquefasciatus* and *Aedes* spp ratio was minimally low in March, followed by a distinct rise during the monsoon and post-monsoon months. Though there were site specific differences the pattern remained more or less the same. Plastic habitats also recorded a peak during the monsoon and post-monsoon months for all the species concerned with a sharp decline in the summer and winter months (Fig 1).

The results of three way factorial ANOVA revealed that the abundance varied with the types of habitats, sampling months and species of the mosquitoes (Table 2A). Post hoc Tukey test was noted to vary between the habitats (Table 2B). Results of ANOVA on pupal weight revealed significant variation between species, sex and months (Table 3A). However dependence of pupal weight on habitat types did not vary ($F = 0.89$) as well as the interaction between the habitat types and species ($F = 0.50$) and habitat, species and sex ($F = 0.51$) were noted to be non significant, possibly due to the fact that basic nature of the container to serve as potential breeding center of the mosquitoes remained same in all the habitat types. The results of three way ANOVA on wing length showed significant variation with the sex and sampling months though it did not vary with the habitat types ($F = 2.16$). Variations in pupal weight ($t_{species\text{-}pw} = 6.49$, $t_{sex\text{-}pw} = 9.68$; df = 1443, $p < 0.05$) and wing length ($t_{species\text{-}wl} = 32.22$, $t_{sex\text{-}wl} = 69.83$; df = 1443, $p < 0.05$) were prominent between the species and sexes.

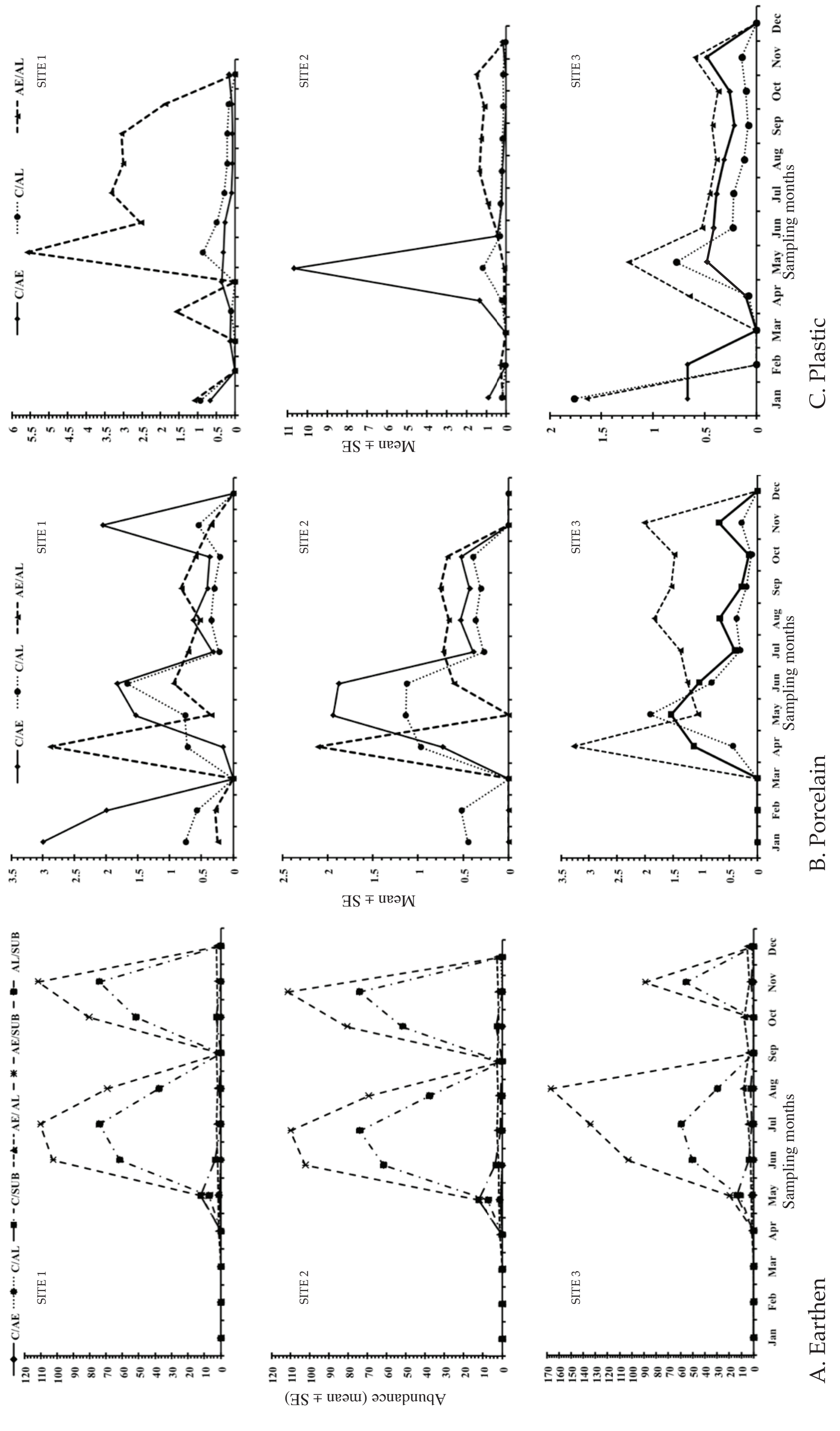

Fig 1–Variation in relative abundance of ratio of *Culex quinquefasciatas* (C), *Ae. aegypti* (AE), *Ae. albopictus* (AL) and *Anopheles subpictus* (SUB) encountered in different container habitats in three sites in Kolkata during a period of one year (2009-2010).

Table 1
Characteristic features of the larval habitats sampled.

Habitats	Volume of water sampled	Water height	Width /diameter	pH
Porcelain, plastic	<100 ml	2-10 cm	8-20 cm	7.4-7.8
Earthen	<500 ml	11-14 cm	7-12 cm	7.2-7.5
Sewage drain	>1,000 ml	30-60 cm	60-90 cm	6.8-7.2

Table 2
Results of three way factorial ANOVA (A) on abundance of different co-occurring mosquito species in different container breeding habitats, taking months, habitats and species as variables and post hoc Tukey test (B) between different habitats. Values marked in bold are significant at $p < 0.05$ level.

A. Abundance

Sources of variation	Sum of squares	df	Mean square	F	Partial η^2	Noncent parameter
Month (M)	555,962.48	11	5,0542.04	**240.82**	0.18	2,648.98
Habitat (H)	836,501.98	2	418,250.99	**1992.83**	0.25	3,985.67
Species (S)	304,486.49	2	152,243.25	**725.39**	0.11	1,450.78
M* H	817,496.75	2	37,158.94	**177.05**	0.24	3,895.11
M * S	323,212.64	22	14,691.48	**70.00**	0.11	1,540.01
H * S	553,336.25	4	138,334.06	**659.12**	0.18	2,636.47
M * H * S	600,192.62	44	13,640.74	**64.99**	0.19	2,859.73
Error	2,554,630.46	12,172	209.88			
Total	7,657,349.42	12,279				

B. Post hoc Tukey test : Habitats
Studentized range q = [|(I-J)|/SE] (df = 12,279, 2)

(I) 1=earthen, 2=porcelain, 3=plastic	(J) 1=earthen, 2=porcelain, 3=plastic	SE	q
Earthen	Porceain	0.32	**16.75**
Earthen	Plastic	0.32	**15.85**
Porcelain	Plastic	0.36	**0.89**

Individuals of *Ae. aegypti* was noted to be heavier than *Ae. albopictus* (Fig 2A and 3A) and the females of both species weighing more than the males (Fig 2B and 3B). Variations in wing length and pupal weight were also prominent between the habitats. However in both species the pupal weight and the wing length were noted to be more higher in case of plastic containers. Perhaps the water retention ability of different habitats played a critical role in maintaining the traits of the dengue vectors.

The degree of dimorphism was prominent for both *Ae. aegypti* and *Ae. albopictus* based on the wing length (Fig 4). A variation in the degree of dimorphism

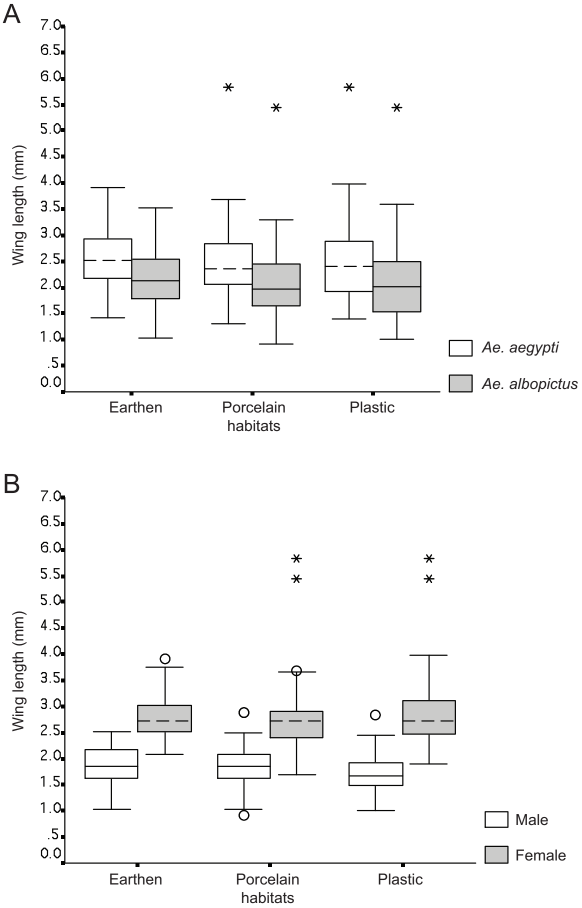

Fig 2–Box-plot representation of variation in wing length of *Aedes* spp between habitats (A) and sexes (B). The circle marks are extreme values and the box represents the lower (25%) and upper (75%) quartile with the mean values marked as black line.

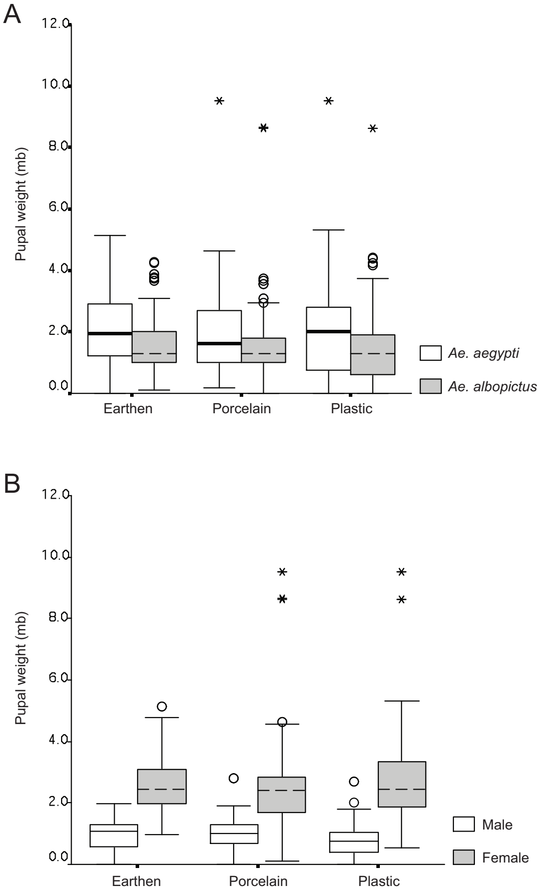

Fig 3–Box-plot representation of variation in pupal weight of *Aedes* spp between habitats (A) and sexes (B). The circle marks are extreme values and the box represents the lower (25%) and upper (75%) quartile with the mean values marked as black line.

was noted depending on the nature of the habitats with respect to the sampling months. For both species the degree of sexual dimorphism was highest in the plastic containers, possibly due the higher retention ability of water in those containers.

DISCUSSION

Entomological observations of several container breeding mosquito vectors have been a principal aspect to understand the population dynamics and continuance at a spatiotemporal scale (Focks and Alexander, 2006). Information in this regard is essential in predicting the population status of the vector mosquitoes and thus the chances of mosquito borne diseases. Based on the data of population variation suitable deduction of the population could be outlined. This has been validated

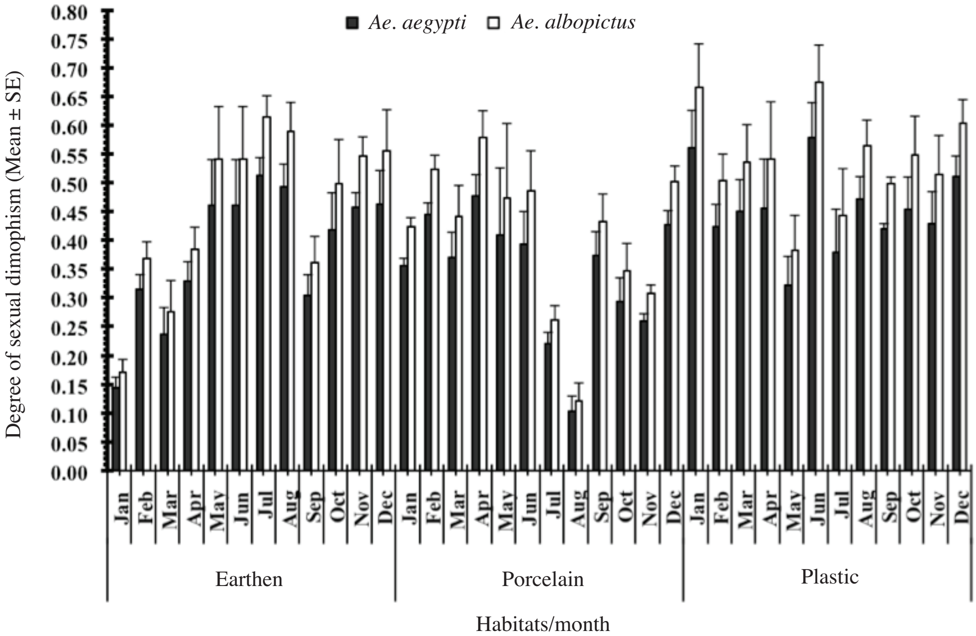

Fig 4–Variation sexual dimorphism in *Ae. aegypti* and *Ae. albopictus* based on wing length in different container habitats.

through several studies around the globe (Tun-Lin *et al*, 1996; Strickman and Kittayapong, 2003; Focks and Alexander, 2006). However, a comprehensive account of the different survey methods and its utility in the vector management policies suggest a transfer from the standard indices for entomological monitoring of the mosquito vectors, which has been employed in the studies carried out in recent years (Banerjee *et al*, 2010, 2013b). In the present study an attempt was made to emphasize the importance to monitor different container habitats that serve as a potential biotope for different mosquito vectors apart from *Ae. aegypti* and *Ae. albopictus*, thereby highlighting the possible population fluctuation and life historical attributes of the mosquito vectors. The wing length was used as an indicator so that the adult size and population growth could be assessed. In case of insects and mosquito in particular, the adult life history features are correlated with the pupal weight and wing length, which in turn is dependent on the prevailing conditions during larval development. Our study reveals that the containers with restricted resources produced pupa with low pupal weight and consequently emerged as adults having smaller wing length, thereby having lower fitness in terms of longevity and fecundity. Both *Ae. aegypti* and *Ae. albopictus* exhibited this pattern, for the number of containers surveyed during the study period. The impact of available resources on wing length could be extended to the sexual dimorphism, which is important in mate choice, successful mating and survival. The changes in the degree of sexual dimorphism with the type of containers is a possible indication that the vector mosquitoes are able to make a trade-off with the available resources, such that in situations with restricted access to the amount of resources they are able to pro-

Table 3
Results of factorial ANOVA on variation in pupal weight (four way) (A) and wing length (three way) (B) of *Aedes* spp. Values marked in bold are significant at $p < 0.05$ level.

A. Pupal weight: variables considered -different container habitats, species, months and sex of the individual.

Sources of variation	Sum of squares	df	Mean square	F	**Partial** η^2	Noncent parameter	Observed power
Habitat (H)	0.91	2	0.46	0.89	0.001	1.78	0.20
Species (SP)	99.23	1	99.23	**192.56**	0.129	192.56	1.00
Sex (S)	1,002.43	1	1,002.43	**1,945.29**	0.599	1,945.29	1.00
Month (M)	29.56	11	2.69	**5.22**	0.042	57.37	1.00
H * SP	0.52	2	0.26	0.50	0.001	1.01	0.13
H * S	16.19	2	8.10	**15.71**	0.024	31.42	1.00
SP * S	47.32	1	47.32	**91.84**	0.066	91.84	1.00
H * SP * S	0.52	2	0.26	0.51	0.001	1.01	0.13
H * M	81.82	22	3.72	**7.22**	0.109	158.79	1.00
SP * M	3.59	11	0.33	0.63	0.005	6.97	0.36
H * SP * M	16.83	22	0.76	**1.48**	0.025	32.65	0.95
S * M	32.17	11	2.92	**5.68**	0.046	62.43	1.00
H * S * M	110.59	22	5.03	**9.75**	0.142	214.60	1.00
SP * S * M	3.59	11	0.33	**0.63**	0.005	6.97	0.36
H * SP * S * M	16.85	22	0.77	**1.49**	0.025	32.69	0.95
Error	669.91	1,300	0.52				
Total	2,127.04	1,443					

B. Wing length: Variables considered here -Different container habitats, months and sex of the individual.

Sources of variation	Sum of squares	df	Mean square	F	**Partial** η^2	Noncent parameter	Observed power
Month (M)	6.60	11	0.60	**4.23**	0.033	46.49	0.999
Habitat (H)	0.61	2	0.31	2.16	0.003	4.32	0.443
Sex (S)	347.29	1	347.29	**2,446.10**	0.643	2,446.10	1.000
M * H	30.70	22	1.40	**9.83**	0.136	216.26	1.000
M * S	6.03	11	0.55	**3.86**	0.030	42.46	0.998
H * S	4.98	2	2.49	**17.54**	0.024	35.08	0.999
M * H * S	24.04	22	1.09	**7.70**	0.109	169.32	1.000
Error	194.79	1,372	0.14				
Total	614.50	1,443					

duce adults that can survive long and add to the permanence and dispersal of the favor mosquito population for the next favorable season. However it would be suitable to appraise this proposition through prospective studies involving the different life history features under changeable environmental conditions. Nonetheless, the important finding is that different types of container habitats influence the immature abundance of *Ae. aegypti* and *Ae. albopictus* as well as produces pupa and thus adults having variable biomass and wing length respectively, thereby affecting the individual fitness.

The variations in the immature abundance were prominent with the type of containers sampling months, and the mosquito species. Till date, empirical evidences suggest that artificial containers and cemeteries serve as a perfect site for breeding of *Aedes* spp, *Cx. pipiens, Ochlerotatus, Culex, Toxorhynchites, Culiseta, Armigeres, Lutzia, Uranotaenia,* and *Tripteroides* (Hribar *et al*, 2001;Vezzani, 2007; Vezzani and Albicócco, 2009) and promote competitive displacement of *Ae. aegypti* by *Ae. albopictus* (Juliano *et al*, 2004). Occurrence of *Anopheles* spp had rarely been reported from the containers (Forattini *et al*, 1998). The present findings also support this and highlight the co-occurrence of *Culex quinquefasciatus, Ae. aegypti, Ae. albopictus* and *Anopheles subpictus* in a number of containers. In urban areas, the landscape pattern acts as a barrier for dispersal of *Aedes* mosquitoes thus it is more probable that within restricted areas where habitats are available dispersal is limited, and the immature abundance will be high (Tun-Lin *et al*, 1995, 1996; Strickman and Kittayapong, 2003). Water storage in containers in residential and non-residential areas provides facilitated *Aedes* abundance (Lambdin *et al*, 2008). In the present study, the immature abundance and its variations in different months follow similar pattern.

During rainy season the container habitats had higher chances to be filled with water, limit the adult dispersal and thus further enhance the chance of higher density of larvae in the habitats (Banerjee *et al,* 2013a, b; Aditya *et al,* 2009). It has been observed that variation in water storage containers leads to a corresponding variation in abundance of *Aedes* spp. In rural areas in Vietnam, a positive correlation between the numbers of household water storage containers and abundance of *Ae. aegypti* immature was found (Nguyen *et al,* 2011). The relation between the types of containers utilized by the mosquito vectors and immature load in Kolkata follows a similar pattern. Thus it appears that socioeconomic and geographical settings of the tropical cities follow a common pattern (Arunachalam *et al,* 2010). In American Samoa, immature productivity differed in the larval habitats depending on the size and material of the container type (Lambdin *et al,* 2008). 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. The results of the present study also reflect similar pattern where the plastic containers are more productive than other container types both in terms of harboring the vectors as well as with higher values of sexual dimorphism based on wing length. Considering Kolkata and the type and amount of waste generated, it is more probable that the earthen, porcelain and plastic waste materials generated from household or otherwise accelerates the availability of potential larval habitats and their utility as oviposition sites by *Aedes* as well as other mosquitoes.

Our study reflects that the pupal weight and wing length can be used as indicators of the abundance of the *Aedes* mosquitoes, thereby serving as a surrogate to assess the chances of the dengue and other mosquito borne diseases in Kolkata. The individual and population level fitness of the principal vectors of dengue can be evaluated from the type of different container habitats. The present study emphasises that unrestricted use and disposal of the daily-use containers not only impose a burden to the environment and at the same time create a residence for the dengue and malaria vectors. Apart prioritizing the monitoring of discarded containers, as a part of successful intervention and management strategies, inclusion of tires as possible and potential breeding sites of the mosquitoes should also be done. This proposition is applicable for similar other 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 explore other possible habitats to note the population dynamics of *Aedes* as well as other coexisting mosquito genera in relation to the varying numbers of habitats available.

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