Indian J Med Res 142 (Supplement), December 2015, pp 87-94
DOI:10.4103/0971-5916.176632

Pupal productivity & nutrient reserves of *Aedes* mosquitoes breeding in sewage drains & other habitats of Kolkata, India: implications for habitat expansion & vector management

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Received October 15, 2015

***Background & objectives*: The quality of breeding sites is reflected through the pupal productivity and the life history traits of *Aedes* mosquitoes. Using nutrient reserves and pupal productivity of *Aedes* as indicators, the larval habitats including sewage drains were characterized to highlight the habitat expansion and vector management.**

***Methods*: The pupae and adults collected from the containers and sewage drains were characterized in terms of biomass and nutrient reserves and the data were subjected to three way factorial ANOVA. Discriminant function analyses were performed to highlight the differences among the habitats for sustenance of *Aedes* mosquitoes.**

***Results*: Survey of larval habitats from the study area revealed significant differences (*P*<0.05) in the pupal productivity of *Aedes* among the habitats and months. Despite sewage drains being comparatively less utilized for breeding, the pupae were of higher biomass with corresponding adults having longer wings in contrast to other habitats. The nutrient reserve of the adults emerging from pupae of sewage drains was significantly higher (*P*<0.05), compared to other habitats, as reflected through the discriminant function analysis.**

***Interpretation & conclusions*: The present results showed that for both *Ae. aegypti* and *Ae. albopictus*, sewage drains were equally congenial habitat as were plastic, porcelain and earthen habitats. Availability of *Aedes* immature in sewage drains poses increased risk of dengue, and thus vector control programme should consider inclusion of sewage drains as breeding habitat of dengue vector mosquitoes.**

Key words *Aedes* mosquito - energy reserves - larval habitats - pupal productivity - sewage drain

The dengue vectors *Aedes aegypti* and *Ae. albopictus* exploit a wide range of habitats for breeding. The size and shape of such habitats vary extensively ranging from tree-holes[1], discarded tyres and containers[2,3] to water storage tanks[4]. Pupal productivity varies with the available amount of water and resources for larvae[5], which is determined by the size and shape of the *Aedes* breeding habitats[6]. It has been suggested that the extent of competition among developing larvae is influenced by the water content[7] and resource availability[8] in the larval habitats. Thus, the habitat type is an important parameter for successful breeding and subsequent emergence as adults. Life history traits, if adult mosquitoes are dependent on the varying levels of feeding and resource acquisition at the larval stage and thus, water and food resources of larval habitats, carry immense significance in the mosquito life cycle[9]. Hence, the choice and selectivity of oviposition habitat is a crucial factor for continuity of mosquitoes population. For *Aedes* mosquitoes, the pattern of oviposition habitat selection seems to be more generalized rather than selective in terms of habitat permanence and food resource availability, thereby promoting the cosmopolitan presence of *Aedes* mosquitoes worldwide[10].

Although entomological monitoring has established *Aedes* as container breeding mosquitoes[3,10,11], but in selected instances cesspits and sewage systems have also been found to be exploited by *Aedes*[12-15]. Consistent availability of *Aedes* mosquitoes in cesspits and septic tanks over a period of time in different geographical locations suggests a possible change in oviposition behaviour and breeding preference of *Aedes* mosquitoes[14,15]. The availability of *Aedes* in relatively less known habitats[1,12] poses a risk for continued disease transmission, since most of the dengue vector control strategies are based on the established container larval habitats. Exclusion of sewage and cesspits may allow continued breeding and availability of the vectors.

In this study, an assessment of the pupal productivity and energy reserves of *Aedes* mosquitoes from sewage drain habitats was made using Kolkata, West Bengal, India as a model geographical area. The ability of adult mosquitoes to transmit disease depends on the features of the individuals constituting the population. Life history traits serve as proxy markers to comment on the possible variations among individual mosquitoes and their disease transmission potential[16]. Thus, the present study included appraisal of the nutritional reserves and life history traits, along with the pupal productivity of *Aedes* in different larval habitats in Kolkata, India.

Material & Methods

The present study was conducted between January 2009 and December 2011 in the Entomology and Wildlife Biology Laboratory of the department of Zoology, University of Calcutta, Kolkata, India. Evaluation of the life history traits and nutritional reserves was performed under room temperature with a relative humidity of 70 per cent and above, mostly between June 2009 and February 2011.

Scheme for sampling Aedes breeding habitats: Entomological monitoring[11] of the different containers of daily domestic usage and sewage drains as larval habitats of *Aedes* mosquitoes was carried out on monthly basis from selected sites of Kolkata during the three consecutive years (2009-2011). The swage drain water was analyzed with the help of a multi-parameter water analyzer (Multi-parameter PCSTestrTM 35; Eutech Instruments, Singapore; Oaklon®). At each site, the *Aedes* mosquito larval habitats were chosen randomly[11] and 20 numbers of each habitat - earthen pots, porcelain and plastic containers, and sewage drains per month were considered.

Depending on the amount of water volume, the immature from the containers were collected either pulling with a pipette (if > 100 ml approximately)[1] or transferring the whole content (if < 100 ml) in a separate specimen containers (Tarson® specimen container, India, 100 ml capacity)[6]. Sewage drains were sampled using short plankton net (7 or 9 cm diameter, 200 µm mesh size) moving horizontally and dredging through the bottom sediment as well. For all containers and sewage drains excess water was flushed and sieved with the help of plankton net (200 µm mesh size, rectangular in shape 30 x 15 cm) or using a circular plastic net (10 mm mesh size) to collect remaining immature, if any. The collected immature stages of *Aedes* mosquitoes were kept in the specimen containers with 75 ml water and covered with nylon net (10 mm mesh size), and brought to the laboratory for count and recording. Although the sampling design included all the possible habitats, the differences in proportional numbers of positive habitats could be a possible source of sampling error. Several other mosquitoes belonging to genera *Culex, Anopheles, Armigeres, Lutzia*, and *Toxorynchites* were encountered during sampling, but for the present study, only *Ae. aegypti* and *Ae. albopictus* were considered for analyses.

Laboratory parameters for assessment of the life history traits and nutritional reserves: Following collection of both larvae and pupae, and subsequent segregation, *Aedes* pupae were reared in plastic trays (15 x 11 x 3 cm) to obtain adults for evaluation of nutritional reserve. Individual pupa of *Aedes* spp. was weighed to record the pupal weight (wet weight up to nearest 0.1 mg using Mettlar-Toledo® Al-104 balance, Hong Kong), following which, each pupa was placed in individual vials, and allowed to emerge as adult. The sex and species of the adults were identified based on appropriate keys[17]. Following natural death of *Aedes* adults, the wing length (WL) of adults was measured to the nearest 0.1 mm using a dissecting stereo microscope (Olympus® SZX, Olympus Corporation, Tokyo, Japan) fitted with a graduated eyepiece (Erma®, Japan) (appropriate magnification, scaling and conversion of eyepiece to mm was followed). The adults were sacrificed for biochemical analysis for assessment of nutrient reserves such as amount of glycogen, sugar and lipid in *Ae. aegypti* and *Ae. albopictus* using single individual at a time[18,19].

Statistical analysis: To comment on the variations in the abundance of *Aedes* mosquitoes based on habitat types and months, the data on relative abundance of *Aedes* sp. were onsidered for three-way factorial ANOVA using month, species and habitat as variables[20]. The data on pupal weight and wing length of both the species and of either sex were also subjected to three-way factorial ANOVA, taking species, sex and habitat types as variables. For both *Ae. aegypti* and *Ae. albopictus*, correlation among life history traits and sex was estimated. The data obtained on the life history traits of *Ae. aegypti* and *Ae. albopictus* [*i.e.* pupal weight, PW (in mg), wing length, WL (in mm), amount of glycogen, GLY (in mg), amount of sugar, SUG (in mg), amount of lipid, LPD (in mg) in males and females] were used as explanatory variables for discriminating the habitats as dependent variables. The discriminant function analysis was performed to justify the habitat based differences in the life history traits of the mosquito species. DA would reflect the quality of sewage drain habitats against known habitats exploited by *Aedes* mosquitoes at a proximate level. The statistical analyses were performed following Zar[20] using XLSTAT software[21].

Results

The number of individual *Ae. aegypti* and *Ae. albopictus* varied seasonally between the different habitats *viz*. various types of containers and sewage drains. The monsoon and post-monsoon months, from June to November, were noted to be most productive in sustaining the dengue vectors irrespective of the habitats. However, in sewage drains *Ae. albopictus* was observed more as compared to *Ae. aegypti* (Fig.1). Analysis of the swage drain water indicated *p*H (7.78 ± 0.07), temperature (30.66 ± 0.97°C), electrical conductivity (2.754 ± 0.3 mS/cm), total dissolved solids (1.956 ± 0.23 ppt) and salinity (1.598 ± 0.18 ppt), similar to the gathered rainwater in mosquito larval habitats described elsewhere[14,15]. The immature productivity was found to decrease in winter and summer seasons. The results of 3-way factorial ANOVA revealed that the abundance varied significantly ($P<0.001$) with the types of habitats, sampling months and species of the mosquitoes (Table I). The results of post-hoc Tukey test indicated significant ($P<0.001$) variations between all habitat pairs except between porcelain containers and sewage drain interaction. In both the species variations in the life history traits and energy reserves were prominent between the sexes and across the habitats

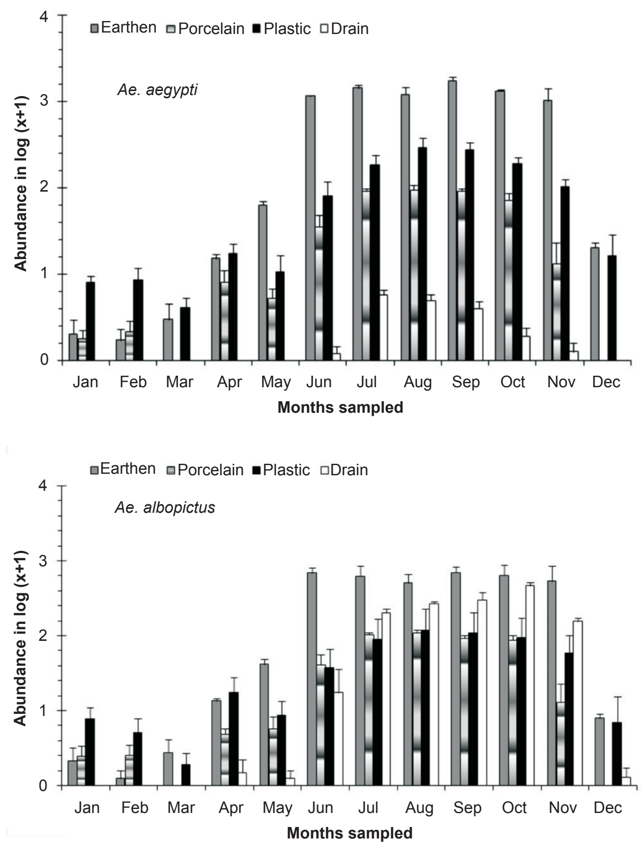

Fig. 1. Variation in the mean abundance [log (x +1) transformed] of *Aedes* sp. in different habitat types observed in Kolkata, India during the study period (January 2009-December 2011). Values are mean ± SE (n=60).

(Fig. 2). Irrespective of habitats the mean pupal weight of males was 2.04 ± 0.25 mg (range 1.21 - 2.51 mg) while the mean weight of female pupae was 3.42 ± 0.25 mg (range 2.87 - 4.02 mg). As noted in the variation in the pupal weight, the mean wing length of male was 3.08 ± 0.21 mm (3.65 - 3.65 mm); compared to this, mean wing length of females was 33.44 ± 0.09 mm (3.28 - 3.67 mm). The glycogen and sugar contents of the males (0.12 ± 0.02 and 0.05 ± 0.01 mg, respectively) were also found to be lower than the females (0.18 ± 0.02 and 0.13 ± 0.007 mg, respectively). Similarly, for *Ae. albopictus*, males were lower in pupal weight (1.29 ± 0.05 mg; range 1.16 - 1.42 mg), and smaller in wing length (2.04 ± 0.12 mm; range 1.73 - 2.6 mm) than the females (pupal weight 2.29 ± 0.09 mg; range 2.07 - 2.46 mg; wing length 2.71 ± 0.09 mm, range 2.48 - 2.93 mm). The glycogen and sugar contents of the males (0.21 ± 0.04 and 0.15 ± 0.01 mg, respectively) were also found to be lower than the females (0.23 ± 0.03 mg and 0.20 ± 0.01 mg, respectively). Results of ANOVA on life history traits (pupal weight and wing length) and energy reserves (glycogen, sugar and lipid contents) revealed significant (P<0.001) variation between species, sex and types of habitats (Tables II and III). Species-specific variation and dependence of the pupal weight, wing length as well as the energy contents on the habitats was evident. However, interaction between the *Aedes* species (SP) and larval habitat (H) (Table II) and sex of the *Aedes* mosquitoes (Table III) did not impact the variation in wing length and glycogen content, respectively. The non-significance may be attributed to the independent variation of the variables. In all cases of pupal weight, wing length, glycogen, sugar and lipid content variation, the results of post-hoc Tukey test indicated significant differences between the habitat types. For both the sexes of either *Ae. aegypti* and *Ae. albopictus,* the different life history traits were correlated (Table IV).

Differences among the habitats were reflected in the biplot suggesting qualitative differences manifested in terms of *Aedes* life history traits (Fig. 3). Discriminant function analysis for *Ae. aegypti* indicated that the first two factors (F1 and F2) accounted for >99 per cent of variations of the data enabling discrimination among the four habitat types (sewage drains, earthen, plastic and porcelain containers) (Fig. 3A). The corresponding canonical correlations for F1 and F2 were 0.998 and 0.992, respectively for both the species. The same was apparent in *Ae. albopictus*, where factors F1 and F2 justified >99 per cent discrimination among the habitat (Fig. 3B). The corresponding canonical correlations for F1 and F2 were 0.914 and 0.758, respectively. For both the species, Fisher's distance varied significantly (P<0.05) among all the habitat pairs.

Perhaps the water retention ability and resource availability of different habitats played a critical role in determining the trait values of dengue vectors. The sewage drain contributed towards production of adults having greater pupal weight and wing length and with higher values of energy reserves. Individuals of *Ae. aegypti* were noted to be heavier than *Ae. albopictus* and the females of both the species weighed more than the males. The same was true for wing length variation and nutrient reserve contents.

Discussion

The ability to breed in a wide variety of artificial containers facilitates the geographical range expansion and extensive spread of *Ae. aegypti* and *Ae. albopictus*[10,11,22]. The quality of the larval habitats determines the pupal productivity and adult features of *Aedes* mosquitoes[8,13], which in turn are linked to the disease transmission potential[5,7,16]. Food resources in the larval habitats[8,9], and competitions[5,7] among developing individuals determine the adult size and thus fitness of *Aedes* mosquitoes. The numerical abundance of one species of *Aedes* over the other varies with the quality of the larval habitats[23,24]. This was evident in the present study where *Ae. albopictus* was found in greater number in the sewage drains compared to *Ae. aegypti*. The size of the adults of both the mosquito species emerging from sewage drains was bigger than those emerging from rest of the larval habitats.

Table I. Variance ratio (F) and corresponding significance levels of three-way factorial ANOVA on the abundance of *Aedes* immature using months, habitat types and species as variables

Sources of variation	df	F	P value
Species (S)	3	4015.63	0.001
Habitat (H)	3	3309.48	0.001
Month (M)	11	1295.38	0.001
S x H	9	1027.33	0.001
S x M	33	276.84	0.001
H x M	33	205.64	0.001
S x H x M	99	81.47	0.001
Error	29548		
Total	29739		

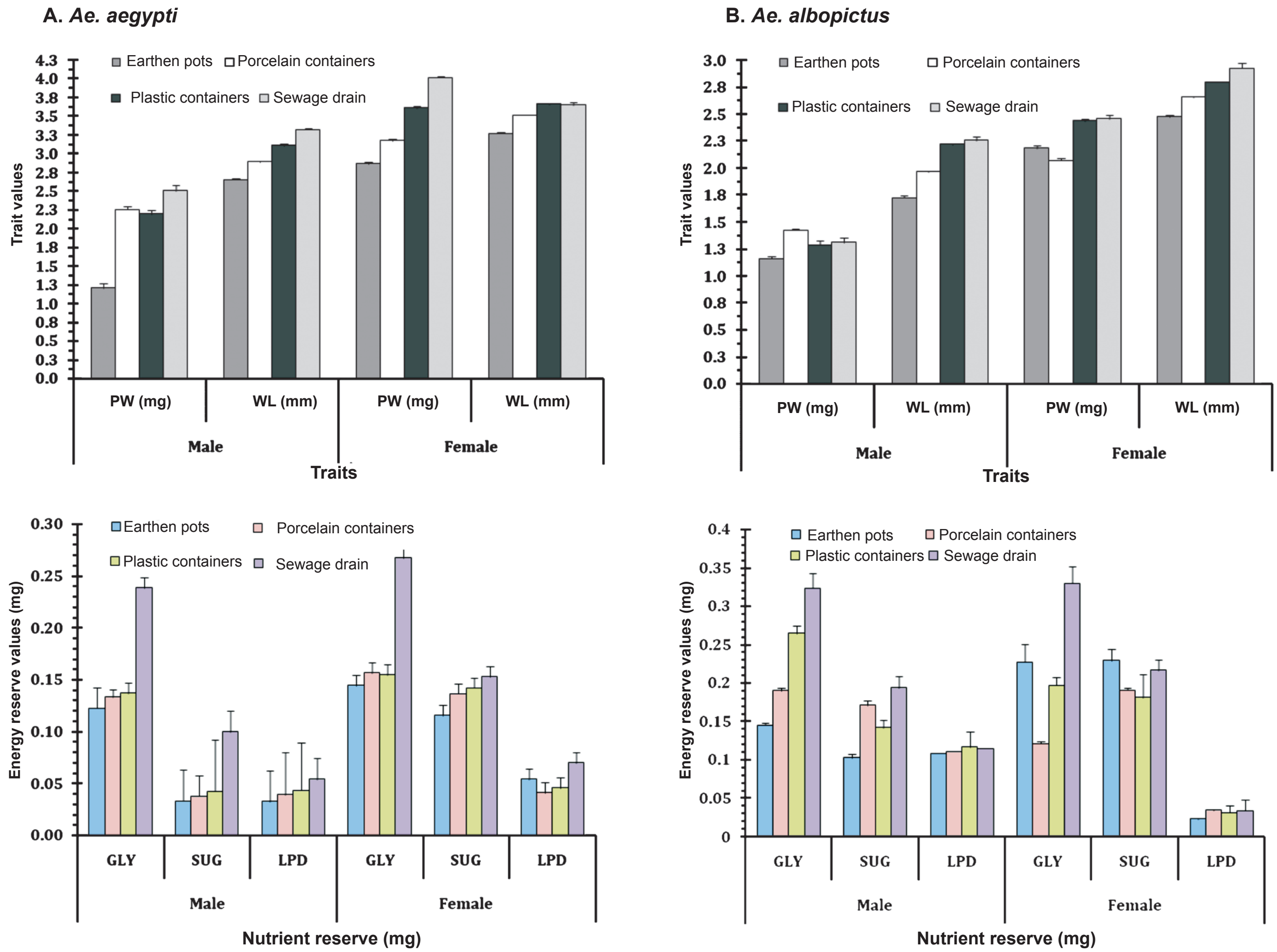

Fig. 2. Habitat-based variation in different life history traits of *Ae. aegypti* **(A)** and *Ae. albopictus* **(B)** [pupal weight (PW in mg), wing length (WL in mm), glycogen (GLY in mg), sugar (SUG in mg) and lipid (LPD in mg)] content in individual mosquito. Values in each bar are mean ± SE (n=60).

Table II. Variance ratio (F) and corresponding significance levels of three-way factorial ANOVA on life history traits namely pupal weight and wing length of *Aedes* mosquito considering species, sex and habitat types as explanatory variables

Sources of variation		Pupal weight		Wing length	
	df	F	*P* value	F	*P* value
Species (SP)	1	3935.69	0.001	4058.75	0.001
Sex (S)	1	6306.33	0.001	122.11	0.001
Habitat (H)	3	416.88	0.001	261.34	0.001
SP x S	1	167.09	0.001	1414.87	0.001
SP x H	3	198.69	0.001	0.25	0.86118
S x H	3	77.97	0.001	70.28	0.001
SP x S x H	3	8.31	0.001	73.6	0.001
Error	944				
Total	959				

Table III. Results of three way factorial ANOVA on energy reserves namely glycogen sugar, and lipid of *Aedes* mosquito considering species, sex and habitat types as explanatory variables

Sources of Variation	df	Glycogen		Sugar		Lipid	
Species (Sp)	1	F	*P* value	F	*P* value	F	*P* value
Sex (S)	1	126.31	0.001	590.99	0.001	5951.66	0.001
Habitat (H)	3	1.34	0.2474	382.09	0.001	13066.8	0.001
Sp x S	1	166.51	0.001	34.07	0.001	373.54	0.001
Sp x H	3	13.03	0.0003	18.51	0.001	21829.2	0.001
S x H	3	11.23	0.001	3.05	0.001	209.65	0.001
Sp x S x H	3	13.73	0.001	16.19	0.001	55.9	0.001
Error	944	12.94	0.001	16.46	0.001	89.57	0.001
Total	959						

Table IV. Pearson correlation matrix between the life history traits of male **(A)** and female **(B)** individuals of *Ae. aegypti* and *Ae. albopictus*. The values marked bold are significant at *P*<0.001 level. [pupal weight (PW in mg), wing length (WL in mm), glycogen (GLY in mg), sugar (SUG in mg) and lipid (LPD in mg)]

A. Male				
Ae. aegypti				
Variables	PW	WL	GLY	SUG
WL	**0.315**			
GLY	**0.494**	-0.112		
SUG	**0.348**	**-0.644**	**0.650**	
LPD	**0.727**	0.053	**0.633**	**0.672**
Ae. albopictus				
Variables	PW	WL	GLY	SUG
WL	**0.193**			
GLY	0.056	**0.818**		
SUG	0.122	**0.530**	**0.749**	
LPD	**0.349**	**0.516**	**0.250**	0.065
B. Female				
Ae. aegypti				
Variables	PW	WL	GLY	SUG
WL	**0.941**			
GLY	**0.805**	**0.868**		
SUG	**0.738**	**0.729**	**0.572**	
LPD	**0.590**	**0.688**	**0.839**	**0.442**
Ae. albopictus				
Variables	PW	WL	GLY	SUG
WL	**0.456**			
GLY	0.111	**0.219**		
SUG	**-0.208**	**-0.234**	**0.570**	
LPD	**0.246**	**0.740**	-0.039	**-0.255**

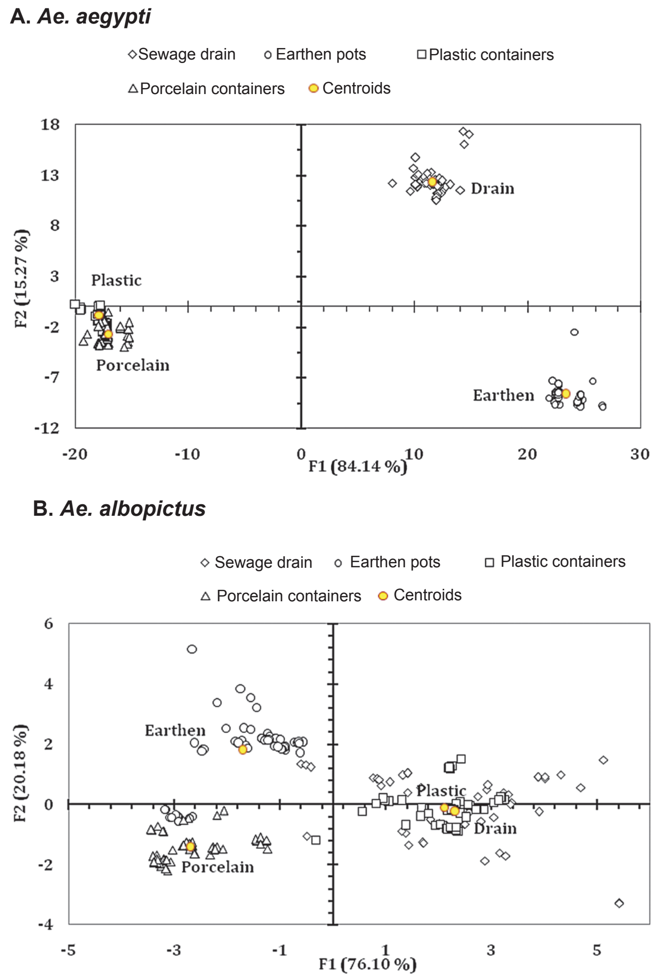

Fig. 3. Biplot representation of the ordination of the abundance of **(A)** *Ae. aegypti* and **(B)** *Ae. albopictus* in the different habitats surveyed as explained by the life history traits and energy reserves. For *Ae. aegypti* Wilks' $\lambda = 0.00002$; $F_{30, 667} = 948.054$; $P<0.001$. For *Ae. albopictus* Wilks' $\lambda = 0.056$; $F_{30, 667} = 37.163$; $P<0.001$.

Possibly the resource content of the sewage drains was higher than other habitats, which contributed to the development of large mosquitoes with higher nutrient reserves (indicated by pupal weight and wing length). Larval effort to acquire and assimilate energy is reflected through the corresponding pupal weight, nutrient reserve and adult body size[23,24]. As a consequence, correlation between pupal weight, nutrient (glycogen, lipid and sugar) reserves, and wing length (body size) of mosquitoes was observed for both *Ae. aegypti* and *Ae. albopictus* in the present study. From disease transmission perspective, female *Aedes* mosquitoes with bigger body size than males will provide better biotope for the parasite and viruses to thrive. Inclusion of pupa weight and wing length[5,7,16] as additional parameters will enhance the existing entomological surveillance of dengue vectors[10,11].

In terms of abundance of immature of *Aedes* mosquitoes, the sewage drains were relatively less exploited compared to the other habitat types. The pupal weight, adult wing length and energy reserves indicated that sewage drains were equally congenial habitats as the established larval habitats like earthen, plastic and porcelain containers in the study area. Earlier studies[2,3,12] have shown that the availability of the containers is linked to household waste generation, and the subsequent pupal productivity in the containers varies with relative density and resources present for larval development[8,9,24,25]. As sewage drains are not restricted in resource availability, these seem to be more favourable habitat for *Aedes* mosquitoes to thrive. However, container habitats with lower density of immature or higher nutrient reserve may provide equal opportunity to yield bigger mosquitoes. Our observations suggested that the sewage drains were utilized under conditions when the relative abundance of *Aedes* remained high (June to November) coinciding with monsoon and post-monsoon seasons. It is possible that addition of rainwater may have led to an overflow of water from the containers or reduce the organic matter content of the container habitats rendering them less effective as larval habitat. Alternatively, to avoid competition, and gain access to nutrients resources, breeding in water logged sewage drains was a better choice. Further monitoring of *Aedes* larval habitats including sewage drains can substantiate these observations.

Breeding of dengue vectors in unconventional habitats like sewage drains, underground water tanks[4], and septic tank and cesspits[13-15], poses a concern for vector management. Following conventional strategy for *Aedes* control, if containers and smaller habitats are only targeted, as an alternative sewage drains and similar water bodies like cesspit and septic tanks may be utilized for breeding, resulting in comparatively fit individuals with higher reproductive success. From sewage drains, less number of individual *Aedes* mosquitoes with higher reproductive success may compensate for the loss due to check on container habitats. Continued water availability in sewage drains, septic tanks and cesspits make them permanent habitats for breeding in contrast to the container habitats, which are dependent on rain water or other sources. Although *Aedes* mosquitoes are known to occupy smaller habitats that are prone to drying, the risk to invasion in sewage drains and subsequent adaptation may increase the relative abundance in different geographical areas. Entomological monitoring for vector management may thus include sewage drains as habitat to reduce possibilities of breeding and sustenance of *Aedes* mosquitoes in the concerned geographical area.

Acknowledgment

The authors thank the respective Heads of the department of Zoology, University of Calcutta, Kolkata and The University of Burdwan, Burdwan for the facilities provided including DST-FIST. The first author (SB) acknowledges the financial assistance provided by the Council of Scientific & Industrial Research (CSIR), New Delhi, through Senior Research Fellow (SRF), in carrying out the work. The last author (GA) acknowledges the University Grants Commission (UCG), New Delhi for providing financial assistance through UGC Research Award.

***Conflicts of Interest*:** None.

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