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Seasonal prevalence of *Aedes aegypti* in urban and industrial areas of Dibrugarh district, Assam

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Abstract. In Dibrugarh district, one of the most populous districts of Assam, there is a potential risk of dengue virus transmission by the mosquito *Aedes aegypti* during the autumn, in the urban and industrial areas of the district. The seasonal patterns of oviposition activity and abundance of breeding sites of this vector were studied in two areas of the district. From January 2010 to December 2010, we examined a total of 767 ovitraps and 6562 water filled containers. Both study areas showed high percentages of positive ovitraps (urban: 18.2% and industrial: 45.08%) and positive breeding sites (urban: 22.8% and industrial: 53.5%) for *Aedes aegypti* with higher abundance in the post monsoon season. In industrial areas, percentages of breeding sites and positive ovitraps were high as compared to the percentages in the urban areas. The percentages of breeding sites and the positive ovitraps are high in autumn when compared to the percentages of the other seasons. In both the areas and with both the methodologies the highest infestation levels were registered in October (ovitraps: 29.3% and 65.1%, breeding sites: 27.5% and 65.2%). This highest abundance took place after heavy rainfall. A sharp decline in oviposition activity was observed when monthly room temperature decreased to16°C with low rainfall (38mm).

INTRODUCTION

Dengue is an acute viral infection with potential fatal complications. Emergence and reemergence of frequent dengue haemorrhagic fever in the Indian subcontinent has become a severe problem in the recent years. The first epidemic of clinical dengue like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern coast of India in 1963–1964 (Sarkar *et al.*, 1964, Chattergee *et al.*, 1965; Carey *et al.*, 1966). The first major epidemic of the DHF occurred in 1953-54 in the Phillippines followed by a quick global spread of epidemics of DF/DHF (Rigau-Perez *et al.*, 1998). DHF was occurring in the adjoining countries but it was absent in India for unknown reasons as all the factors were present. The DHF started simmering in various parts of India since 1988. The first major widespread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi (Dar *et al.*, 1999) and Lucknow (Agarwal *et al.*, 1999) and then it spread to all over the country (Shah *et al.*, 2004). New Delhi, the capital of India, located in the northern region of the country, experienced seven major outbreaks between 1967 and 2003 (Broor *et al.*, 1997; Gupta *et al.*, 2005). Then in 2006, another major outbreak occurred with more than 11,000 reported cases and 165 reported fatal cases (WHO, 2009). Rapid growth of the population and sudden climatic changes has contributed to the increase in cases of DF/DHF in India (Kukreti *et al.*, 2008). The causative agent of dengue is DENV (DENV-1, DENV-2, DENV-3 and DENV-4), a single-stranded, positive sense RNA, enveloped virus, is a member of the genus *Flavivirus* of the Flaviviridae family. It is generally transmitted to humans via *Aedes aegypti* and *Aedes albopictus* mosquitoes (Dutta & Mahanta, 2006).

Aedes aegypti (L) and *Ae. albopictus* (Skuse), the main dengue vectors, were found to be breeding in different container habitats of the north-eastern states (Dutta & Mahanta, 2006) and with the increased industrialization and urbanization of the region, the breeding grounds and the environment are becoming more and more suitable for their rapid multiplication. DENV activity has been documented in Assam (Rodrigues & Dandawate, 1977), Arunachal Pradesh (Rodrigues & Dandawate, 1977) and Nagaland (Dutta & Mahanta, 2006). Entomological survey carried out in different time periods reveals the prevalence of potential dengue vectors in this region (Khan *et al.*, 1996; Dutta *et al.*, 1998, 1999, 2004). A comprehensive entomological survey conducted during 2004–2005 in the seven states of the northeastern (NE) region of India revealed that the region is rich in known dengue vectors, viz. *Ae. aegypti* and *Ae. albopictus* (Dutta *et al.*, 2006).

In a viral sero-survillance study by the Regional Medical Research Centre (ICMR), Dibrugarh, Assam, since 1992, dengue viral antibody had been detected from Dibrugarh, North Lakhimpur, Dhemaji and Golaghat districts of Assam. During our study period itself, several tests were found positive for dengue by RMRC, Dibrugarh and the distribution of all the four DENV serotypes was found in North East Region. Thus the presence of dengue virus in the area together with high infestation levels of the vector pose an actual risk of dengue outbreak in the district as well as in the other districts of Assam in coming years.

Reproduction of *Ae. aegypti* populations from tropical and subtropical zones occur all year round and their abundance can either be associated with rainfall regimens (Moore *et al.*, 1978, Chadee *et al.*, 1991, 1992; Kalra *et al.*, 1997; Micieli & Campos, 2003) or not (Sheppard *et al.*, 1969, Barrera *et al.*, 1997). Assam is situated in the subtropical region where temperature varies in different seasons and heavy rainfall occurs in a particular period, which is highly suitable for proliferation of different mosquito species.

So, this year long survey was conducted to find out the seasonal prevalence and oviposition of dengue vector, *Ae. aegypti* in urban and industrial areas of Dibrugarh district depending on temperature fluctuations and rainfall. The adult mosquito is directly affected by temperature, relative humidity and rainfall, but larval life is mainly affected by rainfall and water temperature (Micieli & Campos, 2003). In this study, temperature of all wet containers' water could not be taken due to resource constraint. The seasonal density here was calculated based on the rainfall only. This study was a part of detailed entomological surveillance activities carried out in urban and industrial areas of Dibrugarh district taking different urban and industrial containers as the study model. The other objective of the study was to see whether *Ae. aegypti* population has established here in great density and also to know the effect of temperature and rainfall on the density of dengue vector mosquito *Ae. aegypti* to evaluate its seasonal breeding pattern.

MATERIALS AND METHODS

Study area

Dibrugarh district (27p 282 N 94p 552 E), Assam, is located at a mean altitude of 108 m (354 ft.) above sea level, in the bank of river Brahmaputra. It covers an area of about 3381 km^2. Population of Dibrugarh district as per 2010 census is about 1.4 crore. Climate is subtropical monsoon climate with mild winter and warm and humid summer. The average rainfall of the Dibrugarh is 2185.7 mm annually. The average annual temperature in Dibrugarh is 22.9p c (last ten years average).

The areas included under municipality are taken as urban areas which are highly populated with rapid urbanisation and industrialisation. Project sites of Brahmaputra Cracker and polymer Limited (BCPL), Dibrugarh, Oil India Corporation, Dulijan and various small and large factories and industries are selected as industrial locations. The areas included in the study are highly populated with rapid urbanisation and industrialisation. As a consequence of rapid urbanisation and industrialisation with high population density with unplanned developments as any other districts of the third world countries lead to a highly polluted environment with the absence of proper drainage system. As a result, Dibrugarh city as well as different areas of the district constantly face severe water logging after each heavy shower. As such the environment is highly suitable for the proliferation and spread of diseases and promotes infestation by mosquitoes and other pests. Population density is more in the urban areas living in a total unhygienic condition. Moreover due to shortage of pipe water facilities, people store water in big containers. Most of the people are employed in Government or private organisations or in industries or some are daily workers and as agricultural activity is totally absent, which have changed the feeding habits of the dwellers and as such more containers of different kinds are used and carelessly discarded increasing solid waste pollution and thus increasing *Aedes* breeding sites. In the ongoing projects of the industrial areas, open drains, large water reservoirs, different types of iron equipments and pipes store more or less water serving as grounds for *Aedes* proliferation.

The study was conducted in the containers present in different urban and industrial areas of Dibrugarh district. In the areas, containers were selected based on their contrasting environmental features and *Ae. aegypti* container indexes (percentage of water holding containers infested with larvae or pupae) (PAHO, 1994). Household containers mainly include waste domestic containers, plastic mugs, buckets, bottles, coconut shells, old unused tyres and battery boxes, various cans, old water tanks, flower vases, earthen pots, bamboo stumps, containers of different food items etc. which were mostly present outside the main houses. These containers of urban and suburban areas of Dibrugarh town are found to be infested mainly with *Ae. albopictus* (when larvae are collected, reared and identified as adults immerged). On the other hand, from the industrial locations, mainly from the ongoing project sites, small and large factories of the town, different containers were randomly selected which are mainly found to be infested with *Ae. aegypti*.

Variations of abundance of *Ae. aegypti* were calculated by two techniques:–

a) Monitoring of breeding sites and
b) Monitoring of oviposition activity

The monitoring of breeding sites consisted of monthly inspections of randomly selected containers (any receptacle capable of containing water), which could not be resampled within a one month period to avoid pseudo replications. The water in each container was filtered with a fine mesh strainer. All pre-imaginal stages were transferred to a beaker after counting, labelled according to date, time of collection and area and reared in tap water with food (biscuit powder and yeast powder in the ratio of 1:1) till adults get emerged. After adult immergence, adults were identified as *Ae. aegypti* and data were managed accordingly. The frequency of occurrence of other mosquito species was not assessed but records for *Ae. albopictus* were maintained separately.

In the urban and suburban areas household containers were examined monthly from the beginning of January 2010 until the end of December 2010. A total of 780 households in the urban areas and 456 households in the suburban areas were surveyed for *Aedes* breeding sites.

In the industrial areas, the containers were examined in the same time that is from the beginning of January 2010 to December 2010 which was present in open areas as well as in the adjacent sites to the industrial premises.

Oviposition was monitored monthly using tyres as ovitraps that were homogeneously distributed in both urban and industrial areas. Tyres were selected as ovitraps because through our investigations it was found that tyres are the most preferred habitat for both *Ae. albopictus* and *Ae. aegypti*. Each tyre had a capacity of 1.5 L and also contained 1.5L of water. Each ovitrap was examined monthly; larvae and pupae were collected out from ovitraps with the help of small cups. Water from the tyre containing preimaginal stages were transferred to another container with the help of small cups and spoons and reared till adult emergence and species identification was confirmed using adult dichotomous keys of Darsie & Morris (2000). Each ovitrap was not resampled within a one month period to avoid pseudo replication and after sampling, each time the tyres were totally emptied, washed and replaced with fresh water. So, there was a least chance of introducing *Aedes* breeding sites instead by removing and destroying the preimaginal stages, *Aedes* proliferation was minimised.

In urban areas a total of 401 ovitraps and in the industrial areas 366 ovitraps were inspected monthly from the beginning of January 2010 until the end of December 2010.

Data Analysis

The percentage of *Ae. aegypti* breeding sites was defined as the percentage of water holding containers infested with at least one larvae or pupa. Percentages of positive ovitraps (with larvae) were compared between urban and industrial sites, respectively, with the t test for two independent proportions. Such comparison was undertaken using mean values obtained during the period when both the sites were simultaneously studied (breeding sites: January 2010-December 2010; ovitraps: January 2010-December 2010). Additionally, separate t tests were performed for each month because we got data for the species in all the months during our study period in both the urban and the industrial sites.

Seasonality was studied in each area by comparing the infestation levels among the four seasons as well as among the twelve months of the year 2010 with a χ^2 test for multiple proportions (Fleiss *et al.*, 1981). A descriptive analysis of the fluctuations of the abundances of the species in the two separate environmental settings was made using monthly values of breeding sites and oviposition activity.

Finally, we evaluated the association of mosquito abundance with monthly precipitation and temperature, which were calculated from the data provided by the Meteorology Department, Tea Research Centre, Dikom, Assam.

RESULTS

A total of 2556 breeding sites of *Ae. aegypti* were found among the 6562 water filled containers examined. In urban areas 708 containers were found positive for *Ae. aegypti* out of 3111 total wet containers observed. In industrial areas, a total of 1848 positive breeding sites were found out of total 3451wet containers. In case of positive ovitraps a total of 73 positive ovitraps were found in urban areas out of total 401 ovitraps but in the industrial areas a total of 165 positive ovitraps were found out of total 366 ovitraps. The percentage of breeding sites for *Ae. aegypti* in industrial areas (53.5%) is significantly higher than that in the urban areas (22.8) with t value 7.65. This difference of infestation of *Ae. aegypti* in the two different environmental settings was significant during all months studied.

The difference in the percentages of positive ovitraps in the two different environmental settings is also significant (t value 14) with higher values in industrial areas (45.08%) than in the urban areas (18.2%).

In regard to seasonality, the percentages of breeding sites for the species in both the environmental settings showed significant differences among the months(urban areas: χ^2=19.7 and in industrial areas: χ^2=46.23 respectively).In both the environmental settings, the percentages of breeding sites and percentages of positive ovitraps for *Ae. aegypti* was significantly higher in post monsoon season (percentage of breeding sites: 27.05% and 59.7%, percentage of positive ovitraps: 27.0% and 59.0% respectively for urban and industrial areas) than in summer, monsoon and winter ($p<0.01$). The percentages of positive ovitraps also showed significant differences

Table 1. Seasonal percentages of positive breeding sites for *Aedes aegypti* in urban and industrial areas of Dibrugarh district

Seasons	In urban areas		In industrial areas		% of breeding sites	
	No. of +ve breeding sites for *Ae. aegypti*	Total wet containers	No. of +ve breeding sites for *Ae. aegypti*	Total wet containers	Urban areas	Industrial Areas
Winter	94	515	185	595	18.25	30.76
Summer	162	650	379	658	24.9	57.6
Monsoon	221	1092	788	1364	20.2	57.8
Post Monsoon	231	854	498	854	27.05	59.7

Table 2. Seasonal percentages of positive ovitraps for *Aedes aegypti* in urban and industrial areasd of Dibrugarh district, Assam

Seasons	In urban areas		In industrial areas		% of positive ovitraps	
	No. of +ve ovitraps for *Ae. aegypti*	Total wet containers	No. of +ve ovitraps for *Ae. aegypti*	Total wet containers	Urban areas	Industrial Areas
Winter	14	100	29	93	14	31.2
Summer	15	104	40	90	14.4	44.4
Monsoon	15	89	37	83	16.8	44.6
Post monsoon	29	108	59	100	27	59

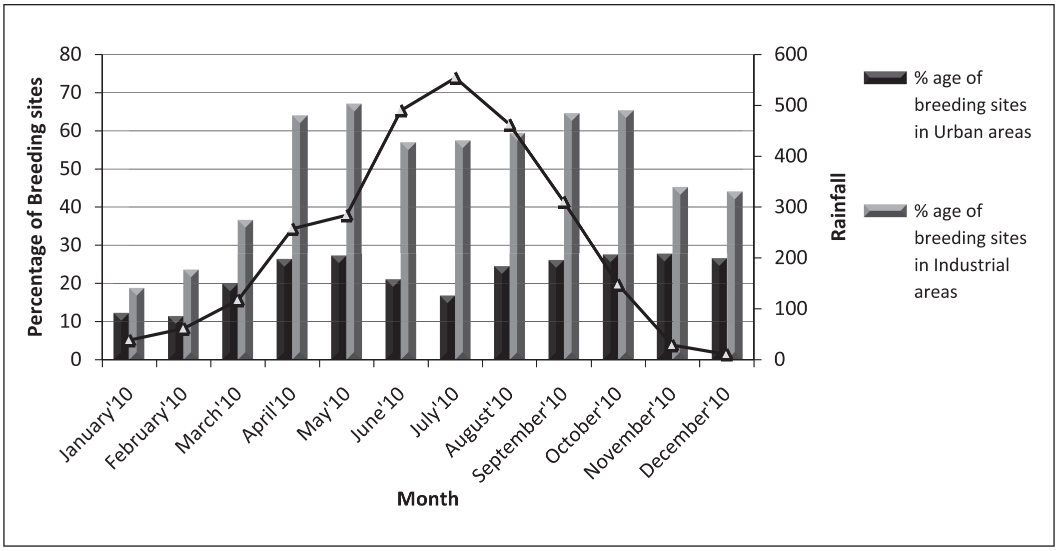

Figure 1. Monthly percentages of breeding sites for *Aedes aegypti* in urban and industrial sites of Dibrugarh district; period - January 2010-December 2010

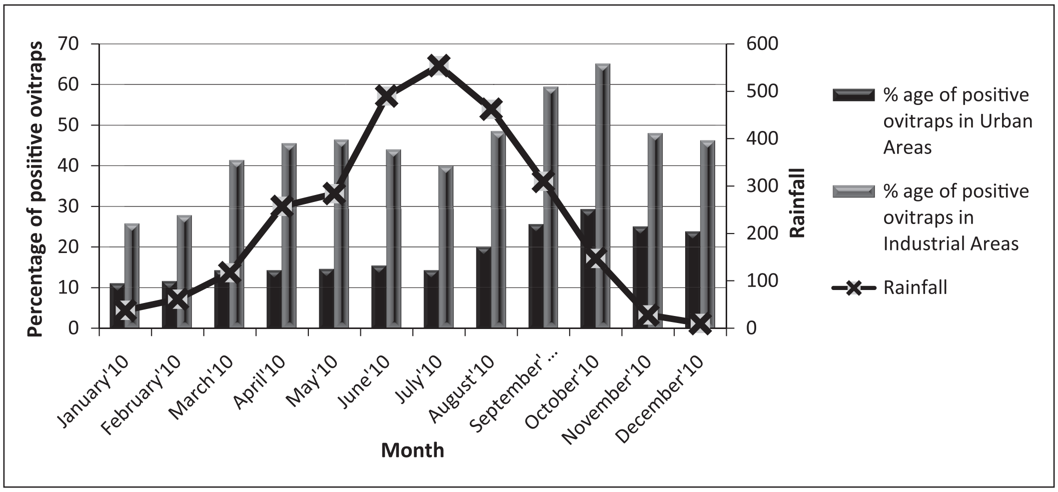

Figure 2. Monthly percentages of positive ovitraps for *Aedes aegypti* in urban and industrial sites of Dibrugarh district; Assam, period: - January 2010- December 2010

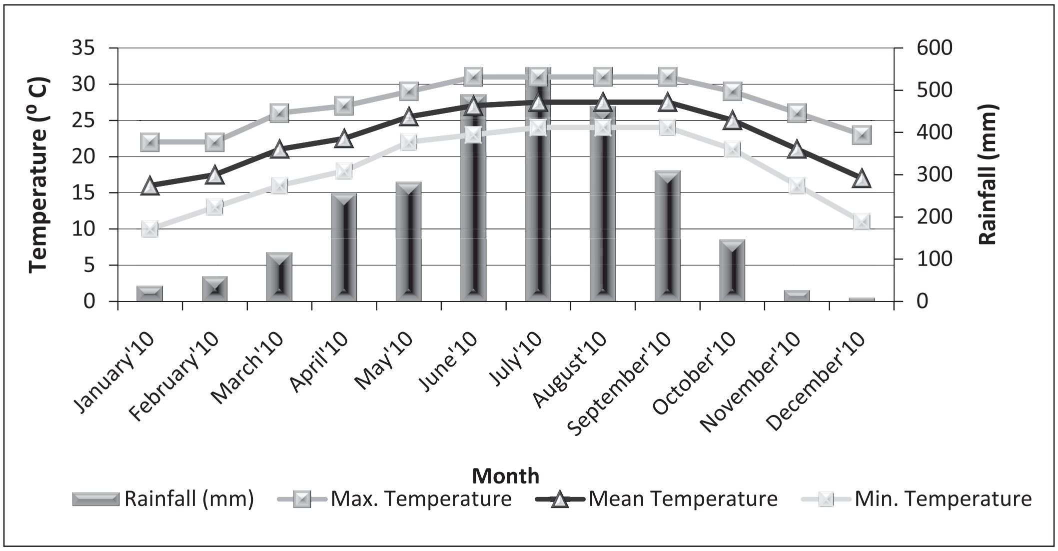

Figure 3. Meteorological conditions (temperature and precipitation) in Dibrugarh, Assam during the period January 2010-December 2010 (National Meteorology Service)

among months for *Ae. aegypti* (urban areas: χ^2=22.9, industrial areas: χ^2=30). In both the areas the values obtained in the post monsoon season were significantly higher than those in the other seasons (p<0.01).

The presence of immature stages was recorded throughout the year. Maximum infestation of breeding sites was reached in the month of October for both the type of areas (urban: 27.5%, industrial: 65.2%).

Oviposition was monitored from January 2010 till December 2010. The percentages of positive ovitraps increased gradually from January to May and then showed a sharp decline in June and July. The highest oviposition activity was registered in the month of October for both urban and industrial areas (urban: 29.3%, industrial: 65.1%).

In general terms, the highest proliferation of *Ae. aegypti* occurred during the months with favourable conditions, after heavy rainfall. The relationship of increasing larval proliferation with meteorological conditions are shown in the Figure 3 where the seasonal pattern of abundance of *Ae. aegypti* was fairly close to the variations in rainfall which results in the formation of suitable habitat for *Aedes* proliferation. Highest percentages of breeding sites and positive ovitraps were recorded during the months of September, October and November after heavy rainfall from June to September and the lowest percentages were recorded in the months of January and February (percentage of breeding sites: 15.55% and 17.5%, percentage of positive ovitraps: 18.4% and 19.7%) having low temperature (16p c and 17.5p c) after a dry period. Overall, during the study period, we found a very high percentage of *Ae. aegypti* population (percentage of breeding sites: 38.15%, percentage of positive ovitraps: 31.64%) which may have resulted into several dengue positive cases and the first dengue death in Dibrugarh, Assam.

DISCUSSION

The seasonal prevalence of dengue vector, *Ae. aegypti*, has been recorded in the urban and industrial areas of Dibrugarh district of Assam. The mosquito population dynamics of this study shows similar seasonal pattern with other studies related with *Aedes* breeding world wide with a little variation. Vezzani *et al*. (2004) found the highest *Ae. aegypti* density with accumulated rainfalls above 150 mm. Micieli & Campos (2003) observed the close relation of the highest peak of *Ae. aegypti* population with high rainfall, and the population decreased for the months with less rainfall. During the present study period, we got a very high percentage of *Aedes* population with an annual average rainfall of 2758 mm, which is much higher than the average rainfall of the last ten years (2185.7 mm) and the highest density was found in the post monsoon season. The highest rainfall was noted during the months of June, July and August (490 mm, 554 mm and 462 mm respectively) but the larval population was not found to be highest, as expected, during these months, since, due to heavy rain, most of the containers were washed away with rain water, heavy rain fall may also create problem in adult dispersal and mating. Data for *Ae. albopictus* was also obtained from the areas studied. The detailed data regarding seasonality of *Ae. albopictus* was presented in an international seminar and also were published in a journal. A very high level of infestation by *Ae. albopictus* was obtained from the district (% of breeding sites=34.06 and % of positive ovitraps=34.2).

In regard to population dynamics, since mean temperature above 17p c, that are required for successful hatching (Christophers, 1960), is registered throughout the year in the district, except in the month of January, the temperature of the study area is highly suitable for *Aedes* proliferation.

Toma *et al*. (1982) found the greater larval abundance of *Ae. albopictus* in July and August in Japan and the U.S.A. Akram & Lee (2004) recorded peak occurrence of *Ae. albopictus* from May to July (34.0%, 35.1% and 30.9%) in South Korea when the rainfall was higher than the other seasons. They also observed that the population showed more variation in August as the month was marked with heavy rain. At mid-latitudes, reproduction of mosquito population was discontinued as a result of low winter temperature; abundances would be mainly regulated by temperature rather than precipitation (Campos & Macia, 1996; Schweigmann *et al*., 2002). In Northern Argentina, reproduction of *Aedes* population probably occurs all year round due to high temperatures, as for example in Corrientes city (Borda *et al*., 1999) and Salta Province (Micieli & Campos, 2003). In our present study also we got a year round high proliferation of *Aedes* mosquitoes in the study areas with the highest percentage in the month of October. Some authors (Moore *et al*., 1978; Toma *et al*., 1982) indicated that *Aedes* abundance would be mainly regulated by temperature rather than precipitation. Taushid *et al*. (2007) suggested that rainfall is the only determining factor for *Aedes* proliferation where temperature is always above the marginal level. But in our present study we found that rain fall, when it is too heavy and continuous, may also be a regulating factor for *Ae. aegypti* proliferation, as heavy rains would have significantly reduced adult production and immatures might have been washed away. So the present study indicates that heavy rainfall in the monsoon or rainy season prepares the habitats for *Ae. aegypti* proliferation in the post monsoon season in areas like Dibrugarh, where the temperature is highly suitable for mosquito proliferation all year round. *Aedes aegypti* density in the industrial sites seems bimodal (both from container and ovitrap surveys), high before and after the monsoon as compared with urban area where the breeding is almost constant throughout the year. This may be due to the constant availability of some of the water filled containers. People living in urban areas store water in containers, water present in the sanitary flash etc. can also serve as *Aedes* breeding sites. The main sites for oviposition in the urban areas are the tyres and battery boxes. Since the materials of these containers are good insulators heat cannot pass into the inside water, as a result, water does not evaporate easily. So, these sites store water almost throughout the year providing suitable oviposition sites. During rain also, they do not overflow quickly and most of the tyres are dumped in shady places. But in the industrial areas, containers mainly made up of iron or steel or other good conductors which facilitates easy evaporation during dry season, if present in open place, they fill up quickly during rain and during heavy showers of monsoon, they also overflow as the open reservoirs, big containers and drains of ongoing industrial project sites.

The seasonal fluctuations of abundances described in this study may allow in determining the most appropriate periods for the implementation of control measures to prevent dengue outbreak in near future. The elimination of containers in the month of January and in the time of heavy rainfall would help to reduce vector population efficiently in the areas with similar climatic conditions as there in Dibrugarh district. However, according to Scott *et al.* (2000), the components of *Aedes* can vary among and within communities as well as among years at the same location, and care should be taken when extrapolating results from one site to another and from one year to the next. Consequently, a longitudinal study covering several consecutive years in different areas of the district as well as in the country is needed to confirm our results.

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