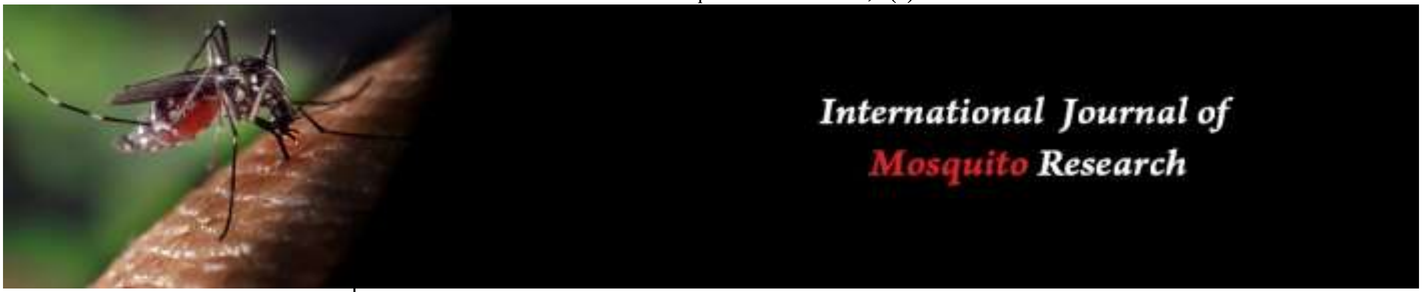

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Ecological and social determinants of *Aedes aegypti* and *Aedes albopictus* larval habitat in northeastern India

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Abstract
Dengue, the arboviral threat to public health affecting millions of people globally, is transmitted by the bite of female *Aedes aegypti* and *Aedes albopictus*. The factors contributing to *Ae.* spp. abundance is variable, region-specific, and needs to be identified region-wise for effective vector control programs. In this study, based on dengue fever case statistics from the previous years, we selected two representatives of natural (forest and riverine) and two urbanized (Oil industrial and tea-estates) areas of upper Brahmaputra valley and dengue prone Kamrup district in the lower Brahmaputra valley of Assam for mosquito surveillance through container search. The count of both species was the highest in the urbanized regions having higher container habitats. All the entomological indices, House index, Container index, and Breteau index were higher than the WHO criteria of dengue sensitive areas from pre-monsoon to post-monsoon seasons. The temperature was the most prominent driver having a significant correlation with entomological indices with R^2 values ranging from 0.825 to 0.965 in urbanized areas and 0.723 to 0.801in natural areas, followed by rainfall and humidity. Response survey of inhabitants of the study sites revealed the status of awareness and practice regarding the vector habitats. The results indicated that the combined action of urbanization, social factors, and changes in meteorological factors have primarily contributed to the large population size of dengue vectors throughout the year. Adaptive expansion of the *Aedes* vectors warrants the adoption of necessary precautionary measures to prevent colonization by *Ae. aegypti* and *Ae. albopictus* in urbanized areas to prevent *Aedes*-borne diseases, dengue, chikungunya, and zika.

Keywords: Dengue, breteau index, temperature, urbanization

1. Introduction

Dengue, the arboviral threat to public health affecting 50-100 million people globally, is transmitted by the bite of female *Aedes aegypti* and *Aedes albopictus* [1, 2]. The disease is caused by the flavivirus DENV I-IV, and its seroprevalence in India is about 56.9% among the laboratory-confirmed dengue cases [3]. In the last two decades, the country has witnessed the geographical extension of the disease prevalence [4]. The burden of dengue infection in India based on seroprevalence study is reported to be heterogeneous, with evidence of low transmission in northeastern regions [5]. However, the literature also speaks about the under-report of dengue cases [6]. For the prevention and control of epidemics in mosquito-borne diseases, vector control is an important option [7, 8]. Previously, *Ae albopictus* was considered a rural vector breeding primarily in tree holes, bamboo stumps, water holding pits of forest habitats, and *Ae. aegypti* as an urban vector breeding mostly in artificial habitats such as containers, pots, tires, and water storage tanks [9, 10]. However, due to human behavior and activities, there is a shift in habitat types which may allow adaptation of the species to different habitats [11, 12, 13].

Mosquitoes are susceptible to climatic factors, but the effect depends on the species and microclimates [14, 15]. The dependence on meteorological parameters is often associated with other factors like human behavior, trade, industrialization, economic condition, and habitat ecology. A recent retrospective analysis on dengue occurrence in the capital city of India has attributed lack of access to tap water as one of the risk factors [16]. There is a need for region-specific surveillance to determine the localized contributing factors for developing a generalized concept of vector habitat expansion which will provide critical information for further research to prevent vector colonization and disease incidences.

The upper Brahmaputra valley of Assam, consisting of seven districts, has different ecological habitats comprising natural habitats in reserved forests, the bank of Brahmaputra River and its tributaries, national parks, rural and urbanized areas. The region is a gateway to the different states of northeast India and a place of attraction for tourists from all over the globe. Studies involving the combined effect of different categories of risk factors for dengue-endemic regions are scanty [17, 18, 19]. The present study aimed to determine the meteorological, habitat ecology, and social contributing factors for the *Aedes* vector population in selected natural and urbanized habitats of the Brahmaputra valley of Assam, located in northeast India.

2. Materials and methods
2.1 Survey area
The districts, Tinsukia (27.48 N, 95.36 E) and Dibrugarh(27.47 ^{0}N, 94.91 ^{0} E), are located in the easternmost parts of Assam and serve as a gateway to adjoining states Arunachal Pradesh and Nagaland situated in northeast India. The districts with a population of about 14 lakhs are fast-growing and a significant center for trade and commerce, mainly for the sector of oil, gas, and Tea. The districts Jorhat (26.75 N, 94.20 E) and Sivasagar (26.45 N, 95.25 E), lying in the middle part of the upper Brahmaputra valley of Assam, are rich in tea gardens and oil industry. Kamrup district (26.31 N to 91.59E) is located in the lower Brahmaputra valley of Assam and recorded with the highest dengue cases (Plate 1). All the districts are also endowed with natural habitats.

Plate 1: Google earth map showing dengue vector surveillance in different selected locations of Brahmaputra valley of Assam, India

2.2 Survey methods
2.2.1 Selection of study sites
Data were collected for the incidence of dengue confirmed cases of the preceding three years from District Malaria Offices. Based on the districts reporting many cases, we performed an entomological survey from June 2020 to May 2021 in the oil industry, tea estates, forest, and riverside areas of Dibrugarh and Tinsukia district. We used the areas under the oil industry and tea gardens as urbanized areas and forest and riverside areas as natural areas. The natural areas had very scattered thin human settlements. The survey in Jorhat, Sivasagar, and Kamrup districts was carried out post-monsoon to collect the minimum essential information for comparison (Figure 1). G.P.S. locations and environmental parameters were recorded from each survey area: ambient temperature, rainfall, and relative humidity. We also collected the meteorological data from the Regional meteorological Department, Guwahati, Assam, from 2015 to 2021 for detecting the changes in the seasonal meteorological scenario of Assam.

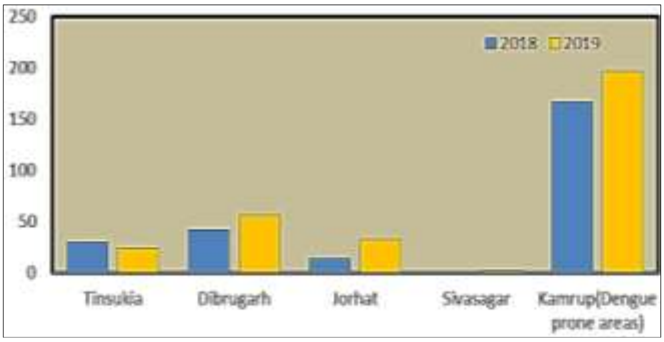


Fig 1: Trend of positive dengue cases of last three years

2.2.2 Immature collection and identification
To determine the ecological habitat-wise prevalence and seasonal variation of *Ae.* spp, the immatures were collected from houses and peridomestic areas by container search using the dipping method. In the container search method, every site was visited fortnightly during pre-monsoon (March-May), monsoon (June-Aug), post-monsoon (Sept-Nov), and winter (Dec-Feb), covering 150 houses per season. The collected immatures were brought to the laboratory, observed under a microscope for larval characters, counted, and kept in water containing plastic trays till the emergence of the adults. Food provided was slurry prepared with soybean and yeast (1:1). The adults were identified following morpho-taxonomic keys [20].

2.2.3 Sociodemographic survey
Besides container surveillance, sociodemographic surveillance was carried out in 50 houses per study area. The survey was based on the structured framework of questionnaires which included the number of members in a household, their educational qualification, their occupations, source of drinking water, knowledge about identification of dengue larval stages and breeding habitats, and access to awareness programs.

2.3 Computation of Entomological Indices and statistical analysis
During the survey using container search, all kinds of containers were examined. The water containers were marked as "Wet containers," and those detected with mosquito immatures were marked as "Positive Containers." Similarly, the houses with positive containers are labeled "Positive houses." The houses for the survey were selected by systematic random sampling technique. The larvae count per season was used to calculate the entomological indices, *viz*. Container Index (CI), House index (HI), Breteau index (BI) as per the guidelines of the World Health Organization [21].
We used the entomological indices and the larval counts of *Aedes* mosquitoes as response variables. The meteorological factors, minimum and maximum temperature, relative humidity and rainfall, and the habitats were considered predictor variables. We performed Scatter plot analysis and constructed bar diagrams in Graph Pad Prism v.5. Pearson's correlation studies predicted meteorological and response variables, the larval count.
3. Results
3.1 Larval Prevalence

During the survey carried out from 2020 to 2021 in industrial (Oil), tea estates, forest, and riverine areas of the districts Dibrugarh and Tinsukia, we encountered 135,155 mosquito immatures, out of which the highest appeared in the industrial area (55,716), followed by tea estates (49,947). Both the species were in high abundance from Pre-monsoon to post-monsoon, and the count was the lowest during winter (Dec-Feb) in all the study sites. In the survey carried out in Jorhat and Sivasagar districts during post-monsoon, we encountered 8105 and 6838 *Ae.* larvae from urbanized areas (Industrial areas and tea-estates) and natural areas (Forest and riverside areas). A total of 3523 and 357 *Ae. albopictus* and *Ae. aegypti* larvae were collected from the urbanized area and 4112 and 223 from the natural areas of the Jorhat district. A total of 3567 and 478 *Ae. albopictus and Ae. aegypti* were collected from the urbanized areas and 2511 and 282 respectively from the natural areas of Sivasagar districts. In the survey carried out post-monsoon in Kamrup District, we encountered 9178 *Ae*. larvae, out of which, the total larval counts of *Ae. aegypti* in Maligaon, Dispur, Six mile, Panikhati, Industrial area (OIL) and Sonapur areas were 779, 860, 185, 483, 189 and 1311whereas in case of *Ae. albopictus*, the total larval counts were 940, 1216, 144, 1078, 493, and 1500 respectively. During post-monsoon, the total larval count was the highest for *Ae. aegypti* in Kamrup and for *Ae. albopictus* in the urbanized area of upper Brahmaputra valley (Fig 2).

Accordingly, in Dibrugarh and Tinsukia District, the HI for *Ae. aegypti* was the highest (36-44%) in all seasons in the Industrial Area, and among the seasons, the highest HI (26-44%) occurred during post-monsoon (Sep-Nov). The HI for *Ae. albopictus* was the highest during post-monsoon (Sep-Nov) in the Tea estates (68.66%), followed by the industrial area (66%). For *Ae. aegypti*, BI was the highest for all the seasons in the industrial area. For *Ae. albopictus*, the highest BI, was recorded in the industrial area during monsoon (192.0) followed by post-monsoon (156.66) and in Tea estates during post-monsoon (150.66) followed by pre-monsoon (120.66). During pre-monsoon, the BI of the Industrial area (118) was close to Tea estates. In all the seasons, the CI for *Ae. aegypti* was the highest in the industrial area ranging from 16.47 to 29.11%. It was the highest (29.11%) in the industrial area during pre-monsoon (Mar-May) and the lowest in Tea estates during monsoon (June-Aug). In the case of *Ae. albopictus*, the CI was the highest (63.95%) in the Tea estates, followed by the Industrial area (56.01%) during the pre-monsoon (Mar-May) and was the lowest (33.56%) in the riverside area during Monsoon (June-Aug).

During post-monsoon In Jorhat district for *Ae. aegypti*, the CI, HI, BI was 18.81%, 26%, 41 and for *Ae. albopictus* was 34.51%, 45.0%, 71.0 respectively in urbanized areas while in natural areas CI, HI, BI for *Ae. aegypti* was 19.4%, 22.0%, 35.0 respectively and for *Ae. albopictus* was 43.32%, 50.0%, 51.0 respectively. In Sivasagar district, the CI, HI, BI for *Ae. aegypti* was 30.74%, 28.0%, 56.0 and for *Ae. albopictus* was 85.3%, 46.0%, 77.0 respectively in urbanized areas, and the CI, HI, BI for *Ae. aegypti* was 17.82%, 19.0%, 32.0 and for *Ae. albopictus* was 32.09%, 35.0%,58.0 respectively in natural areas. From the survey carried out during post monsoon in Kamrup, we recorded the HI, CI, BI in Maligaon area for *Ae.aegypti* as 40.0%, 31.0%, 58.0 and for *Ae. albopictus* 40.0%, 36.26%, 66.0 respectively, in Dispur area, the HI, CI, BI for *Ae. aegypti* as 44.0%, 36.84%, 56.0 and for *Ae. albopictus* as 44.0%, 40.78%, 62.0%, in Six mile for *Ae. aegypti* as 18.0%, 18.18%, 20 and for *Ae. albopictus* as 22.0%, 23.63%, 26 respectively, in Panikhati for *Ae. aegypti* as 32.0%, 25.0%, 40.0 and for *Ae. albopictus* as 36.0%, 33.75, 54.0. In case of industrial area(OIL) the HI, CI, BI for *Ae. aegypti* was 16.0%, 18.0%, 20.0 and for *Ae. albopictus* 28.0%, 30.07%, 34 and in case of Sonapur (Kamrup rural) the HI, CI, BI for *Ae. aegypti* was 50.0%, 33.64%, 72.0 and for *Ae. albopictus* 54.0%, 42.99%, 92.0 respectively (Fig 3).

During post-monsoon, the HI for both the species was higher in the urbanized area of Upper Brahmaputra valley than natural area, and HI for *Ae. aegypti* was the highest in Kamrup district. The BI for both species was also the highest in the urbanized area. The BI of Kamrup was higher than in the natural area in the case of *Ae. aegypti* and lower in case of *Ae. albopictus*. The CI in the case of *Ae. aegypti* was the highest in urbanized areas of Upper Brahmaputra valley, followed by Kamrup. In the case of *Ae. albopictus*, it was the lowest in Kamrup (Fig4).

The overall container distribution in Dibrugarh and Tinsukia District was 32% for industrial areas, 25% in Tea Estates, 22% in the riverside area, and 21% in the forest (Fig 5). The analysis of the container types revealed that in all the studies, maximum positive containers belonged to plastic types ranging from 59 to 167 numbers in all seasons (Figure6).

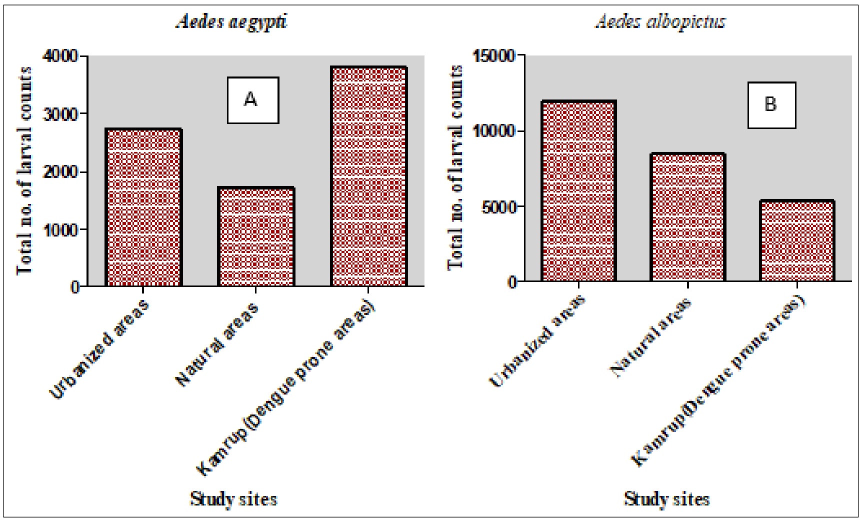


Fig 2: Larval count of *Aedes agypti* (A) and *Aedes albopictus* (B) of urbanized (Industrial areas and Tea-estates), natural areas (Forest areas and riverine areas) of upper Brahmaputra valley, and Kamrup (dengue prone areas of lower Brahmaputra valley)

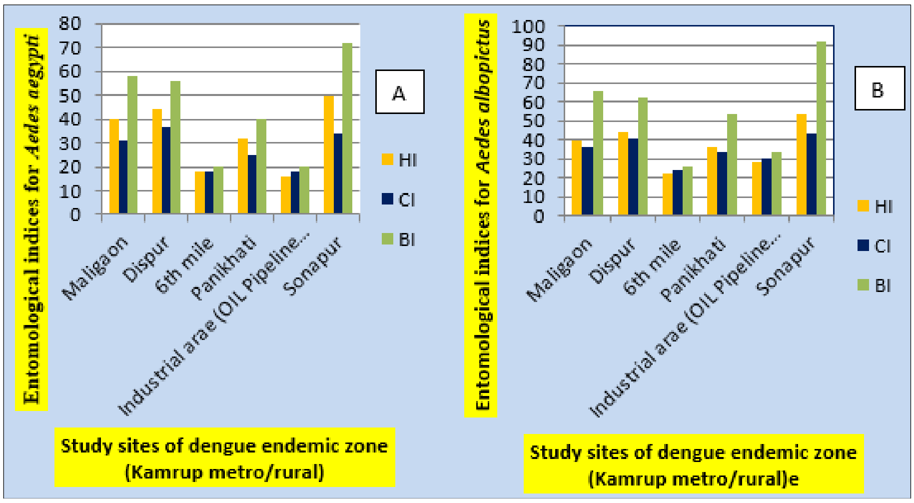


Fig 3: Variation of House Index (HI)%, Container Index (CI)% & Breteau Index (BI) for *Aedes aegypti* (A) and *Aedes albopictus* (B) of different dengue-endemic areas of Kamrup metro/ rural in the post-monsoon season

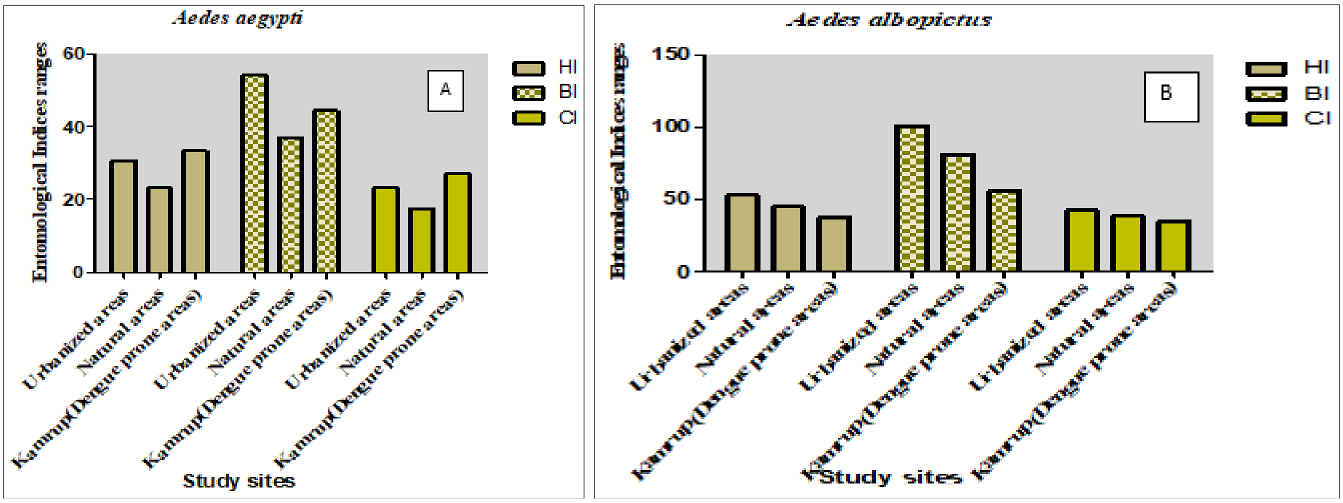


Fig 4: Variations of entomological indices of *Ae. aegypti* (A) and *Ae. albopictus* of urbanized, natural areas of upper Brahmaputra valley and Kamrup dengue prone areas of lower Brahmaputra valley

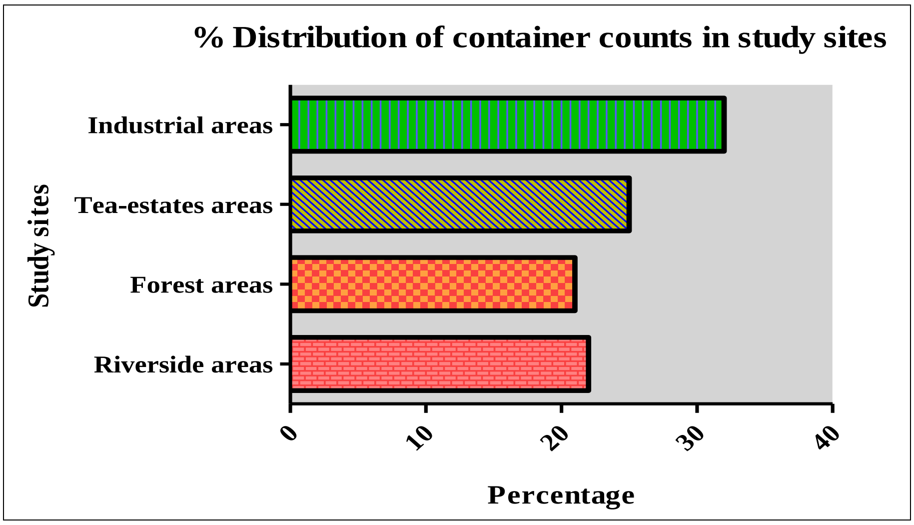


Fig 5: Overall distribution of container counts in study sites

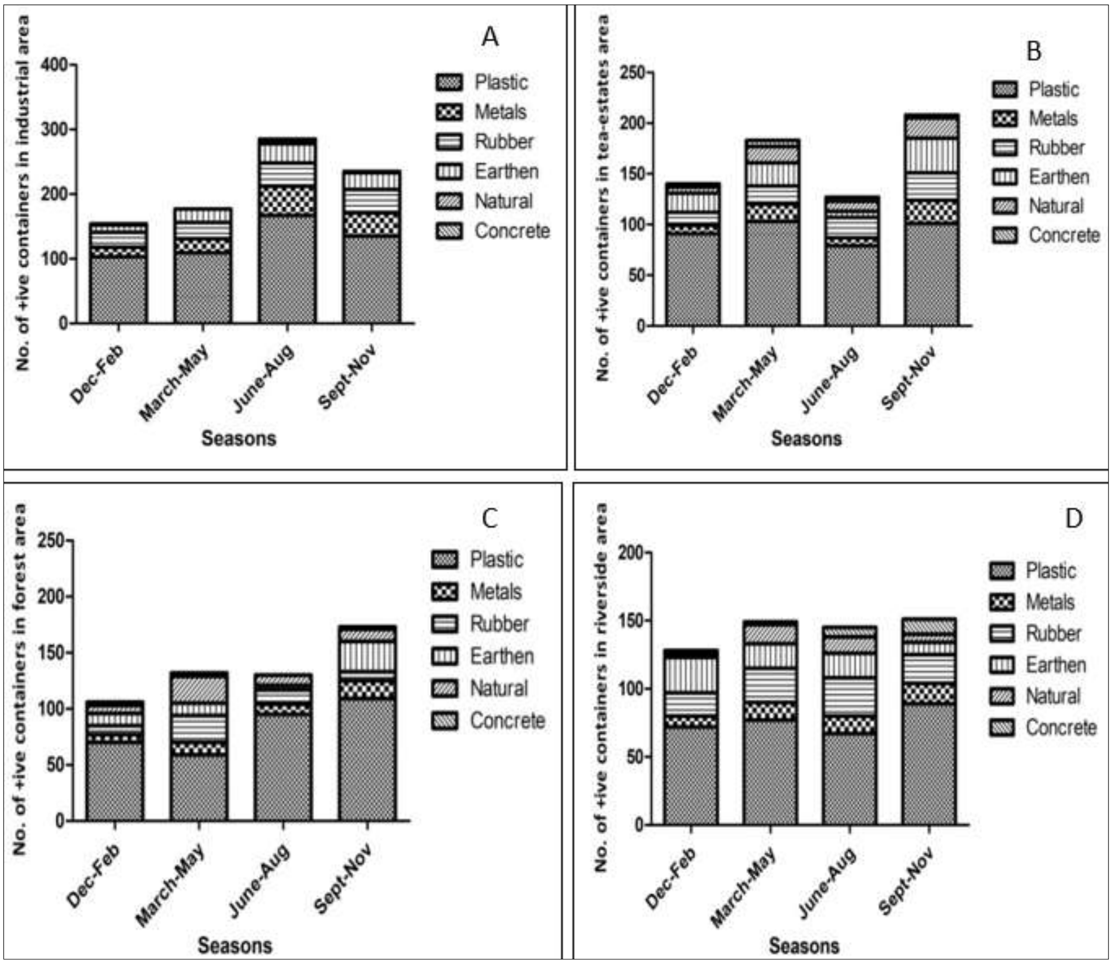


Fig 6: Seasonal distribution of container types in industrial (A), tea-estates (B), forest area (C) & riverside area (D)

3.2 Influence of Meteorological parameters

The data of meteorological parameters showed that the temperature ranged from 8 ˚C in January to 37 ˚C in September, rainfall ranged from 14 mm in December to 484 mm in July. Humidity ranged from 64% in January to 90% in June and July (Figure 7). Further, the data representing the changes in meteorological parameters over six years in the state of Assam showed an increase in temperature with the highest record in August and December during 2019-2020. Simultaneously, the precipitation was the highest in April, July, and September, and Relative humidity was the highest of all the years every month during the study period (Suppl.Figure1).

Pearson's correlation studies showed a significant positive correlation between maximum temperature and CI, HI, BI (R2=0.965, 0.821, 0.865 P≤ 0.01) in urbanized areas and also in (R2= 0.801, 0.726, 0.723 P≤ 0.01) in natural areas. The correlation coefficient between CI, HI, BI, and precipitation for urbanized areas were 0.625, 0.328, and 0.504, respectively, and for natural areas were 0.454, 0.269, and 0.316, respectively. The correlation coefficient between CI, HI, BI, and humidity for urbanized areas were 0.526, 0.542, and 0.597 for natural areas were 0.401,0. 50, 0.40 respectively.

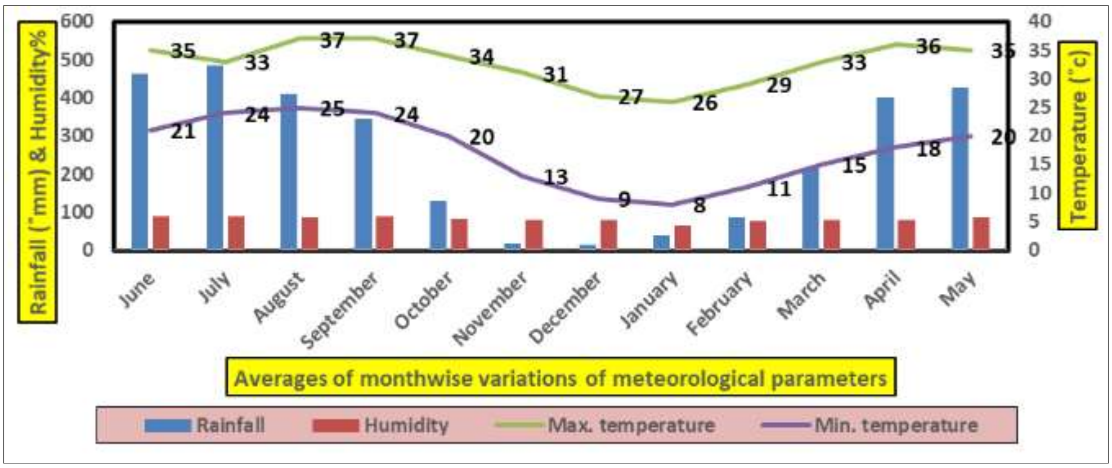


Fig 7: The averages of monthly variations of meteorological parameters during vector surveillance (June 2020 to May 2021)

3.3 Sociodemographic survey

We recorded that the percentage of male respondents was more during the survey. Access to tape water was poor in Tea estates, forest, and riverine areas. Regarding knowledge on dengue vectors and their breeding habitats, the least informed were the respondents of tea-estates areas (20%), and the best informed were the respondents of industrial areas (70%) (Figure8).

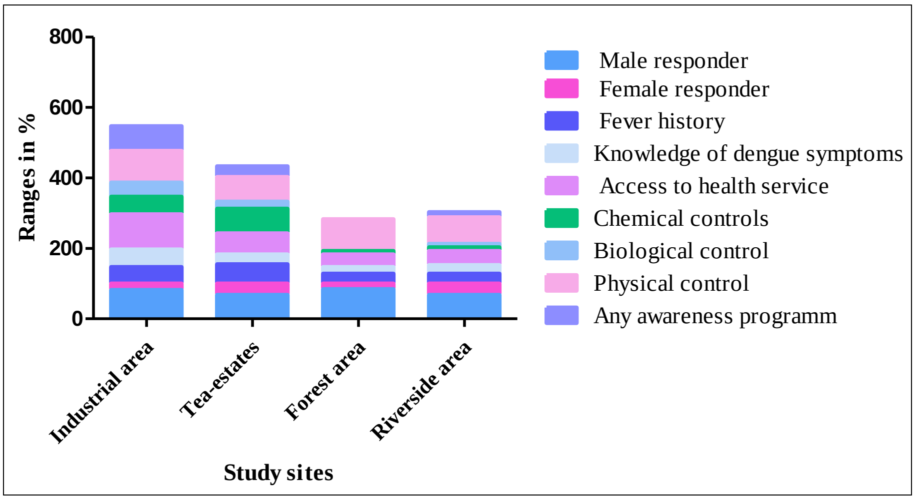


Fig 8: Sociodemographic status of study areas for dengue vector surveillance

4. Discussion

Among the northeast states of India, the first outbreak of dengue fever occurred in 2010 in Guwahati (District Kamrup), Assam, the gateway to the surrounding states of northeastern India. From the adjoining sub-urban areas of the lower Brahmaputra valley were reported 237 confirmed dengue cases. In the subsequent years, it spread to the different areas of the upper Brahmaputra valley. In 2012 and 2013, maximum cases were reported (1056 and 4526) [22]. Among different districts of the upper Brahmaputra valley of Assam, Dibrugarh and Tinsukia reported the highest positive cases of dengue fever from 2017 to 2019.

In the present study, we conducted a detailed study regarding the effect of meteorological parameters, habitat, and social attributes in Dibrugarh and Tinsukia District, and based on the finding of the highest prevalence of dengue vectors during post-monsoon, we surveyed the vectors in the other study sites of upper Brahmaputra valley and Kamrup district during post-monsoon only. The ecology of the natural environment is disrupted by anthropogenic activities, which bring changes to insect population dynamics and size. We recorded the highest dengue vectors in the urbanized area (oil industry and tea-estates). The survey sites of the tea estates were the peridomestic areas of the tea garden workers having no proper drainage and sanitary system compounded by a poor state of awareness about mosquito habitat and disease transmission. The area under the oil industry covers residential areas, construction and production sites, drilling areas, and markets. A higher larval count in the urbanized area could result from multifunctional human activities like trade, travel, industrial activities in the tea and oil sector, and an increase in human population density compared to the natural areas (Riverside and forest areas). The highest count of *Ae. aegypti* in the Kamrup district can be correlated to the highest dengue cases in the District.

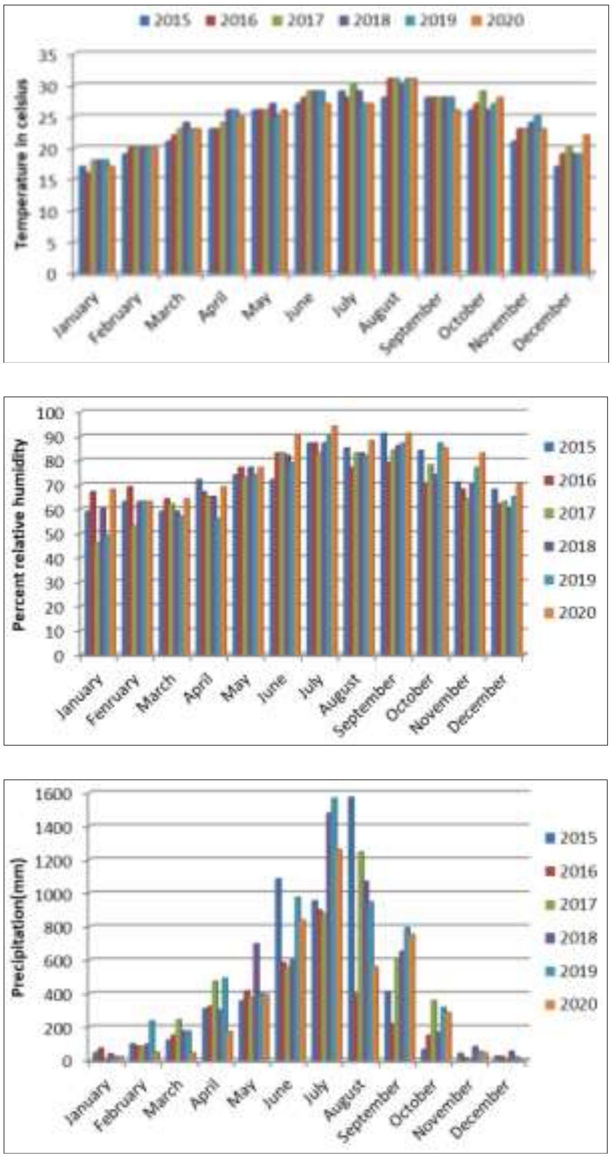
Regarding the seasonal prevalence of the dengue vectors, we found that the entomological indices HI, CI, BI, and vector count were higher in all seasons except winter. Our findings are consistent with studies carried out in Indonesia, Vietnam, South Korea, and Myanmar, where HI, BI, and vector populations were higher during the rainy season than during the winter season [23, 24, 25, 26]. In search of a plausible reason for the high entomological indices recorded in our study during pre-monsoon and post-monsoon, we examined the meteorological parameters of the last six years in the state of Assam (Suppl.Figure1). The examination revealed an increasing trend of temperature over the years. The humidity also increased during 2019-2020. The average precipitation during pre-monsoon was similar to that of post-monsoon during 2019-2020, which indicated a positive interactive influence of precipitation and temperature on enhancing the larval abundance. The persistence of precipitation from pre-monsoon to post-monsoon (March to November), along with a temperature range of 13-37 ^{0}C, can be suitable for Ae. mosquito's growth and development.

On the other hand, the minimum larval population in the winter correlated with low temperature and rainfall (8 ˚C and 14mm). We also found a significant positive correlation between temperature and entomological indices in scatter plot analysis in corroboration. Similar findings are reported by studies carried out in Indonesia, where they encountered minimum mosquito larvae in the dry season compared to the wet season [26]. Less rainfall means that a reduced amount of water is retained in containers, adversely affecting mosquito breeding. We observed in the present study a high relative humidity from pre-monsoon to post-monsoon seasons, which correlated with high vector density. Similar findings have been reported by studies carried out in other parts of southeast Asia [27, 28]. One earlier study reported that the vector density positively correlates with high humidity only [29]. In our study, during the winter season, when all the three meteorological parameters, humidity, temperature, and rainfall, were at their minimum, vector count was also recorded to be minimum. Thus, in conformity with certain other studies [30, 31, 32, 33]. We also observed that all three meteorological parameters predominantly influenced the vector density. Therefore, the present concerns regarding the effects of climatic change can be expected to apply to *Ae. spp* population and disease incidence.

The HI, CI, and BI are the commonly used entomological indices in determining the distribution pattern of the dengue vectors. Among the three indices, HI and BI are the most widely used larval indices for detecting dengue vector population density [34, 35]. The container index reflects the proportion of water holding positive container. HI reflects the presence and distribution of the *Aedes* population in a given locality. BI shows a correlation between the numbers of positive containers to the number of houses reflecting the density in the locality. Generally, a condition of HI greater than five and BI greater than 20 indicates a locality as a dengue-sensitive zone [36, 21]. In our study, the HI value ranged from 14.66 to 68.66, BI ranged from 22.6 to 192, and CI ranged from 5.14 to 63.95, and hence both the natural and urbanized areas come under dengue sensitive zones. We also observed the presence of larvae of both species in the same artificial containers, which indicated that both the vectors were well adapted to the artificial containers. The intriguing question in the results was our finding of the dengue sensitivity in the natural habitats with different ecology. The reason may be attributed to the methodology adopted for the study in which container search was done where the natural habitats were encroached by the human population. During winter, the riverside areas serve as good recreational spots leading to increased deposition of plastic containers followed by creating more larval habitats. The analysis reflected the ease of colonization of the encroached natural habitats by the dengue vectors.

Our survey carried out in Jorhat and Sivasagar district with records of medium and the lowest dengue cases, respectively, showed the HI and BI to be lower than those of Dibrugarh Tinsukia districts. The observation indicated a greater chance of disease incidence when the vector population is higher. In areas having consecutive yearly dengue outbreaks, the high population of incriminated dengue vectors correlates with high virus-positive ones [29]. In the case of Kamrup metro/rural, all the three entomological indices were recorded the highest in Sonapur, followed by Maligaon and Dispur, and the least was recorded from Six miles. However, the study sites of Kamrup come under urbanized areas and are comparable with the urbanized area of Upper Brahmaputra valley. Accordingly, all entomological indices of Kamrup were close to urbanized areas in the case of *Ae. aegypti.* The lower indices of *Ae. albopictus* in Kamrup District corroborates with a low larval count of the species in the District as compared to upper Brahmaputra valley. However, the low count of the species of albopictus might be due to our site selection policy based on dengue prevalence records. *Ae. albopictus* is a comparatively less effective vector for the dengue virus, but it is also incriminated as a Chikungunya and Zika.

The study in the upper Brahmaputra valley further revealed that the deposition of artificial containers near households or commercial places such as plastic containers, abandoned tires, flower pots, water storage drums, providing suitable breeding habitats for dengue vectors was high in the urbanized area with the highest recorded in the industrial area followed by tea estates. We attributed the reason for comparatively lower CI in the Tea estates as compared to oil industry areas during summer to the presence of comparatively smaller sized containers (utensils, plastic cups, earthen pots) in the Tea estates used by the garden workers which overflow during summer rains leading to flowing out of the immatures from the containers. The plastic containers constituted the highest proportions of *Ae.* bearing containers. The sociodemographic survey revealed the indiscriminate use of plastic containers and unorganized disposal rooted in either lack of awareness about their contribution towards increasing *Ae.* population or social attitude towards non-compliance. Further, our study reflects a higher population size of *Ae. albopictus* in industrial areas of upper Brahmaputra valley and indicates a shift in *Ae albopictus* population from forest to urban and semi-urban areas. In contrast, earlier studies in many countries reported that *Ae. albopictus* breeds only in natural habitats like tree holes, and bamboo stumps [37, 38]. Out studies supported the reports from other countries regarding *Ae.*'s high adaptability and invasive character [39, 40, 41, 42]. Again, along with high container habitat in the urban environment rooting from human knowledge, attitude, and malpractice, increasing human population size may also contribute to higher vector density dependent on host availability for reproduction. Region-specific identification of the source of container increase, providing awareness about their potential risk, and development of Governmental or industrial management mechanism for adoption of good societal practice can contribute towards the reduction of *Ae*. vector population.



Suppl. Fig 1: Changes in temperature, humidity and rainfall in Assam (India) during 2015 to 2020

5. Conclusion

The study results indicated that the combined action of urbanization, social attitude, and changes in meteorological factors have primarily contributed to the high dengue vector population throughout the year, except for a very narrow winter window. Adaptive expansion of the *Aedes* vectors warrants the adoption of necessary precautionary measures to prevent colonization by *Ae. aegypti* and *Ae. albopictus* in urbanized areas for preventing *Aedes* borne incidence of diseases, dengue, chikungunya, and zika.

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Contribution: Both the authors have equally contributed to the manuscript.

Conflict of Interest: The authors bear no conflict of interest.

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