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Physico-chemical characteristics of breeding habitats in relation to larval density and relative abundance of *Aedes* mosquitoes from Siliguri sub-division, West Bengal, India

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Abstract

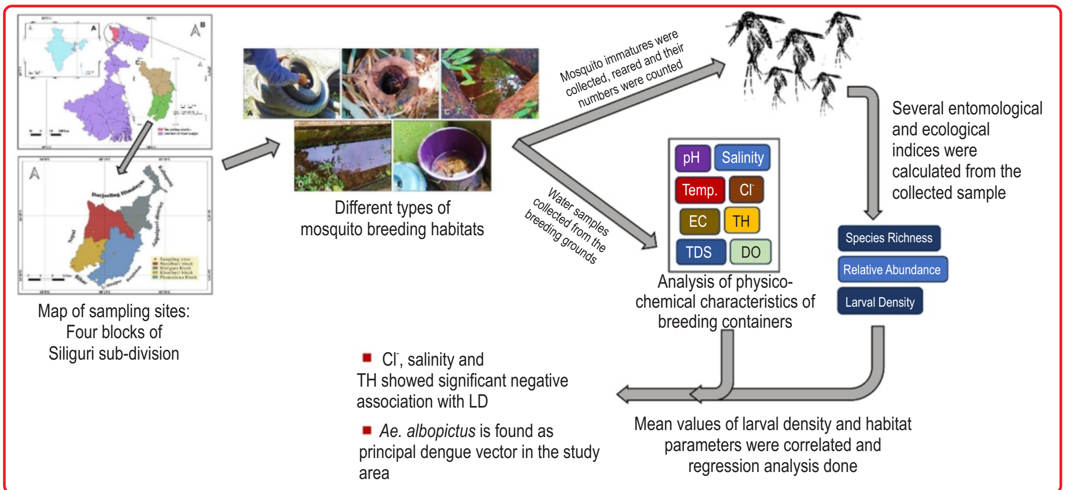
Aim: Characterization of physico-chemical parameters of *Aedes* mosquito breeding habitats from dengue-endemic Siliguri sub-divisional area and correlation of larval density with the habitat characteristics.

Methodology: Natural and artificial water-holding containers found in four blocks, namely- Naxalbari, Matigara, Khoribari and Phansidewa of Siliguri sub-divisional area were surveyed for the collection of *Aedes* mosquito larvae. Water parameters such as pH, temperature, electric conductance (EC), total dissolved solid (TDS), chloride, salinity, total hardness (TH) and dissolved oxygen (DO) were measured and the larval density (LD) indices and relative abundance for *Aedes* spp., were calculated.

Results: Out of 40 habitats, larvae of *Aedes* spp. were found in 20 habitats (50%). *Ae. albopictus* was found as the pre-dominant species (81.24%) in all the positive breeding habitats.Three habitat characteristics, like chloride (r= -0.959; *p*= 0.041), salinity (r= -0.958; p= 0.041)and TH (r= -0.961; p= 0.039) showed significant negative association with larval densityin the study. Whereas the remaining parameters, like-pH, temperature, EC, TDS, were positively correlated and DO showed a negative correlation with larval density. Although none of the later correlations were statistically significant. The larval density of *Aedes* spp. (mean ± SD) was lowest in Khoribari (27.66 ± 21.55) and highest in Phansidewa block (128.33 ± 187.26).

Interpretation: Pre-dominance of *Ae. albopictus* reiterates its importance as a principal dengue vectorin rural, peri- or sub-urban area of the sub-division. Utilizing the obtained data, the efficacy of oviposition traps can be enhanced for locale-specific vector surveillance and development of novel techniques that may deter larval growth, development and survival in an area.

Key words: *Aedes* mosquitoes, Dengue, Larval density, Mosquito-borne viral diseases, Siliguri



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Introduction

A number of mosquito-borne viral diseases of public health concern, like Dengue, Urban yellow fever, Zika and Chikungunya, are caused by the mosquitoes of genus- *Aedes*, especially, *Aedes aegypti* Linnaeus 1762 and *Aedes albopictus* Skuse 1894 (Mbanzulu *et al.,* 2022). Dengue is an acute systemic viral disease, which is transmitted globally both in endemic and epidemic'mosquito-human-mosquito' infection cycles. World Health Organization (WHO) estimates about 400 million dengue infections occur annually, with 96 million clinical manifestation and 5,00,000 hospitalizations (Roy *et al.,* 2022). Dengue is endemic almost in all Indian states that results in more than 1,00,000 infections and 200 to 400 deaths each year (Baruah *et al.,* 2020). During 2022, India reported 2,33,251 infections and 303 deaths (Das *et al*., 2024). By last decade, West Bengal has reported infection rates up to 39.97 % (per lakh population) and maximum 21.66% death rate due to dengue which is about 7.8 % of country's dengue cases during the past two decades (Baruah *et al.,* 2020). In 2022, the total figure of infections was 67,271 and 30 dengue deaths reported in West Bengal (Das *et al*., 2024). Darjeeling is one among the three dengue endemic districts of Northern West Bengal, as the incidences of dengue occur annually in the district (Saha and Saha, 2021).

Dengue vectors- *Ae. aegypti* and *Ae. albopictus* are highly anthropophilic, well-adapted to human societies and breeds efficiently in diverse domestic, peri-domestic and human-made artificial habitats (containers) along with the natural habitats. The mosquitoes critically investigate suitable oviposition sites by visual, olfactory and tactile responses (Rao *et al.,* 2011). High humidity, dark cavities, reflective surfaces, low illumination with dark colour of water, suitable pH, optimum temperature, rich dissolve oxygen, higher electric conductance, total dissolved solid, and low levels of salinity and hardness are preferred by female *Aedes* mosquito (Multini *et al.,* 2021; Chatterjee *et al.,* 2015). Therefore, larval development of vector mosquitoes depend on several ecological parameters, like-nutrient content, pH, temperature, EC, DO, TH, alkalinity, acidity, phosphate content, chloride content, TDS of water holding containers where they reproduce and grow (Multini *et al.,* 2021; Chatterjee *et al.,* 2015; Gopalakrishnan *et al.,* 2013).

Several studies have reported the impact of physico-chemical parameters of breeding containers, such as pH, EC, TDS, salinity and DO on *Aedes* immature productivity, development, body size, longevity, survival, vectorial capacity and ultimately disease epidemiology (Ouedraogo *et al.,* 2022; Multini *et al.,* 2021; Chatterjee *et al.,* 2015; Gopalakrishnan *et al.,* 2013). Adult body size has been found affected by these ecological parameters (Schneider *et al.,* 2011) and larger adults live longer than the smaller ones (Barreaux *et al.,* 2018). Thus, larval environmental parameters may differentially affect different stages of DENV-2 infection (*i.e.,* midgut, dissemination or saliva) via carry-over effects, reported in *Ae. albopictus* (Evans *et al.,* 2018). So far the attempt to control the transmission of DENV-infection and disease occurrence have not been completely successful and there are no licensed vaccines or antiviral treatments against the viruses, the larval source management and vector control may be the most effective sustainable strategy for dengue control in a disease affected area (Mbanzulu *et al.,* 2022). If the breeding-water parameters can be checked and treated suitably utilizing different intervening measures, the mosquito larval population may be reduced and would be useful to manage the locale mosquito population. Understanding how the characteristics of breeding containers contribute to different classical entomological indices, like- larval density index and affect mosquito life-history traits is essential to establish locale-specific surveillance and effective vector control for the prevention and management of outbreak conditions (Mbanzulu *et al.,* 2022). Therefore, the present study was conducted with the aim to obtain a clear knowledge about the physico-chemical characteristics of *Aedes* mosquito breeding sites which is crucial for designing a novel vector control measures in a dengue-endemic area.

Materials and Methods

Study area, Study design and Sampling: The study was conducted in Siliguri sub-division of Darjeeling District, West Bengal, India, located at the southern foot-plain zone of Darjeeling Himalaya and covers a geographical area of 819.61 km^2.The sampling was carried out between October and November, 2022 in four Community Development Blocks (C.D. Blocks) *viz.* Naxalbari (NxlB), Matigara (MtgB), Khoribari (KoiB) and Phansidewa (PasB). Two sampling sites within each block thus, eight sites were selected for field survey (Fig.1). The containers supporting *Aedes* spp., were considered as positive breeding habitats and eggs, larvae and pupae of mosquitoes were collected from the natural breeding habitats like bamboo stumps, tree holes, leaf axils etc., as well as from artificial breeding habitats like plastic containers, earthen pots, household tanks, metal containers, clay pots and glass bottles. From each block, ten such water-holding containers, in total 40 breeding habitats were inspected.

Depending on the habitat size, mosquito immatures were collected by either dipping or pipetting or emptying the container. All mosquito larvae and pupae for each habitat were counted when collected to determine the density. Sampling during the month of October and November is necessary for better understanding of density of Dengue vectors and their abundance in the region as most cases of Dengue out-break reported in the district from late August up to the month of November in the monsoon and post-monsoon period just prior to winter. Water samples were collected in 350 ml clean plastic containers from the positive breeding habitats with proper labelling of date and place and transferred carefully to the laboratory, avoid shaking. In laboratory, water samples were fixed immediately by preserving them at 4°C in a refrigerator to reduce microbial growth. The physico-chemical characteristics of water samples were estimated in the Department of Zoology and Department of

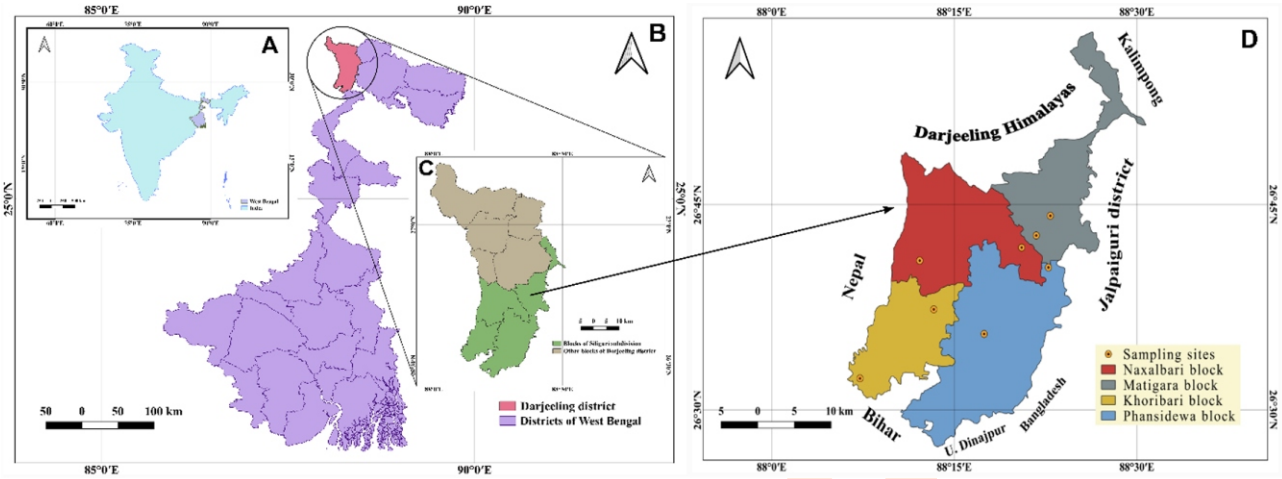

Fig. 1: Map of the study area: (A) India (B) West Bengal (C) Darjeeling district and (D) Siliguri sub-division. Siliguri sub-division comprises Community Development four Blocks: Naxalbari, Matigara, Khoribari and Phansidewa. Two sampling sites were selected for the larval survey in each block.

Chemistry, North Bengal University. The collected water samples from each inspected containers were analysed for the following physico-chemical properties: pH, Temperature (°C), TDS (mg l^{-1}), EC (mS cm^{-1}), Chloride ion concentration (mg Cl-l^{-1}), Salinity (PPM), TH (mg l^{-1}) and DO (%), following the standard methods of APHA (2017). Water temperature and pH were measured on the spot of collection with using a normal mercury-in-glass thermometer (Japson, India) and laboratory pH-meter (model no- kph-01, K. Roy & Company) respectively. TDS and EC were measured with the help of Systronics Conductivity 7DS Meter 308.

Estimation of Entomological Indices: *Ae. aegypti* and *Ae. albopictus* are two major global vectors of Dengue, Zika and urban yellow fever throughout the globe. Therefore, considerable attention needs to be paid for larval surveys and estimation of Entomological Index *viz.,* Larval Density Index (Service 1993; Silver 2008) in disease endemic areas. Entomological indices were calculated to determine the risk posed by the mosquito vectors in Dengue incidences in Siliguri sub-division. Relative abundance and Margalef's Index of Richness (D_{mg}) were additionally calculated for the knowledge of population structure of mosquito vectors in the study area (Margalef, 1957; Curtis and McIntosh, 1950).

$$\text{Larval Density} = \frac{\text{Total no.of individuals of a species in all containers}}{\text{Total no.of positive containers}}$$

$$\text{Relative Abundance (\%)} = \frac{\text{Number of larvae belonging to the species}}{\text{Total number of larvae collected}} \times 100$$

$$\text{Margalef's Index of Richness} = \frac{S\text{-}1}{\ln(N)}$$

where, S= Total no. of species; N= Total no. of individuals

Statistical analyses: The calculated Mean values and Standard Deviations (SD) were used for summarizing the physico-chemical parameters.Correlation with the mean values of physico-chemical characteristics of the breeding-habitats and the larval densities of *Aedes* mosquito species was derived using the Pearson's Correlation Coefficient Test and Linear Regression Analysis. To determine, significant differences (if any) in the physico-chemical characteristics among the different mosquito breeding-habitats of the four C. D. Blocks of the surveyed area, one-way ANOVA has been performed. For, the all-statistical analysis, a 'p' value ≤ 0.05 was considered statistically significant. The statistical analyses were performed using IBM© SPSS© version 21 statical software.

Results and Discussion

In total, 1914 mosquitoes were collected, out of which 1560 were found as *Aedes* spp. In Naxalbari, 855 out of 1005 mosquitoes were identified as Aedes spp., while in Matigara, 237 out of 390 individuals belonged to this genus. Similarly, Khoribari Block contributed 83 *Aedes* mosquitoes out of 118 collected and in Phansidewa Block 385 found as *Aedes* mosquitoes out of 401 individuals captured. Relative abundance of *Aedes* spp. in the four blocks were 85.07, 60.77, 70.34 and 96 % in between October and November, 2022 (Fig. 2). Total nine mosquito species were found among the total larvae collected from Siliguri sub-division. Among them, *Ae. albopictus* reported the highest abundance (81.24%) followed by *Ae. aegypti* (0.26%) (Fig. 2). The highest Margalef's index of richness (D_{mg}) was observed in Naxalbari Block (D_{mg}= 0.867), while the lowest was found in Phansidewa Block (D_{mg}= 0.5). The larval density (LD) (mean ± SD) of *Aedes* spp. in four community Development. Blocks *viz.,* Naxalbari, Matigara, Khoribari and Phansidewa Blocks were 95 ± 78.29, 47.4 ± 46.68, 27.66 ± 21.55 and 128.33 ± 187.26,

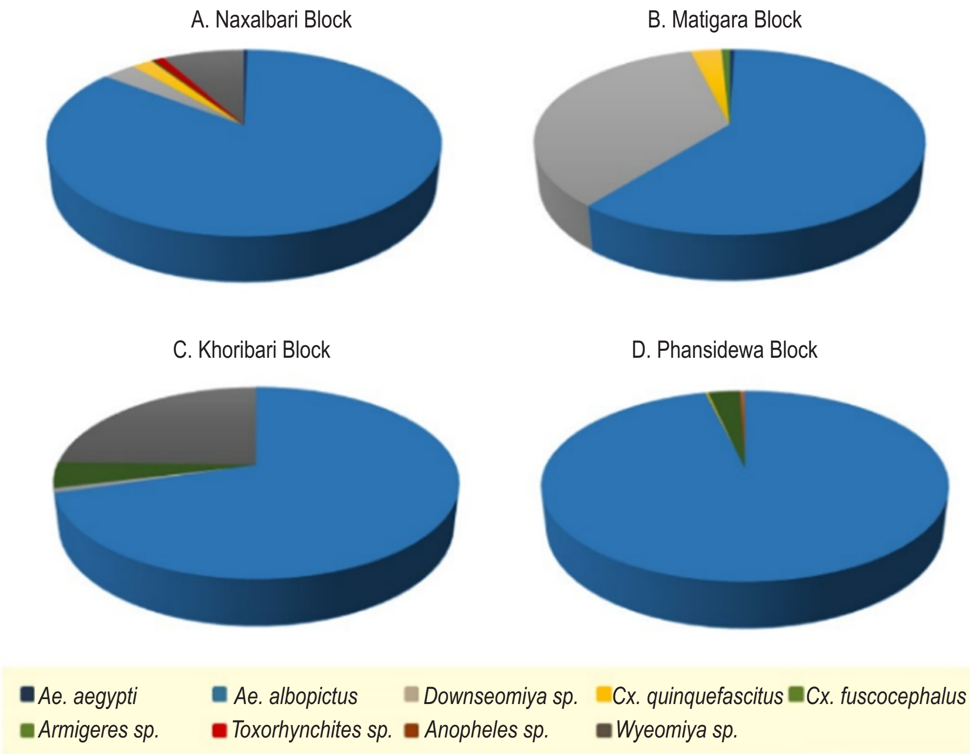

Fig. 2: Pie chart showing Relative Abundance (%) of different mosquito species in the surveyed water-holding containers from Community Development Blocks.

respectively. Larval density among the containers of Phansidewa block showed the highest value (128.33 ± 187.26), while the lowest value was noted (27.66 ± 21.55) in Khoribari Block (Fig. 3). However, ANOVA result showed that there was no significant difference in LD among the four blocks of Siliguri sub-division ($p > 0.05$). *Aedes* mosquitoes, especially *Ae. albopictus* was found in all the 20 PBHs whereas *Ae. aegypti* was present only in two containers with *Ae. albopictus*. Species richness was also low in artificial and natural water-holding containers in the study as D_{mg} was found to be lesser than 1 in all the blocks, which indicates that the containers were supportive only for specific mosquito species, not for all mosquitoes.

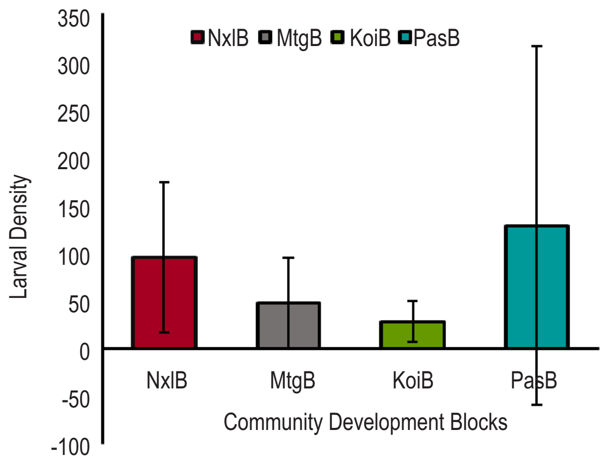

Fig. 3: Larval density of *Aedes* sp., from four Community Development Blocks of Siliguri sub-divisional area. Values are mean ± S.D.

The physico-chemical parameters of habitat water samples (Table 1) showed that the pH ranged from 6.61 ± 2.001 (in Matigara) to 8.316 ± 0.158 (in Phansidewa). The minimum TDS and EC values were recorded in Naxalbari (132.470 ppm and 277.933 μS m^{-1}, while the maximum were reported in Phansidewa (323.033 ppm and 558.167 μS m^{-1}). The Cl^- ion concentration and salinity values were maximum in Matigara (272.885 mg l^{-1} and 450.260 ppm) whereas the lowest was found in Phansidewa (53.115 mg l^{-1} and 87.641 ppm). Khoribari reported the minimum hardness values (121.114 ppm) whereas Matigara reported the maximum values (228.55 ppm). DO in water samples of the breeding-habitats ranged from 0.56% in Phansidewa to 1.488 % in Naxalbari. Pearson correlation test revealed that physico-chemical parameters like Cl^- ion (r= -0.959; p= 0.041) (Fig. 4a), salinity (r= -0.958; p= 0.041) (Fig. 4b) and total hardness (r= -0.961; p= 0.039) (Fig. 4c) had a significant negative correlation with larval density. Other physico-chemical

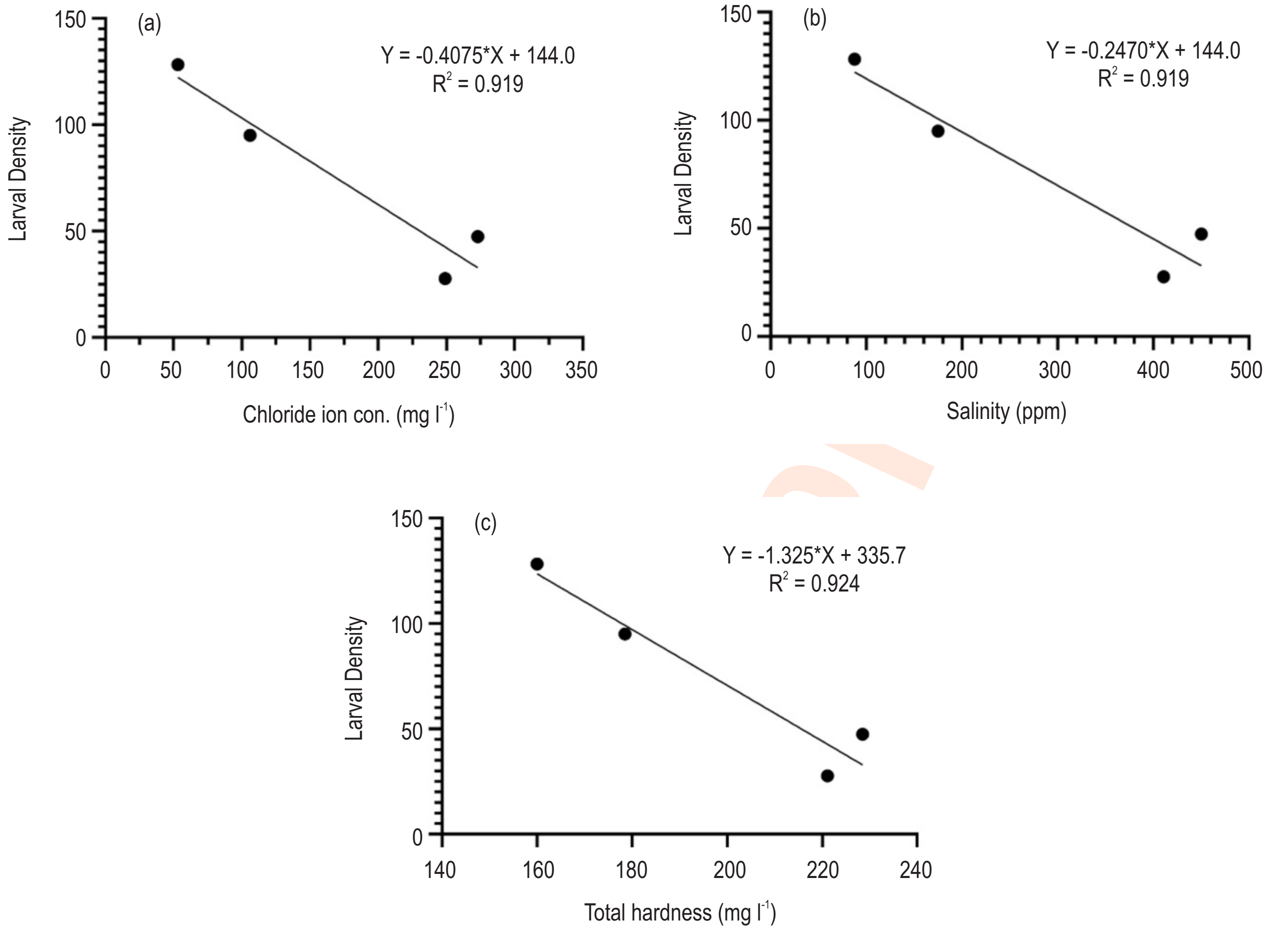

Fig. 4: Correlation between larval density of *Aedes* spp. of positive breeding habitats with three habitat characteristics: (a) larvel density vs Cl^- conc; (b) larvel density vs Salinity and (c) larvel density vs TH.

parameters like pH (r= 0.330; *p*= 0.670), temperature (r= 0.410; *p*= 0.590), TDS (r= 0.515; *p*= 0.485) and EC (r= 0.521; *p*= 0.479) were positively correlated with larval density. Whereas a negative correlation was found between the larval density and dissolved oxygen (r= -0.146; p= 0.854). Although, none of the correlations were statistically significant where *p* values were greater than 0.05.

In the present study, the pH of water samples collected from positive breeding habitats varied from acidic (pH 6.61) to alkaline (pH 8.32). This pH range aligns with the findings from the previous studies (Chatterjee *et al*., 2015; Mbanzulu *et al.,* 2022; Ouédraogo *et al.,* 2022). Although *Aedes* spp. prefer a mild basic pH (7.4) (Afolabi *et al.,* 2010), however, in this study *Aedes* larvae were found to survive at 8.9 pH (Table 1). Survival in such harsh condition may be adaptability to global climate change and resistance development (Chatterjee *et al.,* 2015). Both, *Ae. aegypti* and *Ae. albopictus* grow rapidly at temperature range of 20–30°C.High temperature shortens the developmental period and also results in the production of smaller adults too (Tun-lin *et al.,* 2000; Delatte *et al.,* 2009). Here, *Aedes* larvae were found in temperature (mean) range of 22.66—25.77°C. Among the chemical parameters, EC ranged between of 238.028—558.167 μS m^{-1}, while TDS ranged between 132.470—323.033 ppm (Table 1), respectively. Several studies across the world on larval density of dengue and malaria vectors showed both the negative and positive correlations with water conductivity and TDS (Fillinger *et al.,* 2009; Rao *et al*., 2011; Gopalakrishnan *et al*., 2013; Chatterjee *et al*., 2015; Mbanzulu *et al*., 2022). However, in the study no statistically significant relation of LD has been found with the both parameters (Table 1).

The level of Cl^- ion in haemolymph is crucial for the maintenance of midgut alkalinity (pH 11) of *Ae. aegypti* mosquitoes (Boudko *et al.,* 2001) which is significantly associated with larval development and survival. A significant increase in larval density resulted in the decrease of Cl^- ion, which suggests a statistically significant negative correlation between larval density and chloride ion concentration. In this study. Similar result was

Table 1: Physico-chemical characteristics of different positive breeding habitats from four Community Development Blocks of Siliguri sub-divisional area during October to November, 2022

Physico-chemical characteristics	Blocks			
	Naxalbari	Matigara	Khoribari	Phansidewa
pH	7.51 ± 0.99	6.61 ±2.001	8.2 ±0.58	8.32 ±0.16
Temperature (°C)	25.78 ±2.69	26.3 ±2.109	22.67 ±0.47	25 ±1.41
TDS (ppm)	132.47 ±91.04	255.96 ±235.97	139.95 ±144.08	323.03 ±77.95
ED (µS m^{-1})	277.93 ±157.29	440.23 ±405.88	238.03 ±243.29	558.17 ±135.68
Cl^- ion (mg l^{-1})	106.01 ±102.12	272.88 ±173.02	248.98 ±374.43	53.11 ±29.02
Salinity (ppm)	174.92 ±168.50	450.26 ±285.48	410.82 ±614.81	87.64 ±47.89
Total Hardness (mg l^{-1})	178.54 ±127.85	228.55 ±159.48	121.11 ±68.33	160 ±20
DO (%)	1.49 ±0.86	1.48 ±0.39	0.65 ±0.35	0.56 ±0.13

Numbers of positive breeding habitats in Naxalbari- 9; Matigara- 5; Khoribari- 3; Phansidewa- 3; total number of containers observed- 40. Values are mean ± SD.

found in Delhi, India (Seghal and Pillai, 1970) where Cl^- concentration was low in *Aedes* breeding habitats. In accordance, LD of *Aedes* spp. were recorded highest in Phansidewa block and lowest in Khoribari Block (Fig. 3). Freshwater osmoregulators the like- *Ae. aegypti* and *Ae. albopictus* larva maintain the ionic concentration of their haemolymph according to the concentration of mineral salts dissolved in water (Donini *et al.,* 2007; Multini *et al.,* 2021). The results of this study, showed that the maximum larval density of *Aedes* spp. was found at low salinity level and thus showed a statistically significant negative correlation with larval density (Fig. 3; 4b). The mean water salinity level of PBHs varied from 87.64 to 450.26 ppm in the present study (Table 1). Similar results were also reported by Multini *et al.* (2021), Chatterjee *et al.* (2015) in Kolkata and Gopalakrishnan *et al.* (2013) in Asom, India. Multiple laboratory studies revealed that *Ae. albopictus* larvae preferentially inhabits in lower salinity mediums, the selection of oviposition sites as well as oviposition by *Ae. aegypti* gravid females decrease with increase in salinity level (Navarro *et al.,* 2003; Multini *et al.,* 2021). Although studies by Mbanzulu *et al.* (2022) in Congo and Rao *et al.* (2011) in Kerala, India reported a positive association. larval density of *Aedes* spp. was highest in Phansidewa Block followed by Naxalbari Block (Fig. 3). Total hardness varied from 121.11 to 228.55 mg l^{-1} in the study area (Table 1). The results showed that larval density was maximum in low TH and showed a statistically significant negative correlation with LD (Table 1; Fig. 4c). The correlation is compliant with Gopalakrishnan *et al.* (2013) in Asom. Larval density was highest in Phansidewa Block (LD- 128.33) and lowest in Khoribari Block (LD- 27.67) (Table 1). The larval density and abundance of mosquitoes varied with DO levels in the container-water. Reduced DO levels of water resulted in reduced larval survival and prolonged development time (Silberbush *et al.,* 2015). In the present study, DO ranged between 0.56 to 1.488 and showed a negative association with larval density (Table.1) which is contrasting with the existing studies (Gopalakrishnan *et al.,* 2013;Mbanzulu *et al.,* 2022).

In conclusion, *Ae. albopictus* has was found significantly higher than any other mosquito species in the water-holding containers which suggests the containers provide suitable environment and support the development of *Aedes* larvae in rural and semi- or peri-urban areas of Siliguri sub-division. Wide prevalence of 'Asian tiger mosquito', *Ae. albopictus* at all the surveyed area establishes its importance as a potential vector of Dengue in this region. Larval density indicates a much higher vector-load and higher possibility of DENV virus transmission at local level if outbreaks happen. No such studies have been reported previously from any dengue-endemic districts of Northern West Bengal. From this point of view, the current study has been conducted for the first time and further, more elaborate studies are needed to delicately analyse the associations and to apply this information in vector control programs to manage the locale vector-mosquito population in this region.

Furthermore, an increased Cl^- ion concentration, salinity and hardness of the habitat waters by employing novel techniques can deter oviposition and reduce the growth and survival of immatures in this dengue-prone study area. These relationships can be utilized to enhance the efficacy of oviposition traps used the capturing and monitoring of dengue vectors by modifying the habitat parameters as per the obtained data.

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Authors' contribution: R. Sarkar: Sample collection, methodology, data analysis, original draft and review writing; **S. Das:** Conceptualization, statistical analysis, data analysis and editing; **A. Saha:** Data collection, methodology and editing; **P. Das:** Statistical analysis and editing; **D. Raha:** Data analysis and editing; **D. Saha:** Conceptualization, supervision and editing.

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Data availability: The obtained information has been presented within the article and if needed, the supplementary files will be made accessible on request by the authors.

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