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Abundance of major malaria vectors and their population dynamics in northern districts of West Bengal, India

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Abstract
The northern districts of West Bengal are known to be endemic for malaria for long time. Information regarding vector species composition, and their population dynamics is very limited from this part of the country. The present work was conducted to study the abundance of malaria vector species and their population dynamics during January, 2021 to December, 2022. Entomological survey was conducted on monthly basis. Per dip density and per man hour density was calculated. *Anopheles culicifacies* and *An. stephensi* were found to be the major malaria vectors. No significant difference was observed between the overall abundance of both larvae and adult of *An. culicifacies* and *An. stephensi* (p>0.05). The peak densities were observed between August and October, after which a gradual decline was noted across all districts. A significant positive relationship was recorded between anopheles abundance and various climatic factors. Present study finding may serves as the baseline data for formulation effective vector control measures.

Keywords: Malaria vector, *Anopheles stephensi*, *Anopheles culicifacies*, per dip density, per man hour density, West Bengal

1. Introduction
Malaria continues to pose a significant public health concern across approximately 85 nations, including India, with an estimated 249 million cases reported globally in 2022 [1]. Since the launch of the Global Technical Strategy for Malaria (2016 - 2030), there has been a global increase of 7.39% in malaria cases [1, 2]. Despite this rise, the incidence rate among per 1,000 individuals at risk has declined - from 82 in the year 2000 to 59 in 2020 [1]. Similarly, deaths due to malaria dropped by 50% between 2000 and 2021 [1]. African countries account for about 94% of the world malaria burden, whereas the South-East Asia (SEA) region contributes about 2% [1]. Within the SEA region, India alone is responsible for nearly 70% of malaria cases and about 69% of related deaths annually [3]. Malaria is transmitted through the bite of an infected female Anopheles mosquitoes. The challenge of malaria control in India is very complex due to the country's diverse ecological zones and the presence of multiple vector species complex. Out of 58 identified Anopheline mosquito species from India, 10 are reported to be malaria vectors [4]. These species exhibit different distribution patterns that are ecotype-specific and showed a diverse behavioural trait [5, 6], making the vector control efforts complicated. Several initiatives were employed for vector control but it continues to be a challenge to public health programme officials.
A deep understanding of ecology of vector mosquito, their distribution, life cycle, and population dynamics is very essential for formulation of effective vector control strategies [7]. The climatic factors such as temperature, rainfall and humidity influence vector population density leading to seasonal variation in malaria incidence [8, 9, 10]. Temperature plays a critical role in determining the pace and scale of malaria transmission. It is widely accepted that the rate of parasite development within vectors is temperature-dependent [11-13]. It affects both the speed of larval development and the internal cycle of the *Plasmodium* parasite within the mosquito. Generally, higher temperatures accelerate mosquito growth, increase their feeding frequency, and shorten the time required for the parasite to mature and become transmissible

[14]. Low temperature - especially in highland areas - hindering parasite development and transmission [15]. The link between rainfall and malaria outbreaks has long been acknowledged [16]. Moderate rainfall can increase the number of breeding sites and thus enhances mosquito population, whereas heavy precipitation may wash away larvae from shallow water bodies such as ditches and puddles [17]. Life span of mosquitoes is significantly influenced by relative humidity also [18, 19].

Mosquito abundance in any given region not only depends on climatic factors but also by other like the availability of breeding habitats, vegetation, and rates of evaporation [13, 20, 21]. Humidity, soil moisture and man-made irrigation systems also plays important role on it [22, 23, 24]. Competition among mosquito species may further influence population dynamics [25].

Several studies from different malaria endemic countries showed correlation between climatic factors and mosquito population density [26-31]. Such studies have been under taken in different malaria prone areas of India like Dehradun [32], Rajasthan [33], Visakhapatnam [34], Uttar Pradesh [35] and Varanasi [36]. But such reports from West Bengal, particularly from its northern districts are very scarce. The present study was under taken with the aims to determine the influence of climatic factors on abundance of malaria vectors in northern districts of West Bengal.

2. Materials and Methods

2.1. Study areas: The present study was conducted in five northern districts of West Bengal, namely Darjeeling, Jalpaiguri, Coochbehar, Uttar Dinajpur and Malda. In each study district, one village was selected as study site. The study villages were selected after analysis of last five years malaria cases and in consultation with district health authorities. The study was conducted during January, 2021 to December, 2022. The geographical location and demography of the study villages are given in Table 1.

Table 1: Geographical location and demography of the study villages

District	Block	Village	Location	Nature
Darjeeling	Matigara	Kawakhali	$26^0$41’23”N $88^0$23’36”E	Semi Urban
Jalpaiguri	Sadar	Patkata	26^0 54’70”N $88^0$70’91”E	Semi Urban
Coochbehar	Haldibari	Volarhat	$26^0$33’00”N $88^0$77’00”E	Rural
Uttar Dinajpur	Chopra	Lakhipur	$26^0$36’83”N $88^0$31’25”E	Rural
Malda	English Bazer	Doulatpur	$25^0$08’00”N $88^0$14’60”E	Rural

2.2. Mosquito collections and identification: During the entomological survey, immature stages (larva and pupae) were collected from all potential anopheles mosquitoes breeding sites present surrounding each study village. The potential natural breeding habitats were rainwater pools and puddles, borrow pits, river bed pools, pond margins, sluggish streams, irrigation channels, rice fields, wells and storage water containers, etc. The study team typically spent 2 - 3 hours in each village for collection of immature stages of mosquitoes. To investigate these aquatic habitats, a variety of tools, including different types of dippers and aquatic nets were used. For larval and pupal collection, utensils of varying capacities were utilized - for example, soup ladles with a capacity of approximately 100-150 ml and larger dippers holding up to 500 ml. The mosquito larvae collected from each dip were placed in 500 ml plastic containers for safe transport to the laboratory. Once there, the immature mosquitoes were reared up to third/fourth instar stages and at this point they were identified up to the genus level.

From each of the study villages, indoor resting adult mosquitoes were captured using mouth aspirators (John W. Hock, USA) and flashlight assistance from both human residences and cattle shelters. The collection was carried out for 10 minutes in each household, during dusk (6:00 PM to 8:00 PM) and in morning (8:00 AM to 10:00 AM). This entomological survey was conducted once a month across 60 randomly selected houses in each study village, involving two trained personnel. The mosquitoes collected were placed in clearly labelled containers and then transported to the laboratory for further identification at the species level.

Adult mosquitoes obtained directly from the field were killed by freezing and identified to the species level based on morphological traits, utilizing taxonomic keys and descriptions provided by Nagpal *et al*. (2005) [37] and Tyagi *et al*. (2015) [38]. The total count of mosquitoes collected from each study village was carefully documented. Following species identification, individuals of each mosquito species were counted, and detailed records were maintained for each study site throughout the duration of the research.

2.3. Entomological indices: Per dip density is the measurement parameter of immature stage concentration in different habitats and in different time periods of a year. Per dip density was calculated for estimating the concentration of larvae and pupae in different breeding habitats throughout the year. Per dip density was calculated as follows -

$$\text{Per dip densi} = \frac{\text{Total no. of larve and pupae collected from a habitat}}{\text{Total no. of dips performed}}$$

For calculation of per dip density of Anopheles vector immature stages, site wise month wise collected larvae were reared in the laboratory and allowed to hatch into adults. After hatching Anopheles vectors were identified and counted to calculate the per dip density of Anopheles vectors larvae. The no. of non- Anopheles vector mosquitoes was excluded from this calculation.

Man hour density (MHD) is the measurement parameter of adult mosquito density in different habitats collected by mouth aspirator. MHD for the mouth aspirator collected mosquitoes is calculated as follows -

$$\text{MHD} = \frac{\text{Total no. of mosquitoes collected}}{\text{Total time spent in hours} \times \text{No. of insect collectors}}$$

2.4. Meteorological data collection: During the study period, climate data including maximum and minimum temperature (ºC) and relative humidity (%) were recorded on the day of entomological with the help of digital thermometer and hygrometer from different study villages. The data of monthly average rainfall of each study sites were collected from the website (https:/www.worldweather.com).

2.5. Ethical statement: Prior to beginning the entomological collections, an awareness meeting was conducted at the village level, involving community members along with local health officials and administrative representatives. The purpose and goals of the research were clearly communicated, and residents were encouraged to support the study team throughout the research activities. Consent was obtained from property owners before accessing private homes and land for mosquito collection. The study did not involve any species listed as endangered or protected. Ethical approval for the research was granted by the Institutional Ethics Committee of the Calcutta School of Tropical Medicine, Kolkata, India. Throughout the study period, both adult and immature mosquito stages were collected using standardized entomological procedures.

2.6. Data analysis: Data on the number of mosquito larvae and pupae collected, the total dips taken, and adult mosquitoes captured using mouth aspirators during the survey were systematically entered into an Excel spreadsheet, categorized by collection month and study location. Using this dataset, larval per dip density and man-hour density for indoor-collected adult mosquitoes were computed. Monthly cumulative data on Anopheles vector presence, breeding site positivity, per dip density of immature stages, and overall man-hour density across the three study districts were statistically examined using t-tests. In addition, one-way ANOVA was employed to assess variations in species-specific man-hour densities of adult Anopheles mosquitoes. To evaluate how climatic variables - such as maximum, minimum, and average temperatures, relative humidity, and average rainfall - influenced the population trends of indoor Anopheles vectors, Spearman's correlation analysis was applied. All statistical procedures were conducted using Minitab software (version 19.0).

3. Results

3.1. Species composition: Throughout the 24-month duration of the study, a total of 12,874 female mosquitoes were collected from the surveyed locations, representing 14 species across five genera. The genus *Culex* accounted for the majority of specimens (67.02%), followed by *Anopheles* (16.62%), *Armigeres* (12.70%), *Mansonia* (3.40%), and *Aedes* (0.26%). Within *Culex*, five species were identified, with *Cx. quinquefasciatus* (30.54%), *Cx. tritaeniorhynchus* (15.32%), and *Cx. gelidus* (12.89%) being the most frequently encountered. Four *Anopheles* species were found, among which *An. culicifacies* (8.68%) and *An. stephensi* (6.49%) were dominant. The genus Aedes was represented by two species - *Ae. albopictus* (0.22%) and *Ae. aegypti* (0.04%). Additionally, *Armigeres subalbatus* (12.70%) and *Mansonia uniformis* (3.40%) were the sole representatives of their respective genera (Fig. 1).

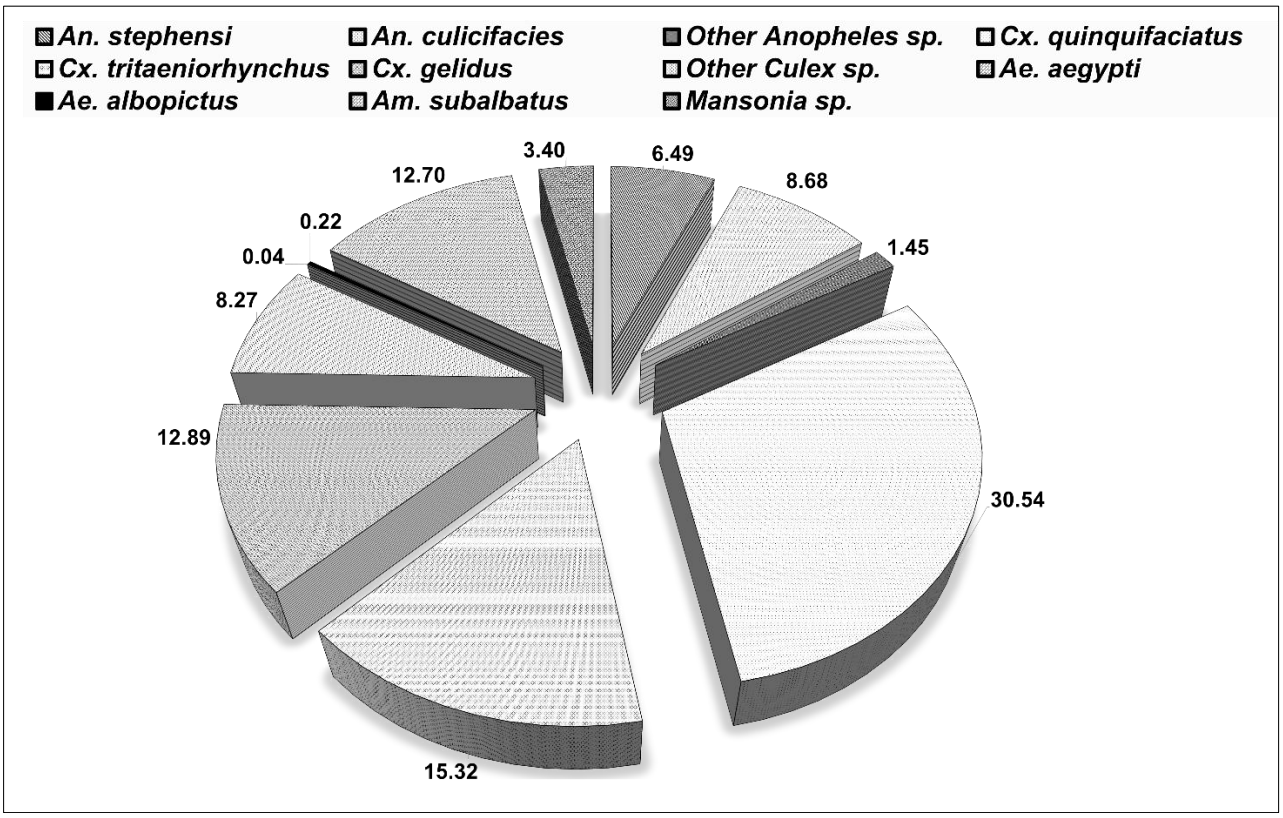


Fig 1: Species composition of mosquitoes collected during the study period

3.2. Major Anopheles vectors in the study areas: Out of 12874 collected mosquitoes, 2140 (16.62%) belonged to four different species of Anopheles vectors and *An. culicifacies* (8.68%) and *An. stephensi* (6.49%) were dominant. (Fig. 2). No significant difference was observed between the overall abundance of *An. culicifacies* and *An. stephensi* ($p > 0.05$).

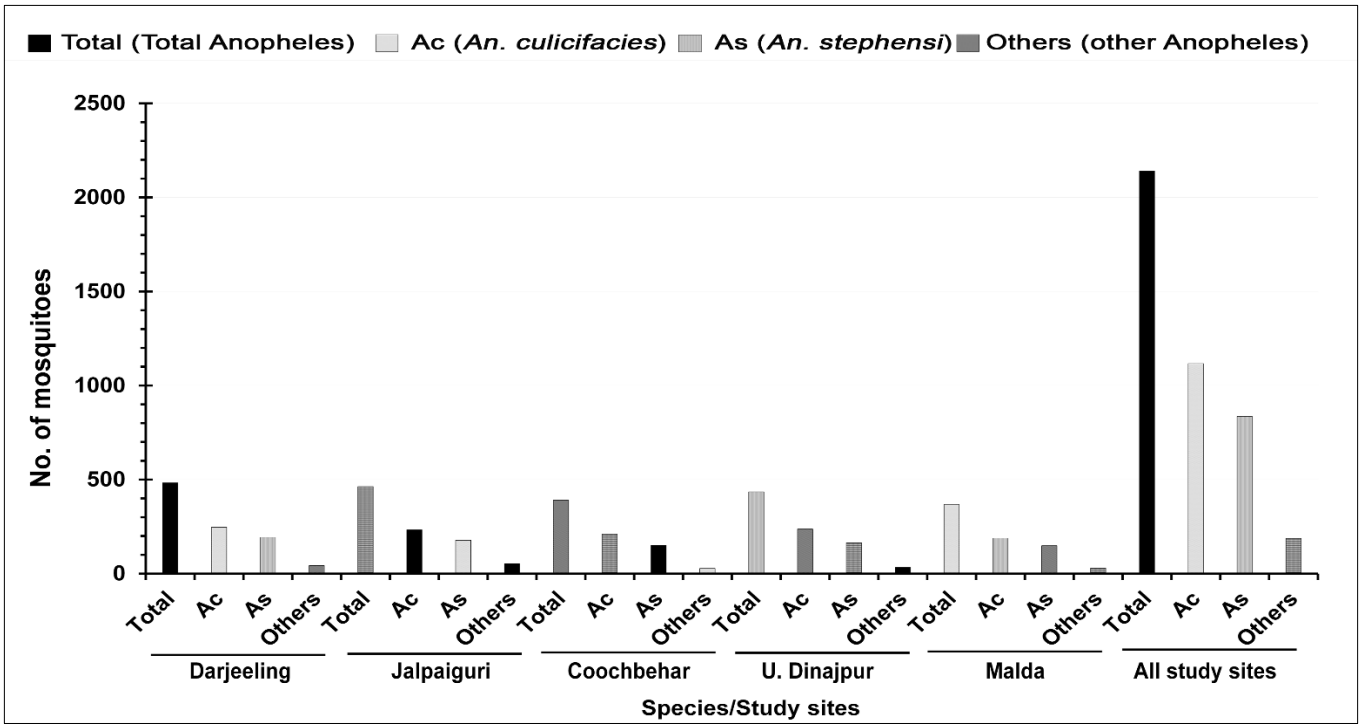
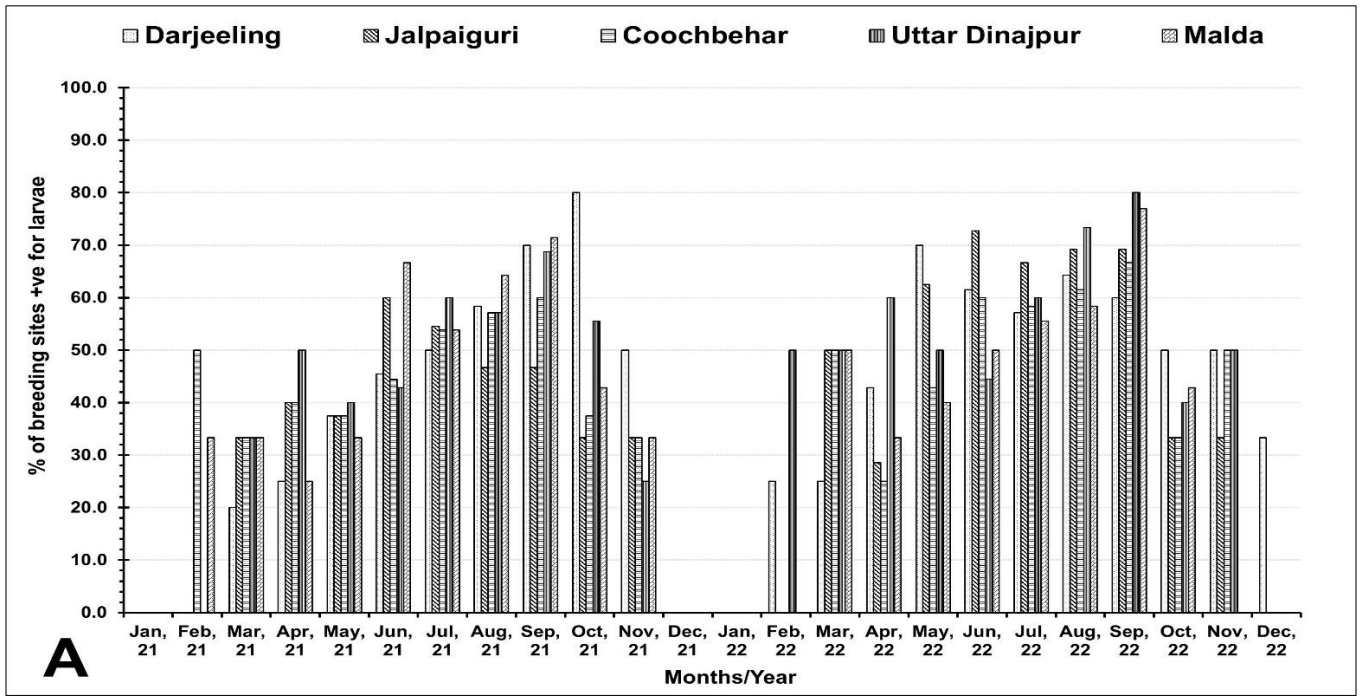


Fig 2: Abundance of Anopheles vector mosquitoes in study sites

3.3. Larval and pupal survey for Anopheles vectors: Throughout the duration of the study, approximately 800 potential mosquito breeding habitats were examined. Frequently encountered larval environments included ponds, abandoned wells, paddy fields, cesspools, cesspits, concrete tanks, culverts, irrigation channels, muddy pools, pools within riverbeds, rain-fed water collections, and areas with slow-moving streams. The percentage of sites found positive for immature mosquito stages was consistent across the five districts surveyed: Darjeeling (49.72%), Jalpaiguri (50.0%), Coochbehar (49.68%), Uttar Dinajpur (54.84%), and Malda (51.06%). Statistical analysis indicated no significant variation in breeding site positivity among the study districts ($p>0.05$). During the colder months, from November through February, breeding site positivity was notably lower but showed a progressive increase with the onset of warmer weather, peaking during the monsoon season. Across all districts, the highest rates of breeding site positivity were observed between August and October. Similarly, the average number of larvae per dip was minimal during winter but rose gradually through the summer, reaching a maximum during the rainy season. Monthly fluctuations in Anopheles vector density per dip across the five districts are illustrated in Fig. 3. Again, no significant inter-district differences were found in per dip densities ($p>0.05$).



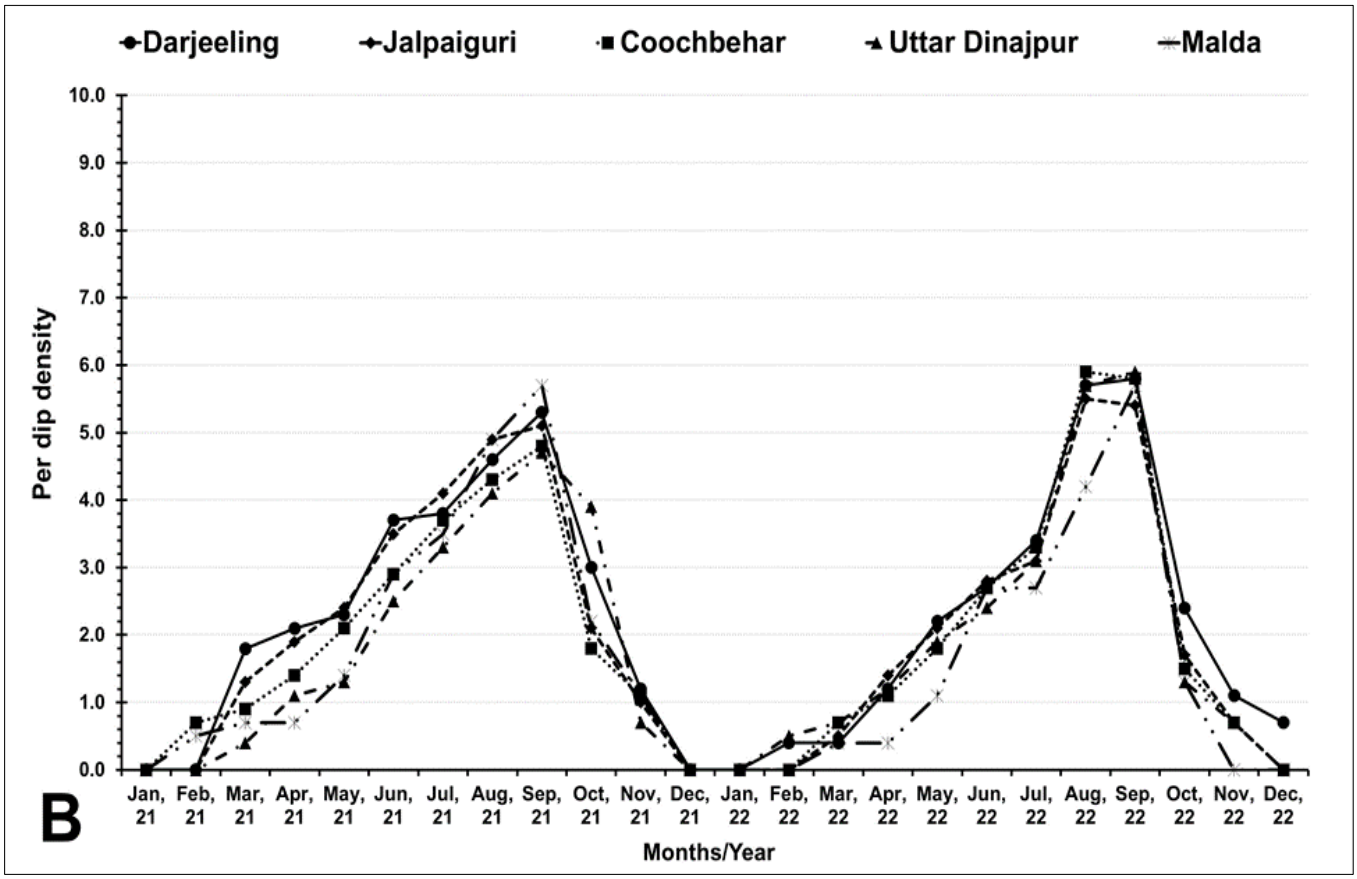


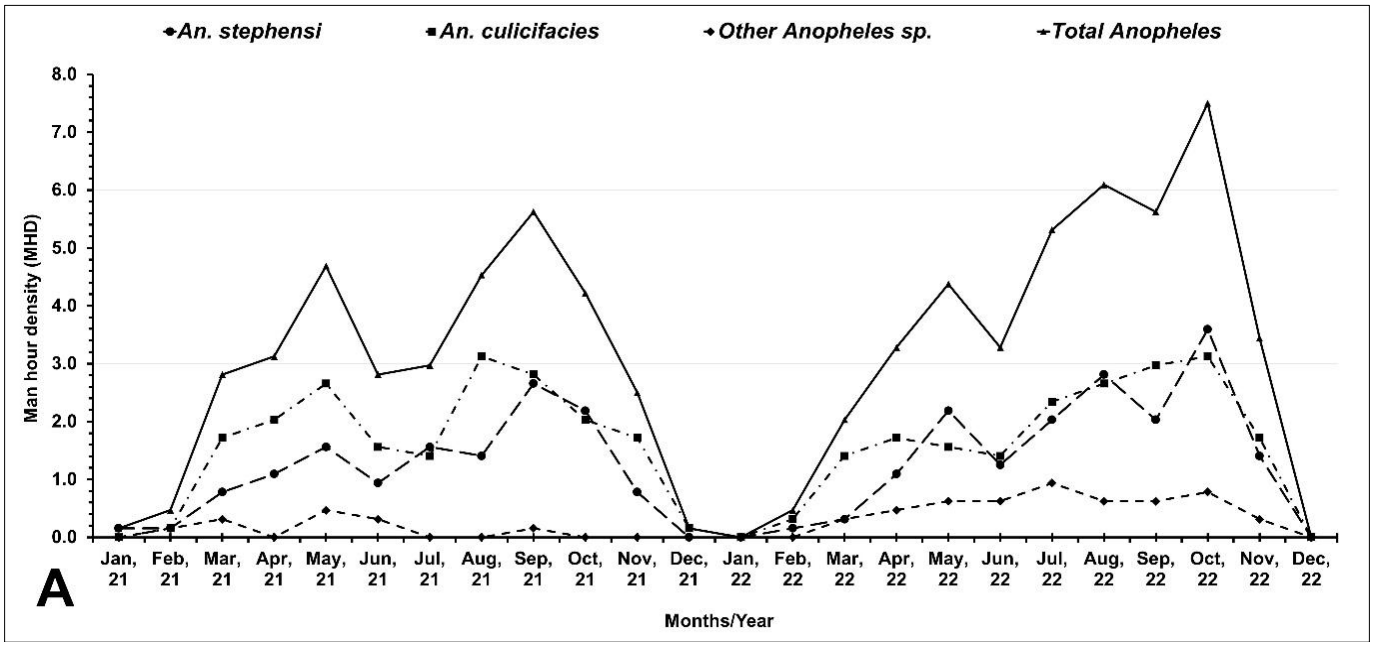
Fig 3: Month wise percentage of breeding site positive (A), per dip density of Anopheles vectors (B) larvae and pupae in the study area

3.4. Adult mosquito survey and man hour density of Anopheles vectors: Throughout the study period, a total of 2140 adult Anopheles mosquitoes using mouth aspirators. The monthly variation in man-hour density of Anopheles species across the five districts surveyed is illustrated in Fig. 4. During the winter months (December to February), the density levels were notably low, followed by a steady rise beginning in March. The peak densities were observed between August and October, after which a gradual decline was noted across all districts. District-specific peaks in man-hour density were recorded as follows: Darjeeling reached its maximum in October (7.5), Jalpaiguri in August (7.5), Coochbehar and Uttar Dinajpur both peaked in September, while Malda recorded its highest value in the same month (5.8). Statistical evaluation revealed no significant differences in the man-hour densities of Anopheles mosquitoes among the five districts (Table 2).

Table 2: Statistical analysis of month wise species wise man hour density of four Malaria vectors in study areas

Study sites	Species[#$*]	Basic statistics of month wise MHD			Within study site (t-Test)
		Mean ± SD	Range	95% CI	
Darjeeling	*An. stephensi*^{A}	1.25 ± 0.99	0 - 3.594	0.84 - 1.68	t = - 1.20
	An. culicifacies^{B}	1.61 ± 1.04	0 - 3.125	1.17 - 2.04	p = 0.238
Jalpaiguri	*An. stephensi*^{A}	1.16 ± 1.03	0 - 3.125	0.73 - 1.61	t = - 1.14
	An. culicifacies^{B}	1.52 ± 1.14	0 - 3.906	1.03 - 1.99	p = 0.262
Coochbehar	*An. stephensi*^{A}	0.99 ± 0.96	0 - 3.281	0.58 - 1.39	t = - 1.35
	An. culicifacies^{B}	1.37 ± 1.01	0 - 3.281	0.95 - 1.81	p = 0.184
Uttar Dinajpur	*An. stephensi*^{A}	1.06 ± 0.98	0 - 2.969	0.65 - 1.48	t = - 1.56
	An. culicifacies^{B}	1.54 ± 1.12	0 - 3.594	1.06 - 2.01	p = 0.125
Malda	*An. stephensi*^{A}	0.97 ± 0.82	0 - 2.344	0.62 - 1.32	t = - 0.98
	An. culicifacies^{B}	1.22 ± 0.96	0 - 3.125	0.81 - 1.63	p = 0.335
Total	*An. stephensi*	0.218 ± 0.18	0 - 0.60	0.14 - 0.29	t = - 1.36
	An. culicifacies	0.291 ± 0.19	0 - 0.60	0.22 - 0.38	p = 0.180

[#]There was no significant difference between month wise per man hour density of *An. stephensi* between five study sites (F = 0.37, p = 0.829)
[$]There was no significant difference between month wise per man hour density of *An. culicifacies* between five study sites (F = 0.51, p = 0.729)
[*]Means that do not share a letter are significantly different by Tukey Test


An. stephensi
An. culicifacies
Other Anopheles sp.
Total Anopheles
Man hour density (MHD)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Jan, 21
Feb, 21
Mar, 21
Apr, 21
May, 21
Jun, 21
Jul, 21
Aug, 21
Sep, 21
Oct, 21
Nov, 21
Dec, 21
Jan, 22
Feb, 22
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Jun, 22
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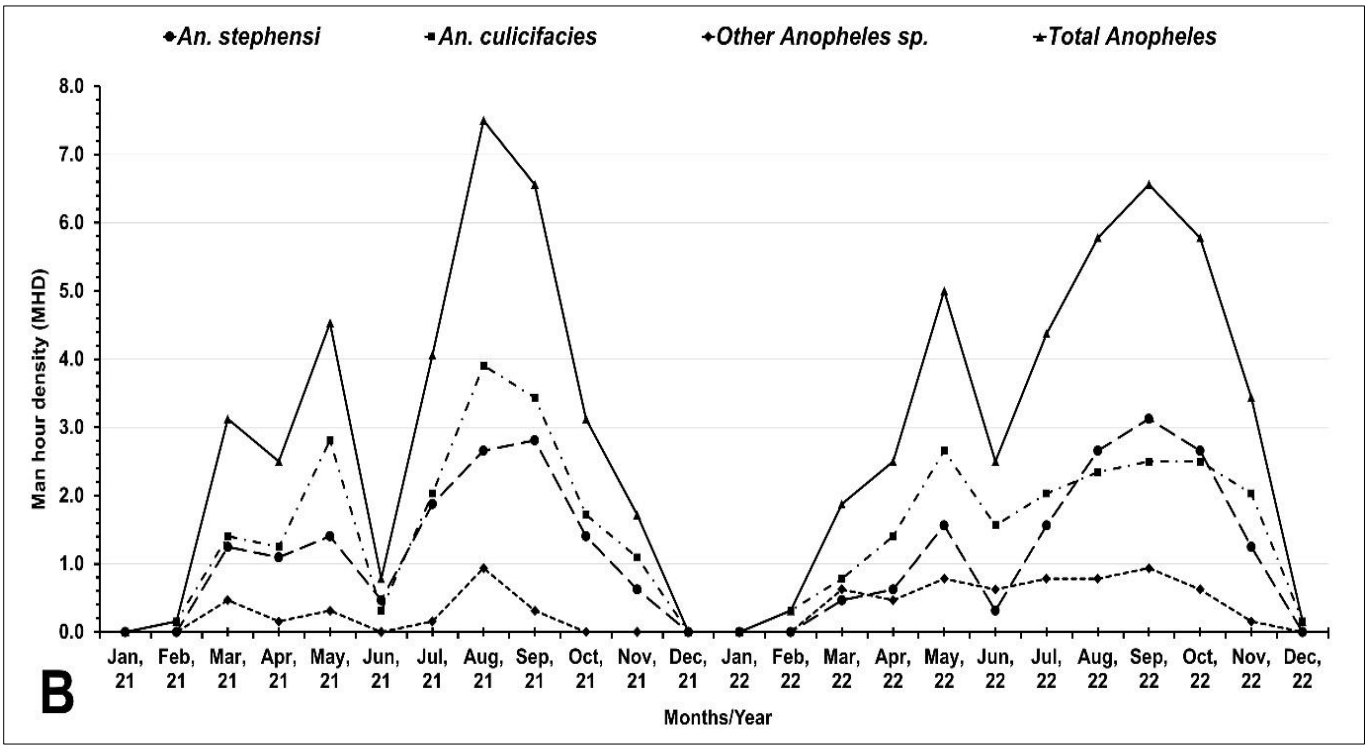
Aug, 22
Sep, 22
Oct, 22
Nov, 22
Dec, 22
Months/Year
A


An. stephensi
An. culicifacies
Other Anopheles sp.
Total Anopheles
Man hour density (MHD)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Jan, 21
Feb, 21
Mar, 21
Apr, 21
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Jan, 22
Feb, 22
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Nov, 22
Dec, 22
Months/Year
B

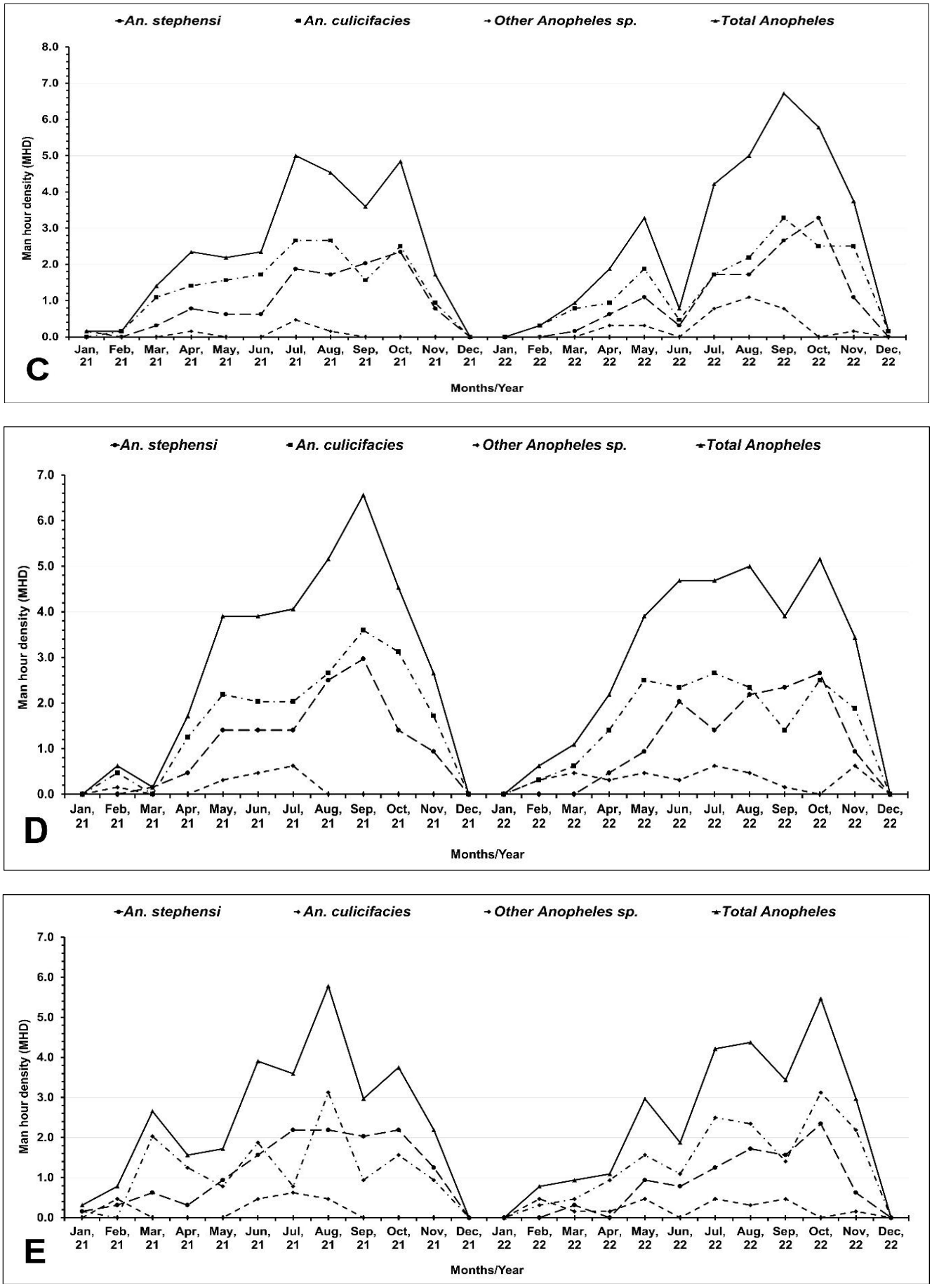

Fig 4: Month wise man hour density of major Anopheles vector in Darjeeling (A), Jalpaiguri (B), Coochbehar (C), Uttar Dinajpur (D) and Malda (E)

3.5. Impact of environmental factors on Anopheles vector population dynamics: Across all surveyed districts, the population of Anopheles mosquitoes reached its peak in August and dropped to its lowest point in January. A steady increase in vector abundance was observed from March through November. Spearman's rank correlation analysis demonstrated a significant positive relationship between Anopheles abundance and various climatic factors-namely, maximum, minimum, and average temperatures, as well as relative humidity and rainfall-in each of the study districts (Table 3 and Fig. 5-9).

Table 3: Analysis of impact of maximum, minimum, average temperature, relative humidity and rainfall on abundance of Malaria vectors

Study sites	Climatic factor	Spearman's correlation analysis	
		ρ	p value
Darjeeling	Max. temp	0.63	<0.001
	Min. temp	0.75	< 0.0001
	Avg. temp	0.79	< 0.0001
	RH	0.56	0.004
	Rainfall	0.52	0.008
Jalpaiguri	Max. temp	0.75	< 0.0001
	Min. temp	0.79	< 0.0001
	Avg. temp	0.78	< 0.0001
	RH	0.42	0.033
	Rainfall	0.43	0.039
Coochbehar	Max. temp	0.46	0.023
	Min. temp	0.64	0.001
	Avg. temp	0.73	< 0.0001
	RH	0.64	0.001
	Rainfall	0.61	0.002
Uttar Dinajpur	Max. temp	0.42	0.039
	Min. temp	0.58	0.003
	Avg. temp	0.80	< 0.0001
	RH	0.82	< 0.0001
	Rainfall	0.70	< 0.0001
Malda	Max. temp	0.49	0.047
	Min. temp	0.60	0.002
	Avg. temp	0.72	< 0.0001
	RH	0.59	0.002
	Rainfall	0.51	0.01

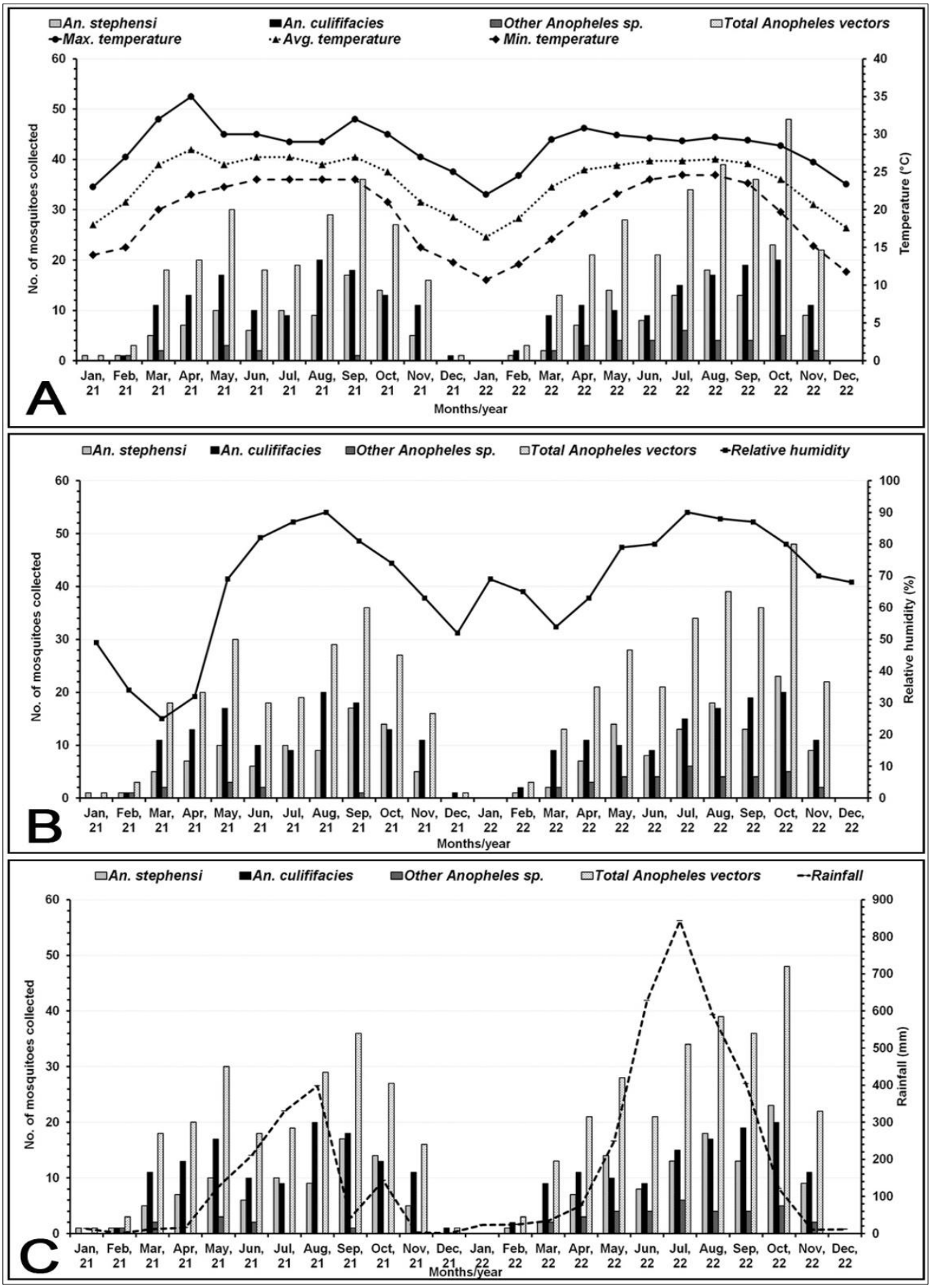

Fig 5: Influence of environmental factors on population density of Anopheles vector species in Darjeeling district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

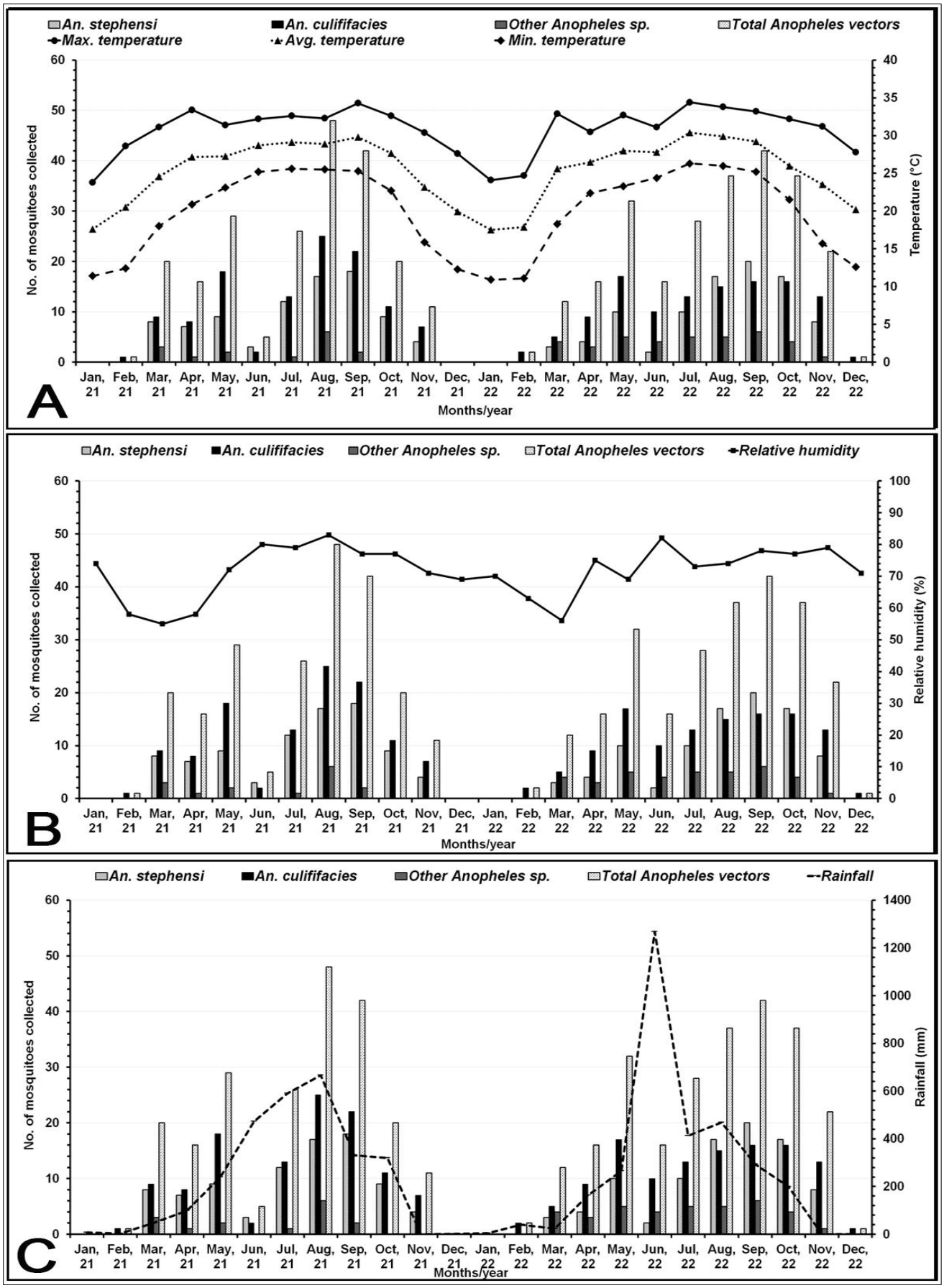

Fig 6: Influence of environmental factors on population density of Anopheles vector species in Jalpaiguri district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

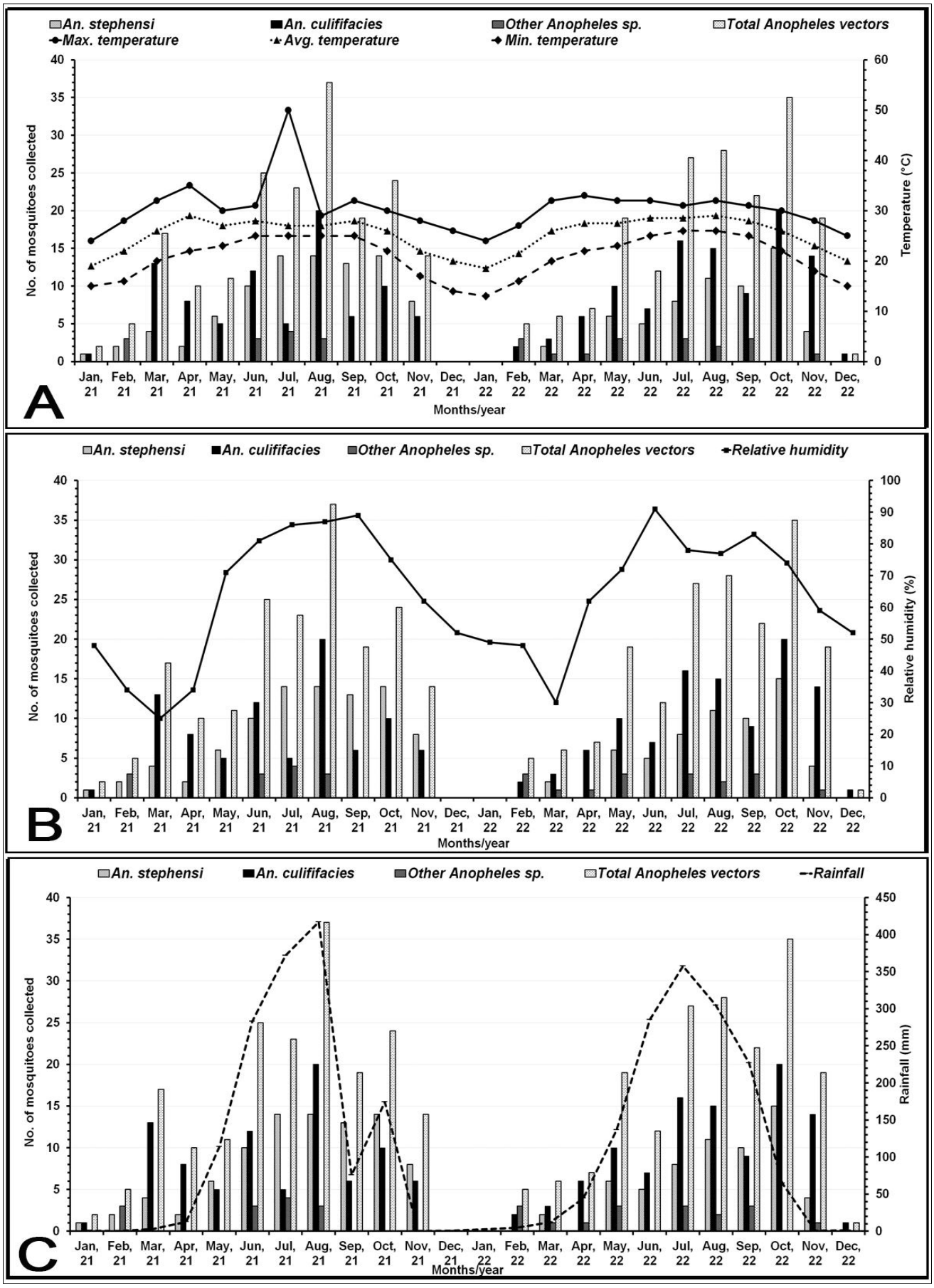

Fig 7: Influence of environmental factors on population density of Anopheles vector species in Coochbehar district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

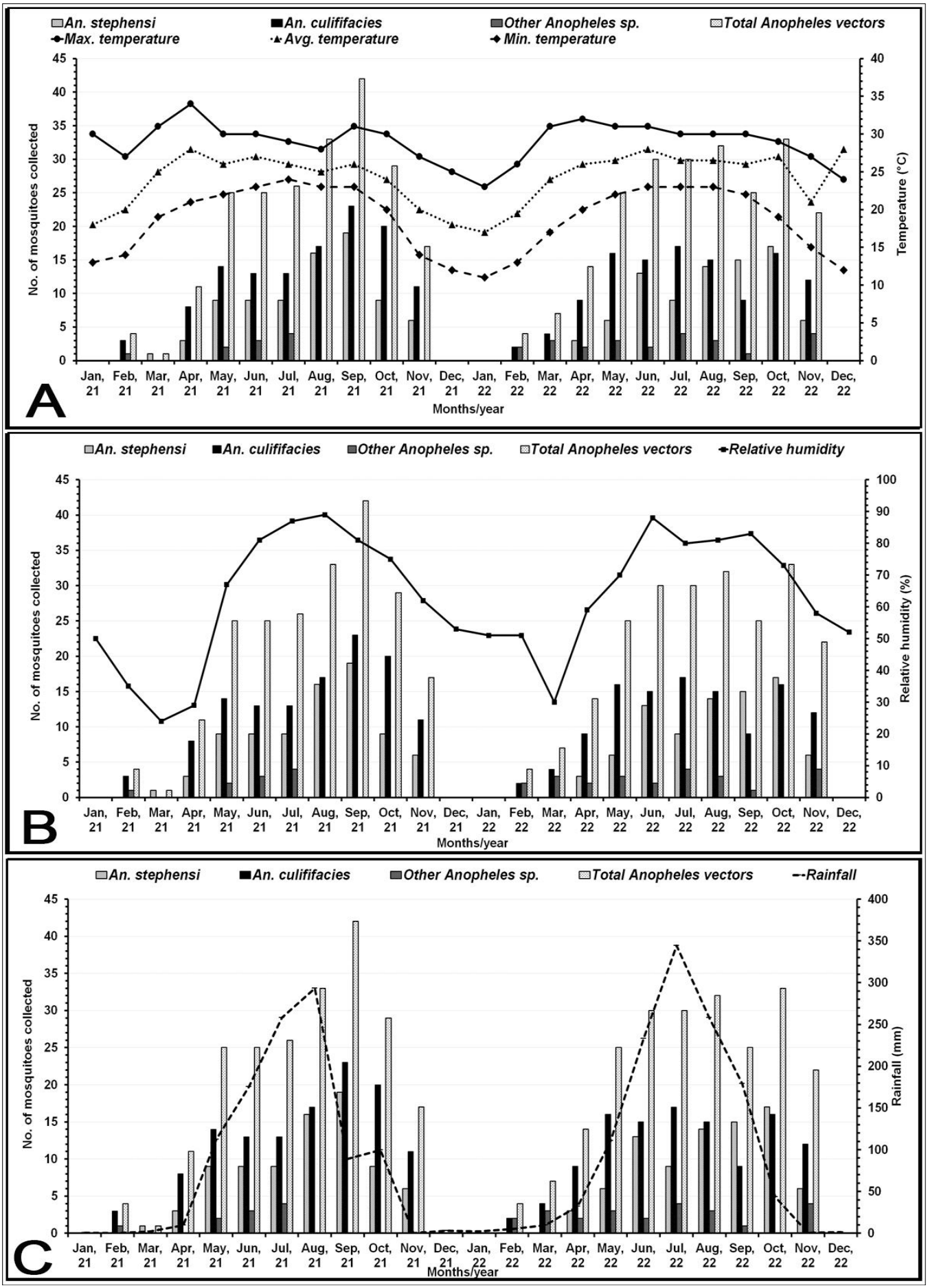

Fig 8: Influence of environmental factors on population density of Anopheles vector species in Uttar Dinajpur district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

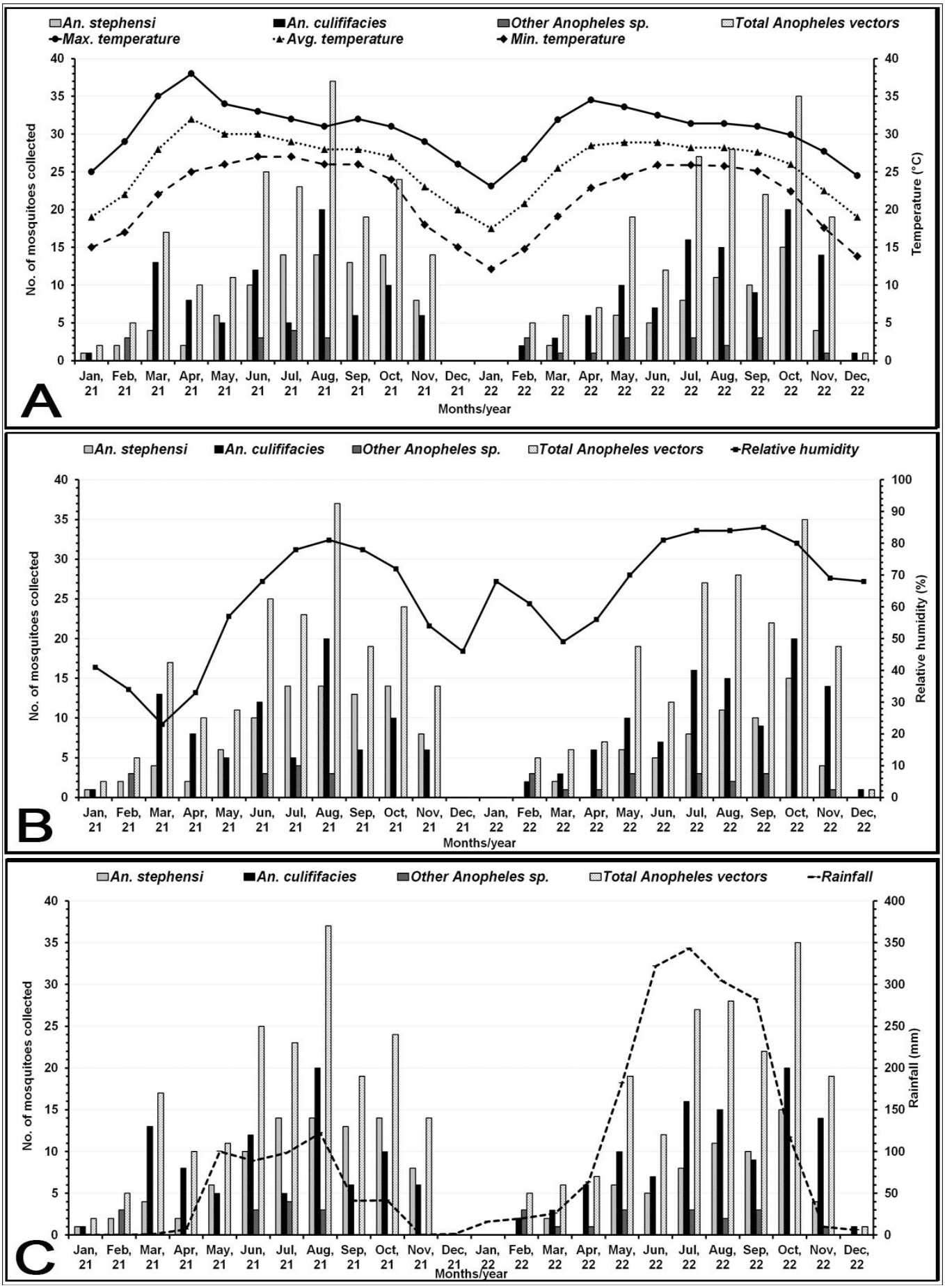

Fig 9: Influence of environmental factors on population density of Anopheles vector species in Malda district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

4. Discussion

This study provides valuable insights into the species composition, seasonal dynamics, and environmental determinants of mosquito populations, particularly *Anopheles* vectors, across five districts in northern part of West Bengal over a 24-month period. A total of 12,874 female mosquitoes, representing 14 species across five genera, were documented, with *Culex* emerging as the most dominant genus. This finding aligns with earlier research indicating the widespread distribution and high adaptability of *Culex* species, especially *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*, in urban and peri-urban settings [39].

Although *Culex* accounted for the majority of collected mosquitoes (67.02%), the presence and abundance of *Anopheles* species - especially *An. culicifacies* and *An. stephensi* - is of particular epidemiological concern as they are the primary malaria vectors in India [5]. Together, these two species represented 15.17% of all collected specimens, and their distribution showed no significant variation in abundance between them ($p>0.05$), indicating their equal contribution to potential malaria transmission risk in the study area.

Larval and pupal habitat surveys revealed a consistent pattern of breeding site positivity across five study districts, ranging from approximately 49% to 55%, suggesting similar environmental conditions conducive to mosquito proliferation. Common breeding habitats such as ponds, paddy fields, and slow-moving streams were in agreement with previously documented larval ecology of *Anopheles* vectors [26]. Seasonal variation was prominent, with lower breeding habitat positivity during the winter months (November-February) and higher rates of positivity during the rainy season, peaking between August and October. These findings are consistent with earlier studies showing that monsoon-driven increases in water availability create optimal conditions for mosquito breeding [40, 41].

The adult mosquito survey further emphasized the seasonal abundance pattern of Anopheles vectors. Man-hour density of *Anopheles* vectors showed a pronounced increase from March onwards, reaching peak levels during the post-monsoon period (August-October). Study district-specific differences in peak months were observed but no statistical significance was observed in overall vector density ($p>0.05$) among five study districts. From this, it was suggested that despite local ecological variability, the broader climatic drivers operate similarly across the study districts. The peak adult densities during late monsoon months corroborate the larval findings and reflect the mosquito life cycle dynamics, where increased larval habitat availability translates into higher adult emergence [42].

Environmental factors were found to significantly influence *Anopheles* population dynamics. Spearman's correlation analysis indicated strong positive correlations between vector abundance and key climatic variables, including maximum, minimum, and average temperatures, as well as relative humidity and rainfall. Similar associations have been reported in numerous studies across India and other tropical regions [30-36]. Temperature and humidity directly affect mosquito development, survival, and biting rates, while rainfall influences the availability of breeding sites.

The study revealed that abundance of two major malaria vector species namely *An. culicifacies* and *An. stephensi* remain low during November to February. A steady rise of population density was observed from March onwards and reached to the peak in August to September. The vector control measures in the form of indoor residual spray with DDT is applied during the month of March and August when the population density starts increasing and reaches its peak, respectively. So, the timings of IRS recommended by NVBDCP is justified by the findings of the present study.

5. Conclusion

Present study confirms the high diversity of mosquito fauna in northern West Bengal and underscores the importance of climatic and ecological factors in shaping vector population dynamics. The dominance of *An. culicifacies* and *An. stephensi*, coupled with their strong association with environmental conditions, highlights the need for targeted vector control strategies that are seasonally adapted and environmentally informed. Further studies for assessing susceptibility status of major malaria vectors against different insecticides is highly recommended.

Conflicts of interest

We have no conflicts of interest concerning the work reported in this article.

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