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Species Diversity and Seasonal Abundance in Relation to Environmental Factors in Different Agro Climatic Zones of Tamil Nadu, India

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Abstract

Background: Dengue emergence was high in several regions of Tamil Nadu, India recently, which can lead to severe health problems in human population. Prevention of this arbovirus requires a clear understanding of transmission cycles and breeding capability, especially of their vectors.

Results: A total of 11,202 specimens (6,337 larvae and 4,865 adults) which belongs to 64 taxonomic categories distributed in 14 genera were collected. Among the collected mosquitoes, morphological identification showed the presence of 64 species: *Aedes* (11), *Anopheles* (11), *Armigeres* (3), *Christophersiomyia* (2), *Culex* (13), *Downsiomyia* (2), *Ochlerotatus* (7), *Heizmannia* (1), *Orthopodomyia* (1), *Fredwardsius* (1), *Lutzia* (2), *Tripteroides* (2), *Uranotaenia* (6), *Toxorhynchites* (2). Significant relationships were observed between abundance and seasonality, with a notable increase in the mosquito's abundance in rainy periods. Diversity indices were revealed with interaction between seasonality and months. High Altitude Zone (HAZ) (3,726) had highest species richness followed by Cauvery Delta Zone (CDZ) (1,774). Larvae of *Ae. aegypti* in containers and *Cx. tritaeniorhynchus* in paddy were omnipresent.

Conclusion: This work highlights the importance of detailed analysis of different mosquito larvae and pupae occurring at different localities and in different habitat types for detailed insight into the agro ecosystem wide fauna. Information on the different species occurring at inventoried aquatic habitats provides interesting ecological insights into species co-occurrence and habitat preferences.

Keywords: Mosquitoes diversity; *Aedes aegypti*; Agro climatic zones; Shannon and berger-parker index; Tamil Nadu

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Introduction

One of the most dangerous animals on earth is a tiny insect: The mosquito. More than 500 million people are currently infected with mosquito-borne diseases, and more than 3 million people die every year from these infections [1]. In addition, currently half of the world's population, about 3.5 billion people, is now at risk for infection from mosquito borne diseases. These diseases profoundly restrict socioeconomic status and development in countries with the highest rates of infection, many of which are located in the tropics and subtropics [2]. Climate change may impact human health and wellbeing in many ways, including by facilitating the spread of many infectious agents. For instance, the changing scenario of major vector-borne diseases (e.g., malaria, leishmaniasis, changes disease) have been linked to several factors, including urbanization and deforestation changing demographics in both developing and developed countries, economic crisis, increased global movement of people and animals [3].

Recently, biodiversity changes have also been linked to shifts in disease risk for humans and wildlife [4-6]. Many environmental variables can have a direct or indirect effect on mosquito oviposition [7] and subsequently on larval distribution, density, and development [8]. Field studies have indicated that, by recognizing key habitat factors, mosquitoes can select suitable habitats for egg deposition [9]. The suitability of a site for mosquito breeding depends on complex interactions of biotic factors such as competitors [10], predators, and food availability [11]. In addition, abiotic factors *viz.* precipitation [12], temperature and water quality parameters also influenced in breeding

[13]. Understanding how these factors influence the abundance and distribution of mosquito larvae would be essential for the design and implementation of integrated vector management programs [14].

The physicochemical characteristics of water bodies are complicated and determine their condition and faunal composition [15]. It includes salts, dissolved inorganic and organic matter, turbidity and presence of suspended mud. Other hydrologic factors that affect pre-imaginal mosquito populations in water are the presence or absence of plants, temperature, light and shade, hydrogen ion concentration, presence of food substances (live or dead), presence of predacious mosquito larvae and other insects [16]. The biological and physicochemical attributes of aquatic environments may alter adult mosquito vector competence. Tree holes structure is determined by a complex set of biological and physical factors that interact to develop a patchy habitat [17]. Altering landscapes through agricultural development can change the physical, chemical, and biological characteristics of larval mosquito habitats, in turn, affecting oviposition and larval survival [18-20]. Understanding the landscape changes in distribution and ecological requirements of larval mosquito populations is important for managing potential breeding habitats and also for predicting vector-borne disease prevalence.

The study of the composition and diversity of mosquitoes, both in natural and anthropically disturbed areas, is of considerable importance due to their role in pathogen transmission to humans and animals [21]. Furthermore, the study of natural environments allows us to know the habitat of those species whose role in transmission cycles remains unknown [22]. The spatial and temporal distribution of culicids is dependent on availability of suitable aquatic habitats where the immature stages can develop. Rivers constitute an important larval habitat for several species of Anophelines of medical importance.

Environmental factors, including human activities that enhance vector densities (irrigation, heavy rains followed by floods, higher than usual temperatures, and formation of ecologic niches enabling the mass breeding of mosquitoes) allow the re-emergence of these mosquito-borne diseases [23]. Understanding of arboviral transmission cycles begins with the correct identification of potential vectors and thorough knowledge of their bioecology in the environment. The goals of this study were to determine diversity, abundance, and seasonal dynamics of potential mosquito vectors. This is the first regional report to investigate the relationships between immature mosquitoes and landscape and environmental habitat characteristics in different agro climatic conditions associated with row crop agriculture during wet and dry hydrologic regimes.

Materials and Methods

Study areas

Survey was conducted in water-holding sites supporting mosquito breeding in urban, semi-urban and rural areas of seven different agro climatic zones, *viz*., Cauvery delta zone, North eastern zone, Western zone, North western zone, High altitude zone, Southern zone and High rainfall zone of Tamil Nadu (78.6569° E and 11.1271° N), India (Figure 1). Cauvery Delta Zone (CDZ) includes Thanjavur, Nagapattinam, Tiruvarur, Tiruchirappalli, Karur, Ariyalur, Pudukkottai and Cuddalore districts. Total area of zone is 24,943 km^2 in which 60.2% of the area are under cultivation and the annual rainfall is recorded as 956.3 mm. North Eastern Zone (NEZ) covered by Kancheepuram, Tiruvallur, Cuddalore, Vellore, Villurpuram and

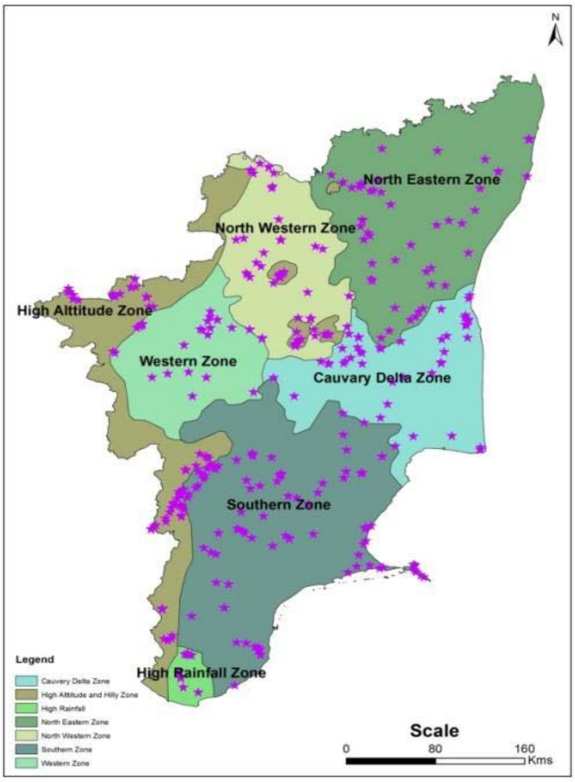


Figure 1: Map showing the mosquito sampling sites in agro climatic zones of Tamil Nadu, India.

Tiruvannamalai districts. Total geographical area of the zone is 32,194 km^2. The areas under cultivation are 50.5% of the total area and the annual rainfall are 1109 mm [24]. Western Zone (WZ) covers Erode, Coimbatore, Tiruppur, Theni, Namakkal (some parts), Dindigul, Perambalur and Ariyalur (some parts) and this zone constitutes an area of 15,678 km^2 and only 42.4% of the area under cultivation. The annual rainfall is recorded as 653.7 mm. North Western Zone (NWZ) covers Dharmapuri, Krishnagiri, Salem and Namakkal (some parts) district excluding hilly areas. This zone covers an area of 18,271 km^2 in which 56.3% are under cultivation. Out of the total area of cultivation, only 23% is irrigated areas. The annual rainfall of the zone is 849 mm. This zone has been identified as moderately drought prone area. High Altitude Zone (HAZ) covers the Nilgiris, Kodaikanal, Shevroy, Elagiri, Javadhi, Kollimalai, Pachamalai, Yercaud, Anamalai, Palani and Podhigaimalai. This zone covers an area of 2,549 km^2. The area under cultivation is only 28.9% of the total geographical area of the zone and the annual rainfall is recorded as 1,857 mm [25]. Southern Zone (SZ) comprises the districts of Madurai, Sivagangai, Ramanathapuram, Virudhunagar, Tirunelveli and Thoothukudi. This zone constitutes an area of 36,655 km^2. This zone is prone to frequent drought. The annual rainfall is 816.5 mm. High Rainfall Zone (HRZ) consists of only Kanyakumari district. The total geographical area is 1,684 km^2, 47.4% of the total area are under cultivation. The annual rain fall were recorded as 1,456 mm.

Collection methods

Mosquito trapping and collections were carried out from January to December 2018. Sampling was made at 30 selected sites of each zone, thrice per month on 10 days interval to the fixed breeding sources of different agro climatic zones of Tamil Nadu, India for overnight trapping periods from 18:00 to 06:00 and in some places

Figure 2: Common breeding habitats found in study areas.

day time collection also adapted (Figure 2). Adult trapping sites were fixed according to human population and blood feeding host and the information on DF, filariasis and malaria cases. It was not always possible to collect mosquitoes repeatedly from the fixed localities for adults. Survey was mainly focused on aquatic preimaginal stages. The priorities were given to natural areas due to anticipated higher specific richness, although urban and agricultural zones were also surveyed.

Immature mosquito sampling

Immatures (larvae and pupae) were sampled with a mosquito larval dipper in breeding sites at ground level (puddles, lakes and other water bodies) by taking 20-40 (500 mL) dip samples based on the standardized larval sampling protocol at each district [26]. Removable breeding sites, such as plastic containers, vases and tires, their contents transferred to plastic trays and the specimens were then collected with pipettes. In natural larval habitats, the immature specimens were collected directly with a suction pipette and/or a mosquito dipper depending on the size of the exposed surface. In case of larval habitats in plants, such as tree holes and the axils of bromeliad leaves, the content was extracted using suction samplers [27]. The collected specimens were transported to the laboratory and then prepared for morphological identification.

Adult trapping

Adults were collected using Carbon dioxide (CO_2) baited Centre for Disease Control (CDC) light traps and Human Landing Collection (HLC) methods. This trap has been chosen for its effectiveness to capture a wide range of mosquito species (not specific to some genera or diurnal/nocturnal mosquito species) and especially for its autonomy (several days on battery backup, until three weeks) [28]. HLC is the effective method for sampling *Aedes* and it is appropriate method for determining human risk of infection [29]. These two methods were combined in order to have representative catch of the different mosquito species present at the farms. For each site, one CDC trap was set close to the cattle shed while trained scouts were collected mosquitoes from study locations. Collectors were dressed in thick clothing materials with hoods, hand gloves and socks. They collected mosquitoes landing on their socks covered legs to minimize exposure to mosquito bites.

Culture and Identification

Field collected immatures were maintained in the laboratory as separately under ideal condition (maintained by incubator) 27°C ± 2°C, 75% to 85% Rh, under 14L:10D photoperiod cycles to minimize the death. Reliable taxonomic identification (species level) of mosquitoes requires adult stage. Therefore, immature collections were reared in the laboratory on a diet of yeast and dog biscuits at 3:1 ratio. The feeding was continued till the larvae were transformed into the pupal stage. The pupae were collected from the culture trays and transferred to plastic containers (12 cm × 12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in 90 cm × 90 cm size mosquito cage for adult emergence. Thirty cages were used for each zones and the cage was made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part covered with a muslin cloth. In bottom of the cage, placed cotton pad soaked in 10% sugar solution is provided in a Petri-dish for a period of three days and after they were killed with chloroform and/or ethyl acetate vapors for adult identification [15]. Sampled mosquitoes were identified based on morpho-taxonomy analysis, by using the electronic identification key of Schaffner et al. [30] and dichotomous identification keys [31-33]. The trinocular stereo zoom microscope with digital system was used for identification with particular reference to the head, thorax, wings and hind legs. The voucher specimens were present in the depository of the Department of Zoology, Annamalai University, Chidambaram.

Meteorological data acquisition

Weather data (rainfall, relative humidity and temperature) were obtained from the Regional Meteorological Centre (RMC), Chennai and Tamil Nadu Agricultural University (TNAU), Coimbatore. Wind conditions data also obtained from source because it's also one of the factors that affect the abundance of mosquitoes trapped

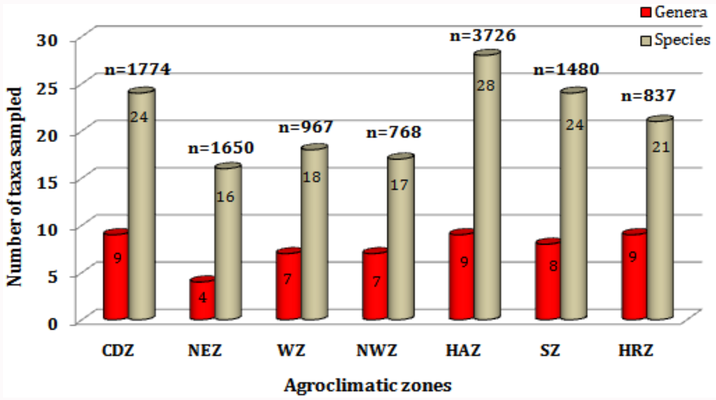


Figure 3: Genera and species of mosquitoes from seven agro-climatic zones of Tamil Nadu.
Numbers within columns indicate number of taxa. Numbers above columns indicate number of individuals identified.

Table 1: Total number of mosquito habitats surveyed, proportion of habitats positive, proportion of immatures and adults collected during January-December 2018.

S. No	Habitat types	Agro climatic Zone	No. of habitats surveyed	No. of habitats with water	Proportion of habitats positive (%)	Total Number of samples collected	Proportion of immatures sampled (%)	Proportion of Adults sampled (%)
1	Rain pool	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	30	30	93.3	529	46.3	53.6
2	Swamp	NEZ, WZ, SZ, HRZ	18	18	83.3	201	58.7	41.2
3	Well	NEZ, WZ, NWZ, SZ	41	36	100	145	37.9	62
4	Used cans	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	152	101	90.1	706	48	51.9
5	Vessels	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	140	82	58.5	81	58	41.9
6	Mud pots	CDZ, NEZ, WZ, NWZ, SZ	36	28	89.2	122	59.8	40.1
7	Tree holes	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	264	156	87.1	845	46.8	53.1
8	River section	CDZ, NEZ, HAZ, SZ, HRZ	45	43	93	241	75.5	24.4
9	Ground pool	CDZ, NEZ, WZ, NWZ, SZ	51	48	85.4	139	58.2	41.7
10	Stream	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	32	32	90.6	463	59.6	40.3
11	Container	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	189	168	91	1022	63	36.9
12	Paddy fields	CDZ, NEZ, WZ, HAZ, SZ, HRZ	58	49	100	894	62.5	37.4
13	Discarded tyres	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	127	74	98.6	1202	54.7	45.2
14	Cement tanks	CDZ, NEZ, WZ, NWZ, SZ, HRZ	88	72	97.2	841	60.2	39.7
15	Public water tanks	CDZ, NEZ, WZ, NWZ, SZ, HRZ	42	41	97.5	653	67.5	32.4
16	Coconut shells	CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ	94	72	97.2	703	58.3	41.6
17	Cattle sheds	NEZ, WZ, NWZ, HAZ, SZ	39	22	86.3	159	54	45.9
18	Leaf axils	WZ, NWZ, HAZ, SZ	168	111	98.1	864	71.8	28.1
19	Animal foot prints	CDZ, NEZ, NWZ, HAZ, SZ	201	184	47.8	369	38.4	61.5
20	Rocky pool	NEZ, WZ, HAZ, SZ	146	95	71.5	386	35.7	64.2
21	Bamboo stump	NEZ, WZ, NWZ, HAZ, SZ	124	104	90.3	637	50	49.9
Total			**2085**	**1566**	**84.9**	**11202**	**56.5**	**43.4**

CDZ: Cauvery Delta Zone; **NEZ**: North Eastern Zone; **WZ**: Western Zone; **NWZ**: North Western Zone; **HAZ**: High Altitude Zone; **SZ**: Southern Zone; **HRZ**: High Rainfall Zone

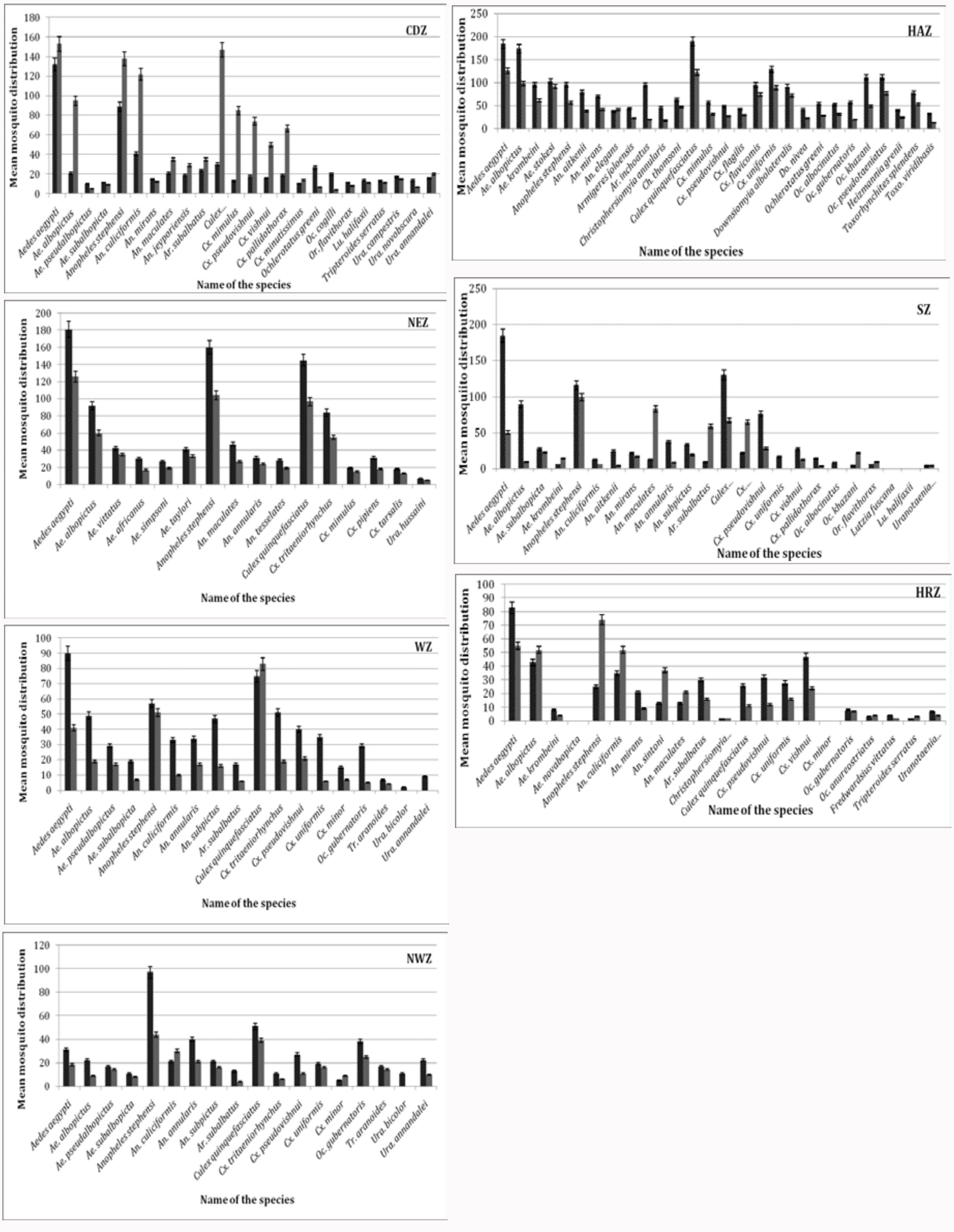


Figure 4: Culicids (immatures and adults) in different agro climatic zones (CDZ, NEZ, WZ, NWZ, HAZ, SZ, HRZ) of Tamil Nadu.
The dark bars with square designs represent mean immatures distribution of the zone; the light bars with 'star' signs indicate the mean adult distribution of the zones. Error bars are the standard errors of the means.

but these data were not used for analysis due to less wind records. Data were pooled by year wise and the mean monthly values were computed for all variables.

Environmental variables

Water samples were collected by monthly during the survey from all selected mosquito breeding sites in all the study areas by small container each 500 mL. Before sample collection containers were sterilized by 70% alcohol. The containers were covered with perforated caps, labeled properly with date and place of collection. The collected water samples carried carefully and transferred to the laboratory. Water temperature (°C), specific conductivity (S/cm), pH levels, and

Table 2: Mean physico-chemical characteristics of mosquito breeding habitats of Tamil Nadu during January-December 2018.

Name of the Zone	Breeding water Temperature (°C)	pH	Turbidity (NTU)	Magnesium (mg/L)	Chloride (mg/L)	Nitrate (mg/L)	TDS (mg/L)	Total Suspended Solids (mg/L)	Phosphate (mg/L)	DO (mg/L)
CDZ	31	7.8 ± 0.3	24.6 ± 1.3	4.05 ± 1.5	10.2 ± 0.1	1.74 ± 0.1	176 ± 2.8	367 ± 9.1	0.79 ± 0.01	6.52 ± 0.5
NEZ	29	7.8 ± 1.2	269 ± 2.8	3.2 ± 0.3	30.3 ± 4.12	0.70 ± 0.03	358.04 ± 7.65	205 ± 6.1	0.25 ± 03.2	40.9 ± 1.02
WZ	28	6.8 ± 0.94	22.7 ± 3.08	7.01 ± 0.3	8.36 ± 0.13	14.0 ± 0.2	226.15 ± 10.27	380 ± 19.1	0.68 ± 0.05	6.21 ± 0.13
NWZ	25	7.1 ± 0.14	187 ± 19	5.1 ± 1.7	26.96 ± 1.36	16.2 ± 2.1	561.07 ± 6.65	368 ± 21	2.15 ± 0.25	3.64 ± 0.08
HAZ	16	6.59 ± 1.08	310 ± 1.39	4.3 ± 0.2	9.1 ± 2.1	11.3 ± 0.5	1.50 ± 0.12	112 ± 3.21	1.48 ± 0.2	2.06 ± 0.1
SZ	30	6.5 ± 0.66	36.2 ± 6.7	3.6 ± 1.7	9.57 ± 0.72	31 ± 2.6	358.04 ± 7.65	178 ± 7.3	0.58 ± 0.38	6.8 ± 0.06
HRZ	25	5.94 ± 0.99	280 ± 0.02	4.6 ± 0.2	11 ± 5.1	14 ± 0.08	140 ± 1.27	638 ± 08.97	0.51 ± 1.3	2.0 ± 0.5

dissolved oxygen (mg/L) measurements were taken in the field using a portable meter (Quanta Hydrolab, Hach Hydromet Inc., Loveland, CO). Within 24 h after collection the breeding environmental habitat variables of total dissolved solids (mg/L), turbidity (NTU), alkalinity, suspended solids in the water column, nitrate (NO_3-N), phosphate (PO_4-P) were analyzed using standard procedure [34] and in tree holes water depth, diameter and tree hole height were also measured.

Statistical analysis

As a measure of species diversity Shannon's diversity index (H') was calculated since it is well accepted that all species at a zone, within and across systematic groups contribute equally to its biodiversity [24]. In addition, Simpson's diversity index (D) [35], species evenness, Brillouin, Menhinick, Margalef and Berger-Parker indices also calculated. Community analysis was carried out during rainy season when majority of the mosquitoes were at the peak of their growth. The faunal structure with environmental variables among sampling sites and effect of seasonality on mosquitoes were subjected multivariate analysis of Principal Component Analysis (PCA) by using PAST version 3.06 [36].

Shannon and Weaver index of diversity: The formula for calculating the Shannon diversity index [37] is $H' = -\Sigma pi \ln pi$

Where H= Shannon index of diversity

pi= the proportion of important value of the ith species (pi=ni/N), ni is the important value index of ith species and N is the important value index of all the species.

Simpson index of Dominance: The equation used to calculate Simpson’s index [38] was $D = \Sigma(pi)^2$

where D= Simpson index of dominance

As D increases, diversity decreases and Simpson’s index was therefore usually expressed as 1- D or 1/D.

Results

A total of 11,202 specimens (6,337 larvae and 4,865 adults) which belongs to 64 taxonomic categories distributed in 14 genera were collected (Figure 3). The species accumulation curve (including both adults and immatures) showed collections during January to December 2018. Of these, Cauvery Delta Zone (CDZ) recorded as 621 immatures and 1,153 adults; North Eastern Zone (NEZ) recorded as 983 immatures and 667 adults; Western Zone (WZ) recorded as 638 immatures and 329 adults; North Western Zone (NWZ) 474 immatures and 294 adults; High Altitude Zone (HAZ) recorded as 2,310 immatures and 1,416 adults; Southern Zone (SZ) recorded as 879 immatures and 601 adults; and in High Rainfall Zone (HRZ) recorded as 432 immatures and 405 adults (Figure 4). Overall a great number of specimens from the *Aedes* were collected followed by *Anopheles* and *Culex*. The least encountered genus in this study was *Toxorhynchites* except Nilgiris biosphere. This genus had a low abundance or absent in all other locations and this might be due to its prolonged life cycle. *Toxorhynchites* spend longer time in developing from egg to adult than the other mosquito species.

Of the mosquitoes identified to species, 98.6% individuals from the total identified breeding discarded tyres, 97.5% from public water tanks, and 93.3% of individuals were from seasonal rain pools. The least individuals were recorded from animal foot prints (47.8%) followed by vessels (58.5%), rocky pools (71.5%). Wells and paddy field habitats recorded 100%. At the zone level, HAZ had the highest abundance and richness of Culicid taxa, with 3,726 individuals followed by CDZ (1,774). The 75.5% of the immatures were observed in river section followed by leaf axis (71.8%) in overall agro climatic zones (Table 1). *Anopheles* larvae have low abundance in Western zones (WZ) of Tamil Nadu and it is only collected during rainy season. The scarcity of *Anopheles* can be attributed to lack of appropriate breeding grounds since *Anopheles* species are known to breed mostly in clean waters devoid of pollutants. The breeding grounds observed during this investigation which includes foul, stagnant, dirty bodies of water presumably unsuitable for supporting larvae of the species. Hence, low abundance of *Anopheles* reported in this study as a result of polluted environment that does not support its breeding.

Analysis of the physico-chemical characteristics of breeding water indicated that the pH ranged from 5.94 ± 0.99 in HRZ to 7.8 ± 0.3 in NEZ and CDZ whereas the Total Suspended Solids (TDS) (mg/L) ranged from 112.9 ± 3.21 in HAZ to 638 ± 8.97 in HRZ of Kanyakumari district. The lowest nitrate (mg/L) values recorded in NEZ (0.70 ± 0.03) and highest in SZ (31 ± 2.6) whereas the highest dissolved oxygen (mg/L) values were recorded in SZ (6.8 ± 0.06) and the lowest in HRZ (2.0 ± 0.5). The turbidity of breeding water ranged from 24.6 ± 1.3 in CDZ to 310 ± 1.39 in HAZ of Coimbatore district and the breeding water temperature was ranged from 25°C at NWZ and HRZ to 31°C at CDZ (Table 2). The pH of 6.5 to 7.0 ± 0.52 is the ideal condition for *Aedes* and *Culex* mosquitoes breeding. The breeding water pH is mainly depending on the meteorological variations. Immediately after rain the pH is in basic, if the pH is at acidic in nature the *Aedes* mosquitoes collected as in less number.

Mosquito breeding in the surveyed containers did commence with less in number until the month of September in particularly forest covers. This is due to lack of rainfall during this month in

Table 3: Climatic variables recorded in the Agro climatic zones of Tamil Nadu during January-December 2018.

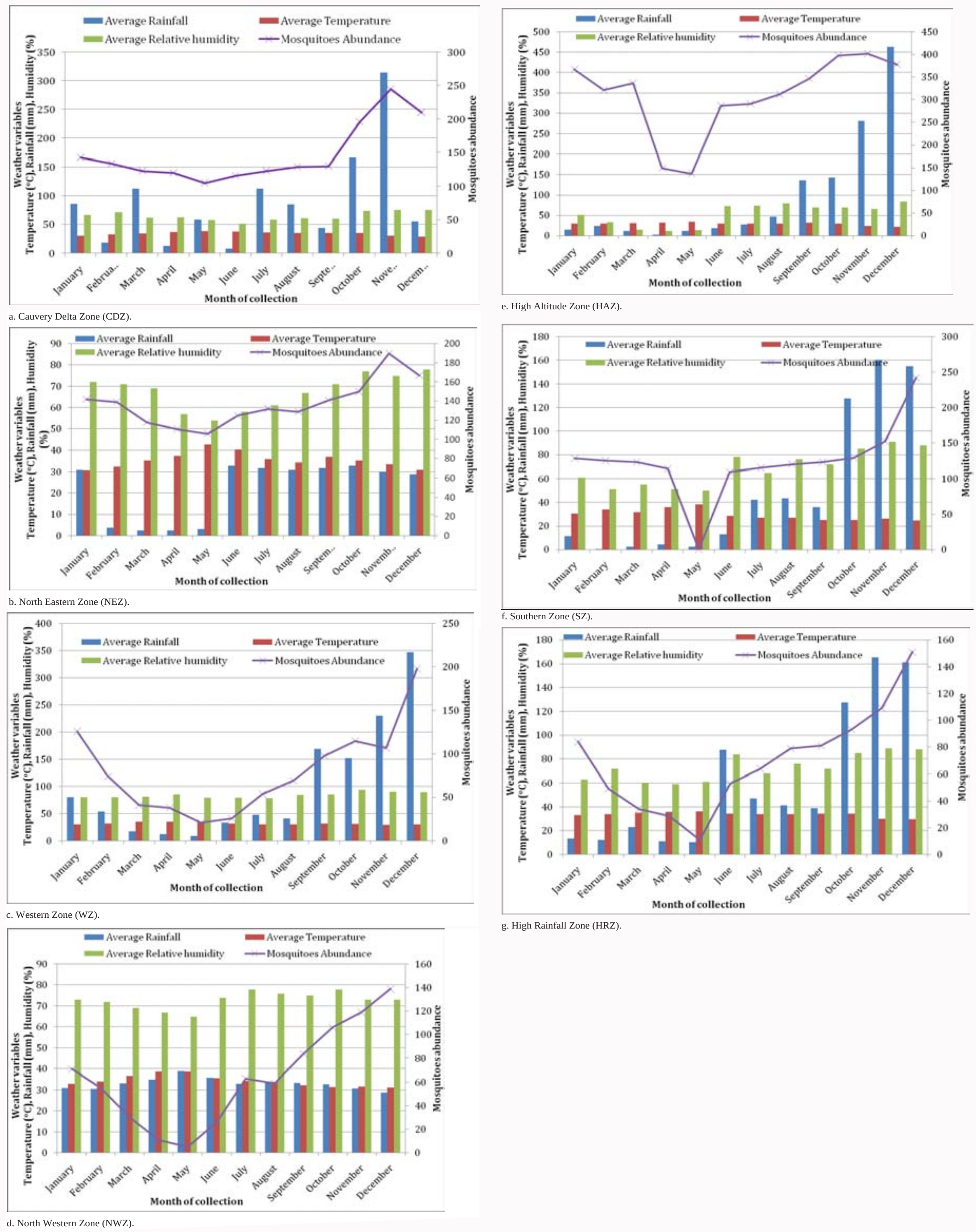
Zone	Weather condition	Month of collection												Average
		Jan'16	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec'16	
CDZ	Temperature (°C)	30.7	33	34.7	36.9	38.9	37.5	36.6	35.3	35.7	35.6	30.9	29.2	34.8
	Rain fall (mm)	86.1	18.82	112.41	13.24	58.8	7.76	112.5	84.99	43.99	166.62	314.49	55.19	97.04
	Relative humidity (%)	67	72	62	63	58	51.5	58.5	61	60	74	76	76	64.91
NEZ	Temperature (°C)	30.9	32.5	35.1	37.4	42.7	40.4	35.8	34.3	36.8	35.1	33.6	31	35.46
	Rain fall (mm)	31	3.9	2.5	2.6	3.3	33	32	31	32	33	30	29	21.94
	Relative humidity (%)	72	71	69	57	54	58	61.1	67	71	77	75	78	67.5
WZ	Temperature (°C)	29.4	31.8	34.5	35.2	34	31.6	30.1	30.1	31.6	30.9	29.2	29.4	31.48
	Rain fall (mm)	79.7	53.4	17.2	12.2	8.1	32.9	47.2	40.6	169.1	151.9	229.6	347.4	99.1
	Relative humidity (%)	80	80	81	85	79	79	78	84	85	93	90	89	83.58
NWZ	Temperature (°C)	32.9	34	36.8	38.7	38.8	35.7	34.3	33.8	32.4	31.4	31.7	31.2	34.3
	Rain fall (mm)	31	30.6	33.3	35	39	35.8	33	34	33.4	32.8	30.9	28.8	33.13
	Relative humidity (%)	73	72	69	67	65	74	78	76	75	78	73	73	72.75
HAZ	Temperature (°C)	29.1	29.8	30.1	31.6	33.6	29.8	29.6	29.5	31.5	29.1	23.6	22.1	29.11
	Rain fall (mm)	15.2	23.29	11.2	2.1	11.2	17.93	27.63	46.66	136.2	143.2	281.43	464.2	98.35
	Relative humidity (%)	51.36	33.1	14.76	11.01	13.93	72.7	73.2	78.93	68.93	69.4	65.63	84.3	50.6
SZ	Temperature (°C)	30.4	33.8	31.5	36.1	38.2	28.5	26.8	26.9	25.1	24.9	26.3	24.7	29.43
	Rain fall (mm)	11.4	0.5	2.3	4.2	2.5	12.8	42.1	43.4	36.1	127.5	160.3	155.1	49.85
	Relative humidity (%)	61	51	55	51	50	78	65	76	72	85	91	88	68.58
HRZ	Temperature (°C)	33.4	33.8	35	36.1	36.2	34.5	34	33.9	34.3	34.3	30.3	29.7	30.93
	Rain fall (mm)	13.4	12.5	23.3	11.2	10.5	87.8	47	41.4	38.9	127.5	165.3	161.1	61.65
	Relative humidity (%)	63	72	60	59	61	84	68	76	72	85	89	88	73.08

agro climatic zones. Natural breeding started in July but less in number and subsequently increased and high peak of mosquitoes were recorded in post monsoon season of late October, November and December months. This indicates that frequency and pattern of rainfall affect the composition and distribution of larvae and pupae in containers, animal foot prints, rocky pools, stream slow flowing, discarded tyres, tree holes, etc. This finding was supported by Gubleret al. [39], Afolabiet al. [40], and Senthamarai Selvan et al. [41]. The breeding temperature observed during the study suggested that mosquitoes breed at water temperature of 27.0°C to 28.0°C. This finding was supported by Afolabiet al. [42], who reported that female mosquitoes preferred water temperature range of 24.7°C to 29.1°C. The pH range of 6.1 to 7.8 supported breeding in all the habitats sampled with relative humidity of 84.30% to 88% (Table 3). The amount of Dissolved Oxygen (DO) present in water range from 2.0 mg/L to 6.5 mg/L.

Temperature is most important factor in the dynamics of mosquito populations. Increase in environmental temperature had observed to causes a decrease in mosquito generation time, longevity and life expectancy and increases the growth rate of vector populations, as well as decreasing the extrinsic incubation period and increasing the length of the pathogen transmission period. In this study temperature fluctuations recorded during the study period was significantly correlated with mosquito abundance. Higher humidity increases the mosquito survival rate. Overall, the different mosquito genera responded differently to weather variables, their response was related to their individual lifecycle.

Mosquitoes are cold-blooded insects whose development and behavior is strongly linked to prevailing climatic conditions such as temperature, rainfall, extreme flooding or drought, relative humidity and wind. A high value of tree hole mosquitoes was obtained in the rainy seasons (September-December), with a lower one in the cold seasons (January-February), and the lowest value was in the hot season (April-June) at all sampling sites (Figure 5). In CDZ mosquitoes were abundantly recorded in the month of November (245) and December (210) whereas in the month of May (105) and June (116) were least recorded. In the HAZ of Western Ghats the highest mosquitoes were recorded in August to December ranged from 312 to 378. According to inventory at HRZ in the month of December (151) more number of mosquitoes were recorded and in the month of April (29) and May (11) were in least recorded (Figure 5a-5g).

Our results suggest that environmental conditions in closed-canopied forests are more favourable than disturbed sites for forest mosquito populations. Silvicultural treatment may impact mosquito abundance through microhabitat (e.g., sunlight levels, microbial biomass, and frequency of drying events) and macrohabitat changes (i.e., removal of over story canopy cover and trees). It has been shown that tree density is an important factor for mosquito abundance in the Western Ghats of Tamil Nadu. Because the shelter wood had the lowest tree regeneration when compared to the clear-cut and control sites, our results are consistent with a role for tree density in driving



a. Cauvery Delta Zone (CDZ).

b. North Eastern Zone (NEZ).

c. Western Zone (WZ).

d. North Western Zone (NWZ).

e. High Altitude Zone (HAZ).

f. Southern Zone (SZ).

g. High Rainfall Zone (HRZ).

Figure 5: Month wise collection of Culicidae in agro climatic zones (a-CDZ, b-NEZ, c-WZ, d-NWZ, e-HAZ, f-SZ, g-HRZ) during January-December 2018.

mosquito abundance. Phytotelm specialists (e.g., *Ae. albopictus*) are dominant across our sites so this tree density effect is most likely related to the presence of oviposition sites. Undisturbed forest with suitable oviposition habitat may serve as a source of emergent mosquitoes. The logged sites we examined were nested within a natural forest, rather than an urban area, and were relatively small in size (2 ha), mosquitoes from nearby intact forest may have been able to rapidly colonize the disturbed sites.

Shannon's diversity index was calculated as a measure of diversity within agro climatic zones. The diversity indices H', and λ appear useful as they incorporate both species richness and evenness into a single value. Shannon's (H') diversity index appears to have more value (3.193) (Table 4). The present study indicates that mosquito species are more diverse in High altitude zones. However, the Shannon's index gave high values in Western Ghats of Coimbatore, Mettupalayam and Nilgiris biosphere followed by Cauvery delta zones

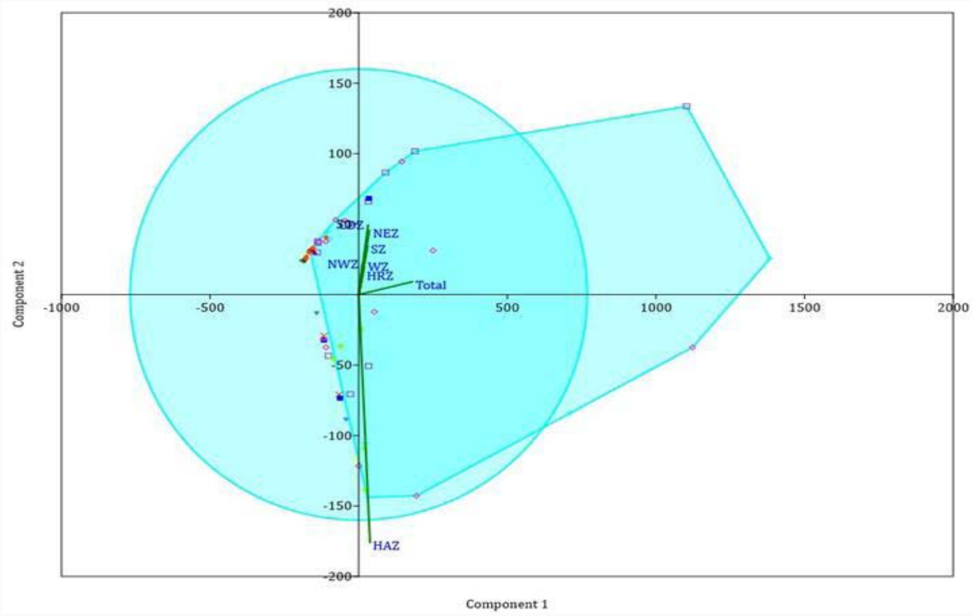


Figure 6: PCA ordination representing the distribution of mosquitoes with altitudinal differences in agro climatic zones of Tamil Nadu.
Plus (Aqua) - Aedes; Square (Blue violet) - Anopheles; Fill square (Blue) - Armigeres; X (Brown) - Christophersiomyia; Diamond (Deep pink) - Culex; O (Yellow) - Downsiomyia; Star (Lawn green) - Ochlerotatus; Triangle (Fuchsia) - Heizmannia; Dash (Coral) - Orthopodomyia; Bar (Dark green) - Fredwardsius; Oval (Maroon) - Lutzial; Fill triangle (Purple) - Tripteroides; Fill triangle (Orange red) - Uranotaenia; Fill inv. tri (Dodger blue) – Toxorhynchites

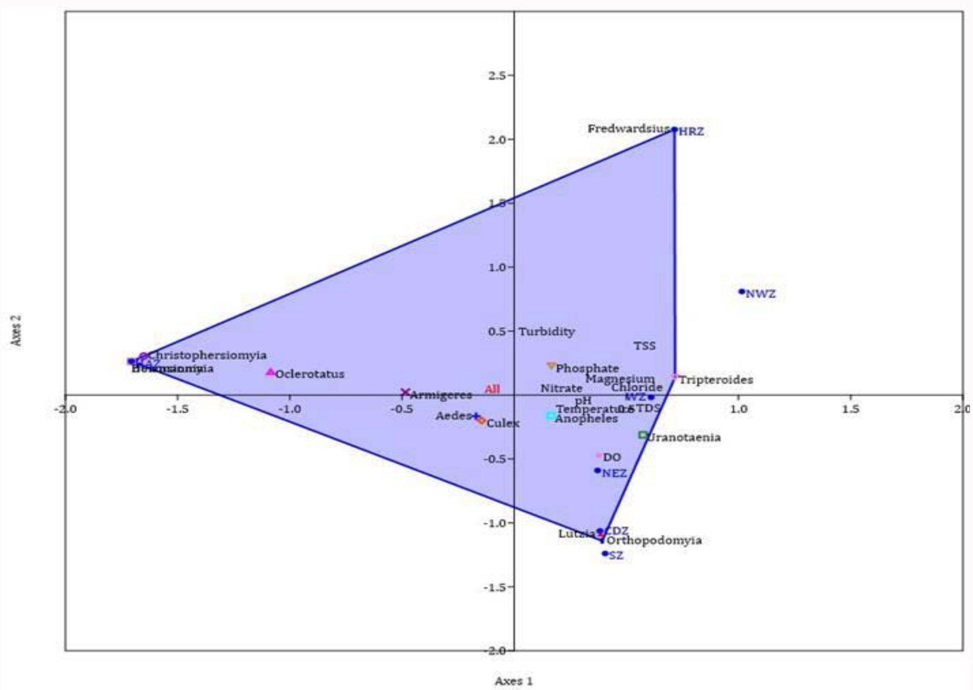


Figure 7: Correspondence analysis showing the relationship between environmental variables and mosquito species distribution in sampling sites of Tamil Nadu.

of Krishnagiri and Tiruchirappalli district localities in particularly forest covers. Simpson's diversity index, 'λ', also gave high value for aforementioned sites. It indicates that the deep forests and human/animal interfered locations are occupied by species with more individuals. Ecological statistics demonstrated that the differences in diversity between species and agro climatic zones.

To study the spatial pattern, seven different zones were selected in Tamil Nadu, which covers Western and Eastern Ghats hill ranges. The Principal Component Analysis (PCA) to measure the relationship between distributions of mosquito species with landscape difference. Cumulative variance was 68.96% for PC1 and 20.50% for PC2. PCA loadings of zone wise variables on the mosquito species distribution in sampling sites exhibited that geographical variance solids were important factors in PC1 and PC2 axes (Figure 6). PCA explained variance among the different landscapes to understand the habitat ecology. The factors were analyzed to determine the unique dimensions existing in the data. Prior to conducting the factor analysis procedure, cleaning of data was done, i.e. removal of outlier, verification of data

Table 4: Diversity indices in agro climatic zones of Tamil Nadu, India.

Name of the Index	CDZ	NEZ	WZ	NWZ	HAZ	SZ	HRZ
Species	24	16	18	18	28	24	21
Individuals	1774	1650	967	768	3726	1480	837
Dominance (D)	0.0797	0.1087	0.088	0.0846	0.0466	0.0887	0.0899
Simpson (1-D)	0.9203	0.8913	0.912	0.9154	0.9534	0.9113	0.9101
Shannon (H')	2.805	2.448	2.61	2.675	3.193	2.698	2.604
Evenness (e^H/S)	0.6885	0.7229	0.7557	0.8064	0.87	0.6186	0.6434
Brillouin	2.768	2.421	2.564	2.618	3.17	2.657	2.548
Menhinick	0.5698	0.3939	0.5788	0.6495	0.4587	0.6239	0.7259
Margalef	3.074	2.025	2.473	2.559	3.283	3.151	2.972
Equitability (J)	0.8826	0.8829	0.9031	0.9255	0.9582	0.8489	0.8551
Fisher alpha	3.924	2.457	3.14	3.3	4.111	4.068	3.91
Berger-Parker	0.1607	0.1861	0.1634	0.1836	0.0837	0.1581	0.1649
Chao-1	24	16	18	18	28	24	22

with raw data. Rotation in factor analysis produced results in a more interpretable form and helped to scrutinize the landscape differences based on their contribution to mosquitoes. Factor analysis is often used to generate hypotheses regarding causal mechanisms and to screen variables for subsequent analysis.

Environmental variables were analyzed in agro climatic zones of Tamil Nadu by using correspondence analysis. In over all, the physical characteristics of temperature, pH, Conductivity, TDS and TSS were chosen for studying the physicochemical variations in breeding water. This resulted; the mosquitoes representing 14 genera, 64 species were distributed in seven different ecological zones and it differ based on the breeding water properties correlated with geographical landscapes. Figure 7 shows changes in mosquito species among physicochemical parameters. Although the first axis of CA explained 60.21% of mosquito variability and all analyzed mosquito species influenced by water parameters revealed by CA results.

Discussion

This extensive study in seven different agro climatic zones of Tamil Nadu involved the capture of large numbers of mosquitoes in more than one hundred sites. The warmer climates in tropical areas allow them to be active all year round, with the ideal conditions being hot and humid with moderate rainfall. In hot climates they are able to be more active, and the rainfall gives them aquatic sites for larval and pupal stages [43]. Many mosquitoes are generalists and choose a variety of oviposition sites, whereas others are specialists and choose unique habitats for laying eggs. The specialist mosquitoes tend to disappear after land use changes (e.g. deforestation), but generalists are able to survive in a wide range of habitats [44,45]. In agro climatic zones of Tamil Nadu there are several types of oviposition sites, which can be categorized into ground water sites or container sites. Ground water sites include rivers, lakes, ground pools and many more. Container sites include artificial containers (tires, bottles, cups, jugs) or natural containers (fallen leaves, tree holes, tree stumps, plant axils).

Agricultural development increases the number of humans working in an area, but can alter environmental conditions so that they favor mosquitoes (e.g. deforestation and water management) [19]. Agriculture can cause sedimentation, which can slow or block streams and decrease the water depth [46]. This provides a larger number of mosquito habitats and increases the water temperature for vector development [19]. Changing landscapes can also significantly affect the microclimate of a habitat, such as temperature, runoff and evapotranspiration. These factors are keys in determining the abundance, survivorship and diversity of mosquito vectors in Cauvery Delta Zone and High Altitude Zone. Some authors suggest that human interference in the natural environment can lead to the elimination and/or modification or substitution of habitats, with the subsequent loss of richness in the existing Culicidae fauna, although the conditions are also favourable for the settling of exotic or opportunist species well-adjusted to disturbed environments [47].

Pemola and Jauhari reported that climatic variables are major predictors for mosquito survival and their establishment. The ratio of rainfall over precipitation/potential evapotranspiration, indicative of the humidity conditions are the driving force for the increase of mosquito populations. Also study report suggests that low or moderate rainfall at regular intervals favors new larval habitats, provides ideal conditions for mosquitoes to breed sustain and increases in density. The high or low rainfall shows both constructive and destructive effects on mosquito populations significantly affects the larval and pupal stages by flushing them out of their aquatic habitat and killing them [48]. In this present study data showed that the post monsoon is the ideal time for mosquito species to amplify and flourish as the rainfall was reduced and filled larval habitats favors oviposition.

Study findings also correlated with Reiter [49] report, during summer, mosquitoes were recorded lowest due to rise in atmospheric and water temperatures while the mosquito habitats were dried out. In this study, during summer (March-May), the mosquito productivity was disturbed and the offspring production was reduced due to high surface water temperature this correlated with the findings of Kolivras [50]. The small and transient habitats such as ditches, puddles and pools are dried off, whereas in ponds the water level decreases gradually, but the perennial habitats (water tanks, pond and drainage canals) are major water source, where the majority of mosquitoes breed. Temperature is inversely related to geographical landscape and the air temperatures are as lower than the habitats, water temperatures a vital factor that favors the existence of mosquitoes in high altitude areas. Temperature over 40°C or lower than 0°C are limiting factor that has lethal effects on the mosquito adults and the survival rates [51]. Pascual et al. [52] studies supports to our studies, the climate driven response temperature dependent population dynamic model demonstrates that a small change in mean ambient air temperature of just 0.5°C could translate into a 30% to 100% increase in mosquito abundance.

Monsoon season brings precipitation and mosquito density is highly associated with levels of rainfall, moderate rains may prove beneficial to mosquito breeding and abundance, but may destroy breeding sites and flush out the mosquito larvae when it is excessive. Gimnig et al. [53] have also reported that, heavy rainfall may flush out the predators and pathogens that may have previously colonized the same habitats and this could increase the mosquito larvae survival rate. Romoser and Lucas [54] also suggested that pupal life stage is very important, as the emergence of adult mosquitoes determines the productivity of a breeding site. The pupal diving behaviors during heavy pelting rains help to avoid drowning and being flushed away. Rainfall may result in larval stress and consume more energy, which may affect life history traits such as development time, survival and adult size.

Humidity can greatly influence the transmission of vector-borne diseases. Rainfall increases the near-surface humidity associated with rainfall enhances mosquito flight activity and host-seeking behavior. Mosquitoes can desiccate easily and survival decreases under dry conditions and saturation deficit (similar to relative humidity) is one of the most critical determinants of climate/disease [46,55]. Rainfall provides breeding sites for mosquitoes to lay their eggs, and ensures a suitable relative humidity of at least 50% to 60% to prolong mosquito survival. Relative humidity below 60% shortens the life span of the mosquitoes. In our study we recorded mosquitoes in high peaks at 84% to 88% of relative humidity. The peaks in relative humidity coincided with higher amounts of rainfall and led to increases in larvae in the rainy months.

Teklu et al. [56] noticed that mean larval density of *Anopheles* larvae was greater in slightly turbid and shallow aquatic habitat with emergent aquatic vegetation and with relatively open sunlit conditions. While Minakava et al., reports supported our findings that *Anopheles* larvae prefers to breed with relatively less shade, muddy substrate and slight turbidity. The seasonal change in oviposition of *Aedes* mosquitoes is based changing in weather patterns, and the availability of breeding sites. Chadee [57] reports that *Aedes* sp. breeds mostly in smaller containers depending on the temperature ovipositing eggs and cryptic microhabitants. *Ae. aegypti* and *Ae. Malayensis* prefers less vegetation, while *Ae. albopictus* commonly breeds in all types if natural containers like tree holes, pitcher plants, plant axils, coconut shells, wooden logs etc., [35]. Senthamarai Selvan et al. [58] and Chen et al. [59] reported *Ae. ageypti* and *Ae. albopictus* prefer to breed in the concrete drainage with clear stagnant water can also become a potential habitat with fallen tree leaves and other debris. *Ar. subalbatus* breed commonly in polluted water collections, foul smelling and stagnant water as well as in container habitats like tree holes and bamboo stumps [60]. Our finding reports that the species of *Toxo. splendens* and *Toxo. minimus* were highly recorded in HAZ of Nilgiris district.

During our survey we recorded malaria vector *An. Stephensi* almost in all rural areas wherever we surveyed in agro climatic zones. The said species were recorded in previous studies done by Tyagi et al. [61], in slow running river breeding. The species *Cx. quinquefasciatus* was identified frequently in CDZ, HAZ and NEZ, were in Cauvery Delta Zones of Tanjore and Trichirappalli have large number of paddy field which is supported to *Cx. Quinefasciatus* breeding. The genus of *Culex* are among those that have adapted well to habitats created by human interference. The accumulation of organic matter, household garbage and other waste materials, drainage systems which favors the emergence of *Culex* sp., *Armigeres* sp., *An. subpictus* and *An. vagus* which are endophilic and highly anthropophagic and gain importance as vectors of human diseases. *Culex* sp. is associated with unshaded, warm, vegetated waters in flooded pastures and swamps [62]. The predominantly ground water breeding *Cx. quniquefasciatus* occurred widely in diverse habitat types, is abundant in turbid water, and organic rich water [63] and occurs only occasionally in natural containers such as tree holes and bamboo stumps [64].

Culex sp. vector density is positively associated with monsoon months. The trend may lead to increase in vector abundance in post-monsoon months, during NEM (middle of October) in CDZ. Kaliwal et al. [65] reported that the first peak in adult density during July with the onset of monsoon rains in the month of June. The subsequent decline in the adult density in August and September months can be attributed to flushing of drains, caused due to physical impact of heavy rains. Temporary patterns were exhibited by *Cx. tritaeniorhynchus* occur during the post-monsoon season [66]. *Cx. tritaeniorhynchus, Cx. vishnui* abundance was lowest in the hot and dry season (April-May) and highest in the cool and wet season (October-December) [67]. Two peaks of larval density of *Cx. tritaeniorhynchus* was observed in October and May. *Cx. Vishnui* mainly recorded during the post-monsoon months of November and December [68].

Seasonal variations in the larval population of mosquito species closely followed the fluctuations in rainfall with zero population during the driest three months (March-May). Mani et al. [69] supported our findings, the peak in vector density and virus activity during the north-east monsoon period, October-December. *Anopheles* and *Aedes* mosquitoes increases in abundance shortly after the rain fall (began in June), were mostly collected in June-July (South West monsoon). Physicochemical factors that influence oviposition, survival rate and the distribution of mosquito species include salts dissolved organic and inorganic matter, degree of eutrophication, turbidity, presence of suspended mud, temperature, light, shade, hydrogen ion concentration and presence of food substances. Larval control can be achieved through larval habitat management by altering physiochemical properties of breeding habitats [69-71]. Understanding the impact of these parameters will lead to better decision making in relation to mosquito control activities.

The association between climatic variables and abundance of adult mosquitoes indicated that production of mosquitoes was affected more by relative humidity and temperature recorded during the previous month than the precipitation registered during the specific study period. In Tamil Nadu, a combination of unique environmental and demographic factors was more likely as an underlying cause of conditions appropriate for human transmission than was the presence of a particular bridge vector species. Epidemiologic and ecologic research on a sub country level has been proposed as a priority for nationwide development to elucidate the spatial patterns of vector-borne disease risk.

Apart from environmental factors, altitudinal features/landscape changes also play an important role in mosquito distributions, especially weak fliers with very short dispersal distances such as *Ae. aegypti*. While elevation was not significant because it was accounted for by other variables, land use was the second most important variable. Specifically, three categories (urban areas, forested areas, and open agricultural areas) showed strong positive or negative associations with presence of mosquitoes. Urban areas increased the probability of presence, which was in agreement with a previous study demonstrating strong *Ae. Aegypti* affinity to urbanized environments in Western zone. Forested areas might be less susceptible to *Cx. quinquefasciatus* invasions, whereas agricultural areas are mostly open crop or pasture fields with little protective cover and lack of container habitats for *Ae. aegypti* larval production. Urban areas with the surrounding suburban envelopes were, therefore, considered the future "hotspots" of high *Ae. aegypti* activity if located within the predicted range.

Summary

Totally 11,202 mosquitoes were identified during a one-year survey January to December 2018. *Ae. aegypti* was the most predominant species in agro climatic zones, collected as larvae and adults. It was continuously abundant and active throughout the year and *Lutziafuscana*, *Ae. Novabopicta* was rarely reported during the study. On the coastal areas of high rainfall zone, *An. Stephensi* was very common, and proved to be a complex of species as two forms were detected. One of the vectors of malaria, *Anopheles culiciformis*, was collected during April to August, from Kanyakumari and coastal districts. Its breeding sites were restricted to fresh and clean water in pools and wells. Most of these breeding sites were in the populated areas.

Conclusion

Environmental changes caused by human increases the abundance of opportunistic and exotic species such as *Cx. Quinquefasciatus* and *Ae. aegypti*. The data provided by this research could contribute to potential disease control programs and they reinforce the need to conduct further studies on the biology of these species since continuous or bigger environmental changes caused by human activity could cause new epidemiological scenarios. It is necessary entomological and control monitoring by health authorities to detect new species possibly involved in pathogen transmission in Tamil Nadu, India and whose role has not yet been revealed. Similarly, this type of baseline information allows detection of changes in the distribution or abundance of species and detection of introduced species of vectors that have extended beyond their natural distribution or biogeographic areas (termed invasive species), and which can cause environmental, economic, and human health impacts. In particular, understanding the link between environmental factor, landscape and vector population is central in the design of mosquito control measures to prevent disease outbreaks, and in understanding where and when the risk of mosquito-borne disease is to be highest.

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