International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2024; 11(2): 30-36

https://www.dipterajournal.com
Received: 18-12-2023
Accepted: 23-01-2024

Rushikesh G Pawar
Department of Zoology, Karnataka University, Dharwad, Karnataka, India

Pulikeshi M Biradar
Department of Zoology, Karnataka University, Dharwad, Karnataka, India

Corresponding Author:
Rushikesh G Pawar
Department of Zoology, Karnataka University, Dharwad, Karnataka, India

Diversity and abundance of mosquitoes in different larval habitats of Uttara Kannada District of Karnataka, India

Rushikesh G Pawar and Pulikeshi M Biradar

DOI: https://doi.org/10.22271/23487941.2024.v11.i2a.765

Abstract
Mosquitoes are important vectors of diseases like malaria, dengue, chikungunya etc. A study was conducted to document mosquito diversity in Uttara Kannada district of Karnataka. Mosquito larvae were collected from different habitats using standard dipping method during the study period from January, 2017 to December, 2019. A total of 3784 mosquito larvae belonging to 27 species and 6 genera were recorded *viz., Aedes, Anopheles, Armegeres, Culex, Toxorhynchites and Uranotaenia.* The genera *Culex* was the most dominant with 1731 individuals followed by *Anopheles.* Among all the species recorded, Cx. *quinquefasciatus* was found to be the most predominant with 12.26% relative abundance followed by *Cx. Tritaeniorhynchus* 11.65%. Paddy fields had highest individuals (679) inhabiting in them, followed by Swamps and Ponds with 492 and 451 individuals respectively. The Shannon diversity reveals highest species diversity in swamps with H value 2.611, consisting of 16 different species and lowest species diversity in streams with H value of 1.143 having only 5 species. The Berger-Parker index is seen highest in drainage water habitats (0.4531) and lowest in swamps (0.1382) indicating abundance of the most dominant species i.e. *Cx. quinquefasciatus* is the dominant species and is found highest in drainage water (226) and least in swamps (36).

Keywords: Diversity, Abundance, Mosquito, Habitat, Uttara Kannada and Karnataka

Introduction
Mosquitoes are widespread insect vectors and are easily found in various habitats, such as fresh water, paddy fields, stagnant water, and sewage water. Mosquitoes provide a serious threat to human health worldwide. They are the primary carriers of many parasitic and viral illnesses that affect humans and other domestic animals. Many diseases and illnesses, like dengue, malaria, arboviruses, filariasis, and western equine viruses, are transmitted by different species of mosquitoes [1]. With the use of pictorial identification keys larvae and adult mosquitoes may be classified and grouped according to their morphological features, adult male and female species can be distinguished by looking at their mouth parts like antennae, palps and wings. The mosquito's life cycle typically consists of aquatic stages like egg, larva and pupa, and terrestrial adult stage. According to Foley *et al.,* (2007), the most species-rich countries and the number of species were highest in Brazil (447), Indonesia (439), Malaysia (415), Thailand (379), India (338). Checklist of Indian mosquitoes, 2014 provided 393 species belonging to 49 genera and 41 sub-genera, of these, less than 10% of the mosquitoes (31 species) are known to be disease carrying vectors in India [2]. The updated catalogue of Indian mosquitoes suggests that of the total 3541 mosquito taxa in the world, India accounts for more than 12% with 404 species and subspecies belonging to 50 genera and 2 subfamilies [3]. Checklists are accessible from Southern India, including 31 species of *Anopheles* mosquitoes from eastern slopes of the Western Ghats, 119 species from the Nilgiri hills and 124 species from the phytotelmatic habitats of the Western Ghats hills of Karnataka, Kerala and Tamil Nadu [4]. Surveys on mosquitoes diversity are usually limited to only certain parts of the Western Ghats (Mishra *et al.,* 1984; Tewari *et al.,* 1987; Ninge Gowda and Vijayan 1992; Geeverghese *et al.,* 1994; Urmila *et al.,* 1999; Gokale *et al.,* 2000; Sathish Kumar *et al.,* 2004; Sathish Kumar and Vijayan 2005; Fakoorziba *et al.,* 2006; Bhuyan *et al.,* 2013; Selvan

et al., 2015; Sajith *et al.*, 2016). Mosquito diversity has been recorded from various regions of Karnataka like Mysore, Nagarhole (Rajiv Gandhi National park), Mandya, Kundapur, Bellary, Manglore, Dakshin Kannada, Kodagu (Pushpagiri Wildlife Sanctuary) and Dharwad [4-15]. According to Das *et al.* (2006), different mosquito species of *Culicidae* family may be found in a variety of habitats. In epidemiological studies, it is critical to have information on the diversity of mosquito species in a given location [16]. The present state of the mosquito fauna in diverse habitats in various geographical locations must be assessed to meet the difficulties of vector-borne diseases in the future. No previous studies have been conducted in Uttara Kannada district of Karnataka. Hence, the main objective of the present study was to record the diversity and abundance of mosquito species at different larval habitats in the study area.

Methodology

Study area: Uttara Kannada District is located between 13º55' to 15º32' N and 74º05' to 75º 05' E, it is the 5th largest district of Karnataka State with 10,291 km² of area and 12 Talukas *viz*. Karwar, Ankola, Kumta, Honnavar, Bhatkal, Sirsi, Siddapur, Yellapur, Mundgod, Haliyal, Supa(Joida) and Dandeli. The district has varied geographical areas with thick forest, perennial rivers, abundant flora, fauna and a long coastal line of about 140 km in length. It is bordered by the state of Goa and Belgaum District to the north, Dharwad District and Haveri District to the east, Shivamogga District and Udupi District to the south and the Arabian sea to the west (Fig-1). The city of Karwar is the administrate headquarters of the district. In its 10.25 lakh hectares of total land, 8.28 hectares is of Forest land and only about 1.2 lakh hectares of land is under agriculture / horticulture practice. The cultivable land in the District is approximately 10 percent, as the forests dominate the total area. Apart from the natural resources, the district has also got some man-made wonders, some important among them are Hydro Electric Power Projects such as Supa Dam, Kadra Dam, Kodasalli and Gerusoppa Dam and a Nuclear Power Project Kaiga Atomic Power Station (KAPS). Uttara Kannada District receives heavy rainfall during monsoon season. The important rivers flowing in the district are Kali River, Gangavali River, Aghanashini River, Sharavati River, Venkatapur River, Varada River, Bedti River. The study area map was created by QGIS software (Version 3.32.3).

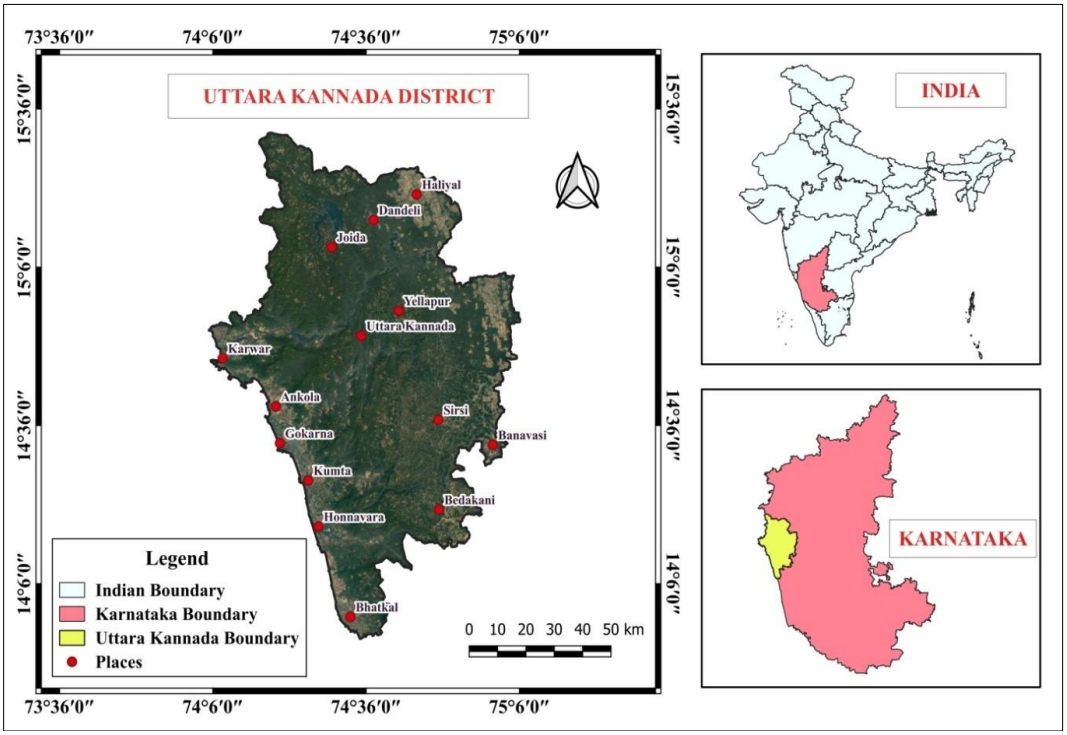


Fig 1: Map showing geographical location of Uttara Kannada district in Karnataka and India.

Sampling

A preliminary random sampling was conducted with two to three visits every week to locate the larval habitats. After the identification of larval habitats with highest probable density, every fortnight visit is carried out for mosquito larva sample collection during the period from January, 2017 to December, 2020 using a dipper with 500 ml volume and 1m handle. Egg, larval and pupal stages were collected from nine major habitats such as Water Tanks, Drainage Water, Tyres, Plastic Containers, Paddy Fields, Tree Holes, Swamps, Ponds and Streams. Most of the collection was done from varied habitats at dawn and dusk between 5:00 am - 8:00 am and 5:00 pm - 8:00 pm respectively. The collected individuals were stored in plastic jars with perforated lids and brought to laboratory for further studies. The larvae collected were transferred into the bowls and kept in mosquito cages. Egg and early larval stages were reared in laboratory conditions by feeding dog biscuit and yeast in 3:1 ratio until they attain 4th instar stage, which are most suitable for identification. Pupae collected were allowed to emerge as adult and are used for identification.

Identification

The morphological features of late 3rd instar, early & late 4th instar larvae and adult mosquitoes are considered for identification process. The larvae underwent starvation for 24hrs before identification; if they were alive during the

identification process, the larvae were exposed to chloroform vapours. The pupae, which emerged as adults were also collected with the help of aspirator and are killed in a test tube containing cotton swab dipped in diethyl ether. All the specimens are identified using standard dichotomous key, pictorial key, catalogue provided by ample of research literatures and web sources [3, 17-27].

Data Analysis

The percentage Relative Abundance (R.A) of each species is calculated using below formula:

$$R.A. = \frac{\text{No.of individuals in a species}}{\text{Total no.of Individuals in all species}} X\ 100 \qquad \text{Eq. (1)}$$

The criteria for Relative Abundance status of a species was adopted from Trojan (1992) [28]. According to which, a species is said to be Dominant, if R.A. is above 5%, Sub-dominant is between 1-5% and if R.A. is less than 1% it is said to be satellite.

All the major diversity indices such as Dominance, Simpson, Shannon, Evenness, Margalef and Berger-Parker indices were calculated by using the software Paleontological Statistics (PAST) version 4.03. The Pearson's correlation among habitats along with rarefaction with 95% confidence level were also calculated using same software.

Results and Discussion

The present study documented a total of 3784 individuals belonging to 6 different genera and 27 species. Among all the genus recorded, *Culex* had highest number of individuals (1731) belonging to 11 species followed by *Anopheles* comprising 1110 individuals belonging 8 species, *Aedes* recorded 706 individuals with 4 species, *Armegeres* with 159 individuals having 2 species, *Toxorhynchites* and *Uranotaenia* had only one species each with 47 and 31 individuals respectively (Table 1). The genus *Culex* had the highest distribution of species (41%) followed by *Anopheles* (29%) and *Aedes* with 15% (Fig.-2).

Table 1: Name of the genus, with no. of species and total no. of individuals

Sl. No.	Genus	No. of species	Total no. of individuals
1.	*Aedes*	4	706
2.	*Anopheles*	8	1110
3.	*Armegeres*	2	159
4.	*Culex*	11	1731
5.	*Toxorhynchites*	1	47
6.	*Uranotaenia*	1	31
Total		27	3784

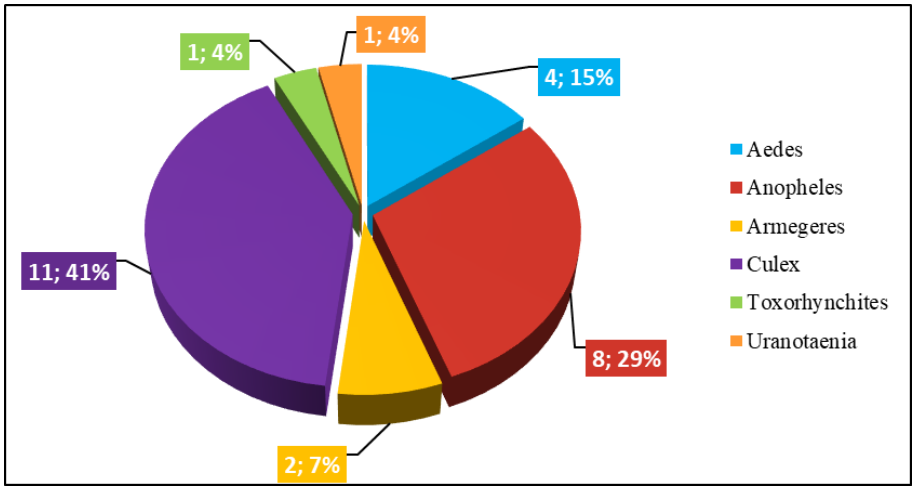


Fig 2: Pie chart displaying no. of species with genus and their percentage distribution.

During the study period, *Cx. quinquefasciatus* species displayed highest Relative Abundance (12.26%) followed by *Cx. tritaeniorhynchus* (11.65%) and *Ae. aegypti* (9.06%), whereas *Ae. vexans* displayed least Relative Abundance of 0.60%. Considering the Relative Abundance of species; out of the 27 species recorded, 7 species had more than 5% R.A. and are identified as Dominant (*Ae. aegypti, Ae. albopictus, An. barbirostris, An. stephensi, Cx. quinquefasciatus, Cx. tritaeniorhynchus* and *Cx. vishnui*), 15 species with R.A. between 1-5% are identified as Sub-dominant (*Ae. vittatus, An. annularis, An. jamesi, An. karwari, An. maculatus, An. subpictus, An. vagus, Ar. subalbatus, Cx. gelidus, Cx.infantulus, Cx. malayi, Cx. mimulus, Cx. pseudovishnui, Cx. uniformis* and *T. splendens*) and the rest 5 species with R.A. of below 1% are considered as Satellite species (*Ae. vexans, Ar. flavus, Cx. minor, Cx. sitiens* and *U. atra*) (Table-2). Attaullah *et al.*, 2021[1] recorded 14 species from Malakand and Dir Lower; of which 9 species were Dominant, 4 species were Sub-dominant and 1 species was Satellite species. Relative Abundance of all these species is displayed in a line graph (Fig.-3).

Table 2: No. of individual collected with respect to different genus and species along with % R.A. and their dominance during the study period (January, 2017 - December, 2019)

Sl. No.	Genus	Species	2017	2018	2019	Total	R.A. (%)	Relative Abundance Status
1.	*Aedes*	*Ae. aegypti*	102	128	113	343	9.06	Dominant
2.		*Ae. albopictus*	81	96	87	264	6.97	Dominant
3.		*Ae. vexans*	9	8	6	23	0.60	Satellite
4.		*Ae. vittatus*	25	27	24	76	2.00	Sub-dominant
5.	*Anopheles*	*An. annularis*	34	67	51	152	4.01	Sub-dominant
6.		*An. barbirostris*	74	98	84	256	6.76	Dominant
7.		*An. jamesi*	14	16	17	47	1.24	Sub-dominant
8.		*An. karwari*	25	28	23	76	2.00	Sub-dominant
9.		*An. maculatus*	19	22	17	58	1.53	Sub-dominant
10.		*An. stephensi*	89	96	91	276	7.29	Dominant
11.		*An. subpictus*	39	45	48	132	3.48	Sub-dominant
12.		*An. vagus*	31	47	35	113	2.98	Sub-dominant
13.	*Armegeres*	*Ar. flavus*	9	8	10	27	0.71	Satellite
14.		*Ar. subalbatus*	34	57	41	132	3.48	Sub-dominant
15.	*Culex*	*Cx. gelidus*	48	59	43	150	3.96	Sub-dominant
16.		*Cx. infantulus*	14	21	12	47	1.24	Sub-dominant
17.		*Cx. malayi*	17	11	13	41	1.08	Sub-dominant
18.		*Cx. minor*	8	12	13	33	0.87	Satellite
19.		*Cx. mimulus*	32	47	35	114	3.01	Sub-dominant
20.		*Cx. pseudovishnui*	24	33	27	84	2.21	Sub-dominant
21.		*Cx. quinquefasciatus*	141	164	159	464	12.26	Dominant
22.		*Cx. sitiens*	8	11	13	32	0.84	Satellite
23.		*Cx. tritaeniorhynchus*	133	161	147	441	11.65	Dominant
24.		*Cx. uniformis*	27	38	29	94	2.48	Sub-dominant
25.		*Cx. vishnui*	76	72	83	231	6.10	Dominant
26.	*Toxorhynchites*	*T. splendens*	14	18	15	47	1.24	Sub-dominant
27.	*Uranotaenia*	*U. atra*	8	13	10	31	0.81	Satellite
	Total		1135	1403	1246	3784	99.86	

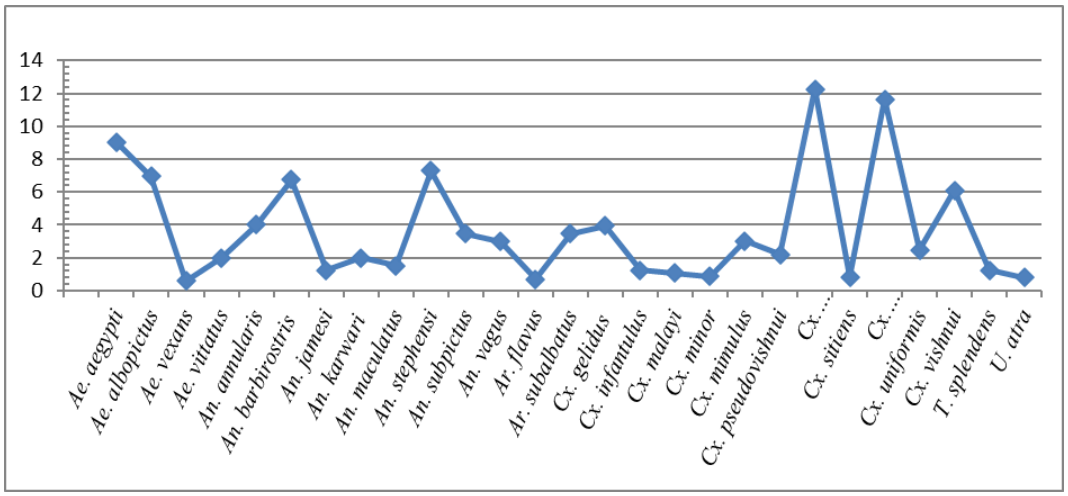
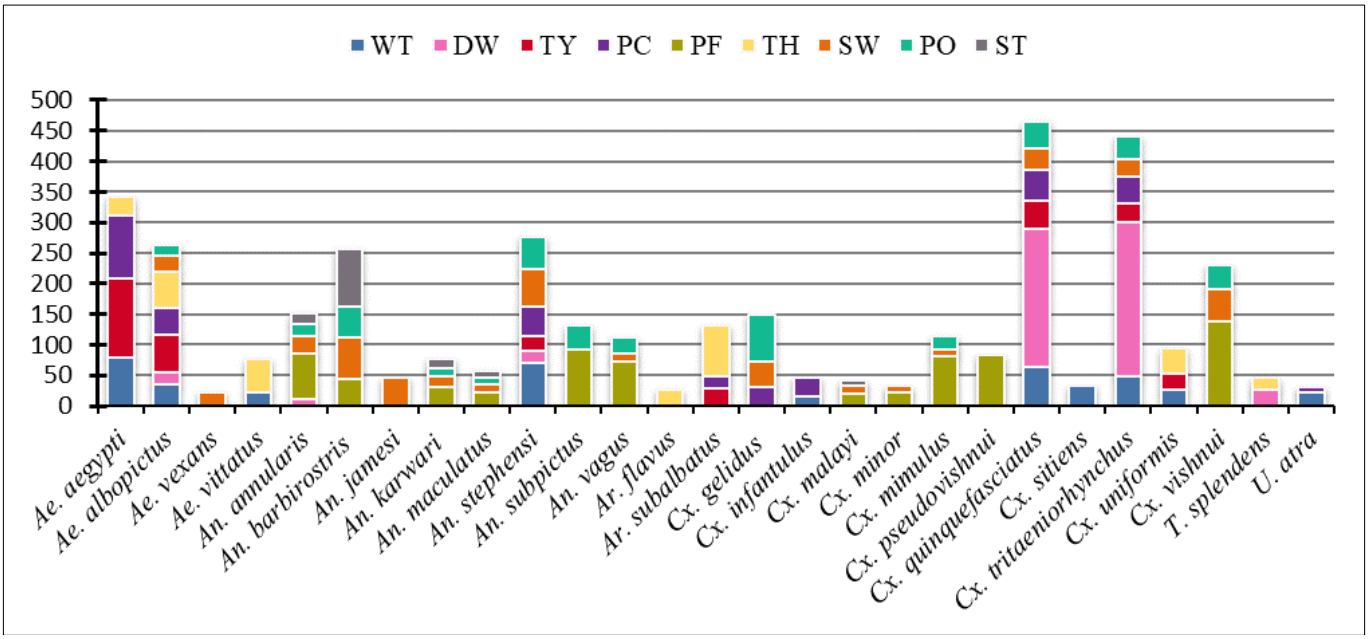


Fig 3: Line graph showing Relative Abundance of different species in the study area.

Among all larval habitats (9) surveyed, the swamps (SW) supported richest species diversity with 16 species is because swamp has high organic matter (vegetation), which provides abundant food resource, hideouts from predators and optimum water parameters to developing larvae and might act as excellent breeding ground for adults. Likewise, the ponds supported 13 different species, paddy fields with 11 species, water tanks with 10, plastic containers having 9, tyres and tree holes supported 6 species of mosquitoes. In contrast, the least larval species diversity was found in streams supporting with only 5 species; this might be due to most of the mosquito species are adapted to stagnant water bodies. One more interesting thing is 5 species of mosquitoes are found in only one type of habitat *viz*., *Cx. pseudovishnui* is found in paddy fields, *Cx. sitens* are recorded from water tanks, *An. flavus* exclusively found in tree holes and swamps are the only habitat most preferred by *Ae. vexans* and *An. jamesi*. Whereas *Ae. albopictus* was most widespread and found in 7 habitats except paddy fields and streams (Table-3). Similar studies have been reportd by Prasad *et al.*, (2021) [4]. *Cx. quinquefasciatus*, *An. stephensi* and *Cx. tritaeniorhynchus* are recorded form 6 habitats each, *An. annularis* was found in 5 different habitats, *Ae. aegypti*, *An. karwari*, *An. barbirostris and An. maculates* were collected from 4 different habitats. 7 species *viz*., *An. vagus*, *Ar. subalbatus*, *Cx. gelidus*, *Cx. malayi*, *Cx. mimulus. Cx. uniformis* and *Cx. vishnui* were

found to be inhabiting in 3 habitats, the remaining six mosquito species were recorded from only 2 type of habitats.The most abundant species *Cx. quinquefasciatus* (464 individuals) predominates in drainage water bodies, similarly, more than 50% recorded *Cx. tritaeniorhynchus* species are found co-exist with *Cx. quinquefasciatus* in drainage water habitats. The stacked column graph (Fig.-4) shows occurance and abundance of all the species in different habitats. The previous studies from the state of Karnataka reported diversity of mosquitoes from different regions that shows Karnataka has wide range of species richness. Prasad *et al.,* (2021) reported a total of 37 species belonging to 12 genera from Dakshin Kannada district and *Culex* was the dominating genus [4]. The present study also showed highest species richness in *Culex* genus only with 11 species. Krishna (2018) studied mosquito diversity in Pushpagiri Wildlife Sanctuary of Kodagu district reported about 25 species belonging to 6 genera, this study also revealed *Culex* as dominating genus with 9 species followed by genus *Anopheles* with 7 species [5]. Urmila *et al.,* (1999) have studied mosquito diversity in different larval habitat of University of Mysore, Manasagangotri campus, Mysore and reported 22 species belonging to 7 genera [7]. Kumar *et al.,* (2004) documented 60 species belonging to 10 genera from Rajiv Gandhi National Park (Nagarhole), Karnataka, this study also revealed *Culex* as dominant genus with 21 species [8]. Rajavel *et al.,* (2006) [11] have recorded 26 species belonging to 11 genera from mangroves of Kundapur, Karnataka. Kanojia (2007) [12] have reported the Ecological study on mosquito vectors with respect to Japanese encephalitis virus in Bellary district, Karnataka and collected a total of 120113 mosquitoes belonging to 24 species under 5 genera. *Cx. tritaeniorhynchus* was the dominant species with a contribution of 70.3%. Similarly, recent study from Dharwad district by Channi and Biradar (2023) have recorded a total of 2658 individuals belonging to 22 species and 4 genera [15].



WT- Water Tanks; DW- Drainage Water; TY- Tyres; PC- Plastic Containers; PF-Paddy Fields; TH- Tree Holes; SW-Swamps; PO-Ponds; ST- Streams.

Fig 4: Distribution of different species in various habitats of the study area.

Table 3: Occurance of number of different mosquito species larvae in various habitats of the study area

Sl. No.	Species	WT	DW	TY	PC	PF	TH	SW	PO	ST	Total
1.	*Ae. aegypti*	79	0	129	103	0	32	0	0	0	343
2.	*Ae. albopictus*	35	19	63	43	0	59	27	18	0	264
3.	*Ae. vexans*	0	0	0	0	0	0	23	0	0	23
4.	*Ae. vittatus*	22	0	0	0	0	54	0	0	0	76
5.	*An. annularis*	0	12	0	0	73	0	29	20	18	152
6.	*An. barbirostris*	0	0	0	0	43	0	68	51	94	256
7.	*An. jamesi*	0	0	0	0	0	0	47	0	0	47
8.	*An. karwari*	0	0	0	0	31	0	17	14	14	76
9.	*An. maculatus*	0	0	0	0	22	0	13	11	12	58
10.	*An. stephensi*	71	20	23	49	0	0	61	52	0	276
11.	*An. subpictus*	0	0	0	0	93	0	0	39	0	132
12.	*An. vagus*	0	0	0	0	72	0	14	27	0	113
13.	*Ar. flavus*	0	0	0	0	0	27	0	0	0	27
14.	*Ar. subalbatus*	0	0	29	20	0	83	0	0	0	132
15.	*Cx. gelidus*	0	0	0	31	0	0	41	78	0	150
16.	*Cx. infantulus*	15	0	0	32	0	0	0	0	0	47
17.	*Cx. malayi*	0	0	0	0	19	0	13	0	9	41
18.	*Cx. minor*	0	0	0	0	21	0	12	0	0	33

19.	*Cx. mimulus*	0	0	0	0	82	0	11	21	0	114
20.	*Cx. pseudovishnui*	0	0	0	0	84	0	0	0	0	84
21.	*Cx. quinquefasciatus*	63	226	46	51	0	0	36	42	0	464
22.	*Cx. sitiens*	32	0	0	0	0	0	0	0	0	32
23.	*Cx. tritaeniorhynchus*	49	251	31	44	0	0	29	37	0	441
24.	*Cx. uniformis*	27	0	26	0	0	41	0	0	0	94
25.	*Cx. vishnui*	0	0	0	0	139	0	51	41	0	231
26.	*T. splendens*	0	26	0	0	0	21	0	0	0	47
27.	*U. atra*	21	0	0	10	0	0	0	0	0	31
Total		414	554	347	383	679	317	492	451	147	3784

WT- Water Tanks; DW- Drainage Water; TY- Tyres; PC- Plastic Containers; PF-Paddy Fields; TH- Tree Holes; SW-Swamps; PO-Ponds; ST-Streams.

Table – 4 represents the diversity indices of mosquito where, the Shannon Diversity Index reveals highest species diversity in Swamps with H-value 2.611, consisting of 16 different species and lowest species diversity was in Streams with H-value of 1.143 having only 5 species. Tree hole habitats exhibited highest Evenness value of 0.9094, which indicates the tree holes have uniform distribution of mosquito species. The Simpson Diversity Index also shown highest (1-D = 0.9167) in swamps, this indicates the richest habitat among all the habitats. Margalef's Index found to be highest in Swamps (2.42) indicating that it has high richness along with more evenness in distribution of species, of mosquito fauna in these habitats, whereas, lowest Margalef's Index is exhibited in Drainage water habitats. The Berger-Parker Index is found highest in the Drainage water habitats (0.4531) and lowest in swamps (0.1382), this index indicates abundance of the most dominant species across habitats i.e. Abundance of *Cx. quinquefasciatus* is highest in drainage water (226) and lowest in swamps (36) (Table-3).

Table 4: Various diversity indices with respect to distribution of mosquito species in different habitats of the study area

Diversity Indices	WT	DW	TY	PC	PF	TH	SW	PO	ST
Taxa_S	10	6	7	9	11	7	16	13	5
Individuals	414	554	347	383	679	317	492	451	147
Dominance_D	0.1271	0.3768	0.2137	0.1492	0.1222	0.1708	0.08328	0.09801	0.4434
Simpson_1-D	0.8729	0.6232	0.7863	0.8508	0.8778	0.8292	0.9167	0.902	0.5566
Shannon_H	2.17	1.187	1.743	2.039	2.223	1.851	2.611	2.431	1.143
Evenness_e^H/S	0.8756	0.546	0.8161	0.8537	0.8395	0.9094	0.8511	0.875	0.627
Margalef	1.494	0.7915	1.026	1.345	1.534	1.042	2.42	1.964	0.8015
Berger-Parker	0.1908	0.4531	0.3718	0.2689	0.2047	0.2618	0.1382	0.1729	0.6395

WT- Water Tanks; DW- Drainage Water; TY- Tyres; PC- Plastic Containers; PF-Paddy Fields; TH- Tree Holes; SW-Swamps; PO-Ponds; ST-Streams.

The Richness S Individual rarefaction plotted by PAST software of all the species with 95% confidence limit shows highest number of species (16) were found in Swamps and lowest number of species (5) in Stream habitats (Fig.5).

The same figure also represents Paddy fields exhibit highest number of individuals (679) and lowest number of individuals in Streams (147).

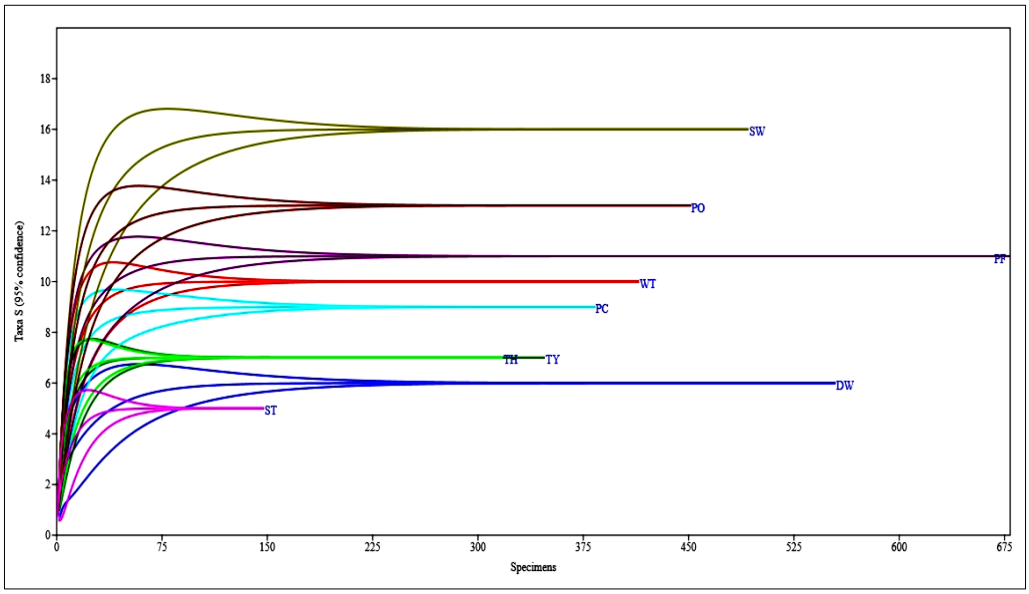


Fig 5: Richness S of Individual rarefaction of all the species across different habitats in the study area

Summary and Conclusion

The present study generated baseline data on the mosquito species composition, diversity and distribution across different breeding habitats in Uttara Kannada district of Karnataka state. It resulted in recording of 27 species belonging to 6 genera with habitat segregation and variable abundance patterns. Swamps were most diverse habitats supporting 16 species followed by Ponds and Paddy field habitats. *Cx. quinquefasciatus* was the predominant species with greater abundance in Drainage water habitats. The diversity indices indicated moderate species diversity and even distribution with low dominance.

The findings provide insights into the ecology of mosquito vector species, which will be useful for targeted surveillance and control of mosquitoes in this region. The diverse mosquito fauna calls for habitat based vector management strategies to curb mosquito density and disease transmission. The extensive breeding of mosquito vectors such as *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Ae. aegypti* is in proximity to human dwellings, poses risk of several mosquito borne infectious diseases and need priority action. Overall, the study highlights the need for regular monitoring of mosquito diversity and population in different habitats to guide intervention measures for integrated mosquito vector management.

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