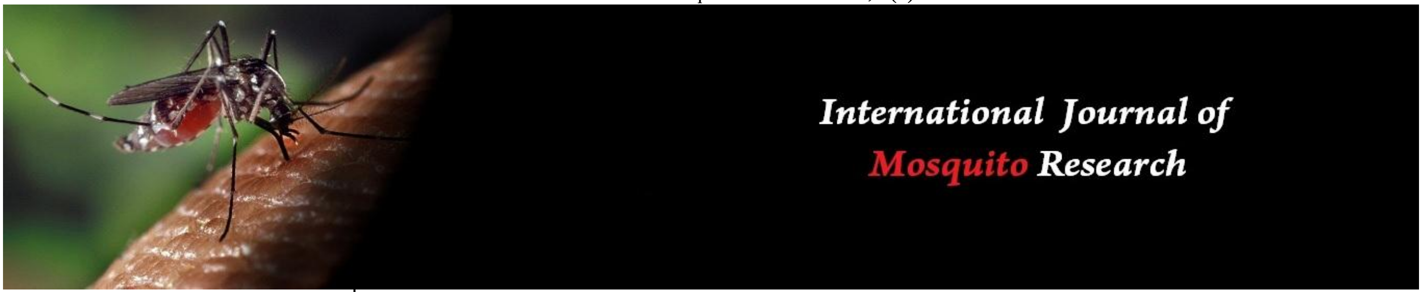
International Journal of Mosquito Research 2019; 6(2): 27-31



ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2019; 6(2): 27-31

Received: 13-01-2019
Accepted: 16-02-2019

Bisu Singh
Department of Zoology, School of Life Sciences, Sikkim University, Sikkim, India

Jiwan Gurung
Department of Zoology, School of Life Sciences, Sikkim University, Sikkim, India

Aparajita Chakraborty
Department of Zoology, School of Life Sciences, Sikkim University, Sikkim, India

Correspondence
Bisu Singh
Department of Zoology, School of Life Sciences, Sikkim University, Sikkim, India

Study of the mosquito larval diversity in the artificial breeding habitat of Gangtok, Sikkim

Bisu Singh, Jiwan Gurung and Aparajita Chakraborty

Abstract
With the growing urbanization, the number of discarded containers and construction of drains has increased considerably in Gangtok which may provide a conducive breeding habitat for mosquito larva. Therefore, the present study was undertaken to investigate the mosquito larval diversity in the artificial breeding habitat of Gangtok. A random sampling was done from the discarded buckets, tanks, tyres, drains at seven different sites during the months of July to October 2017. A total of 247 mosquito larvae was collected from seven sampling sites. The species observed in the different sampling sites were *Aedes albopictus, Aedes atropalpus, Ochlerotatus japonicus, Culex pipiens, Culex territans, Ochlerotatus taeniorhynchus,* and *Ochlerotatus trivittatus.* Among the observed species *Ochlerotatus japonicus* was found to have the maximum abundance (32%). Correlation analysis tests showed the significant positive correlation between the larval abundance and the volume of water (r=0.94, P=0.002). As per the Shannon-Wiener index, highest diversity was observed at Fifth mile (1.37) and least in Gangtok town (0.44). Pielou's Evenness index indicated that species were evenly distributed in all the sites except Gangtok town (0.63). Bray-Curtis index revealed the highest species similarity between Ranipool and Tadong (0.82). The present study gives elementary information about the species diversity of mosquitoes in the artificial breeding sites of Gangtok. The presence of potent disease vectors like *Culex* and *Aedes* shows the vulnerability of this region for the outbreak of mosquito-borne diseases in the near future as Gangtok is a famous tourist destination.

Keywords: Artificial habitat, Gangtok, Mosquitoes

1. Introduction
Gangtok is the capital city of Sikkim and is a popular hill station of North East India. It has a temperate climate throughout the year with an annual rainfall of about 2739 mm (Census of India. Sikkim. 2011) [7]. As such, water gets accumulated in the containers scattered in different localities which are being discarded by the locals and the tourists (Kumar *et al.* 2009) [14]. With the growing urbanization, the number of discarded containers is increasing day by day in Gangtok which provides suitable breeding habitat for mosquitoes. Studies revealed that with the extended urbanization around the world, preference of mosquitoes for artificial containers for breeding has increased to a large extent, which implies that the distribution of larval mosquitoes is greatly influenced by human ecology (Chen *et al.* 2009) [8]. Thus, many of the studies have been conducted to investigate the mosquito larval diversity in artificial breeding sites. Studies have reported that the species of mosquitoes such as *Aedes aegypti*, *Aedes albopictus*, *Aedes niveus*, *Culex quinquefasciatus*, *Anopheles* and *Culex* generally breed in artificial containers (Chen et al. 2009; Adeleke *et al.* 2008; Dass *et al.* 1998; Suganthi *et al.* 2014) [8, 1, 10, 21]. Further, it has been observed that in the urban areas the environment has a major effect on the incidence of *Aedes albopictus* larvae (Li *et al.* 2014) [15]. In addition, studies have also observed the existing and disappearing species of mosquitoes along with their pattern of distribution (Asha *et al.* 2014)4[4]. It has been reported that artificial containers provide a good habitat for the vector of Dengue (Rajesh *et al.* 2013) [18]. So far, the study of mosquito diversity in the Himalayan region of Sikkim has been meagre. A survey conducted on the haematophagous arthropods in the Himalayan regions of West Bengal and Sikkim revealed the existence of 29 species of mosquitoes (Bhat 1975) [6]. Similarly, Aditya *et al.* (2006) studied the mosquito larval habitats (temporary pools and cemented water storage tanks) and their temporal variation in the town of Darjeeling, which revealed the existence of four mosquito genera belonging to *Aedes*, *Armigeres*, *Culex* and *Toxorhynchites* with

significant difference in temporal variation in their relative and absolute numbers (Aditya *et al.* 2006) [2]. In the same study, H' index was found to be between 0.87 and 1.53 and the species evenness was observed between 54.03 and 95.03% (Aditya *et al.* 2006) [2].

As far are the review of the literature is concerned only one study has been conducted so far to study mosquito diversity in Gangtok, Sikkim (Bhat 1975) [6]. However, the previous study had a general approach and did not emphasize on the artificial breeding habitats. Hence, the present study was specifically focused to investigate the mosquito larval diversity and species composition in artificial breeding sites of Gangtok.

Materials and Methods

The study was conducted across various altitudinal gradients between Ranipool and Gangtok during the months of July to October 2017. The study sites considered for the present investigation were Ranipool (932m), Six Mile (1084m), Intel (1124m), Fifth Mile (1170m), Lumsey (1173m), Tadong (1348m), and Gangtok town (1643m).

A random sampling from the water bodies harbouring mosquito larvae was done from the various discarded artificial containers such as tins, motor vehicle tyres, closed tanks, barrels, large flower pots and drains. The collected samples were brought to the laboratory of the Department of Zoology, Sikkim University for identification of the larva and further analysis. Among the collected larvae, the fourth instar larvae were selected and fixed in 10% lactophenol and observed under the microscope for identification. The identification of the larva was made following the keys given by Tyagi et al. (2015) [22], Cutwa *et al.* (2006) [9], Dehgan *et al.* (2016) [11], Andreadis *et al.* (2005) [3] and Barraud (1934) [5].

The statistical analysis was performed using SPSS software version 23, IBM Crop. Pearson correlation analysis was performed to test the correlation between the abundance of mosquito larvae and the volume of water, larval mosquito species and elevation of breeding sites. The P value < 0.05 was considered statistically significant. Mosquito species similarity between the various sampling sites was calculated using the Bray-Curtis similarity index which is an abundance-based similarity index in Estimate S software. Differences in community composition between different areas were analyzed using the Shannon–Wiener diversity index (1949) [20]. Further, the evenness index was calculated using the method given by Pielou (1969) [17], to assess the presence of significant differences between the Shannon–Wiener diversity indices.

Results

The details of species composition of mosquito larvae at different sampling sites are presented in table 1. In the present study, a total of 247 mosquito larvae was collected belonging to seven species namely *Aedes albopictus, Aedes atropalpus, Ochlerotatus japonicus, Culex pipiens, Culex territans, Ochlerotatus taeniorhynchus,* and *Ochlerotatus trivittatus.* Among the identified larval species *Ochlerotatus japonicus* was observed to have the highest abundance (32%) occurring in four study sites out of the seven. Further, *Aedes albopictus* had the second highest abundance (23%) occurring in four study sites, however, its number was less as compared to *Ochlerotatus japonicus*. The most common breeding habitat for *Aedes albopictus* was found to be motor vehicle tyres where its highest number was recorded. These two most abundant larval species were followed by *Culex territans* (17%), *Culex pipiens* (12%), *Aedes atropalpus* (6%), *Ochlerotatus taeniorhynchus* (5%) and *Ochlertotaus trivitattus* (5%).

Pielou's evenness index suggested species were evenly distributed in all the sites except Gangtok town (0.63) (table 2). Shannon–Wiener index for species diversity was high in Fifth mile (1.37) and least in Gangtok town (0.44). However, the values of Shannon–Wiener index did not differ significantly between the study sites ($F = 0.872$, $P = 0.550$) (table 3). The correlation analysis test showed that there was a significant positive correlation between the larval abundance and the volume of water ($r = 0.94$, $P = 0.040$). However, no significant correlation was observed between the larval mosquito species and the elevation of the study sites. Bray-Curtis index for species similarity showed the highest species similarity between Ranipool and Tadong (0.82)(table 4).

Discussion

The present study is the first-time approach to investigate the diversity of mosquito larvae especially focusing on the artificial habitats of Gangtok. From the present study, it is quite evident that there is an ample number of man-made mosquito breeding sites present in and around Gangtok. These artificial habitats are mostly temporary in nature and are created mostly for a brief period of time mainly as a result of rainfall and human activities. In the present study, it was observed that *Ochlerotatus japonicus* was the dominant breeder in the containers followed by *Culex territans* and *Aedes albopictus. Ochlerotatus japonicus* was found to breed in almost all types of artificial containers, with its highest number found in discarded tanks. *Culex* and *Aedes* larvae, identified in the present study, were also reported in the study by Bhat (1975) [6], in the Himalayan region of West Bengal and Sikkim. However, unlike the study by Bhat (1975) [6], the present study showed the existence of *Ochlerotatus* larvae in the highest incidence. The difference in the results could be due to the fact that the present study was conducted on the artificial breeding habitats of Gangtok however in the study by Bhat (1975) [6], larval mosquitoes were collected from the variety of habitats including paddy fields, isolated pools along streams and roadside channels, forest seepage pools, etc. The present findings were also in accordance with the work by Aditya *et al.* (2006) [2], which was carried out in Darjeeling where both *Aedes* and *Culex* larvae were reported. However, in the study of Aditya *et al.* (2006) [2], the species of *Ochlerotatus* was not observed. Moreover, in the same investigation presence of *Armigeres* and *Toxorhynchites* larvae were noted by Aditya *et al.* (2006) [2], which could not be replicated in the present study. The non-agreement of these results may be due to the fact that the present study was specifically carried out in the artificial containers present in and around Gangtok while Aditya *et al.* (2006) [2], conducted the investigation on all possible lentic habitats of mosquitoes distributed around Darjeeling (cemented temporary pools, stagnant streamside pools, etc.) with no particular emphasis on the artificial mosquito breeding sites. In addition, Aditya *et al.* (2006) [2], made the investigation in the sites at the altitude of 2134 m while in the present study, the altitude of the study sites ranged from 932 to 1643 m. In accordance with the present investigation, *Ochlerotatus* larvae were also found to be the dominant breeders in the artificial breeding sites in the study by Vezzani (2007) [23]. The agreement of the results is probably due to the presence of similar artificial containers, rainfall and other conditions for breeding. Another study conducted by Rao *et al.* (2011) [19] reported that the *Aedes* mosquitoes are highly adapted to lay eggs in the artificial containers, showing its high incidence. Accordingly, the results of the present investigations also show that *Aedes* larvae could occupy a variety of artificial containers. Further, in the studies conducted by Dom *et al.* (2013) [12], Das and Mariappan (1998) [10] and Suganthi *et al.* (2014) [21] *Aedes* and *Culex* mosquitoes were observed to have a high preference for breeding in artificial habitats which is in agreement with the present study. However, no incidence of *Ochlerotatus* larvae was reported in these studies which do not corroborate with the present study. Another study by Mohan *et al.* (2014) [16] reported the coexistence of two or more mosquito larvae species of *Anopheles, Culex* and *Aedes* in the same sampling site. However, the coexistence of the mosquito species was not observed in the present study which may be due to the absence of enough phytoplankton to sustain the two species in microhabitats.

The Pearson correlation analysis revealed a significant correlation between the larval abundance and the volume of water. This may be due to the fact that the increase in the surface area of the artificial container along with an increased volume of water could accommodate more larval species. However, the correlation analysis test performed between the larval mosquito species and the elevations of the breeding sites showed no significant value between the two, which indicates that the larval species composition of the mosquito had no variation with the change in the elevation of the breeding sites. Shannon–Wiener index revealed that species diversity was high in Fifth mile (1.37) and least in Gangtok town (0.44). Low species diversity in Gangtok town can be due to the fact that it lies at a higher altitude and temperature is lower than Fifth mile. The Bray-Curtis similarity index showed the highest species similarity was between Ranipool and Tadong (0.82) as both the sites harboured the same species namely *Culex territans and Ochlerotatus japonicas,* with a comparable value of abundance which may be due to the presence of a similar type of artificial containers.

In the present study vegetation, community of another organism in the mosquito breeding habitat were not studied. Besides, the mosquito diversity was studied only in the urban areas of Gangtok, and most importantly, study was carried for a very brief period of time, which can be considered as the limitations of the present study. Considering these limitations, the present study gives first-hand information on the diversity of the mosquito larval species in the artificial breeding habitat of Gangtok.

Studies of mosquito biodiversity help to understand the epidemiological aspects of potential arbovirus vector species (Silva *et al.* 2013) [13]. Since Gangtok is one of the favorite tourist destinations of people all over India and abroad, the presence of potent disease vectors like *Culex* and *Aedes* makes this place quite vulnerable to the outbreak of the different mosquito-borne diseases in the near future. The present study may be helpful for implementation of appropriate mosquito control programs based on the prevalent species of mosquitoes. Future studies for longer time durations including more samplings from different areas considering the altitudinal gradient will throw the light on the prevalence of mosquitoes in the artificial breeding sites of this region.

Table 1: Species composition of mosquito larva and their distribution at various sampling sites.

Mosquito species observed	**Type of artificial container**	**Study sites, elevations and number mosquito larvae collected**							**Total no. of mosquito larva**	**Total vol. of water (ml)**
		Ranipool (932 m)	**Sixth mile (1084m)**	**Entel (1124)**	**Fifth mile (1170 m)**	**Lumsey (1173m)**	**Tadong (1348 m)**	**Gangtok town (1643 m)**		
Aedes albopictus	Large flower pot, Tyres, Drain, Discarded water tank	0	8	25	15	8	0	0	56 (23%)	660
Aedes atropalpus	Drain	0	0	14	0	0	0	0	14 (6%)	200
Culex pipiens	Tyres	0	0	0	15	0	0	16	31 (13%)	700
Culex territans	Drain, Tyres, Discarded water bucket	10	0	0	20	0	11	0	41 (17%)	670
Ochlerotatus japonicas	Discarded tank, Discarded bucket, Garbage tank, Discarded water bucket	25	17	0	23	0	15	0	80 (32%)	1080
Ochlerotatus taeniorhynchus	Discarded plastic bucket	0	0	0	0	0	0	13	13 (5%)	250
Ochlerotatus trivitattus	Tyres	0	0	0	0	12	0	0	12 (5%)	240

Table 2: Shannon-Weiner diversity and Evenness of mosquitoes larvae.

	Shanon- Wiener Diversity Index	Pielou's Evenness Index
Ranipool	0.60	0.86
Sixth mile	0.63	0.90
Entel	0.65	0.94
Fifth mile	1.37	0.99
Lumsey	0.67	0.97
Tadong	0.68	0.98
Gangtok town	0.44	0.63

Table 3: ANOVA for Shannon–Wiener index of different sites.

	Shanon- Wiener Diversity Index	F	P-value
Ranipool	0.60	0.872	0.550
Sixth mile	0.63		
Entel	0.65		
Fifth mile	1.37		
Lumsey	0.67		
Tadong	0.68		
Gangtok town	0.44		

Table 4: Mosquito species similarity between seven localities of Gangtok, Sikkim. Species shared (above diagonal) and species similarity based on Bray-Curtis similarity index, an abundance-based similarity index (below diagonal).

SITE	Ranipool	6th mile	Entel	Lumsey	5th mile	Tadong	Gangtok town
Ranipool	-	1	0	0	2	2	0
6th Mile	0.567	-	1	1	2	1	0
Entel	0	0.25	-	1	1	0	0
Lumsey	0	0.356	0.271	-	1	0	0
5th Mile	0.611	0.51	0.268	0.172	-	2	1
Tadong	0.82	0.58	0	0	0.525	-	0
Gangtok town	0	0	0	0	0.294	0	-

Acknowledgement
The authors would like to thank Mr Sailendra Dewan and Mr Kishor Sharma for analysis.

References

1. Adeleke MA, Mafiana CF, Idowu AB, Adekunle MF, Sam-Wabo SO. Mosquito larval habitats and public health implications in Abeokuta, Ogun State, Nigeria. Tanzania Journal of Health Research. 2008; 10(2):103-7.
2. Aditya G, Pramanik MK, Saha GK. Larval habitats and species composition of mosquitoes in Darjeeling Himalayas, India. Journal of Vector Borne Diseases. 2006; 43(1):7-15.
3. Andreadis TG, Thomas MC, Shepard JJ. Identification guide to the mosquitoes of Connecticut. Connecticut Agricultural Experiment Station, 2005.
4. Asha AV, Aneesh EM. Diversity of mosquito species (Diptera: Culicidae) at Irinjalakuda, Thrissur with special reference to their breeding habitats. International Journal of Current Microbiology and Applies Sciences. 2014; 3(2):536-41.
5. Barraud CP. The fauna of British India including Ceylon and Burma. Taylor and Francis, Red Lion Court, London, 1934.
6. Bhat HR. A survey of haematophagous arthropods in Western Himalayas, Sikkim and Hill Districts of West Bengal: records of mosquitoes collected from Himalayan region of West Bengal and Sikkim with ecological notes. Indian Journal of Medical Research. 1975; 63(2):232-41.
7. Census of India. Sikkim. District census handbook north, west, south and east districts. Village and town wise primary census abstract (PCA). Directorate of census operations Sikkim. Series-12 Part XII-B, 2011.
8. Chen CD, Lee HL, Stella-Wong SP, Lau KW, Sofian-Azirun M. Container survey of mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia, 2009.
9. Cutwa MM, O'Meara GF. Photographic guide to common mosquitoes of Florida. Florida Medical Entomology Laboratory, University of Florida, 2006.
10. Dass K, Mariappan P. A study on diversity of mosquito fauna in Thanjavur urban, Tamilnadu, India. Environmental Science. 1998; 2(1):7-16.
11. Dehghan H, Sadraei J, Moosa-Kazemi SH, Abolghasemi E, Solimani H, Nodoshan AJ *et al*. A pictorial key for Culex pipiens complex (Diptera: Culicidae) in Iran. Journal of arthropod-borne diseases. 2016; 10(3):291.
12. Dom NC, Ahmad AH, Ismail R. Habitat characterization of Aedes sp. breeding in urban hotspot area. Procedia-Social and Behavioral Sciences. 2013; 85:100-9.
13. Silva JD, Lopes CM, Guimarães AÉ, De Mello CF, Alencar J. Diversity of Mosquitoes at the Itatiaia National Park, State of Rio De Janeiro, Brazil. Southeast Asian Journal of Tropical Medical and Public Health. 2017; 33(4):270-5.
14. Kumar S, Bhattacharyya JK, Vaidya AN, Chakrabarti T, Devotta S, Akolkar AB. Assessment of the status of municipal solid waste management in metro cities, state capitals, class I cities, and class II towns in India: An insight. Waste management. 2009; 29(2):883-95.
15. Li Y, Kamara F, Zhou G, Puthiyakunnon S, Li C, Liu Y *et al*. Urbanization increases Aedes albopictus larval habitats and accelerates mosquito development and survivorship. PLoS neglected tropical diseases. 2014; 13:8(11):e3301.
16. Mohan S, Banerjee S, Mohanty SP, Saha GK, Aditya G. Assessment of pupal productivity of Aedes and co-occurring mosquitoes in Kolkata, India. Southeast Asian Journal of Tropical Medicine and Public Health. 2014; 45(6):1279.
17. Pielou EC. An introduction to mathematical ecology. An introduction to mathematical ecology, 1969.
18. Rajesh K, Dhanasekaran D, Tyagi BK. Survey of container breeding mosquito larvae (Dengue vector) in Tiruchirappalli district, Tamil Nadu, India. Journal of Entomology and Zoological Studies. 2013; 1(6):88-91.
19. Rao BB, Harikumar PS, Jayakrishnan T, George B. Characteristics of Aedes (Stegomyia) albopictus Skuse (Diptera: Culicidae) breeding sites. Southeast Asian Journal of Tropical Medicine and Public Health. 2011; 42(5):1077-82.

20. Shannon CE, Weaver W. The Mathematical Theory of Communication. The University of Illinois Press, Illinois, 1949, 144.
21. Suganthi P, Govindaraju M, Thenmozhi V, Tyagi BK. Survey of mosquito vector abundance in and around tribal residential areas. Journal of Entomology and Zoology Studies. 2014; 2(6):233-9.
22. Tyagi BK, Munirathinam A, Venkatesh A. A catalogue of Indian mosquitoes. International Journal of Mosquito Res. 2015; 2(2):50-97.
23. Vezzani D. Artificial container-breeding mosquitoes and cemeteries: a perfect match. Tropical Medicine & International Health. 2007; 12(2):299-313