Studies on the maintenance of 'self-sustained' mosquito vector population in Vaigai river, South India

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Study of mosquito species diversity and its relationship with ecosystems is essential to understand disease epidemiology and to develop control measures of vector populations in human-dominated ecosystems. In the present study, the structure and composition of immature mosquito population and associated ecological parameters were analysed in three different ecosystems (urban, semi-urban and rural) along Vaigai river, Madurai, Tamil Nadu, India. Average larval density was higher in urban than the semi-urban and rural sites during the study period. In 2012–13, immature mosquito diversity was higher in rural site during pre-monsoon and monsoon seasons, whereas mosquito larval diversity was greater in semi-urban site during the post-monsoon season. In 2013–14, mosquito larval diversity was found to be high in semi-urban site during monsoon and post-monsoon seasons. Culicine species dominated the urban areas, while Anopheline species dominated the rural and semi-urban sites during the study period. Among the mosquito species, *Culex tritaeniorhynchus*, *Cx. geldius*, *Cx. vishnuvi*, *Cx. quinquefasciatus*, *Cx. bitaeniorhynchus*, *Anopheles subpictus* and *An. culicifacies* were prevalent throughout the year. Filamentous algal-dominated sites showed a higher number of mosquito species (23 species) than other hydrophytes-dominated sites. Maximum number of sewage inlets was recorded in urban than the other two sites. As a result, the highest turbidity level was recorded in the urban site followed by semi-urban and rural sites. The present study shows that the immature mosquito population is maintained with the help of ecological parameters in all the study sites throughout the year. Therefore, it is essential to take steps to eradicate the mosquito vectors from the Vaigai river basin to avoid disease outbreak in the region.

Keywords: Animal husbandry, aquatic vegetation, larval density, sewage inlets, water quality.

RIVER ecosystems throughout the world have been profoundly altered by a wide range of human impacts, especially in developing countries[1,2]. Most of the rivers in the Indian subcontinent have also been heavily impacted by human interventions over the last few decades, which mainly include water pollution[3], intensive water abstraction, extensive physical channel modifications, and alteration of natural flow regimes due to the construction of dams[4]. River Vaigai flows through the heart of the Madurai city, which is the second largest city in Tamil Nadu, South India. This historic river has been heavily impacted by water pollution[5], extensive physical-habitat modifications, fragmentation in its main channel due to reduced longitudinal connectivity and altered flow regimes[6]. These physical habitat modifications coupled with other kinds of disturbance along its entire stretch have created a variety of disturbed habitats, viz. aquatic, semi-aquatic and terrestrial habitats within the river bed depending on the levels of urbanization[7–9]. Due to these changes the river bed is being used for various purposes such as grazing land, washing ground[10], open toilet[11], etc. These changes resulted in various ecological and health problems related to groundwater pollution[12], proliferation of exotic weeds[9] and changes in the vegetation pattern[7]. The earlier reports have suggested that modified river ecosystems provide suitable habitats for mosquitoes[13]. Mosquito acts as a vector for various diseases such as Japanese encephalitis, malaria, dengue, chikungunya, filariasis, etc. Outbreaks of vector-borne diseases depend on the abundance of adult mosquito vector populations[14]. Eradication of vector-borne diseases requires control of adult vector populations, which depends on the bionomics of the mosquito. Adult vector population is highly influenced by density and diversity of immature mosquito populations[15]. Such populations thrive in aquatic habitats which include both natural aquatic habitats such as pools, lakes, ponds, swamps, rivers, rock pools, water in tree holes, and artificial aquatic habitats such as tyres and containers[16,17]. Lentic water bodies are highly preferred by adult mosquitoes, especially the gravid female mosquitoes for their oviposition[16]. These habitats provide constant supply of food and protection from predators to immature mosquito populations[18,19]. Adult mosquito population is positively influenced by plant community as it provides shelter and food to adult mosquitoes, which help increase their survival rates and support the vectorial

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capacity of adult female mosquitoes[20,21]. Animal and human population distributions collectively or independently support mosquito populations by providing blood meal for the mosquitoes[22]. Though the modified river ecosystem plays a vital role in the maintenance of mosquito vector population, information on the influence of various biotic and abiotic factors on immature mosquito vector population structure in different ecosystems along Vaigai river is not available in the literature. Therefore, the present study analyses the relationship between immature mosquito diversity and composition with various ecological parameters such as vegetation structure, water quality and animal husbandry at different ecosystems (urban, semi-urban and rural) along the Vaigai river.

Materials and methods

The River Vaigai originates from Cumbum valley of the Western Ghats and flows through Madurai for about 11 km in the urban stretch and 127 km upstream as well as 145 km downstream. In order to understand the ecology of mosquito population, three different study sites, viz. urban (Madurai city; 9°55′N and 78°07′E), semi-urban (Solavandan; 10°01′N and 77°57′E) and rural (Thenoor; 9°59′N and 78°00′E) were selected based on the level of urbanization. In each site, the river bank covering a linear distance of 5 km was selected for the study.

Through 15 randomly placed 1 sq. m quadrats for herbs and 5 sq. m quadrats for shrubs in each study site along the river bank, the terrestrial plant density (no. of individuals/m^2) and species richness (total number of species) in each site were studied. The vegetation cover (herb and shrub community) was recorded by randomly placed line transects ($n = 5$) across the river edges in each study site. The percentage of vegetation cover was calculated as the ratio of the total length occupied by the vegetation along the line transect to the total length of the line transect. Dominant aquatic and semi-aquatic species were identified and recorded in the river beds through visual observation in all the study sites. Immature mosquito population density was estimated on every alternative day in all the study sites using the standard dipping method. The immature mosquitoes were collected in isolated water pools within river banks and along the main stream of river beds covered by filamentous algae and other aquatic vegetation. A total of 771 immature mosquito samples (no. of dips/habitat was calculated based on 3–10 dips depending on the larval population) were collected from all the sites during the entire study period (2012–14). The immature mosquitoes were collected and transferred to the laboratory, and emerged adults were collected and identified with help from the Centre for Research in Medical Entomology, Madurai. Mosquito diversity was calculated using the Simpson dominance index, Shannon–Wiener diversity index[23], evenness index[24] and species richness[25]. Filamentous algae were collected from the study sites where the immature mosquitoes were sampled and they were transported to laboratory in a plastic container and species identification was done using Nikon microscope (40×). To study the algal species composition, water sample ($n = 30$ sample) was collected randomly from the urban, semi-urban and rural sites along the Vaigai river. The physico-chemical characteristics of water were analysed for parameters such as pH, temperature, conductivity, salinity, dissolved oxygen, total dissolved solids (TDS) and turbidity ($n = 9$ per parameter) in each study site using water analysing kit (Systronics, 371) in aquatic habitats where immature mosquitoes were bred. In each study site, the number of cattle sheds and sewage inlets was visually counted in a 3 km stretch. To estimate the number of milching animals and other domesticated animals found within the riverbed and banks, visual counts were made by conducting three 10 min walks with an average rate of ≈98 footsteps/min randomly at each site. The study was carried out in August and September (before the onset of the northeast monsoon), November and December (during the monsoon) and post-monsoon (May and June) from 2012 to 2014.

Data analysis

The data were subjected to appropriate statistical analysis. Thus, one-way ANOVA was performed to analyse the influence of seasons and sites on mosquito larval density. The Student's *t*-test was used to analyse the difference between two consecutive years of sampling. All the statistical analyses were performed using SPSS 20.0.0 statistical package.

Results

Vegetation analysis

The vegetation cover was higher in the urban site compared to the semi-urban and rural study sites. Maximum number of terrestrial herbaceous species was recorded in the semi-urban site followed by the other two sites. However, density of terrestrial herbaceous species/m^2 was higher in the urban study site compared to the other two sites. The urban site was dominated by *Cynodon dactylon*, *Amaranthus spinosus*, *Croton sparsiflorus*, *Parthenium hysterophorus* and *Prosopis juliflora* whereas the semi-urban and rural sites were dominated by *Saccharum spontaneum*, *Cyperus rotundus*, *P. juliflora*, *Ipomea carnea* and *Arundo donax* (Table 1). In the case of the aquatic vegetation recorded in all the study sites, *Eichhornia crassipes*, *Azolla* sp. and *Lemna* sp. were the free-floating hydrophytes. Among them, *E. crassipes* was recorded only in the urban site, whereas *Azolla* sp. and *Lemna* sp. were found in all the three sites. *Ipomea aquatia*,

Table 1. Characteristics of terrestrial herbaceous vegetation at different study sites along Vaigai river, Madurai, Tamil Nadu

Parameters	Urban	Semi-urban	Rural
Soil type (observed)	Alluvial	Sand	Sand
Percentage of vegetation cover/m^2	90	40	60
Percentage of palatable grass/m^2	74	12	7
Total no. of species/m^2	14	17	14
Density/m^2	287.5 ± 1.77	91.63 ± 1.96	67.3 ± 1.35
Dominant species	*Cynodon dactylon* *Croton sparsiflorus* *Amaranthus spinosus* *Prosopis juliflora* *Parthenium hysterophorus*	*Saccharum spontaneum* *Cyperus rotundus* *Arundo donax* *Prosopis juliflora* *Ipomea carnea*	*Saccharum spontaneum* *Cyperus rotundus* *Prosopis juliflora* *Ipomea carnea* *Arundo donax*

Table 2. Aquatic, semi-aquatic, filamentous and micro-algae at different study sites along Vaigai river

Type of vegetation	Urban	Semi-urban	Rural
Free-floating hydrophytes	*Eichhornia crassipes* *Azolla* sp. *Lemna* sp.	*Azolla* sp. *Lemna* sp.	*Azolla* sp. *Lemna* sp.
Attached floating hydrophytes	*Marselia* sp.	–	–
Emerged hydrophytes (semi-aquatic plants)	*Ipomea aquatica* *Cyperus rotundus* *Polygonum glabrum*	*Cyperus rotundus* *Polygonum glabrum*	*Cyperus rotundus* *Typha* spp.
Submerged hydrophytes	*Ottelia alismoides*	–	–
Filamentous algae	*Spirogyra weberi* *Oscillatoria terebriformis* *Scytonema simplex* *Phormidium molle*	*Chaetophora incrassate* *Oscillatoria terebriformis* *Phormidium retzii* *Oscillatoria tenuis* *Lyngbya birgei* *Tolypothrix tenuis* *Pithophora affinis* *Spirogyra acqualis* *Scytonema simplex* *Oedogonium crassum* *Oscillatoria salina*	*Spirogyra weberi* *Chaetophora incrassate* *Oscillatoria tenuis* *Calothrix fusca* *Closterium moniliferum* *Cladophora* sp.
Micro-algae	*Aphanocapsa grevillei* *Nitzschia acuta* *Closterium acerosum* *Navicula cuspidata var ambigua* *Navicula pupula* *Navicular rhynchocephalia* *Navicula minuscule* *Euglena polymorpha* *Fragilaria pinnata* *Cocconeis placentula* *Pinnularia fasciata* *Navicula gracilis* *Nitzschia palea*	*Nitzschia minuta* *Nitzschia amphibia* *Closterium incurvum* *Navicula laterostrata* *Cymbella naviculiformis* *Microcystis aeruginosa* *Scenedesmus acuminatus* *Nitzschia tryblionella* var. *Levidensis* *Pandorina morum* *Diatoma hiemale* *Selenastrum minimum* *Synedra berolinensis* *Synedra ulna* *Navicula cuspidata* var. *ambigua* *Achnanthes auckiana*	*Cocconeis placentula* *Pinnularia fasciate* *Chroococcus tenax* *Hantzschia distincte-punctata* *Nitzschia tryblionella* var. *levidensis* *Nitzschia palea* *Nitzschia actinostoloides* *Navicula pygmaea* *Navicula minuscule* *Navicula cuspidata* var. *ambigua* *Scenedesmus arcuatus* *Chlorella vulgaris*

C. rotundus and *Polygonum glabrum* were recorded as emerged hydrophytes in the urban site, whereas *Typha* sp. was present only in the rural site and *C. rotundus* was recorded in all the study sites. *Ottelia alismoides* was recorded as submerged hydrophytes in the urban site. Maximum number of filamentous algae was found in the semi-urban site followed by the rural and urban study sites. Maximum number of micro-algal species was found in the semi-urban site and the minimum in the rural study site (Table 2).

Mosquito diversity

A total of 80,706 immature mosquitoes were collected from all the study sites during the entire study period. During the first year of the sampling period (2012–13), mosquito larval density was significantly higher in the urban site during monsoon season compared to the other two seasons ($F = 9.51$; $P = 0.000$). In the semi-urban site, mosquito larval density was significantly influenced by both monsoon and post-monsoon seasons than pre-monsoon season ($F = 3.43$; $P = 0.035$). However in the rural site, mosquito larval density showed significant increase during post-monsoon season ($F = 8.14$; $P = 0.000$). During the second year of the sampling period (2013–14), the semi-urban site showed significantly higher mosquito larval density during the pre-monsoon season ($F = 16.81$; $P = 0.000$) than the other two seasons. In the urban and rural sites, we found no significant influence of seasons on mosquito larval density ($F = 3.13$; $P = 0.047$ for urban site, and $F = 0.708$; $P = 0.495$ for rural site). Between the two years of the sampling period, the semi-urban site showed marked difference in mosquito larval density during pre-monsoon ($t = 4.74$; $P = 0.000$) and monsoon ($t = 2.18$; $P = 0.031$) seasons. In the rural site, pre-monsoon season had a significant influence on mosquito larval density between the two study years ($t = 2.63$; $P = 0.009$). Such difference was not observed in the urban site during the seasons between the two years (Figure 1).

A total of 32 immature mosquito species belonging to the genera *Anopheles*, *Culex*, *Lutzia*, *Mansonia*, *Fredwardsius* and *Aedeomyia* were collected from the study sites in all sampling seasons during the study period (Tables 3 and 4). Of these, 15 mosquito larvae were found to be malarial vectors, 11 species were Japanese encephalitis vectors, 2 species were dengue vectors, 2 species were filariasis vectors and 2 species were non-vectors. Maximum mosquito diversity (28 species) was recorded during the first year compared to the second year (21 species). Among the mosquito species, *Anopheles minimus*, *An. jamesi*, *An. varuna*, *An. maculates*, *An. aitkeni*, *An. aconitus*, *An. lesteri*, *An. kochi*, *Culex sitiens*, *Lutzia vorax* and *Mansonia indiana* were recorded only during the first year, whereas *An. stephensi*, *Cx. infula*, *Lt. fuscana* and *Stegomyia aegypti* were noted only during the second year. *Anopheles* species diversity (16 species) was higher in the first year compared to second year (nine species). *An. culicifacies*, *An. vagus*, *An. subpictus*, *An. pallidus*, *An. peditaeniatus*, *An. barbirostris*, *An. annularis* and *An. splendidus* were recorded in both years. Although *Culex* species diversity was similar during both years (eight species), species composition of *Culex* differed between the two years of the study period. *Culex tritaeniorhynchus*, *Cx. geldius*, *Cx. vishnuvi*, *Cx. quinquefasciatus*, *Cx. bitaeniorhynchus*, *Cx. fuscocephala* and *Cx. pseudovishnui* were found common during the both years. Mosquitoes such as *Aedeomyia catasticta* and *Fr. vittatus* were also found during both the years.

Site-wise mosquito diversity

Mosquito species dominance differed among the study sites. Species of Culicine were dominant in the urban site, whereas species of Anopheline were dominant in the other two study sites. During the entire sampling period, diversity of mosquito species was higher (24 species) in the rural site compared to the semi-urban (18 species) and urban (16 species) study sites. In general, the mosquito species composition in semi-urban site was a mixture of species composition of the urban and rural sites (Tables 3 and 4).

Season-wise mosquito diversity

During the first year of the sampling period, *Cx. tritaeniorhynchus* was the dominant species in the urban site during pre-monsoon and monsoon seasons, while *Cx. geldius* was the co-dominant and its population showed an

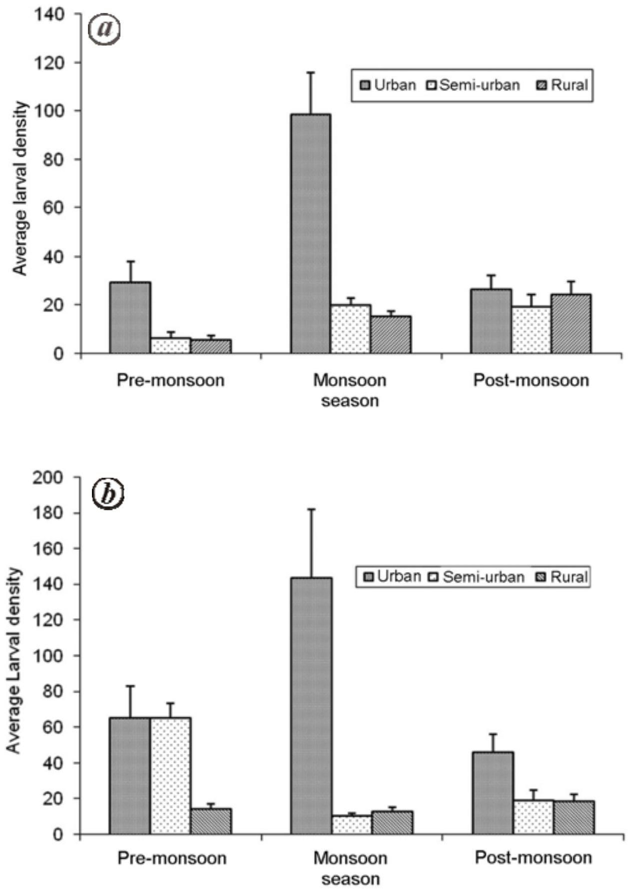

Figure 1. Average larval density (mean ± SE) in different study sites during the pre-monsoon, monsoon and post-monsoon season along the Vaigai river. ***a***, For the year 2012–2013; ***b***, for the year 2013–2014.

Table 3. Mosquito species composition (%) and vector status in urban, semi-urban and rural study sites in the pre-monsoon, monsoon and post-monsoon season during 2012–13 in Vaigai river

		Species composition (%) – pre-monsoon (August and September 2012)			Species composition (%) – monsoon (November and December 2012)			Species composition (%) – post-monsoon (May and June 2013)		
Species	Possible vectors for	Urban	Semi-urban	Rural	Urban	Semi-urban	Rural	Urban	Semi-urban	Rural
Anopheles culicifacies	Malaria	–	40.49	34.7	–	46.79	48.46	–	7.6	9.13
Anopheles vagus	Malaria	–	2.05	–	0.19	1.82	0.59	–	2.8	1.43
Anopheles subpictus	Japanese encephalitis	15.26	25.56	20.2	11.80	30.15	12.26	47.6	34.0	40.72
Anopheles minimus	Malaria	–	–	5.8	–	–	–	–	–	–
Anopheles jamesi	Malaria	–	–	0.6	–	–	–	–	–	0.51
Anopheles varuna	Malaria	–	–	0.98	–	0.85	–	–	–	–
Anopheles maculates	Malaria	–	–	–	–	0.50	–	–	–	–
Anopheles pallidus	Malaria	–	–	–	0.13	–	1.88	–	0.2	0.31
Anopheles aitkeni	Malaria	–	–	–	0.06	–	–	–	–	–
Anopheles aconitus	Malaria	–	–	–	–	0.22	–	–	–	–
Anopheles peditaeniatus	Japanese encephalitis	–	–	–	–	–	0.42	–	–	–
Lutzia (Meta) vorax	Malaria	–	–	–	0.09	–	–	–	–	–
Anopheles barbirostris	Japanese encephalitis	–	–	–	–	–	2.67	–	0.2	0.82
Anopheles annularis	Malaria	–	–	–	–	–	1.29	–	–	0.41
Anopheles splendidus	Malaria	–	–	–	–	–	0.68	–	–	–
Anopheles lesteri	Malaria	–	–	–	–	–	0.21	–	–	–
Anopheles kochi	Malaria	–	–	–	–	–	0.11	–	–	–
Culex tritaeniorhynchus	Japanese encephalitis	58.86	18.20	15.3	38.06	7.11	10.63	12.6	13.9	10.05
Culex bitaeniorhynchus	Japanese encephalitis	–	7.57	5.1	–	5.60	5.49	–	2.2	13.54
Culex sitiens	Japanese encephalitis	–	–	0.22	–	–	–	–	–	–
Culex gelidus	Japanese encephalitis	23.54	2.45	0.6	36.90	0.18	–	0.7	7.6	0.31
Culex vishnui	Japanese encephalitis	–	3.68	0.6	6.16	4.56	1.76	37.7	9.4	18.15
Culex fuscocephala	Japanese encephalitis	–	–	–	–	–	0.80	–	–	–
Culex quinquefasciatus	Japanese encephalitis	2.34	–	–	6.61	–	–	1.0	22.1	0.21
Mansonia Indiana	Malayan filariasis	–	–	–	–	2.22	12.75	–	–	–
Fredwardsius vittatus	Yellow fever	–	–	15.9	–	–	–	–	–	4.2
Aedeomyia catasticta	Non-vector	–	–	–	–	–	1.86`	–	–	–
Culex pseudovishnui	Japanese encephalitis							0.4	–	0.21

Table 4. Mosquito species composition (%) and vector status in urban, semi-urban and rural study sites in the pre-monsoon, monsoon and post-monsoon season during 2013–14 in Vaigai river

		Species composition (%) – pre-monsoon (August and September 2013)			Species composition (%) – monsoon (November and December 2013)			Species composition (%) – post-monsoon (May and June 2014)		
Species	Possible vectors for	Urban	Semi-urban	Rural	Urban	Semi-urban	Rural	Urban	Semi-urban	Rural
Anopheles subpictus	Japanese encephalitis	18.66	80.47	32.18	21.36	28.35	9.75	46.24	38.43	76.58
Anopheles vagus	Malaria	–	1.06	1.97	0.33	10.55	7.2	–	9.81	0.42
Anopheles culicifacies	Malaria	–	1.34	22.85	–	27.69	30.93	–	–	3.59
Anopheles annularis	Malaria	–	0.05	–	–	0.22	–	–	–	–
Anopheles barbirostris	Japanese encephalitis	–	0.19	–	–	2.64	2.74	–	0.78	–
Anopheles peditaeniatus	Japanese encephalitis	–	–	1.72	0.06	0.44	1.69	–	–	–
Anopheles pallidus	Malaria	–	–	0.25	–	–	0.42	–	–	–
Anopheles splendidus	Malaria	–	–	0.25	–	–	–	–	–	–
Anopheles stephensi	Malaria	–	–	–	–	0.44	–	–	–	–
Culex pseudovishnui	Japanese encephalitis	0.23	–	0.25	0.82	–	0.21	–	–	–
Culex gelidus	Japanese encephalitis	2.31	2.04	–	3.19	2.42	0.21	1.33	2.75	–
Culex tritaeniorhynchus	Japanese encephalitis	5.18	5.37	16.22	1.65	5.05	0.42	–	–	–
Culex quinquefasciatus	Japanese encephalitis	14.52	0.6	0.74	59.47	1.32	–	1.99	12.16	–
Culex vishnui	Japanese encephalitis	58.41	6.85	11.54	11.42	14.07	8.1	50.00	31.76	8.23
Culex bitaeniorhynchus	Japanese encephalitis	–	1.94	2.45	–	6.59	20.34	–	4.31	0.63
Culex infula	Japanese encephalitis	–	–	–	1.26	0.22	–	–	–	–
Culex fuscocephala	Japanese encephalitis	–	–	–	–	–	–	0.22	–	–
Lutzia fuscana	Non-vector	–	0.09	–	0.11	–	1.48	–	–	–
Fredwardsius vittatus	Yellow fever	0.69	–	9.58	–	–	14.4	–	–	10.55
Stegomyia aegypti	Dengue	–	–	–	0.33	–	–	0.22	–	–
Aedeomyia catasticata	Non-vector	–	–	–	–	–	2.11	–	–	–

Table 5. Mosquito diversity measures at different study sites in the pre-monsoon, monsoon and post-monsoon season during the sampling period along the Vaigai river

		2012–13			2013–14		
Index	Study site	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
Simpson dominance index, *C*	Urban	0.463	0.303	0.384	0.656	0.414	0.464
	Semi-urban	0.275	0.321	0.204	0.381	0.196	0.275
	Rural	0.221	0.198	0.237	0.201	0.203	0.606
Shannon–Wiener diversity index, *H′*	Urban	0.957	1.370	1.087	1.196	1.208	0.865
	Semi-urban	1.496	1.456	1.801	0.823	1.873	1.487
	Rural	1.769	1.935	1.739	1.421	1.834	0.822
Species richness (Margalef's index), D_{Mg}	Urban	0.522(4)	0.913(9)	0.726(6)	0.888(7)	1.465(11)	0.818(6)
	Semi-urban	1.048(7)	1.266(11)	1.318(9)	1.302(11)	1.961(13)	1.083(7)
	Rural	1.692(11)	2.324(16)	0.763(14)	1.768(12)	1.891(14)	0.812(6)
Evenness, *E*	Urban	0.690	0.624	0.607	0.615	0.504	0.483
	Semi-urban	0.755	0.607	0.819	0.343	0.730	0.764
	Rural	0.738	0.698	0.658	0.572	0.695	0.459

increasing trend during the monsoon season. *An. subpictus* was the predominant species during post-monsoon season in the urban site. In the semi-urban and rural study sites, *An. culicifacies* was the dominant species during pre-monsoon and monsoon seasons but *An. subpictus* was predominant in the post-monsoon season. *Mansonia indiana* was recorded during the monsoon season in both the semi-urban and rural sites. *Lt. vorax* was found only in the urban site during monsoon season.

During the second year of the sampling period, *An. subpictus* was the dominant species in the semi-urban site during all seasons whereas *An. culicifacies* was more dominant in the monsoon season than pre- and post-monsoon seasons in the rural site. *Cx. bitaeniorhynchus* was the co-dominant species during monsoon season in the rural site. *Cx. vishnuvi* was the dominant species in the urban site during pre- and post-monsoon seasons. *Cx. quinquefasciatus* was predominant during the monsoon season in the urban site. *Fr. vittatus* was recorded in the pre-monsoon season, whereas *Stegomyia aegypti* was recorded in the post-monsoon season in the urban site.

Aedeomyia catasticta was recorded only in the rural site during monsoon season in both the years. Among the mosquito species, *Cx. tritaeniorhynchus*, *Cx. geldius*, *Cx. vishnuvi*, *Cx. quinquefasciatus*, *Cx. bitaeniorhynchus*, *An. subpictus* and *An. culicifacies* were prevalent throughout the year. Therefore, these are the most important species with respect to the spread of vector-borne diseases in all the study sites.

Diversity, species richness and evenness of mosquito species

During the first year of the sampling period, the rural site had greater diversity during pre-monsoon ($H' = 1.769$; $S = 11$; $D_{Mg} = 1.692$) and monsoon seasons ($H' = 1.935$; $S = 16$; $D_{Mg} = 2.324$) than the other two sites, while during the post-monsoon season the semi-urban site had greater diversity ($H' = 1.801$; $S = 9$; $D_{Mg} = 1.318$; $E = 0.819$) compared to the rural site ($H' = 1.739$; $S = 14$; $D_{Mg} = 0.763$; $E = 0.658$). In the urban site, mosquito diversity was least in all the seasons compared to the other two sites (Table 5).

During the second year of the sampling period, the semi-urban site had greater diversity during monsoon ($H' = 1.873$; $S = 13$; $D_{Mg} = 1.961$) and post-monsoon seasons ($H' = 1.487$; $S = 7$; $D_{Mg} = 1.083$). During pre-monsoon season, the rural site ($H' = 1.421$; $S = 12$; $D_{Mg} = 1.768$) had greater diversity than the other two sites (Table 5). Urban site had greater diversity index ($H' = 1.196$; $E = 0.615$) compared to semi-urban site ($H' = 0.823$; $E = 0.343$) during the pre-monsoon season.

Hydrophytes and mosquito diversity

We found a strong association of mosquito species with hydrophytes colonized in the study sites. Out of 32 mosquito species, 25 were strongly associated with various hydrophytes in all the study sites. Among the hydrophytes, filamentous algae-dominated sites showed higher mosquito diversity (23 species) than the other hydrophytes (Table 6). Species of *Anopheles* (14 species) were positively coupled with filamentous algae; however, *An. aitkeni* was recorded in a Lemna-dominated site. Most of the *Anopheles* species preferred thick mat of filamentous algae for their habitats. *Cx. bitaeniorhynchus*, *Cx. fuscocephala*, *Cx. infula* and *Aedeomyia catasticta* were also associated with thick mat of filamentous algae. Distribution of *Cx. bitaeniorhynchus* and *Aedeomyia catasticta* was directly influenced by that of S*pirogyra* sp. and *Cladophora* filamentous algae. *Cx. gelidus*, *Cx. quinquefasciatus*, *An. subpictus*, *Cx. tritaeniorhynchus* and

Table 6. Presence (+)/absence (–) matrix for different mosquito species in various aquatic vegetation-dominated sites in Vaigai river

	Dominant vegetation				
Species	*Eichhornia crassipes*	*Ipomea aquatic*	*Marselia* sp.	*Lemna* sp.	Filamentous algae
Anopheles culicifacies	–	–	–	–	+
Anopheles vagus	–	–	–	–	+
Anopheles subpictus	+	+	+	+	+
Culex quinquefasciatus	+	+	+	+	+
Culex tritaeniorhynchus	+	+	+	+	+
Culex bitaeniorhynchus	–	–	–	–	+
Culex gelidus	+	+	+	+	+
Anopheles varuna	–	–	–	–	+
Culex vishnui	+	+	+	+	+
Anopheles maculatus	–	–	–	–	+
Anopheles pallidus	–	–	–	–	+
Anopheles aconitus	–	–	–	–	+
Anopheles barbirostris	–	–	–	–	+
Anopheles annularis	–	–	–	–	+
Anopheles splendidus	–	–	–	–	+
Mansonia indiana	–	–	–	–	+
Aedeomyia catasticta	–	–	–	–	+
Anopheles jamesi	–	–	–	–	+
Culex pseudovishnui	+	–	–	–	–
Anopheles peditaeniatus	–	–	–	–	+
Culex fuscocephala	–	–	–	–	+
Anopheles aitkeni	–	–	–	+	–
Anopheles lesteri	–	–	–	–	+
Culex infula	–	–	–	–	+
Anopheles stephensi	–	–	–	–	+

Table 7. One-way ANOVA results for physio-chemical characteristics of water in different study sites during the sampling period 2012–13 along Vaigai river

Parameter	Urban	Semi-urban	Rural
pH	8.54 ± 0.233[a]	8.38 ± 0.155[a]	8.38 ± 0.224[a]
Dissolved oxygen (ppm)	6.07 ± 1.075[a]	5.09 ± 0.514[a]	4.79 ± 0.391[a]
Conductivity (ms)	2.68 ± 0.232[c]	0.96 ± 0.115[a]	1.56 ± 0.134[b]
TDS (ppt)	1.43 ± 0.117[c]	0.49 ± 0.544[a]	0.85 ± 0.672[b]
Salinity (ppt)	1.58 ± 0.131[c]	0.55 ± 0.599[a]	0.94 ± 0.747[b]
Temperature (°c)	29.11 ± 0.513[a]	30.44 ± 1.041[a]	29.01 ± 1.062[a]
Turbidity (NTU)	43.41 ± 7.561[b]	7.99 ± 2.735[a]	3.15 ± 0.721[a]

Values are represented as mean ± SE. Values having different superscripts within rows are significantly different at $P < 0.05$.

Cx. vishnui species preferred different aquatic vegetation habitats such as *E. crassipes*, *Lemna* sp., *Marselia* sp., *I. aquatica* and also filamentous algae.

Physio-chemical analysis of water

Water quality plays a vital role in mosquito diversity in all the study sites. Salinity level was recorded maximum during the first year in all the study sites (Table 7). Turbidity level had increased in all the study sites during the second year compared to the first year (Table 8). The highest turbidity level was recorded at the urban site followed by semi-urban and rural sites in both years of the sampling period.

Animal husbandry and sewage inlets

Maximum number of sewage inlets was recorded in the urban site compared to the semi-urban and rural sites. Greater number of cattle sheds was found in the urban site compared to semi-urban and rural study sites along the river (Table 9). Cattle sheds in the urban site comprised of cow (41.0%), goat (24.4%), buffalo (12.8%), sheep (5.1%), horse (3.9%) and pig (12.8%). In the semi-urban site it was cow (70.0%), pig (30.0%), and in the rural site it was cow (83.3%) and sheep (16.7%). The density of domesticated animal was found to be high in the urban site compared to the semi-urban site and rural study sites. In the urban site, it was dominated by buffalo (34.7%) (Figure 2 *f*) and goat (32.3%) followed by horse (13.4%), cow (12.6%), pig

Table 8. One-way ANOVA results for physio-chemical characteristics of water in different study sites during the sampling period 2013–14 along Vaigai river

Parameter	Urban	Semi-urban	Rural
pH	7.93 ± 0.06[a]	8.07 ± 0.04[ab]	8.23 ± 0.11[b]
Dissolved oxygen (ppm)	9.60 ± 0.080[c]	6.33 ± 0.25[b]	4.44 ± 0.16[a]
Conductivity (ms)	1.96 ± 0.12[c]	0.76 ± 0.05[b]	0.48 ± 0.047[a]
TDS (ppt)	0.11 ± 0.06[b]	0.03 ± 0.03[a]	0.02 ± 0.03[a]
Salinity (ppt)	1.05 ± 0.07[b]	0.41 ± 0.03[a]	0.29 ± 0.03[a]
Temperature (°C)	30.29 ± 0.27[b]	28.65 ± 0.29[a]	30.04 ± 0.21[b]
Turbidity (NTU)	68.29 ± 8.80[b]	17.20 ± 2.40[a]	12.30 ± 2.66[a]

Values are represented as mean ± SE. Values having different superscripts within rows are significantly different at $P < 0.05$.

Table 9. Animal husbandry and sewage inlets data at different study sites along Vaigai river

	Study site		
Parameter	Urban	Semi-urban	Rural
Cattle sheds (total no./site)	78	10	6
Milching animals (cows and buffaloes) (total no./site)	60	26	4
Other domesticated animals (Total no./site)	58	12	6
Sewage inlets (Total no./site)	21	5	5

(5.5%) (Figure 2 *g*), and sheep (1.5%). Domesticated animals observed in the semi-urban site were mainly cow (68.4%) and pig (31.6%) while in the rural sites it was cow (40%), pig (40%) and sheep (20%).

Discussion

The freshwater ecosystems in India have been heavily impacted by various human activities in the past. These modifications resulted in the loss of biodiversity, habitat reduction, changes in the water quality and new ecological niches. These modified ecological niches are readily occupied by vector mosquitoes[26]. Water quality plays an important role in mosquito larval density and diversity in all the sites. Water quality was strongly influenced by sewage inlet into the river in all the sites. Sewage inlets were higher in the urban site than the other two sites. In the urban site, sewage inlets provide constant supply of polluted water with rich organic matter and act as a permanent breeding source during all seasons for the mosquitoes. As a result, average larval density was maximum in all the seasons in the urban site than the other two sites during the sampling period. The turbidity level in water was high in the urban site due to sewage inlets, which led to increase of *Culex* larval density in the urban site compared to the semi-urban and rural sites. As a result, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. vishnui* and *An. subpictus* were recorded as the dominant species in the urban site during the entire period of study. *Culex* sp. prefers breeding in habitats with high turbidity level than *Anopheles* sp.[17]. In contrast, *Anopheles* larval density was high in the semi-urban and rural sites which have low turbidity level in water. Low water turbidity is an important factor required to find the breeding habitat of *Anopheline* mosquitoes[15]. However, in our study, *An. subpictus* was found in highly turbid water and adapted to breeding in polluted water. The larval density of *An. gambiaes* is associated with high turbidity[27] and this species can breed in polluted water[28]. Polluted water also influences the species composition of phytoplanktons communities[29] and supports proliferation of exotic weeds[30]. Polluted water helps *Eichhornia crassipes* to grow vigorously in the urban site. In semi-urban and rural sites filamentous algae are the dominant species due to low water pollution. This modified aquatic vegetation plays an important role in the diversity and density of immature mosquitoes. In the present study, mosquito diversity was higher in rural and semi-urban sites compared to urban site. Surface of the aquatic habitat in the rural and semi-urban sites is covered by filamentous algae which provide shelter to the mosquito larvae, thereby preventing them from predators. Cole *et al.*[31], and Sunish and Reuben[32] reported that the filamentous algal-dominated habitats have high nutrient values in the water, which helps the immature mosquito populations and also provide shelter and protection from predators and water waves. Thick mat of *Spirogyra majuscula* provides food and shelter to *An. pseudopunctipennis*, which also acts as barrier to the predator fish *Poecilia sphenops*[33]. Filamentous algae also act as the preferred site for breeding mosquitoes (Figure 2 *d*). Therefore, we found greater mosquito diversity (23 species) in the algal-dominated sites than the macrophytes-dominated sites. In this study, most of the *Anopheles* species (14 species) were strongly associated with *Spirogyra*. Earlier studies also reported that green algae act as a major food source to the Anopheline larvae and that there was a large number of Anopheline species in algal-dominated sites[34–36]. Our result is supported by the finding of Savage *et al.*[37], who reported that filamentous algae release organic volatile compounds in water, which act as

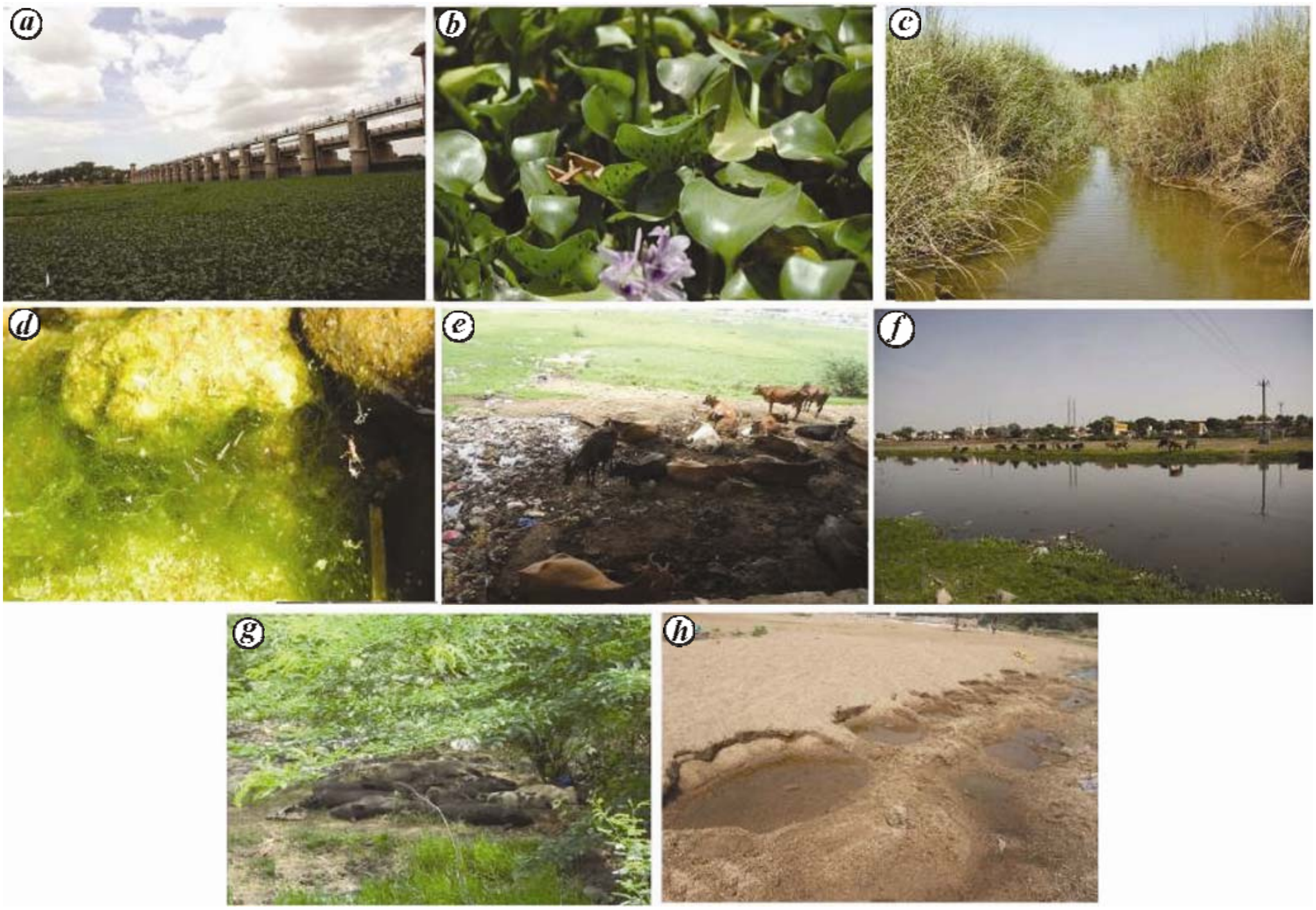

Figure 2. Favourable environment for breeding mosquitoes in the Vaigai river ecosystem. ***a***, ***b***, *Eichhornia crassipes*-dominated sites. ***c***, *Saccharum spontaneum* which provides shade along the edges of the aquatic habitat. ***d***, Filamentous algae act as habitat for mosquito larvae. ***e***, Cattle shed and sewage inlet along the river beds. ***f***, Grazing animals within river beds. ***g***, *Prosopis juliflora* acts as a habitat for pig pathogen reservoirs. ***h***, Drinking water fountains act as microsites for breeding mosquito.

a clue for gravid *An. pseudopunctipennis* to detect the oviposition sites. *Cx. bitaeniorhynchus* and *Aedeomyia catasticta* are strongly associated with *Spirogyra* and *Cladophora* sp. Composition of *Cx. bitaeniorhynchus* directly depends on the distribution of *Spirogyra*. *Cx. bitaeniorhynchus* larvae use *Spirogyra* sp. as their food, and larval density decreases when the abundance of *Spirogyra* species decreases[38]. In the present study we found low larval density in algal-dominated sites compared to hydrophytes-dominated sites. This may be due to the limited space available within the mat for the larval population. Similar result was also observed by Femandez-Salas *et al.*[39] in filamentous algae-dominated sites. *E. crassipes* rendered predator less habitat in urban environment which increases the larval density (Figure 2 *a*). During the monsoon season, all the study sites showed increased mosquito diversity and density as a result of newly formed breeding habitats (small isolated pools). Habitats dominated by *Lemna* sp. during the rainy season are found suitable. Terrestrial plant species also support immature and adult mosquitoes in all the study sites. In semi-urban and rural sites, the dense growth of plant species such as *S. spontaneum* (Figure 2 *c*), *C. rotundus*, *A. donax* and *P. juliflora* created micro-habitats by providing shelter along the edges of aquatic habitat where the immature mosquitoes could breed. These findings concur with those of Opoku *et al.*[40], who reported that bank vegetation such as grasses and weeds provide shade and shelter for immature populations of *An. melas* and *Cx. thallassius*. Homogenous vegetation of *E. crassipes*, *A. spinosus* and *C. sparsiflorus* acts as resting places and major food sources for adult mosquitoes within the river in the urban site (Figure 2 *b*). Similar observation was reported in *E. crassipes* and *Lemna gibba*, which rendered ideal breeding habitats by acting as food source and resting place for the adult population, and protection from predators and water waves for the immature population[41–43]. Margins of the riverbeds were covered by palatable grass which acts as an ideal breeding ground for mosquitoes in urban sites.

Maximum number of animal sheds (Figure 2 *e*) and grazing animals in the urban site is also one of the reasons for the increase in *Culex* diversity. Adult female mosquitoes may get constant supply of blood meals from animal sheds in river banks in the urban site, which tends to increase the larval density and diversity of *Culex* mosquitoes. *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. gelidus* and *Cx. vishnui* prefer to feed on the blood of cattle and pigs[22,44,45]. *Cx. quinquefasciatus* is highly anthropophilic, feeding on humans[46,47].

Thus we conclude that the human-modified river ecosystem through its ecological parameters such as terrestrial and aquatic vegetation, water quality and animal husbandry along and within the riverbeds helps maintain self-sustained mosquito vector populations along the Vaigai river. Therefore, it is essential to take steps to eradicate the mosquito vectors from the Vaigai river basin to avoid disease outbreaks in the region.

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