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Larval habitats and species composition of mosquitoes in Darjeeling Himalayas, India

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Abstract

Background & objectives: A preliminary survey of larval mosquito habitats and temporal variation in mosquito diversity in the hill town of Darjeeling, India was made during 2003, for a qualitative and quantitative assessment of mosquito distribution.

Methods: The possible larval habitats of mosquitoes were surveyed and the species diversity in the sites positive for mosquito larvae was noted. Bi-weekly sampling from a particular habitat was carried out to reveal the temporal variation in mosquito species.

Results: A good number of lentic aquatic habitats were found to be hosting mosquito immatures, though difference in the physical and biological features of these habitats was prominent. Altogether, immatures of six mosquito species, belonging to four genera — *Aedes, Armigeres, Culex* and *Toxorhynchites* were noted with significant difference in temporal variation in their relative and absolute numbers. A positive correlation (r = + 0.707) was found between population of the prey mosquito immatures and the population of immatures of *Tx. splendens.* The species diversity index (H') for the mosquitoes remained between 0.87 and 1.53. The evenness components ranged between 54.03 and 95.03% and differed significantly.

Interpretation & conclusion: In the present study, the aquatic bodies could be categorised into six types depending on the size and structural complexity that may account for the observed variation in the species composition of the larval habitats. In addition to this, other factors like temperature, rainfall and other related climatic attributes may be responsible for the observed species variation, which needs to be confirmed through further studies.

Key words *Aedes* – *Armigeres* – *Culex* – mosquito larval habitats – *Toxorhynchites*

Introduction

Mosquitoes exploit almost all types of lentic aquatic habitats for breeding. The immature stages of mosquitoes thrive in these aquatic bodies along with conspecifics and heterospecifics – forming the larval mosquito community. The resources in terms of food, predators and competitors present in the habitat determine the population status of larval mosquitoes, both qualitatively and quantitatively[1, 2]. Composition of organisms in these ensembles depends on size and type of aquatic bodies[1]. Besides, association of natural enemies in these habitats influences the selection of oviposition site by the mosquitoes thereby limiting the mosquitoes to breed[3]. Moreover, the intraguild species composition and interaction in the larval mosquito communities influence the adult population at a particular time and space[1-8]. Evaluation of larval mosquito habitats in terms of species composition and resources help to understand the bio-ecology and related control measures of pests and vector mosquitoes more appropriately.

In view of these and in continuation of the studies on mosquitoes in the hills and foothills of Darjeeling-Sikkim Himalayas, a survey on the mosquito larval habitats was carried out on selected localities of Darjeeling town, India. Several species of mosquitoes have been reported earlier from this region[9-13], however, little is known about the larval habitats or the aquatic predators associated with the mosquito immatures. The present study was aimed to make a preliminary estimate of the possible larval habitats and species composition therein as well as temporal variation of the mosquito species to substantiate the mosquito distribution in this part of Himalayas.

Material & Methods

Study area: The survey was carried out between June through October for two consecutive years (2003 and 2004), from different localities of Darjeeling town (altitude 2134 m above sea level), India. During this period, in Darjeeling the average annual rainfall remained between 104 and 798 mm, temperature 18–23°C, and the relative humidity 75 and 89%. At least 20 numbers of each of the following type of lentic aquatic bodies were considered per month in a year, at random from these localities. These were considered as probable mosquito larval habitats: (i) cemented temporary pools (cemented walls); (ii) cemented open water storage tanks (meant mainly for rain water harvesting); (iii) household water storage tanks (large plastic containers to buckets); (iv) stagnant stream side pools, (v) temporary roadside ditches; and (vi) clogged sewage drains. The structural complexity in terms of water holding capacity, vegetation, amount of detritus and species composition are different in these habitats.

Methodology: The water bodies were surveyed and subsequently sampled using the plankton net of appropriate diameter depending on the size of the habitat. The water bodies were also dredged using long-handle plankton net (10 or 15 cm diameter, 20 µm mesh size) for the benthic organisms based on the amount of detritus and vegetation. If the habitat was found positive for the mosquito larvae, the numbers were recorded and kept in a separate container for eclosion. Also, the number and type of other organisms present were noted to make a quantitative assessment. At least three samples were considered for each of the positive sites. Throughout the study period three cemented temporary pools were selected for sampling. Rest of the habitats varied in numbers and location. Thus the temporary pools, with cemented walls, located in the Lloyd's Botanical Garden, Darjeeling, were taken as fixed sampling units.

To assess the temporal variation of the mosquitoes, five cemented tanks (water storage) in and around Darjeeling Government College campus were considered. Bi-weekly sampling[14-16] of these tanks using plankton net (15 cm diameter; 20 µm mesh size) was done. Mosquito immatures collected from each sampling (3 samples/tank) were allowed to eclose to adult stage. The adults were identified using appropriate keys[17,18], though the data were recorded in respect to the immatures. The data obtained on the immatures of mosquitoes (or number eclosed to adults) were subjected to statistical analyses following Zar[19]. Two-way ANOVA was conducted to justify the difference in abundance of mosquitoes over time. One-way ANOVA followed by Tuckey test was performed to find out significant difference in population of different species of mosquitoes over months. Also, Shannon-Wiener diversity index (H') was calculated to note the variation in the larval mosquito temporally. Regression equations and correlation coefficients were calculated on the population of immatures of prey and predator mosquito species sampled during the months.

Results

All the different types of habitats surveyed were found to be positive for mosquito immatures, though the numbers varied between the months and the habitat types. In the temporary pools, maximum number of species was encountered, followed by the cemented water storage tanks. Immatures of *Culex* mosquitoes were found in all habitats excepting the stream pools. Least number of species was found in temporary ditches. The number of water bodies found positive for mosquito immatures along with the biota are presented in Table 1. Immatures of six different species belonging to four genera were encountered. These were *Aedes w-albus* Theobald, 1905; *Armigeres subalbatus* Coquillett, 1898; *Ar. theobaldi* Barraud, 1939; *Culex bitaeniorhynchus* Giles, 1901; *Cx. quinquefasciatus* Say, 1823; and *Toxorhynchites splendens* Wiedemann,1819. The cemented water storage tanks were found to host all these species of mosquitoes. In rest of the habitats one or more species of mosquitoes were absent. The number and species of mosquito larvae and pupae encountered habitat-wise are shown in Table 2.

The temporal variation in the abundance of different mosquito species in the cemented water storage tanks is presented in Table 3. The maximum number of mosquito larvae was encountered during the month of September ranging between 179 and 398 per sampling-day, while least number of mosquito larvae was sampled in June (72 to 138). Mosquito species diversity was maximum in September and minimum in June. In the cemented water storage tanks, populations of the dytiscid beetle *Rhantus sikkimensis* Regimbart, 1899 (Coleoptera: Dytiscidae) unidentified acari, crustaceans, tadpoles of *Bufo himalayanas*, bugs of the family Gerridae and immatures of *Chironomus* Meigen, were present. However, data on the mosquito immatures only were noted. Two-way ANOVA conducted on these data revealed that between the months the total number of mosquito immatures (larva and pupa) did not vary significantly, but variation in mosquito species was significant (between months $F_{4, 20} = 1.594$, NS; between species, $F_{5, 20} = 4.475$, $p < 0.01$). The diversity index (H') did not vary between the months ($\chi^2 = 0.2935$, df = 4, NS), but the evenness aspect of diversity varied significantly ($\chi^2 = 18.205$, df = 4, $p < 0.005$) supporting the variation of abundance of the immatures of different mosquito species in the months (Table 3). The numbers of the predator and prey mosquito immatures over time are shown in Fig 1.

The Tukey test revealed significant differences in abundance of various species of mosquitoes as given in Table 4. *Cx. quinquefasciatus* was predominant species and its abundance was significantly different from other mosquito species. The regression equation and the correlation coefficient of the sample and the population estimates of prey and the predator mosquito immatures in respect to their abundance in the sampling days are given in Table 5. The population of *Tx. splendens* immatures was found to be positively correlated with the population of immatures of other prey mosquito species ($r = + 0.707$, df = 13, $p < 0.005$), however, the population of individual prey mosquito species varied temporally.

Discussion

From the survey it is evident that the hill town of Darjeeling provides ample habitats for the mosquitoes to breed and thrive. These habitats are tempery in nature and exist for a brief period of time in a year. Some of these habitats also host other aquatic organisms, many of which are predators of mosquito larvae. In general, the features of the habitats noted in the present study remain qualitatively same as that of other similar habitats[1-8]. Compared to other aquatic bodies, the cemented temporary pools containing maximum resource, in terms of detritus, veg-

Table 1. Characteristics and biota of mosquito larval habitats in Darjeeling, India (Sampling period June through October 2003–04)

						Diptera					Hemiptera	Coleoptera	Odonata	Others			
Habitat type	Nos. of sites found +ve [Range, mean ± S.E.]	Algae	Detritus	Vegetation	Structural complexity	*Chironomus*	*Aedes*	*Armigeres*	*Culex*	*Toxorhynchites*	Gerridae	Dytiscidae		Mayfly larvae	Acari	Crustacea	Tadpoles
Temporary pools	3 – 3 3 ± 0	+	+	+	Complex	+	+		+	+	+	+	+	+	+	+	+
Cemented tanks	8 – 15 11 ± 2.55	+	+		Complex	+	+	+	+	+		+	+		+	+	+
Household tanks	1 – 19 12.2 ± 6.87	+	+		Simple	+			+								
Temporary ditches	0 – 3 1.8 ± 1.09		+		Simple				+								
Stream pools	6 – 12 9.8 ± 2.28	+		+	Simple	+	+				+			+			
Sewage drains (Stagnant)	0 – 3 2.2 ± 1.3	+	+		Simple	+			+		+						+

Table 2. Larval habitats and species of mosquito immatures encountered from Darjeeling during the sampling period

Habitat type	Mosquito species					
	Ae. w-albus	*Ar. subalbatus*	*Ar. theobaldi*	*Cx. bitaeniorhynchus*	*Cx. quinquefasciatus*	*Tx. splendens*
Temporary pools	+			+	+	+
Cemented tanks	+	+	+		+	+
Household tanks					+	+
Temporary ditches					+	
Stream pools	+					
Sewage drains (Stagnant)					+	+

Table 3. Numbers (Range, mean ± S.E.) of mosquitoes enclosing from pupa sampled from Darjeeling between 2003 and 2004

Mosquito species	Months				
	June	July	August	September	October
Ae. w-albus	42–70 (59.17 ± 11.18)	29–76 (58.67 ± 19.81)	29–72 (49.17 ± 17.66)	9–23 (17.17 ± 5.95)	29–75 (43.83 ± 17.51)
Cx. quinquefasciatus	30–68 (46.33 ± 15.45)	42–100 (73.17 ± 18.84)	86–159 (128.83 ± 26.6)	118–249 (209 ± 56.7)	29–51 (36.67 ± 9.85)
Cx. bitaeniorhynchus	0–11 (3.5 ± 2.22)	0–15 (7.83 ± 6.37)	72–118 (90.5 ± 21.07)	15–48 (27.17 ± 13.38)	60–108 (84.83 ± 20.19)
Ar. subalbatus			15–110 (53.67 ± 39.99)	5–15 (11 ± 3.74)	0–41 (13.17 ± 20.42)
Ar. theobaldi			12–48 (27.33 ± 13.68)	13–32 (21.83 ± 7.57)	0–32 (16 ± 13.56)
Tx. splendens		10–23 (16.5 ± 4.51)	12–34 (20.17 ± 8.26)	19–31 (24.83 ± 3.87)	10–17 (13.5 ± 2.59)
Shannon-Weiner Diversity index (H’)	0.87	1.1	1.59	1.17	1.53
Evenness index (H_{even})	0.54	0.68	0.98	0.72	0.95

etation and algae offered maximum species of different guild to coexist. Similar observations were made in the urban pools in Malindi, Kenya[2], and the rice-fields in Sulawesi, Indonesia[5] and Madurai[6], Central Gujarat[7] and Jabalpur [8] in India, where the structural complexity of the habitat was positively correlated with species richness.

However, in terms of the mosquito species only, the cemented water storage tanks were found to be more congenial hosting all the mosquito species sampled and stand to be model mosquito larval habitat in Darjeeling. The larger temporary cemented pools in the Botanical Garden, had higher number of predatory insect groups and thus could allow less number

Table 4. Results of Tukey test with respect to the abundance of immatures of different mosquito species in Darjeeling

Source of variation	SS	df	MS	F-value
Total	185486.73	89		
Between mosquito species	80392.23	5	16078.45	12.85 $p < 0.0005$
Error	105094.5	84	1252.13	

Rank of sample	Sample mean
1. *Cx. quinquefasciatus*	98.77
2. *Ae. w-albus*	45.6
3. *Cx. bitaeniorhynchus*	42.77
4. *Ar. subalbatus*	15.57
5. *Tx. splendens*	15
6. *Ar. theobaldi*	14.4

Standard error (S.E.) = 9.13 [MS_{error}/N ; here N = 15 sampling unit]

Comparison	$q_{84,5}$
1 vs. 2	5.82*
1 vs. 3	6.13**
1 vs. 4	9.11**
1 vs. 5	9.18**
1 vs. 6	9.24**
2 vs. 3	0.31 NS
2 vs. 4	3.29 NS
2 vs. 5	3.35 NS
2 vs. 6	3.42 NS
3 vs. 4	2.98 NS
3 vs. 5	3.04 NS
3 vs. 6	3.11 NS
4 vs. 5	0.06 NS
4 vs. 6	0.13 NS
5 vs. 6	0.07 NS

*$p < 0.025$; ** $p < 0.001$; NS = Not significant.

of mosquito immatures to settle. Other than these two, the rest of the water bodies surveyed faced disturbance time-to-time as habitats for growth of mosquito and other aquatic organisms. Also, in case of household water storage tanks, the waters were regularly changed, thus decreasing the resources available for mesocosm formation at a particular point of time. Despite all these, *Cx. quinquefasciatus* immatures were recorded from these tanks and clogged sewage drains reflecting its diverse adaptive nature. It seems that among the various mosquito species, only the *Ae. w-albus* is adapted to breed in the stagnant stream pools formed by the trapped water between the rocks reflecting its ability to survive in this specialised habitat with low detritus and higher algal growth.

The mosquito species noted in the cemented water storage tanks were recorded earlier [9-11], however, the difference in the population level of mosquito immatures in the months can be attributed to the environmental variables that influence both the biology and the availability of resources in the habitats. It can be assumed that the habitats are formed first at the onset of the monsoon in Darjeeling followed by the establishment of different organisms in the same. From the abundance of prey and predator mosquito immatures as observed in the cemented pools, it appears that among the prey mosquitoes *Ae. w-albus* and *Cx. quinquefasciatus* may be the first to colonise, keeping apart rest of the organisms that form the mesocosm. Also, the absence of the predator mosquito larvae in the initial period of sampling probably indicates that at least a minimum level of prey mosquito species was required to sustain the population of the predator mosquito larvae in the habitat. It is to be noted that the predator colonisation is influenced by the presence of prey species[1] and structure of the habitat[2,3]. Thus the predator mosquito larva *Tx. splendens* establishes in the habitat at a later stage as observed in the study. Though the significant difference in abundance of the immatures of different prey mosquito species is noted in respect to

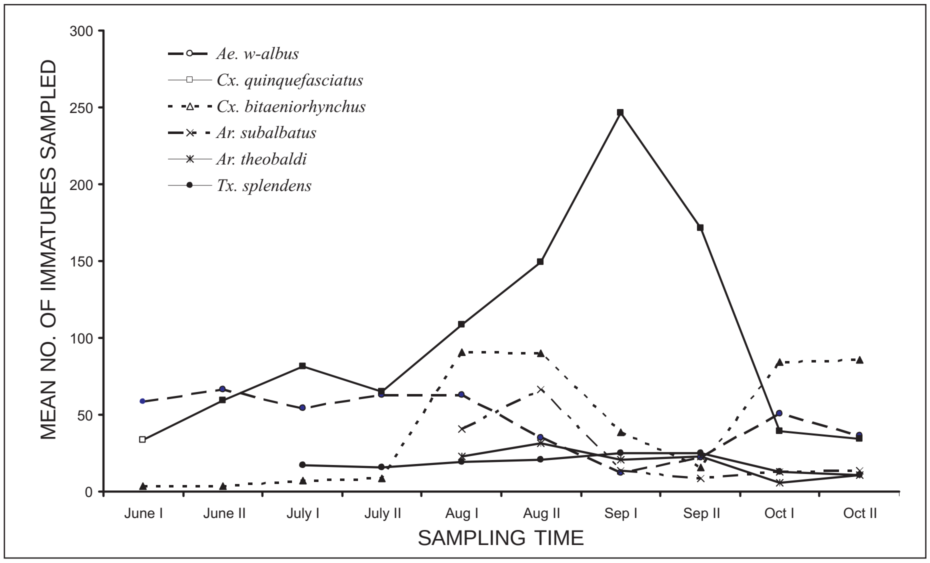

Fig. 1: Numbers of prey and predator mosquito immatures collected from cemented open water storage tanks (n =4) of Darjeeling Government College campus during 2003–04

Table 5. Correlation coefficients and regression equations on the immatures of prey and predator mosquito species sampled from Darjeeling

Mosquito species	df	Correlation coefficient (r)	$t = r.\sqrt{n-2}/\sqrt{1-r^2}$	Regression equation $y = a + bx$	$F_{\alpha(1)\ 1,13}$ value
Tx. splendens [y = Predator] vs.					
Ae. w-albus	13	(–) 0.4908 $p < 0.05$	2.33 $p < 0.05$	$y = 25.99 - 0.24x$ $r^2 = 0.24$	4.13 NS
Ar. subalbatus	13	(+) 0.344 NS	1.41 NS	$y = 13.14 + 0.12x$ $r^2 = 0.11$	1.75 NS
Ar. theobaldi	13	(+) 0.634 $p < 0.02$	3.83 $p < 0.005$	$y = 9.51 + 0.38x$ $r^2 = 0.4$	5.28 $p < 0.05$
Cx. bitaeniorhynchus	13	(+) 0.327 NS	1.322 NS	$y = 11.73 + 0.08x$ $r^2 = 0.11$	1.56 NS
Cx. quinquefasciatus	13	(+) 0.708 $p < 0.005$	5.01 $p < 0.001$	$y = 5.16 + 0.1x$ $r^2 = 0.49$	13.08 $p < 0.005$
Overall [x = prey]	13	(+) 0.707 $p < 0.005$	5.11 $p < 0.001$	$y = 0.45 + 0.07x$ $r^2 = 0.5$	13.03 $p < 0.005$

NS–Not significant.

the months as well as between the species (Table 4), the choice of the predator mosquito for one or the other prey mosquito species could not be deduced. However, the structural complexity of the habitats is also expected to change with the growth of the aquatic vegetation and algae through the rainy season, creating a more complex scenario and gradual invasion by other organisms including different mosquito species. At this stage, other than the mosquito immatures, presence of immatures of chironomid larvae as well as the predatory dytiscid beetle *R. sikkimensis*, tadpoles and other groups of the aquatic insects were noted which can influence the population of all mosquito species thriving in the habitat. It needs to be mentioned here that in rice-fields[5,6,20] and other such similar temporary habitats[3,21-23], predator colonisation occurs in succession, with the age of the habitat. The predator number is also found to increase proportionately with the increase in prey population, in these temporary habitats.

Selection of oviposition site by mosquitoes depends on the presence of aquatic predators of mosquito immatures[3, 20-23]. The mosquito *Culiseta longiareolata* Macquart exhibits predation dependent oviposition in pools, where females oviposit with a certain probability in the presence of predators[21]. In another study it was found that the mosquitoes *Cu. longiareolata* and *Cx. laticinctus* Edwards oviposited less in pools containing different densities of *Notonecta maculata* Fabricius, compared to pools where the predators were absent[22].

In the cemented water tanks and the temporary pools surveyed, where the structure of the habitat is complex, the presence of the resources and the predators are expected to determine the population level of immatures of mosquitoes and the possible alternative prey forms—the immatures of chironomid midges. Thus the ratio of the predator and prey mosquitoes noted during the months (Fig. 1), could probably be a stable outcome of the interactions. Though the correlation coefficient value support this, further field observations in this regard including the multiple prey and predator species in the habitats need to be evaluated to elucidate the prey-predator dynamics and oviposition habitat selection by the mosquitoes. It may also be assumed that the structurally simple habitats had less amount of resource to support large mosquito population as has been noted in larval habitats like bamboo stumps[24] and containers[1, 25-27]. Thus few mosquito species prefer to oviposit in these habitats and available numbers of mosquito immatures were unable to sustain the predator population.

Since the study is restricted to the urban environment, mosquito larval habitats in the forested and rural areas of Darjeeling district may differ in structure and characteristics. Nonetheless, the present findings provide a preliminary assessment of the mosquito larval habitats of Darjeeling-Sikkim Himalayan region. Further studies would disclose the influence of the biotic and abiotic components of the habitats to the abundance of adult mosquitoes. If the potential treat to human health by prevalence of the mosquito species in urban areas of Darjeeling and control of breeding in temporary water bodies using biological control agents are highlights, it would add to applied value of the present findings.

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