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RESEARCH ARTICLE

SURVEY AND EPIDEMIOLOGY OF TREE HOLE BREEDING MOSQUITOES IN ANNAMALAI UNIVERSITY CAMPUS, TAMILNADU, INDIA

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ABSTRACT

Water-filled tree holes are important temporal ecosystem for a number of different aquatic and semi-aquatic organisms. The study of tree-hole breeding mosquitoes was carried out in the Annamalai University campus, Tamilnadu, India between June 2013 - February 2014, by using standard entomological procedures. The tree holes are sampled for various mosquito species around the campus. Types of mosquito species encountered, their relative abundance, as well as genera varied significantly during the study. Three genera of mosquitoes: *Aedes*, *Culex*, *Anopheles* were recovered while 14 species of mosquitoes encountered include: *Aedes aegypti*, *Ae.albopictus*, *Ae.africanus*, *Ae.simpsoni*, *Ae.taylori*, *Culex quinquefaciatus*, *Cx.nebulosus*, *Cx.tritaeniorhynchus*, *Cx.pseudovishnui*, *Cx.pipens*, *Anopheles stephensi*, *An.sundaicus*, *An.culiciformis and An.fluviatilis*. Most of the mosquitoes showed preference to tree holes for their oviposition. Tree hole mosquitoes are studied by α-biodiversity like Margalef index (D_{Mg}), Simpson's index (λ), Shannon-Wiener (H'), Pielou's index (J'). The presence of *Ae.aegypti* and *Ae.albopictus* indicate that the study areas were at risk of dengue fever epidemic. The presence of *Anopheles* and *Culex* species ensured endemicity of malaria and filariasis, while the recovery of *Ae.aegypti* in this region suggests a possible outbreak of dengue fever in future if not properly controlled.

INTRODUCTION

Mosquitoes are the most important among the other arthropod vectors of human diseases. Mosquitoes act as vectors of protozoan, helminth and viral agents (Dutta *et al*., 2010). Although mosquito-borne disease currently represents a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Fradin and Day, 2002). Phytotelmata, plant held aquatic habitats in tree holes are one of the important classes of aquatic ecosystems (Kitching, 2000). Tree holes are segregated habitats with many interrelations of the contained organisms. In the water contained tree holes, there may have several types of bacteria, molds, algae, and many types of animals. The mosquito larvae form an important part of the animal life of the tree hole but are only links in the complex food cycle. In their aquatic stages, different species of mosquitoes may occupy the same habitat and form part of a single guild (Jenkins and Carpanter, 2013). Early researchers recorded and attempted to control the vectors or suspected vectors of dengue fever in these habitats paid little attention to the bionomics during the past few decades, most of the research on mosquitoes has been concerned with malaria vectors that in the Chidambaram region did not concern tree holes. Mosquitoes have been more thoroughly studied and better known taxonomically than any other insect order, with the possible exceptions of a few Lepidoptera (Anosike *et al*., 2007). The disparity of knowledge between the Culicidae and other entomofauna is especially apparent in the tropics where mosquitoes are the primary vectors of malaria parasite, filariasis, dengue and many other arboviruses (Anosike *et al*., 2007 and Jebanesan *et al*., 2012a). Surveys are essential for the planning, operation and evaluation of any effective mosquito control program, weather for the prevention of these biting insects to a level permitting normal activities without undue discomfort. Tree holes are especially appropriate habitats for comparative studies of population interaction (Bradshw and Holzapfel, 1992). This study was undertaken to examine a tree holes represents a small, discrete and patchy habitat. One of the purposes of this study therefore was to properly identify the types of mosquitoes that breed in tree-holes and evaluate the status of these tree hole breeding mosquitoes in the Annamalai University campus, Tamilnadu, India.

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MATERIALS AND METHODS

The Study Area

Investigation on tree hole mosquitoes was carried out in the Annamalai University campus, Tamilnadu, India from June 2013 - February 2014. The study campus having around 1000 hectors and it was situated in 79.7148° E latitude and 11.3908°

N longitude. During the period, the ecological distribution of various species of mosquitoes inhabiting water filled tree-holes and their allies in the area were sampled.

Climate

The annual rainfall ranges from 150 mm to 207 mm. The main rainy months are June, July, August and September. The annual temperature of the area ranges from 21 ^{0}C to 36 ^{0}C. The occurrence of mosquito species is related to ecological condition.

Criteria for Selection

In 2012, a high number of dengue fever epidemics were reported in Chidambaram, the headquarters of Cuddalore district. Therefore a need to investigate the types of mosquitoes prevalent in this area is necessary.

Methods of Collection

The mosquitoes collected from the each tree hole of the campus has been determined and counted for identification. Total number of mosquitoes collected from each tree hole and month in which collected was noted. The larvae may be collected easily by means of small sieve, siphon or by a rubber suction tube to remove the water from the tree hole. A rubber tube of about one and half inch diameter and three feet in length with an eight inch piece of glass tubing inserted makes a satisfactory siphon (Jenkins and Carpenter, 1946). Adult mosquitoes were collected by using suction-tube. Larva and adult were collected for species identification (Jebanesan *et al*., 2012b).

Identification of Mosquitoes:

The collected tree hole mosquitoes were identified into genera and species according to the keys adopted by following the standard mosquito identification keys and nomenclature (Barraud, 1934, Rao, 1984, and Reinert, 2009). Each labeled Petridish containing several collected mosquitoes were photographed in a magnified form and then identified. Again the microscope is used for further identification with particular reference to the head, thorax, wings and hind legs. The voucher specimens are present in the department of Zoology, Annamalai University, Chidambaram.

Diversity Studies and Statistical Analysis

Diversity studies (alpha diversity) were conducted by calculating classic diversity indexes like,

Margalef Index (D_{Mg}) = (S-1/In N)

Simpson's Index (λ)= $1/\sum (Pi^2)$

Shannon-Wiener ($H^{'}$) = $-\sum pi$ In (Pi)

and

Pielou's Index (J') = $H^{'}/H_{max}$ (or) $H^{'}$/In S

Where,

S = Total number of species or Species richness,
N = Total number of individuals,
In S = natural log of species,
Pi = Frequency of species.

Shannon-Weinner diversity index is commonly used to characterize species diversity in a community, according to both abundance and evenness of the species present (Shannon and Weaver, 1949). Species richness (S) is the number of species present in a community while species evenness (J') indicates the distribution of individuals within the species (Magurran, 1988; Albert Bernues and Ricardo Jimenez, 2013).

RESULTS AND DISCUSSION

Tree holes provide a unique specialized type of ecological habitat which is different from usual breeding places (Jenkins and Carpenter, 2013). An entomological survey has been conducted around the campus for collection of mosquito larvae and adults from different tree holes. During the survey 14 species of mosquitoes in 3 genera were collected from water filled tree holes in Annamalai University campus, Chidambaram from June 2013 – February 2014. Overall results are presented in Table 1.

Table 1. Diversity of tree hole mosquitoes recorded in the study area during the study period (June-2013 to February-2014)

S.No	Name of the Mosquito Species
1	*Aedes* (Stegomyia) *aegypti* (Linnaeus)
2	*Aedes* (Stegomyia) *albopictus* (Skuse)
3	*Aedes* (Stegomyia) *africanus* (Theobald)
4	*Aedes* (Stegomyia) *simpsoni* (Theobald)
5	*Aedes* (Diceromyia) *taylori* (Edwards)
6	*Culex* (Culex) *quinquefasciatus* (Say)
7	*Culex* (Culiciomyia) *nebulosus* (Theobald)
8	*Culex* (Culex) *tritaeniorhynchus* (Giles)
9	*Culex* (Culex) *pseudovishnui* (Colless)
10	*Culex* (Culex) *pipiens* (Linnaeus)
11	*Anopheles* (Cellia) *stephensi* (Liston)
12	*Anopheles* (Cellia) *subpictus* (Grassi)
13	*Anopheles* (Anopheles) *culiciformis* (Cogill)
14	*Anopheles* (Cellia) *fluviatilis* (James)

The three genera are *Aedes*, *Culex* and *Anopheles*, there were more *Aedes* than *Culex* with *Anopheles* as the least and the 14 species of mosquitoes identified as *Aedes aegypti*, *Ae.albopictus, Ae.africanus, Ae.simpsoni*, *Ae.taylori, Culex quinquefasciatus, Cx.nebulosus, Cx.tritaeniorhynchus, Cx.psedovishnui*, *Cx.pipens, Anopheles stephensi, An.subpictus, An.culicifacies* and *An.fluviatilis* were recorded from the various localities of tree holes in the study area (Table 2). Observations on the monthly relative abundance of mosquitoes in the tree holes revealed the presence of 5 species of *Aedes*, 5 species of *Culex* and 4 species of *Anopheles*. Totally 1548 mosquito species was collected from tree holes at study area, the most mosquito abundant months are July, August, December and January due to rain and also these are the critical months for producing mosquito vector-borne diseases. The field observations are made at dusk and dawn time (Table 3). *Ae.aegypti* and *Ae.albopictus* was the most abundant tree hole mosquito species, it represents about 60% of the mosquito larvae, pupae and adult collected from water filled tree holes in study period. *Ae.aegypti, Ae.albopictus* and *An.stephensi* observed in all locations of study period in dusk collection. Different mosquito species showed oviposition preferences for one or more habitats. Also most mosquito species preferred tree hole water for their larval development. The results from this field survey also indicated that, apart from the University main

Table 2. Occurrence of tree hole mosquito species in various localities of the study area during the study period (June-2013 to February-2014)

S.No	Name of the Species	Annamalai University										Total
		Locality-1		Locality-2		Locality-3		Locality-4		Locality-5		
		Adult	Larva	Adult	Larva	Adult	Larva	Adult	Larva	Adult	Larva	
1	*Aedes aegypti*	35	71	14	62	21	49	24	43	30	55	404
2	*Ae.albopictus*	22	20	26	38	11	31	9	21	28	19	225
3	*Ae.africanus*	9	6	10	19	15	0	5	12	14	11	101
4	*Ae.simpsoni*	5	2	6	15	8	3	8	9	6	9	71
5	*Ae.taylori*	7	0	9	11	6	2	2	11	0	7	55
6	*Culex quinquefasciatus*	41	19	17	16	13	16	11	16	21	9	179
7	*Cx.nebulosus*	16	5	11	10	3	1	9	3	0	3	61
8	*Cx.tritaeniorhynchus*	19	9	9	2	0	12	13	9	2	0	75
9	*Cx.pseudovishnui*	26	10	15	9	2	6	10	11	0	11	100
10	*Cx.pipiens*	3	2	0	0	6	3	7	2	1	0	24
11	*Anopheles stephensi*	31	13	17	2	8	4	11	6	16	6	114
12	*An. subpictus*	17	16	8	0	1	0	0	4	3	4	53
13	*An.culicifacies*	14	11	3	0	0	3	0	7	1	2	41
14	*An.fluviatilis*	3	4	5	4	6	8	4	8	2	1	45
	Total	436		338		238		275		261		1548

Locality-1 = Faculty of Science campus, Locality-2 = Faculty of Engineering campus, Locality-3 = Faculty of Arts campus, Locality-4 = Faculty of Medicine cai
Locality-5 = Faculty of Agriculture campus.

Table 3. Season wise diversity of mosquitoes recorded in study area during study period (June-2013 to February-2014)

Mosquito Species	Name of the Months								
	Jun'13	Jul'13	Aug'13	Sep'13	Oct'13	Nov'13	Dec'13	Jan'14	Feb'14
Aedes aegypti	+	+	+	+	+	+	+	+	+
Ae.albopictus	+	+	+	+	+	+	+	+	+
Ae.africanus	+	+	+	+	-	-	-	-	+
Ae.simpsoni	+	-	+	-	+	+	+	-	-
Ae.taylori	-	+	+	+	+	+	-	+	+
Culex quinquefasciatus	+	-	+	+	-	-	+	+	+
Cx.nebulosus	+	+	+	+	-	+	-	+	+
Cx.tritaeniorhynchus	-	+	+	+	-	-	+	+	+
Cx.pseudovishnui	-	+	-	+	+	+	-	-	-
Cx.pipiens	+	-	+	-	+	-	+	-	+
Anopheles stephensi	+	+	+	+	+	+	+	+	+
An. subpictus	-	-	+	+	-	-	+	+	-
An.culicifacies	-	-	+	-	+	-	-	+	+
An.fluviatilis	+	+	-	-	-	+	-	+	-

(+ = Present, - = Absent)

Table 4. Statistical analysis (α-diversity) of the tree hole mosquitoes in Annamalai University campus

Estimation (α-diversity)	Locality 1	Locality 2	Locality 3	Locality 4	Locality 5
Total number of species or Species (S)	14	13	14	14	14
Total number of individuals (N)	436	338	238	275	261
Natural log of species (*In* S)	2.639	2.564	2.639	2.639	2.639
Natural log of Individuals (*In* N)	6.077	5.823	5.472	5.617	5.565
Margalef Index (D_{Mg})	2.139	2.060	2.376	2.314	2.336
Simpson's Index (λ)	8.205	7.991	6.610	8.883	5.715
Shannon-Wiener (H')	2.327	2.289	2.205	2.413	2.060
Pielou's Index (J')	0.882	0.893	0.836	0.914	0.781

administrative building, the campus having lot of trees and some sporadic tree holes. However, in localities where medical, engineering and Agriculture Faculty campuses the trees are present in large numbers there may be enough tree holes to sustain a viable breeding population of mosquitoes.

The natural tree holes of *Azadirachta indica*, *Magnifera indica*, *Delonex rigia*, trees and the species of bamboo, *Bambusa vulgaris* were available in the Faculty of Agriculture campus. Around the campus some of the tree holes are contaminated with dead leaves and some other insects besides mosquito larvae were found. Tree hole inhabitants of the genus *Culex* and *Aedes* are generally plankton feeders. According to the analysis of α diversity indexes (Table 4), the comparison of α biodiversity indices reveals that the most diverse value are present in locality-1 (faculty of sceince campus) like

(D_{Mg}=2.139, λ=8.205, $H^{'}$=2.327, $J^{'}$=0.882) followed by locality-2 like Faculty of Engineering campus (D_{Mg}=2.060, λ=7.991, $H^{'}$=2.289, $J^{'}$=0.893) and the least values present in locality-3 like Faculty of Arts campus (D_{Mg}=2.376, λ=6.610, $H^{'}$=2.205, $J^{'}$=0.836). Mosquito diversity varies across climates like environmental factors and heterogeneity of aquatic environments increase the appearance of colonizable habitats for many species, thereby increasing mosquito biodiversity (Ruben Bueno Mari and Ricardo Jimenez-Peydro, 2011). The analysis of α-biodiversity values indicates that the closest categories in their specific composition and diversity of tree hole mosquitoes. The aim of the representing information through Simpson index and Shannon-Weiner index used for identification and estimation of tree hole breeding mosquitoes in study area. Mosquitoes are responsible for spreading serious human diseases like malaria, japanese encephalitis, yellow fever, dengue and filariasis (Jebanesan, 2013). Tree hole inhabitants of the genus *Culex* are generally regarded as plankton feeders. *Aedes* larvae are capable of browsing and filter feeding, and in the latter mode may overlap in diet with *Culex*. *Ae.aegypti*, *Cx.quinquefasciatus* and *Cx.nebulosus* are also peridomestic breeders found in the tree holes located in the villages and also were domestic containers provide additional habitats for larvae (Anosike *et al*., 2007).

Conclusion

July, August, December and January are the four critical months because of high occurrence of mosquitoes in tree hole water. Presence of *Ae.aegypti*, *Cx.quinquefasciatus* and *An.stephensi* are the responsible factor for mosquito vector borne diseases. The University management should practice Integrated Vector Management (IVM) recommended by WHO (WHO, 2004) in the campus. IVM encourage effective coordination of the control activities at all sectors that have an impact on vector-borne diseases, including health and water. Continuous monitoring and control measures are essential for the prevention of dengue, chikungunya, malaria, filariasis and japanese encephalitis in the University campus.

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