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Research Article

BREEDING HABITAT DIVERSITY AND SPECIES COMPOSITION OF A*nopheles* MOSQUITOES IN TRINCOMALEE DISTRICT, SRI LANKA

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Abstract

Entomological studies on the abundance of malaria vector *Anopheles* mosquitoes have not been studied in some malaria endemic areas of Sri Lanka over past 30 years in view of the security situation. The aim of this study was to explore the habitat diversity and distribution of anopheline species in Trincomalee District in order to prioritize vector breeding habitats for developing timely and cost effective larval controlling measures.

Potential larval habitats for *Anopheles* mosquitoes were surveyed from June 2010 - December 2010, in selected sampling sites in the Trincomalee District; Gomarankadawala, Echchallampaththu, Mollipothana, Thoppur and Padavisiripura, within a radius about 20 km on weekly basis. The species distribution and density were calculated. A total of 3,701 larval specimens representing twelve *Anopheles* species were reported form 19 breeding habitats (Tank margin, main canal, paddy field, vegetative canal, lake, built well, burrow pit, distribution canal, pond, rock pool, canal, un-built well, common well, river margin, sand pool, animal foot print, rain water collection, quarry pit and marshy land). Ten habitats were categorized under structurally complex group based on the presence of biotic communities.

Only *An. subpictus* can be regarded as constant according to Distribution (C) (C= 80.1-100%). *An. nigerrimus*, *An. peditaeniatus*, *An. pallidus* and *An. vagus* were frequent (C= 60.1 – 80%). *An. varuna*, *An. barbirostris*, *An. annularis* and *An. barbumbrosus* were shown as infrequent species (C= 20.1 – 40%) and other namely *An. aconitus*, *An. culicifacies* and *An. jamesii* can be categorized under sporadic appearance (C= 0 – 20%). According to Density (D) criterion, five species (*An. subpictus*, *An. nigerrimus*, *An. varuna*, *An. pallidus*, *An. barbumbrosus*) were within the dominant class (D > 5%). Four species (*An. vagus*, *An. peditaeniatus*, *An. annularis*, *An. aconitus*) were in the subdominant class (1< D <5%). Only *An. jamesii* and *An. culicifacies* were the satellite species (D < 1%).

Keywords: *Anopheles*, breeding, habitats, larvae, survey
Geotags: Sri Lanka, Trincomalee; [Gomarankadawala 8.675755, 80.963944 | Mollipothana 8.432400, 81.044105 | Thoppur 8.406934, 81.322517 | Padavisiripura 8.926175, 80.809228]

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INTRODUCTION

Malaria has been a scourge in Sri Lanka since ancient time. The occurrence of the disease in the country probably dates back several centuries, and it is believed that most of the ancient capitals of the country were abandoned due to epidemics of malaria-like fever (Samarasinghe, 1990). About 10.33 million out of the 18.5 million population live in malarious area (WHO 1997). Malaria was endemic in 2/3rds of the country mainly in the Dry Zone. It has been a leading cause of morbidity and mortality among people in the Dry Zone throughout its known history.

The most prevalent malaria species is *Plasmodium vivax* (70%), the rest of the cases are caused by *Plasmodium falciparum.* Anophelines are the only vectors of human malaria. There are about 422 species of anopheline mosquitoes throughout the world, but only some 70 species are considered to be vectors of malaria under natural conditions; of these, some 40 species are of major public health importance (Gilles et al., 1993). Twenty two anopheline species have been recorded in Sri Lanka (Herath et al., 1986).

The principal vector is *Anopheles culicifacies* and the known secondary vectors are *An. subpictus* and *An. annularis* (Herath et al., 1986). According to the studies conducted by Herath et al., (1983) using ELISA- based evidences have shown a large number of anopheline species to be infected with malaria parasites in addition to *An culicifacies*. These include *An. aconitus*, *An. annularis*, *An. barbirostris*, *An. nigerrimus*, *An. pallidus*, *An. subpictus*, *An. tessellatus*, *An. vagus* and *An. varuna.*

The intensity of and stability of malaria transmission in an area depend on the geographical location and other factors. In stable malaria, the endemicity is not substantially altered by moderate changes in the environment such as temperature, humidity and density of vectors. However, in stable malaria, climatic changes have a pronounced influence on the intensity of transmission with marked yearly fluctuations in malaria incidences. Vector density plays an important role in unstable malaria (Pampana, 1969).

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 (Gautam Aditya et al., 2006). The resources in terms of food, predators and competitors present in the habitat determine the population status of larval mosquitoes, both qualitatively and quantitatively (Carlson et al., 2004). Composition of organisms in these ensembles depends on size and type of aquatic bodies (Carlson et al., 2004). Besides, association of natural enemies in these habitats influences the selection of oviposition site by the mosquitoes there-by limiting the mosquitoes to breed (Eitam et al., 2002)Moreover, the intraguild species composition and interaction in the larval mosquito communities influence the adult population at a particular time and space (Singh and Mishra, 1997).

Mosquito larval habitat ecology is important in determining larval densities and species assemblage. These in turn influence malaria transmission in an area. Understanding larval habitat ecology is, therefore, important in designing malaria control programmes (Oyewole et al., 2009). Hence the objective of the this study is to determine the larval habitat diversity and species composition of *Anopheles* mosquitoes in Trincomalee District special attention with main and other potential vectors of Malaria. In order to of prioritize vector breeding habitats for developing timely and cost effective larval controlling measures under the current malaria elimination program in Sri Lanka.

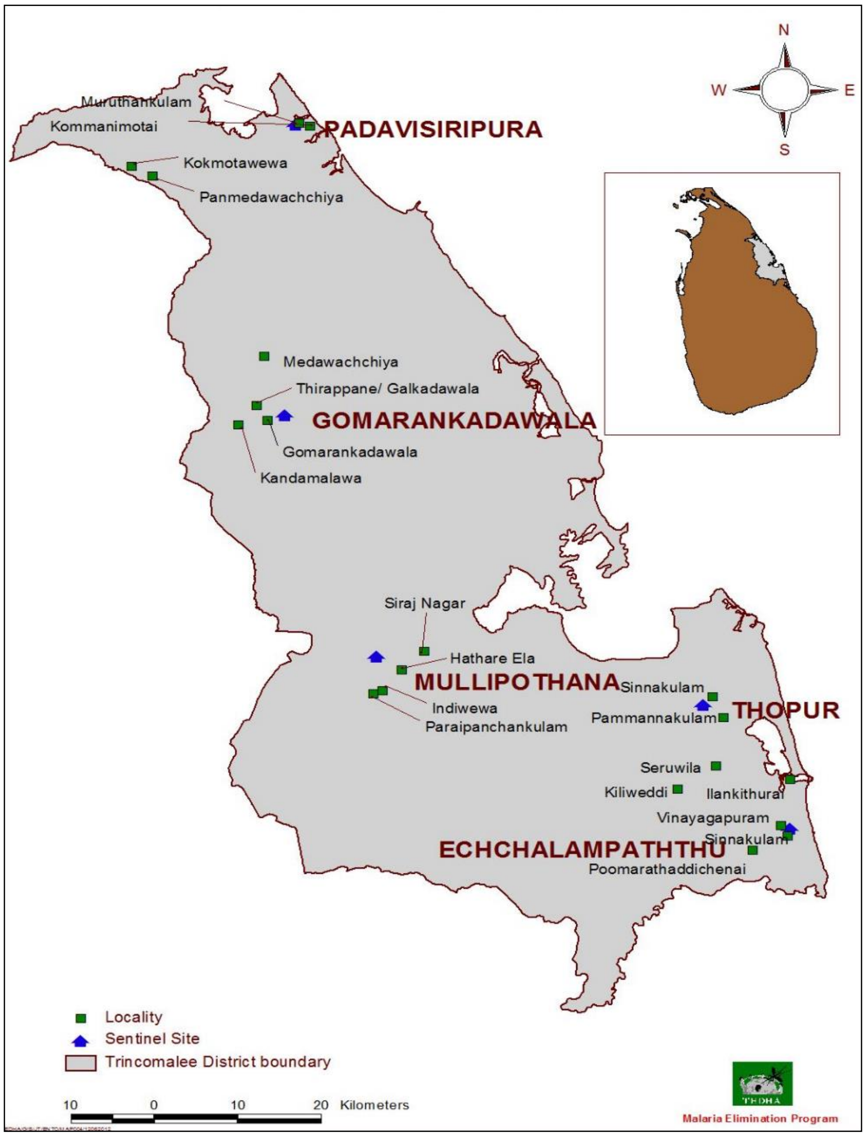

Figure 1. Map showing sentinel sites and localities in the District of Trincomalee

METHODS

Site Selection: Trincomalee District belongs to the Eastern province of Sri Lanka (8.566667°N 81.233333°E). Total area of the District is 7.5 km^2 (2.9 sq mi). According to 2007 population censes data; there are about 101,958 inhabitants. When considering the climate, this district has been categorized under Dry Zone of Sri Lanka, attaining 24.8 °C – 30.7 °C average temperature and 1, 649 mm rainfall annually.

The climatic conditions in Trincomalee are very much favorable for *Anopheles* mosquito breeding. Hence, this area is considered as malaria endemic. Therefore, potential *Anopheles* breeding habitats were identified through a preliminary survey carried out in March to May 2010, in selected sampling sites at Trincomalee District; Gomarankadawala, Ichchallampaththu, Mollipothana, Thoppur and Padavisiripura, which are sentinel sites, having radius about 20 km. In each sentinel site 4 localities (within 5-30 km) were selected forming 20 localities in Trincomalee District (Figure 1).

Sample collection and Identification: Mosquito Larvae were collected regularly on weekly basis from June to December 2010 in identified breeding sites using mettle larval ladles with extensible handles (12 cm diameter, 250 ml capacity). The number of dips and number Anopheline i & ii and iii & iv stages of larvae were recorded while iii & iv *Anopheles* larvae were collected in to clean larval collecting vials. Date of collection, breeding site, sentinel site and locality were marked in each collecting tube. Collected larvae were transported safely to field laboratories established in each sentinel site in the district. Above collected larvae from each sentinel sites were transferred locality and breeding site wise in to net covered plastic basins (30 cm diameter) filled in 1L of well water and fed with fish food dust. These larvae were reared until the adults emerged. Emerged adults were identified to the species level by using achromatic magnifying lens (x 10) and the taxonomic keys prepared by Amarasinghe (1990). During each sampling, a survey of plant taxa and faunal communities were made at each site.

Data analysis: Seasonal dynamics of mosquito larvae populations in the sampling sites was analyzed using the following factors:

Distribution was determined as the percent of sampling sites in which a species was noted, according to the formula:

$$C = \frac{n}{N} \cdot 100\%$$

Where:

C - Distribution, n - number of sites of the species, N - Number of all sites.

The following distribution classes were adopted (Dziêczkowski, 1972):

C1 - sporadic appearance (constancy 0- 20 %)

C2 - infrequent (20.1. 40%)

C3 - moderate (40.1. 60%)

C4 - frequent (60.1. 80%)

C5 - constant (80.1-100%)

Density was expressed as percent of specimens of the species in the whole sample (Dziêczkowski, 1972; Banaszak and Wi.niewski, 1999), according to the formula:

$$D = \frac{l}{L} \cdot 100\%$$

Where:

D- Density, l- Number of specimens of each mosquito species, L- Number of all specimens.

The following density classes were accepted after Trojan (1992):

satellite species (D < 1%)

subdominant species (1< D <5%)

dominant species (D > 5%).

RESULTS AND DISCUSSION

All the different types of habitats surveyed were found to be positive for mosquito immatures, though the numbers varied between the habitat types. Maximum number of species were encountered from tank margins, lakes, ponds, rock pools, and canals including main canals, vegetative canals and distributory canals.

Immatures of *Culex* mosquitoes were found in almost the all habitats except wells, sand pools and rain water collections. Least number of species was found in wells. The number of water bodies found positive for mosquito immatures along with the biota is presented in Table 1.

A total of 12 *Anopheles* mosquito species were observed to be positive in breeding sites surveyed throughout the study period. Among them *An. culicificies* which is considered as the main malaria vector in Sri Lanka was recorded only from tank margins during the study period (Table 2). *An. subpictus, An. aconitus, An. annularis, An. barbirostris, An. nigerrimus, An. pallidus, An. vagus and An. varuna* were observed as the other potential vectors.

During the study a total of 19 breeding site categories were identified in selected areas of Trincomalee District (Table 2). Among them tank margin was the more conducive for *Anopheles* breeding, followed by vegetative canals and burrow pits.

Table 1. Characteristics and biota of mosquito larval habitats in Trincomalee in Sri Lanka.

Habitat Range	Algae	Detritus	Vegetation	Structural complexity	*Chironomus*	*Aedes*	*Armigeres*	*Culex*	*Toxorhynchites*	Gerridae	Mayfly larvae	Acari	Crustacean	Tadpoles
Tank margin	+	+	+	Complex	+	+	+	+	+	+	+	+	+	+
Main canal	+	+	+	Complex	+	+	+	+	+	+	+	+	+	+
Paddy field	+	+	+	Simple	+	-	+	+	-	-	-	-	+	-
Vegetative canal	+	+	+	Complex	+	+	+	+	+	+	+	+	+	+
Lake	+	+	+	Complex	+	+	+	+	+	+	+	+	+	+
Built well	+	-	-	Simple	-	-	-	-	-	-	-	+	-	-
Burrow pit	-	+	-	Complex	-	+	-	+	-	-	-	+	+	+
Distributory canal	+	+	+	Complex	-	+	+	+	+	-	-	+	+	-
Pond	+	+	+	Complex	+	+	+	+	+	+	+	+	+	+
Rock pool	+	+	+	Complex	+	+	+	+	+	+	-	+	+	+
Canal	+	+	+	Complex	+	+	+	+	+	+	+	+	+	+
Un built well	+	+	+	Simple	-	-	-	-	-	-	-	+	-	-
Common well	+	-		Simple	-	-	-	-	-	-	+	+	-	+
River margin	+	-	-	Simple	-	+	-	+	-	-	-	+	-	-
Sand pool	+	-		Simple	-	+	-	-	-	-	+	+	-	+
Animal foot print	+	+	-	Simple	-	+	-	+	-	-	-	-	-	-
Rain water collection	-	+	-	Simple	-	+	-	-	-	-	+	+	-	-
Quarry pits	+	+	+	Complex	+	+	+	+	+	+	-	+	+	+
Marshy land	+	+	+	Simple	+	-	+	+	-	-	-	-	+	-

Table 2. Summary of Immature habitats of mosquito species in Trincomalee District.

Species	Immature habitats											
	An. vagus	*An. nigerrimus*	*An. peditaeniatus*	*An. pallidus*	*An. subpictus*	*An. varuna*	*An. barbirostris*	*An. annularis*	*An. aconitus*	*An. jamesii*	*An. culicifacies*	*An. barbumbrosus*
Tank margin	+	+	+	+	+	+	+	+	+	+	+	+
Main canal	+	+	+	+	+	+						
Paddy field	+	+	+	+	+	+	+					
Vegetative canal	+	+	+		+	+	+	+	+		+	
Lake		+		+	+	+						+
Built well		+	+		+	+					+	+
Burrow pit	+	+	+	+	+	+	+					+
Distributory canal	+	+	+	+	+	+						
Pond		+		+	+							+
Rock pool	+				+							
Canal	+											+
Un built well	+											+
Common well												
Mud pool												
River margin												
Sand pool	+	+	+	+	+							
Animal foot print	+	+	+	+	+		+					
Rain water collection		+	+		+							
Quarry pit		+	+	+								

Only *An. subpictus* can be regarded as constant according to Distribution (C= 80.1-100%). *An. nigerrimus*, *An. peditaeniatus*, *An. pallidus* and *An. vagus* were frequent (C= 60.1 – 80%). *An. varuna*, *An. barbirostris*, *An. annularis* and *An. barbumbrosus* were shown as infrequent species (C= 20.1 – 40%) and others, namely *An. aconitus*, *An. culicifacies* and *An. jamesii* can be categorized under sporadic appearance (C= 0 – 20%). According to Density criterion, five species (*An. subpictus*, *An. nigerrimus*, *An. varuna*, *An. pallidus*, *An. barbumbrosus*) were within the dominant class ;(D > 5%). Four species (*An. vagus*, *An. peditaeniatus*, *An. annularis*, *An. aconitus*) were in the subdominant class (1< D <5%). Only *An. jamesii* and *An. culicifacies* were the satellite species (D < 1%).

Table 3. Occurrence of characteristic mosquito species in the sampling sites

Mosquito species	Distribution (%)	Density (%)
An. subpictus	83.34	42.12
An. nigerrimus	77.78	15.20
An. varuna	44.45	8.85
An. pallidus	66.67	7.85
An. barbumbrosus	38.89	5.68
An. barbirostris	33.34	5.43
An. peditaeniatus	66.67	4.96
An. vagus	66.67	4.96
An. annularis	22.23	3.47
An. aconitus	11.12	1.13
An. jemesii	5.56	0.75
An. culicifacies	11.12	0.13

It has been demonstrated that the availability of water and associated mosquito oviposition behaviour can play an important role in determining the distribution of malaria risk (Service, 1997). In some cases, proximity to water where mosquitoes oviposit increases the risk of malaria, whether or not the eggs develop into adults. In other words, a non-productive site for adult mosquito emergence can be a source for malaria (Giglioli, 1964). More generally, as would be expected, malaria prevalence would be higher close to water bodies. The transmission potential of mosquitoes is maximized when water and humans are both available.

The aim of the research was to investigate the influence of oviposition behaviour on the spatial distribution of *Anopheles* mosquitoes; substantial uncertainty remains about strategic aspects of mosquito behaviour, such as how mosquitoes locate and choose a place to oviposit.

The present study examined 19 potential breeding sites for *Anopheles* mosquitoes (Table 1). Some breeding sites were encountered with biota, representing both faunal (Table 1) and floral communities. Some of the plants communities appeared to be the indicators of the presence of mosquito larvae such as Water lettuce (*Pistia*), Salvinia (*Salvinia molesta*), Hydrilla (*Hydrilla spp.),* Lotus (*Nelumbo spp.*), Water hyacinth (*Eichhornia spp.*) and some grasses.

However, the structural complexity of the habitats is also expected to be changed with the growth of aquatic fauna and flora communities, creating more complex scenario and gradual invasion by other organisms including different mosquito species (Gautam Aditya et al., 2006).

The present study precise that out of 19 types of potential breeding sites for malaria vector mosquitoes were examined, ten of them were categorized under structurally complex group, based on the presence of biota in each habitat (Table 1). Especially, tank margins were noticed as the most conducive for *Anopheles* larval breeding, which might be due to availability of good hiding places against their natural predators.

An. culicifacies is the primary vector of malaria in Sri Lanka, is known to breed primarily in association with stream and river systems. However, this species also breeds in a wide variety of other surface water and habitats in Sri Lanka. (Amerasinghe and Munasinghe, 1988; Amerasinghe and Ariyasena, 1990; Abhayawardana, 1995). It prefers clear, sunlit, fresh waters. Intense breeding of *An. culicifacies* occurs in sand and rock pools of drying rivers and the margins of slow moving water-ways such as streams and irrigation channels. Recent studies shown that *An. culicifacies* breeds in brackish water bodies in Trincomalee District of Sri Lanka (Gunathilaka et al., 2010).

When considering secondary vectors, *An. subpictus* is considered as the highest secondary vector in Sri Lanka. In addition *An. annularis, An. varuna* and *An. tesellatus* are also considered as secondary vectors. The present investigation showed, twelve *Anopheles* species (Table 2 & 3), denoting *An. culicifacies* as the major vector for malaria in Sri Lanka. *An. subpictus* and *An. vagus* were observed as subsidiary vectors. *An. culicifacies* was noticed from tank margins, vegetative canals and built wells in Trincomalee District. *An. vagus* and *An. subpictus* were present in most of the breeding habitats (Table 2), but more predominant in paddy fields, burrow pits, lakes, animal foot prints and marshy lands. Some species were limited to certain breeding habitats (Table 2) because different species may have their own habitat range, depending on the water quality conditions which each species prefer such as Dissolved Oxygen (DO), nutrient level, P^H and temperature etc (Rydzanicz and Lonc 2003).

When considering the abundance of mosquito species, only *An. subpictus* can be regarded as constant. Meanwhile, *An. nigerrimus*, *An. peditaeniatus*, *An. pallidus* and *An. vagus* were frequent. *An. varuna*, *An. barbirostris*, *An. annularis* and *An. barbumbrosus* were shown as infrequent species and others namely *An. aconitus*, *An. culicifacies* and *An. jamesii* can be categorized under sporadic appearance.

According to density criterion, *An. subpictus*, *An. nigerrimus*, *An. varuna*, *An. pallidus* and *An. barbumbrosus* are within the dominant class. *An. vagus*, *An. peditaeniatus*, *An. annularis* and *An. aconitus* were included in subdominant class. Only *An. jamesii* and *An. culicifacies* were the satellite species.

The presence of primary and other potential vectors in these areas may lead to increase malaria incidence in these areas with the presence of parasite. Anyhow we need not banish *Anopheles* entirely; we need only to reduce their numbers below a certain figure. A few of contemporaries have paid attention to this idea of threshold densities of *Anopheles*, some applied it in successful programs of environmental management.

Hence, some work plans should be also implemented to distinguish vector from non-vector species and identifying their actual breeding sites, so that targeted, sustainable vector programs could replace ineffective or inefficient generalized anti-mosquito approaches. Larval density may be a leading indicator of a habitat's importance for malaria control.

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