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Spatial distribution and habitat characterisation of *Anopheles* larvae along the Kenyan coast

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Abstract

Background & objectives: A study was conducted to characterise larval habitats and to determine spatial heterogeneity of the *Anopheles* mosquito larvae. The study was conducted from May to June 1999 in nine villages along the Kenyan coast.

Methods: Aquatic habitats were sampled by use of standard dipping technique. The habitats were characterised based on size, pH, distance to the nearest house, coverage of canopy, surface debris, algae and emergent plants, turbidity, substrate, and habitat type.

Results: A total of 110 aquatic habitats like stream pools (n = 10); puddles (n = 65); tire tracks (n = 5); ponds (n = 5) and swamps (n = 25) were sampled in nine villages located in three districts of the Kenyan coast. A total of 7,263 *Anopheles* mosquito larvae were collected, 63.9% were early instars and 36.1% were late instars. Morphological identification of the III and IV instar larvae by use of microscopy yielded 90.66% (n = 2,377) *Anopheles gambiae* Complex, 0.88% (n = 23) *An. funestus*, *An. coustani* 7.63% (n = 200), *An. rivulorum* 0.42% (n = 11), *An. pharoensis* 0.19% (n = 5), *An. swahilicus* 0.08% (n = 2), *An. wilsoni* 0.04% (n = 1) and 0.11% (n = 3) were unidentified. A subset of the *An. gambiae* Complex larvae identified morphologically, was further analysed using rDNA-PCR technique resulting in 68.22% (n = 1,290) *An. gambiae s.s.,* 7.93% (n = 150) *An. arabiensis* and 23.85% (n = 451) *An. merus*. Multiple logistic regression model showed that emergent plants (p = 0.019), and floating debris (p = 0.038) were the best predictors of *An. gambiae* larval abundance in these habitats.

Interpretation & conclusion: Habitat type, floating debris and emergent plants were found to be the key factors determining the presence of *Anopheles* larvae in the habitats. For effective larval control, the type of habitat should be considered and most productive habitat type be given a priority in the mosquito abatement programme.

Key words *Anopheles gambiae* – habitat characterisation – rDNA-PCR technique – spatial heterogeneity

Introduction

Anopheles gambiae s.s., An. arabiensis, An. merus and *An. funestus* (Diptera : Culicidae) are the most important vectors of human malaria in coastal Kenya[1]. Production of adult *An. gambiae s.l.* occurs in small, temporary, sunlit, turbid pools of water[2]. Mosquito aquatic habitats are often created by human

or animal activities wherein larvae are found in small depressions such as foot or hoof prints, the edges of bore holes and burrow pits, roadside puddles formed by tire tracks, irrigation ditches and other artificial bodies of water[3–6].

The adaptability to environmental changes leading to marked contrasts in vector bionomics has led to the development of various levels of vectorial efficiency for populations of *Anopheles* species in heterogenous environments within the same locality and has thus become an important factor in determination of epidemiology of malaria[7]. Environmental heterogeneities have arisen mainly as a result of human activities which act as a means of constant evolutionary challenge as they provide a source of environmental change to which anthropophilic *Anopheles* have to respond by developing a highly dynamic vector-host relationship.

The extent to which environmental heterogeneity affects patterns of vector production that are important for malaria parasite transmission is unknown[8]. The factors affecting larval survival and the mechanism controlling adult production are also largely unknown for even most important vector species. Appropriate management of larval habitats in sub-Saharan countries, particularly during dry season may help suppress vector densities and malaria transmission[5]. However, the understanding of anopheline larval ecology is limited and insufficient to achieve effective vector control through means of larval control[9]. The main objective of this study was to determine spatial distribution and to characterise the larval habitats and associated environmental parameters, that influence the abundance of *Anopheles* mosquitoes. The results of this study would be useful in planning and implementation of mosquito larval abatement programme along the Kenyan coast.

Material & Methods

Study villages: This study was carried out in nine villages located in Malindi, Kilifi and Kwale districts on the Kenyan coast. In each district, three villages were selected for mosquito larval sampling. In each village, larval habitats were selected for sampling of the mosquito larvae. These villages are a subset of the 30 villages described by Mbogo *et al*[1] and Mwangangi *et al*[10]. These villages were selected-based on the availability of larval habitats, species composition and accessibility to the village during the rain period. The previous study by Mbogo *et al*[1] using adult *Anopheles* populations, more *An. arabiensis* was found in the Malindi area than in Kilifi or Kwale, while *An. merus* was found near the coastline but mostly in Malindi. Higher proportions of *An. funestus* densities were detected in Kwale than in Kilifi and Malindi, and these decreased toward the north. Likewise, vector abundance of *An. gambiae s.s.* decreased from the north in Malindi to the south in Kwale. Similarly, the proportions of *An. funestus* in Kwale decreased from the coastline moving inland. These previous observations of spatial heterogeneity in vector composition aided in village selection from each district.

In brief, the selected villages are located between 2 and 30 km from the coastline. Coastal Kenya has two rainy seasons, April to June and October to November. Mean annual precipitation range from 7.5 cm in the hinterland to 1.2 cm along the coastal belt. Several rivers and seasonal estuaries transect and drain the area. In Kwale, the Umba and Ramisi rivers, and smaller permanent and seasonal streams, drain the area. The Jaribuni and Sabaki rivers drain the Kilifi and Malindi areas, respectively.

In the nine villages, the houses are mainly constructed of wooden pillars and walls are plastered using mud and the roof is made of coconut thatch. In most houses, the windows were unscreened and walls had holes. This facilitated easy movement of mosquitoes in and out of the houses. In these sites, the primary agricultural activities include cash crops such as cashew nuts, coconuts, mangoes, bananas, paw-paws and oranges. The subsistence farming includes grow-

ing of maize, cassava, beans and peas.

Larval sampling and habitat characterisation: A cross-sectional survey was carried out between May and June 1999. From each village mosquito larvae were collected from each larval habitat using the standard dipping technique (350 ml dipper)[11]. The *Anopheles* larvae were separated from the culicine larvae. The *Anopheles* mosquito larvae were classified as early instar stage (I and II) or late instar stage (III and IV). The *Anopheles* mosquito larval age grading was done according to Gillies and Coetzee[12]. The late stage instars were preserved in 75% ethanol and transported to laboratory for morphological identification.

The larval habitats were characterised either visually or by use of hand-held field equipment based on the methodology used by Minakawa *et al*[5]. In brief, canopy cover was defined as the amount of terrestrial vegetation and other objects above the aquatic habitat. Canopy was measured visually by estimating the area of the larval habitat covered by shade as a percentage. Emergent plants included both aquatic and immersed terrestrial vegetation. Plant coverage of a habitat was measured as percentage of surface covered by flora, by placing a square frame (1 x 1 m) with grids above the habitat. Distance to the nearest house (human habitation) was measured using a tape when it was shorter than 100 m. When the distance exceeded 100 m it was estimated. Algae cover and debris were estimated as percentage of the total habitat, using a square grid. All the estimations were done by one person throughout the sampling period to avoid discrepancies. Substrate was classified into muddy, sandy, gravel with soil and artificial substrate without soil (concrete and brick). pH was determined with Corning pH/temperature meter (Corning®). Conductivity was determined by hand-held conductivity meter (Corning®). Dissolved oxygen levels in water were measured by use of hand-held dissolved oxygen meter (Corning®). Turbidity was measured by placing water samples in a glass test tube and holding against a white background and was classified into four levels: clear, low, medium and high.

Anopheles larval identification: The III and IV instar larvae were identified morphologically[12], and those of the *An. gambiae* Complex were further identified into sibling species by rDNA-PCR technique[13].

Statistical analysis: The statistical analyses were done using SPSS software (Version 11 for windows, SPSS Inc., Chicago, IL). Multiple logistic regression analysis was used to test associations of the environmental variables with the occurrence of *An. gambiae* larvae. Occurrence of *An. gambiae* was defined as presence or absence of *An. gambiae* larvae irrespective of the relative abundance and density. Presence of larvae was categorised as one while the absence of larvae was categorised as zero.

Results

A total of 110 aquatic habitats were sampled in the nine villages along the Kenyan coast. A total of 7,263 *Anopheles* mosquito larvae were collected, of that 4,641 (63.9%) were categorised as early instars and 2,622 (36.1%) as late instars. The habitat types sampled included stream pools (n = 10), puddles (n = 65), tire tracks (n = 5), ponds (n = 5) and swamps (n = 25). Table 1 shows the *An. gambiae* larval production from each habitat type. Puddles were the most productive habitat type for *An. gambiae s.l.* larvae. Stream pools produced more larvae in Jaribuni, Mtepeni and Garithe. Tire tracks were found to be important habitats in Jaribuni and Garithe. Ponds were important habitats in Garithe. In Majajani, Mtepeni, Majenjeni and Garithe, swamps produced a high proportion of *An. gambiae* larvae. Overall, there was a significant difference in the *An. gambiae* production from the different larval habitat types ($F_{(4, 105)} = 3.552$, $p = 0.009$). Tukeys HSD analysis further showed that stream pools, puddles and tire tracks were the most productive habitat types for *An. gambiae* larvae. Two way analysis of variance

(ANOVA) showed that village from which *An. gambiae* larvae ($p < 0.001$) were collected was highly significant determinant factor for abundance of the larvae whereas distance to the nearest house was not associated with the relative abundance of immatures of the *An. gambiae* Complex ($p = 0.286$) and the interaction between village and distance was not significant ($p = 0.279$).

Morphological identification of the III and IV instar larvae by the use of microscopy revealed 90.66% (n = 2,377) belonging to *An. gambiae* Complex, 0.88% (n = 23) *An. funestus*, *An. coustani* 7.63% (n = 200), *An. rivulorum* 0.42% (n = 11), *An. pharoensis* 0.19% (n = 5), *An. swahilicus* 0.08% (n = 2), *An. wilsoni* 0.04% (n = 1) and 0.11% (n = 3) were unidentified. Of the *An. gambiae* Complex larvae identified morphologically, a subset (n = 1,891), was further analysed using rDNA-PCR technique resulting in 68.22% (n = 1,290) *An. gambiae s.s.,* 7.93% (n = 150) *An. arabiensis* and 23.85% (n = 451), *An. merus*. The *Anopheles* larval composition in the three districts showed that *An. gambiae s.s.* was predominant in Malindi (north coast) but was found at each of the nine villages sampled. *An. arabiensis* was found mainly in Malindi and in Kwale districts. Malindi and Kilifi had most *An. merus* but none was collected at the south coast. It was further observed that *An. merus* was more in the habitats in the northern coast than in the southern coast region. *An. funestus* and *An. coustani* were mainly found in habitats in Kilifi and Kwale districts. Overall, *An. gambiae s.s.* was the most predominant species (60.48%), followed by *An. merus* (21.14%) and *An. coustani* (9.38%) (Table 2).

Multiple logistic regression model (Table 3) showed that emergent plants ($p = 0.019$), and floating debris ($p = 0.038$) were the best predictors of *An. gambiae* larval abundance in the habitats. Floating debris was an indication of newly formed habitat showing the incoming rainwater while emergent plants were associated with the newly formed habitats. Emergent plants were negatively associated with *An. gambiae* larval abundance in the aquatic habitats.

Discussion

Mosquito larval habitat ecology is important in determining larval densities and species assemblage. This in turn influence malaria transmission in an area. Understanding larval habitat ecology is, therefore, important in designing malaria control programmes. Describing larval habitat characteristics in terms of environmental attributes and identifying relationships between biotic and abiotic factors are important for developing novel methods of vector control

Table 1. *Anopheles gambiae s.l.* larval production from each habitat type in three districts along the Kenyan coast

District	Site	Habitat type					
		Stream pool	Puddle	Tire track	Pond	Swamp	Total
Kilifi	Majajani	0 (0)	53 (57.6)	0 (0)	0 (0)	39 (42.4)	92
	Mtepeni	54 (27.1)	108 (54.3)	0 (0)	5 (2.5)	32 (16.1)	199
	Jaribuni	126 (25.1)	344 (68.4)	33 (6.6)	0 (0.0)	0 (0)	503
Malindi	Garithe	48 (9.7)	337 (67.9)	33 (6.7)	33 (6.7)	45 (9.1)	496
	Majenjeni	0 (0)	392 (90.7)	0 (0)	0 (0)	40 (9.3)	432
	Paziani	0 (0)	192 (90.1)	0 (0)	0 (0)	21 (9.9)	213
Kwale	Amani	0 (0)	274 (100)	0 (0)	0 (0)	0 (0)	274
	Vinuni	7 (100)	0 (0)	0 (0)	0 (0)	0 (0)	7
	Magaoni	2 (1.2)	152 (94.4)	0 (0)	0 (0)	7 (4.4)	161

Figures in parentheses indicate percentage.

Table 2. *Anopheles* mosquito larvae species composition in nine villages along the Kenyan coast based on morphological identification of the late instar larvae

District	Site	Anopheline species								
		An. gambiae s.s.	*An. arabiensis*	*An. merus*	*An. funestus*	*An. coustani*	*An. pharoensis*	*An. rivulorum*	*An. swahilicus*	*An. wilconi*
Kilifi	Majajani	68	6	0	8	65	3	5	2	0
	Mtepeni	51	0	0	2	3	0	5	0	0
	Jaribuni	168	0	224	0	0	0	1	0	0
Malindi	Garithe	218	42	94	0	0	0	0	0	0
	Majenjeni	306	37	87	2	1	2	0	0	1
	Paziani	151	18	44	0	0	0	0	0	0
Kwale	Amani	206	35	0	0	0	0	0	0	0
	Vinuni	6	1	0	2	37	0	0	0	0
	Magaoni	113	16	0	9	94	0	0	0	0
	Total	1,290	150	451	23	200	5	11	2	1

Table 3. Multiple logistic regression analysis showing the key factors for *Anopheles gambiae*

Variable	B	df	Sig	OR	95% C.I. for OR	
					Lower	Upper
Length (m)	0.000736	1	0.37	1.0	0.99	1.0
Depth (m)	–0.01787	1	0.14	0.98	0.96	1.01
Width (m)	–0.00029	1	0.82	1.0	1.0	1.0
Distance (m)	–0.00445	1	0.36	1.0	0.99	1.01
Turbidity		3	0.78			
Turbidity (Clear)	–0.78681	1	0.43	0.46	0.06	3.25
Turbidity (Low)	–0.90819	1	0.45	0.40	0.04	4.30
Turbidity (Medium)	–0.00589	1	0.99	0.99	0.24	4.05
Canopy cover	0.047703	1	0.12	1.05	0.99	1.11
Emergent	–0.02972	1	0.02	0.97	0.95	1.0
Algae	0.02818	1	0.23	1.03	0.98	1.08
Debris	–0.0932	1	0.04	0.91	0.83	0.99
Habitat		4	0.08			
Habitat (Stream pool)	6.267399	1	0.08	527.10	0.53	527.66
Habitat (Puddle)	1.53747	1	0.043	4.65	1.05	20.62
Habitat (Tire truck)	–1.16316	1	0.40	0.31	0.02	4.61
Habitat (Pond)	0.769518	1	0.63	2.16	0.090	51.53
Conductivity	0.01336	1	0.94	1.01	0.73	1.40
pH	0.039984	1	0.89	1.04	0.58	1.88
Constant	–23.2769	1	1.0	7.78E-11		

OR– Odds ratio; C.I.—Confidence interval; Sig—Significant; df—Degrees of freedom.

in communities with a high propensity to harbour *Anopheles* mosquitoes. We have studied the ecology of several larval habitats along the Kenyan coast and identified factors that influence *Anopheles* larval densities and diversity.

In this study, we found habitat type was important in determining the abundance and diversity of *Anopheles* larval composition. *An. gambiae s.s., An. arabiensis, An. funestus, An. coustani,* and *An. rivolurum* were collected from same habitats. There was no habitat, which was found to be having only single species of mosquitoes. There was a habitat partitioning along the Kenyan coast, which implies that the mosquito species share the food resources within the same habitats. The co-existence of mosquito larvae ensures that there is adult mosquito production of all anophelines throughout the year as these species use the same habitats. Further, the present study found more diversity in the *Anopheles* larval composition as compared to the previous studies based on adult mosquito populations. Earlier, studies along the Kenyan coast based on adult collections[1,14] showed that the vectorial composition is made up of *An. gambiae s.s., An. arabiensis, An. merus* and *An. funestus* in the three districts. The present study showed prevalence of more *Anopheles* species consisting of *An. coustani, An. pharoensis, An. rivulorum, An. swahilicus* and *An. wilsoni.* Earlier studies used indoor sampling techniques (Pyrethrum spray collection), which only captured endophilic *Anopheles* mosquitoes. Using larval sampling technique, we collected other *Anopheles* species also, which might be due to difference in the feeding and resting behaviour of these mosquitoes. Further, sampling for adult mosquitoes along the Kenyan coast requires to incorporate both outdoors and indoors sampling techniques to describe accurately the anopheline species composition.

The aquatic habitats in these areas were much varied which made the larval abundance to be significantly different in nine villages. In areas where stream pools and puddles were common, more *Anopheles* larvae were collected. However, swamps were less productive for *Anopheles* larvae. This finding showed that small habitats were more productive for anopheline mosquitoes compared to large larval habitats during the rainy season. The possible explanation as to why *An. gambiae s.s.* larvae frequently occurred in puddles and stream pools might be that the *An. gambae s.s.* females preferentially select small, open habitats for oviposition[15], secondly, larval predation is less prevalent in temporary habitats than it is in large, permanent habitats[16,17], and finally, open habitats that tend to produce more algae (the main food source for *An. gambiae s.s.*), than the shaded habitats[2]. *An. gambiae* may have evolved to exploit these favourable conditions by selecting small and open habitats for oviposition. Stream pools and puddles are shallow and tend to be having lower complexity in terms of debris and vegetation cover. This means that the larval development tends to be faster due to higher temperatures and density remain high due to lower predator risk. The swamps, which are big in size, have higher complexity, that results in higher concentration of other invertebrate species[18], which could be important as predators or competitors for *Anopheles* larvae. Further work is required along the Kenyan coast to describe the predators of mosquito larvae in the habitats and how the habitat complexity affects the survivorship of *Anopheles* larvae in the habitats.

The *Anopheles* larval composition revealed that there was spatial heterogeneity in three districts. *An. merus* was found mostly in Malindi (north coast) and used habitats along the tidal zones. *An. gambiae s.s.* were found in all nine villages sampled and were mostly found in puddle habitat type. Puddles are open shallow and sunlit and mostly found within the homesteads and have been previously shown to be important for *An. gambiae* larval production[5,6,12].

The study showed that emergent plants, floating debris and habitat type are the best predictors of *An. gambiae* larval abundance in the aquatic habitats.

The emergent plants in early stages are associated with anopheline mosquito larvae but when the canopy covers the water surface the *An. gambiae* larvae abundance decline. *Anopheles* mosquitoes are known to be ovipositing in newly formed habitats, which are shallow and sunlit[2,12]. When the habitats age increase, the population of the anopheline larvae tend to decline. We conducted our survey at the early days of the rainy season when most of the rain-associated habitats were developing. Most larvae were collected from stream pools, puddles and tire tracks. These habitats are shallow and sunlit which ensures faster development of mosquito larvae.

In the present study low populations of *An. funestus* larvae were observed, which is a major malaria vector along the Kenyan in coast[1,10,14,19]. *An. funestus* usually breeds in vegetated aquatic habitats, which are more stable and more permanent. *An. funestus* larvae were found mostly in Jaribuni and along the stream pools in River Jaribuni. In Jaribuni, all the *An. funestus* were found in stable stream pools that are prevalent throughout the year along the River Jaribuni. Majajani and Magaoni have stream pools and all the *An. funestus* larvae were collected in these habitats. Previous studies have shown that Jaribuni and the villages of Kwale district harboured more *An. funestus* adults[1]. We hypothesised that most of the aquatic habitats which could have been utilised by *An. funestus* were flooded with flowing water along the streams, consequently the habitats were unavailable for mosquito oviposition. With the subsiding of rains, the stream pools would be more productive for *An. funestus.*

In conclusion, habitat type, floating debris and emergent plants were found to be key factors determining the presence of *An. gambiae* larvae in the aquatic habitats. Integrated Vector Management (IVM) programme should be initiated targeting both the adult mosquitoes and larval stages. For effective larval control, the type of habitat should be considered and most productive habitat type[20] given a priority in the mosquito abatement programme.

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