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Seasonal mosquito larval abundance and composition in Kibwezi, lower eastern Kenya

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Abstract

Background & objectives: Changes in weather patterns especially rainfall affects the distribution and densities of mosquitoes. The objective of this study was to describe mosquito aquatic habitats, to determine larval abundance, species composition, and habitat types found in Kasayani village of Kibwezi division.

Methods: A cross-sectional survey of mosquito larval habitats was conducted in Kasayani village in Kibwezi division to determine species composition, larval abundance, and habitat types found in this village. This survey was conducted during the rainy season in November and December 2006 and during the dry season in February and March 2007. Larvae were collected using the standard dipping technique and a total of 24 habitats were sampled. The primary habitats identified were water reservoir tanks, puddles, temporary pools, and tyre tracks.

Results: A total of 2660 mosquito larvae were collected of which 2140 (80.45%) were culicines, 503 (18.91%) were *Anopheles* and 17 (0.64%) were pupae. For culicines, 1787 (83.5%) were categorized as early instars and 353 (16.5%) were as late instars while in the *Anopheles,* 425 (84.49%) were classified as early instars and 78 (15.51%) were late instars. Morphological identification of the III and IV instar larvae by use of microscopy yielded 16.24% (n = 70) *Anopheles gambiae* complex, 1.16% (n = 5) *An. funestus*, 0.70% (n = 3) *An. coustani*, 42.46% (n = 183) *Culex quinquefasciatus,* 6.26% (n = 27) *Cx. duttoni,* and 33.18% (n = 143) *Ae. aegypti.* Puddles, tyre tracks and pools had highly turbid water while water reservoir tanks had clear water. *Anopheles gambiae* and *Cx. quinquefasciatus* were found in all habitat categories while *Ae. aegypti* were found only in water storage tanks.

Interpretation & conclusion: The mosquito larval densities indicate that the inhabitants of this village are at risk of mosquito-borne diseases including malaria, which is one of the greatest causes of morbidity and mortality in this area. Furthermore, mosquito control measures targeting both the mosquito immatures and adults should be enhanced especially during the rainy season to ensure maximum protection of the inhabitants.

Key words Larval densities – larval ecology – larval habitat – mosquito-borne diseases – mosquitoes

Introduction

Mosquito larval habitats are the locations where many important life-cycle processes take place: oviposition, larval development, adult emergence, resting, swarming and mating[1]. Effective control of malaria through vector management requires information on the distribution and abundance of vectors in the targeted areas. Mosquito larval control is a potentially important target in malaria vector control. Source reduction through modification of larval habitats was the key to malaria eradication efforts in the United States, Italy, and Israel[2]. The suppression and even eradication of malaria from vast areas has been attributed to effective large-scale programmes to kill the immature *Anopheles* species vectors or reduce the amount of suitable habitat for them in proximity to vulnerable human populations[3–6]. The appropriate management of larval habitats during the dry season may help suppress vector densities and consequently, malaria transmission. However, our understanding of anopheline larval ecology in Africa is insufficient and this affects the design and implementation of larval control.

National malaria control efforts in Kenya, as in most African countries, focus on case management and the use of insecticide-treated bednets[7]. Currently in Kenya, there is a scaling-up of insecticide-treated bednets access and use to pregnant women and children under the age of five years[8]. Domestic vector control interventions against adult mosquitoes in the form of insecticide-treated bednets[9] or indoor residual spraying[10], combined with improved access to effective diagnosis and treatment, have enormous potential to Roll Back Malaria and constitute the primary means to achieve the Abuja targets[11]. However, even these highly effective interventions are insufficient to eliminate malaria transmission from most endemic parts of Africa[12,13].

Understanding the factors that regulate the size of mosquito populations is considered fundamental to the ability to predict transmission rates and for vector population control[14,15]. Larval habitats are important determinants of adult distribution and abundance. Although the transient habitats of *An. gambiae* may not be a reasonable target for vector control, an understanding of the dynamics and productivity of larval habitats is required, if efforts to model and predict adult abundance are to succeed. To control mosquitoes, whether adults or larvae, it is crucial to understand the relevant ecology of the target species. This requires the study of not only the fluctuations of the adult populations, but also the factors affecting larval abundance and distribution. While considerable progress towards understanding the aquatic stages of *An. gambiae* sensu lato has been made in recent years[16–23], systematic research on the larval ecology of the main malaria vectors in Africa is still limited and often represents short data collection periods, frequently considering mosquito-infested habitats only. In contrast, more intensive larval ecology studies evaluating and acknowledging options for larval vector control have been conducted in central and southern America on various malaria vectors in the last decade[24–30].

During the rainy season, areas of rain-dependent agriculture have provision of ideal aquatic habitats that support high density of diverse mosquito species including vectors of malaria, filariasis and a number of arbovirus. Thus, there is an urgent need to address the problem of mosquito-borne diseases in these areas in order to reduce the risk of massive public health problems and economic loss due to sickness. Such a goal can only be achieved through integrated control of the diverse mosquito species occurring in these areas. Most of the work done in Kibwezi previously described different aspects of malaria including immunological responses in children under five years of age[31], asymptomatic malaria and nutritional inadequacies (as indicated by stunting)[32], and antimalarial use[33]. Malarial infection reported at the clinical facilities in the area is exclusively due to *Plasmodium falciparum*. There is no active malaria control programme in the area, and no epidemiological or entomological studies of malaria have been conducted previously. Although this area has been described as seasonal malaria transmission[31–34], and mosquito is major problem especially during both long and short rains, no effort has been made to describe the mosquito species composition. The objective of this study was to describe mosquito aquatic habitats, to determine larval abundance, species composition, and habitat types found in Kasayani village of Kibwezi division.

Material & Methods

Study village: The study was done in Kasayani village which lies at 37^{o}36'44'' E and 2^{o} 30'15'' S. This village is in Kibwezi division and is 250 km east of Nairobi City. Kasayani village lies approximately 20 km north of Kibwezi town off the Kibwezi-Kitui Road and the University of Nairobi College of Arid and Range Management is situated at the northern end of this village.

The main physiography of the area is generally flat with very few sections of undulating ground especially where erosion has been rife. The village has an inclination of ±1.22 and it is 1200 m above sea level on a very flat expanse of land. It receives rainfall within two seasons of the year—between March and May, and November and December. Mean annual temperatures range from 27–30 and 18–27°C maximum and minimum respectively. There are high evapo-transpiration and percolation rates, thus during rains, water is quickly lost to the atmosphere and to the ground respectively. However, there are some pools of water collection in some areas which last for a longer period of time. The mean annual rainfall is 500 mm.

The agricultural activities in this area include horticulture (including growing of kales (*Brassica oleracea*), tomatoes (*Solanum lycopersicum*), mangoes (*Mangifera indica*) and hot pepper (*Capsicum* spp), livestock farming (cattle, goats and sheep mostly by free range husbandry) and subsistence farming (including growing of maize (*Zea mays)*, peas (*Pisum sativum)* and beans (*Pisum sativum*) mainly using the rain water.

Habitat type description: Larval habitats which were found within 2 km from the Kasayani village market center were sampled. This included: water reservoir tanks which were mainly rectangular in shape constructed with bricks and cement. They are mainly used to store water during construction and after they are converted to harvest rainwater. They are mainly open and about one meter high. Puddles were mainly small pools of water measuring <0.5 m in length and were mainly found within homesteads. Tyre tracks were mainly found along Kibwezi-Kitui Road. These were tyre marks found on the road as a result of the vehicles using this road. Temporary pools were mainly found on two valleys one on the upper side and other on the lower side of the village. The temporary pools were measuring >0.5 m in length.

Larval sampling: A cross-sectional larval sampling in December 2006 to generate stage-specific estimates of *Anopheles* and culicine larval densities and diversity was done. Further a second round of sampling was done in March 2007 in which all the habitats were re-visited. December is the peak of the rainy season in this area while March is the peak of dry season. Samples were taken using standard dipping techniques with a plastic dipper (BioQuip Products, Inc. California, U.S.A.). From each habitat type 2–10 dips were taken and the mosquito larvae collected and sorted to the sub-families as either anopheline or culicine. The anopheline and culicine larvae were grouped according to instars as early (L1 and L2), late (L3 and L4) and pupae. The late instars were preserved in 75% ethanol and identified morphologically[35, 36].

Data analysis: Statistical analysis was done using SPSS software (Version 15.0 for Windows, SPSS Inc., Chicago, IL). The mosquito larvae were grouped as per sub-family and instars and summed for each larval habitat. Variation in larval densities between habitats was compared by Student *t*-test. One-way analysis of variance (ANOVA) was used to compare the differences in the number of mosquito larvae in different habitats. Where significant differences were detected in ANOVA, the means were separated by Tukey test highly significant difference. Variation in diversity of habitat types was compared using chi-square test. Statistical analyses were done using log transformed ($\log_{10}$ n+1) larval counts to normalize the data. Results were considered significant at $p<0.05$.

Results

The habitat types found during this survey included water reservoir tanks (n = 3), puddles (n = 8), tyre tracks (n = 2) and temporary pools (n = 11) (Table 1). All these habitats were found to be dry at the second round of mosquito sampling. Further, observa-

Table 1. Mosquito larval abundance in the different habitat types in Kasayani village

Habitat type	n*	*Anopheles*		Culicine		Pupae
		Early instars	Late instars	Early instars	Late instars	
Tanks†	3	81	10	1221	289	11
Puddles	8	97	19	231	18	2
Tyre tracks	2	26	6	30	4	0
Pools	11	221	43	305	42	4
Total	24	425	78	1787	353	17

*Number of habitats sampled; †Water reservoir tanks

tion showed that puddles, tyre tracks and temporary pools had very turbid water while water reservoir tanks had clear water. Tanks were found within the homesteads and were mostly used to harvest rainwater during the rainy season but they were all concrete and open. Temporary pools were located at the lower parts of two valleys found transversing the village. One-way ANOVA showed that there was no significant variation in *Anopheles* larval densities ($F_{(1,3)}$ = 1.399, p = 0.272) in the habitats whereas the tanks had significantly higher densities for culicine than other habitat types ($F_{(1,3)}$ = 10.736, p < 0.001). A total of 2660 mosquito larvae were collected, of which 2140 (80.45%) were culicines, 503 (18.91%) were *Anopheles* and 17 (0.64%) were pupae. For culicines, 1787 (83.5%) were categorized as early instars and 353 (16.5%) as late instars while in the *Anopheles* 425 (84.49%) were classified as early and 78 (15.51%) as late instars. Only 17 pupae were collected during this survey and they were predominantly found in water storage tanks. The density of early instars was 5.46-fold (t = 2.65, df =1, p = 0.03) higher than late instars for *Anopheles* while it was relatively similar for culicines where the early instars were 5.21 higher than late instars (t = 1.83, df =1, p = 0.008).

During the sampling, six mosquito species were collected which included *Anopheles gambiae* complex 16.24% (n = 70), *An. funestus* 1.16% (n = 5), *An. coustani* 0.70% (n = 3), *Culex quinquefasciatus* 42.46% (n = 183), *Cx. duttoni* 6.26% (n = 27) and *Ae. aegypti* 33.18% (n = 143) (Table 2). *Anopheles gambiae* and *Cx. quinquefasciatus* were collected in all the habitat types while *Ae. aegypti* were found only in water reservoir tanks. *Anopheles funestus* and *An. coustani* were found only in puddles and pools while *Cx. duttoni* were found in water reservoir tanks, puddles and pools. Chi-square analysis indicated that anopheline and culicine larvae were more likely to coexist in the same habitats than would be expected by chance alone (χ^2= 46.208, p<0.01).

Discussion

This is the first entomological study in this study area which sought to understand the ecologies of mosquito larvae and mosquito species composition in this area. This study found *Anopheles* and *Culex* mosquito species together in the habitats. The habitats which were sampled in this study were formed after the onset of rains.

The habitats which were sampled during the study period included pools, puddles, tanks and tyre tracks.

Table 2. Species diversity in each habitat type

Habitat type	*An. gambiae*	*An. funestus*	*An. coustani*	*Cx. quinquefasciatus*	*Cx. duttoni*	*Ae. aegypti*
Tanks	10	0	0	140	6	143
Puddles	17	1	1	3	15	0
Tyre tracks	6	0	0	4	0	0
Pools	37	4	2	36	6	0
Total	70	5	3	183	27	143

Most of the *An. gambiae* larvae were collected in temporary pools and puddles. *Anopheles* mosquitoes have been shown to frequently occur in small temporary pools, such as pits, tyre tracks, and animal footprints[17,18,37,38]. In general, larval predation of mosquitoes is less prevalent in temporary habitats than it is in large, permanent habitats[39,40]. Because small and sunlit habitats have higher water temperatures, mosquito larval-pupal developmental time may be shortened if the warmer habitat produces more food resources. Culicine larvae were collected in diverse habitat types but were predominantly collected in tanks (water reservoir containers). The *Culex* mosquitoes breed in a wide range of habitats although they have been able to exploit same habitats as *Anopheles* mosquitoes. *Culex quinquefasciatus* is predominantly associated with urban areas but occurring also in rural. This Cosmo-tropical urban mosquito *Cx. quinquefasciatus* preferentially breeds in organically rich water. With both anopheline and culicine larvae occurring in similar habitats, there is a need to have larval control programmes targeting all the habitats available in this area. This would ensure maximum reduction of mosquitoes consequently significantly reducing the risk of mosquito-borne diseases. A potentially important target of malaria vector control is the immature stages of anopheline mosquitoes[4,6]. Source reduction through modification of larval habitats has been an important tool for malaria eradication efforts in the United States, Israel, and Italy[2]. Recently, WHO has issued a Global Framework for Integrated Vector Management (IVM), stressing the importance of evidence-based combinations of vector control methods[41].

Our study collected important vectors for malaria, filariasis and haemorrhagic fevers. These vectors included *An. gambiae, An. funestus* (malaria), *Cx. quinquefasciatus* (filariasis and yellow fever) and *Ae. aegypti* (haemorrhagic fevers). Malaria has been documented as a predominant mosquito-borne disease in this area[31–33,42]. Recently, an outbreak of Rift Valley Fever (RVF) was detected in late 2006 in the Garissa District of the north-eastern Province of Kenya and subsequently spread into other districts of Kenya and into Tanzania in early 2007, where it has caused considerable morbidity and mortality in both human and domestic ruminant populations[43]. Rift valley fever virus (RVFV) has been responsible for numerous outbreaks of severe disease in ruminants in sub-Saharan Africa over the past 70 years[44], and those outbreaks have been associated with excessive rainfall in sub-Saharan Africa[45]. Presence of some vectors incriminated in the transmission of RVFV, dengue fever, yellow fever and other haemorrhagic fevers exposes the inhabitants of this area to high risk of infection in the event of an outbreak. The transmission of malaria has been described in this area as seasonal[7,33,34] where higher number of cases are seen in December and in April coinciding with the peaks of long and short rains. Nevertheless, none of other mosquito-borne diseases has been described in the area despite the presence of their vectors. This calls for the public health advisory teams in this area to be alert especially during the rainy season and intensify the mosquito control campaign utilizing the integrated vector management approaches.

There is need for further entomological studies to describe the adult mosquito populations including their feeding, resting behaviours and role in disease transmission especially malaria which is the main mosquito-borne disease. Due to logistic problems we could not further analyze the *An. gambiae* Complex by polymerase chain reaction to their sibling species. In conclusion anopheline and culicine mosquitoes in these areas breed in temporary pools, puddles, tyre tracks and water reservoir tanks. The mosquito species present in this area predisposes the inhabitants of this area to the risk of infections of mosquito-borne diseases. This calls for an accelerated campaign of mosquito control in this area especially during the rainy season encompassing the integrated vector management approaches.

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