Distribution of Mosquito Larvae Within the Paddy and Its Implication in Larvicidal Application in Mwea Rice Irrigation Scheme, Central Kenya

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DISTRIBUTION OF MOSQUITO LARVAE WITHIN THE PADDY AND ITS IMPLICATION IN LARVICIDAL APPLICATION IN MWEA RICE IRRIGATION SCHEME, CENTRAL KENYA

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ABSTRACT. Distribution of mosquito larvae in inundated rice fields is poorly known despite its profound implications in implementation of vector control programs. Based on oviposition behavior of gravid females and biotic and abiotic conditions of the rice field, distribution of mosquito larvae within the paddy may vary greatly. As a guide to implementation of mosquito vector control program targeting the aquatic stages in the rice fields in Mwea, studies were conducted to determine the distribution of mosquito larvae within the paddy. Twenty-eight cages measuring 50 cm^3 were distributed randomly within the paddy during the transplanting stage of the rice growth cycle, and were examined twice per week up to the flowering stage to determine mosquito oviposition pattern. A total of 17,218 mosquito larvae were collected at the periphery and a further 17,570 at the center of the paddy. These comprised 7,461 larvae from the genus *Anopheles* and 27,327 from genus *Culex*. The number of pupae collected at the periphery was 1,004 and 1.5 times greater than the number collected at the center. Significantly higher counts of *Anopheles* larvae were collected at the center (1.00 ± 0.11) than at the periphery (0.55 ± 0.05) of the paddy during transplanting stage, but the difference was not significant during the tillering stage. In contrast, significantly higher numbers of *Culex* larvae were collected from the periphery (3.09 ± 0.39) than at the center (2.81 ± 0.24) of the paddy. More pupae were also collected at the center than at the periphery of the paddy. These findings indicate the distribution of *Anopheles* and *Culex* larvae in rice fields to be nonrandom; however, for successful achievement of an integrated vector control program targeting the diverse mosquito fauna occurring in rice fields, there is need to target the whole paddy for larvicidal application.

KEY WORDS Rice paddy, cage, transplanting, tillering, periphery, center larval distribution, malaria vector control

INTRODUCTION

Inundated rice fields in Africa provide ideal breeding sites for *Anopheles gambiae* s.s. Giles and *An. arabiensis* Patton, the principle vectors of malaria in Africa (White 1972). Due to their preference for open, sunlit pools (Surtees 1970, Gillies and Coetzee 1987), these vectors rapidly colonize recently flooded fields before declining in abundance as the rice grows and begins to cover the water surface (Snow 1983, Lindsay et al. 1991, Ijumba 1997). Depending on the number of rice cropping cycles, irrigated-rice cultivation may extend the breeding season of these vectors and hence increase the annual duration of malaria transmission.

In an attempt to incorporate vector productivity in malaria vector control programs, several studies have addressed the direct effect of the irrigation system, in particular water management on vector populations. In China (Pao Lingh 1984) and Indonesia (Snellen 1990) intermittent irrigation has been reported to contribute significantly in reduction of mosquito densities and is successfully utilized as a mosquito control strategy. Unfortunately, similar studies in central and western Kenya found intermittent irrigation to be insignificant in mosquito control (Grainger 1947). Periodic flooding of rice fields also resulted in seasonal increase in the populations of *An. arabiensis*, and *An. pharoensis* Theobald (Mukiama and Mwangi 1990, Mwangi and Mukiama 1992). Urgent mosquito control strategies are therefore required in these areas if the benefits accrued to rice cultivation are to be realized.

Proper identification of areas within the paddy where mosquito larval development occurs is an essential prerequisite for the success of any larval control program in a rice agroecosystem. However, studies on dispersion of mosquito larvae within the paddy are very rare. Biotopes occupied by the immature stages of a mosquito species are evidence of the oviposition sites used by females of these species (Clements 1999) and studies of specific larval habitats are therefore good starting points for gaining understanding of oviposition choices of gravid females. The larval distribution within a paddy may vary depending on the ovipositional behavior of gravid mosquitoes and physicochemical and biotic condition of the

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paddy. The objective of this study was to access the larval distribution within a rice field prior to application of mosquito larvicidal control program. The study is designed to generate data, which is key in determining the extent in which the *Bacillus thuringiensis israelensis* de Barjac/*B. sphaericus* Neide would be applied within the paddy in Mwea Irrigation Scheme during the 1st wide-scale mosquito microbial control program in Kenya.

MATERIALS AND METHODS

Study area

The study was conducted in the Mwea Rice Irrigation Scheme (MIS) (00°67′S, 37°35′E), at the Mwea Irrigation and Development Center (MIAD) rice farms. Mwea Irrigation Scheme is located in Mwea division in Kirinyaga District, 100 km northeast of Nairobi. The study area has been described previously (Mutero et al. 2004a, Muturi et al. 2006, Mwangangi et al. 2006a). The Mwea Rice Scheme occupies the lower-altitude zone of Kirinyaga District in an expansive low-lying area characterized by black cotton soil. The annual rainfall varies from a maximum of 1,626 mm to a minimum of 356 mm, with an average of 950 mm per year. The average temperatures are 21.3°C (range: 16.0–26.5°C) and the relative humidity averages 59.5% (range: 52–67%). According to the 1999 national census, Mwea division has an estimated 150,000 persons in 25,000 households. The Mwea Irrigation Scheme is located in the west central region of Mwea division and covers an area of about 13,640 ha. Over 50% of the scheme area is used for irrigated rice cultivation while the remaining area is used for subsistence farming, grazing, and community activities.

Mosquito sampling

Cages measuring 50 × 50 × 50 cm were randomly placed within the paddy to determine the distribution of mosquito larvae in different parts of the paddy. The lower side of each cage was covered with iron sheet up to a height of 30 cm to prevent movement of enclosed immature mosquitoes in and out of the cage. The cages were pushed firmly into the mud, leaving surfaces of about 10–15 cm that would not allow overflow of water out of the cages. The area enclosed by the cage had the normal vegetation found within the paddy, which included the rice plant, *Oryza sativa* L., and any other floating and submerged vegetation such as *Azolla microphylla* Kaulfess. These vegetations are essential for the mosquitoes that may require a surface to rest before the oviposition. The cages were examined twice per week (Monday and Thursday) to examine the oviposition preference of mosquitoes within the paddy. On day 0 (day of placement), the cages were randomly placed at the periphery and center, but no counts of larvae were made. The cages were visited again 4 days later for larval sampling. On every visit, mosquito larvae were collected with the use of standard dipping technique with a 350-ml dipper (Clarke Mosquito Control Service and Supplies, Roselle, IL). From each cage, a maximum of 10 dips were taken, but this varied depending on the amount of water enclosed in the cage. The contents of all dips from each cage were concentrated by passing each dip through a fine mesh. The contents were then backwashed in a white tray. The mosquito larvae in the tray were age graded as early instars (1st and 2nd instars), late instars (3rd and 4th instars) and pupae, counted and scored as either anopheline or culicine (consisting of genera *Culex*, *Aedes*, *Mansonia*). After counting they were returned back to the cages. Recent studies have shown that the immature composition is made of *An. arabiensis*, *An. pharoensis*, *An. funestus* Giles, *An. coustani* Laveran, *An. maculipalpis* Giles, *Culex quinquefasciatus* Say, *Cx. annulioris* Theobald, *Cx. poicilipes* Theobald, *Cx. tigripes* De Grandpre and De Charmoy, *Cx. duttoni* Theobald, *Aedes aegypti* L., *Ae. cumminsii* Theobald, and *Ae. vittatus* Bigot (Mwangangi et al. 2006b, Muturi et al. 2007).

Most paddies in the Mwea Irrigation Scheme measure 40 × 80 m, and consequently the paddies used in this study had similar dimensions. The periphery was defined as 1 m from the edge of the habitat, whereas the center was defined as a distance of 20 m from the edge. Within the paddy 15 cages were placed at the periphery and 13 cages were placed at the center. The cages were placed between the transplanting phase (early vegetative stage) and the reproductive phase. We assumed that the development of rice was homogeneous within the paddy.

Paddy characterization

Rice growth cycle was characterized with the use of the standard agronomic categories for Basmati 217 into 5 categories, namely: field preparation, vegetative phase (consisting of transplanting and tillering, 55 days), reproductive phase (booting, meiosis, heading, panicle development, and flowering, 35 days), ripening phase (30 days), and postharvest phase. The agronomic activities involved in growing of Basmati 217 include application of triple superphosphate basal fertilizers during transplanting, top-dressing with nitrogenous fertilizers (sulphate of ammonia) at day 10 and day 35 posttransplanting, application of herbicides (Satunil) at day 14 posttransplanting and application of the insecticide, fenitrothion. The placement of the

Table 1. The distribution of anopheline and culicine larvae in the cages at the periphery and the center.

Cage position	*Anopheles* only (%)	Culicine only	Both groups present	Both groups absent	Total
Periphery	84 (15.8)	65 (12.2)	193 (36.3)	190 (35.7)	532
Center	74 (15.2)	64 (13.1)	235 (48.2)	115 (23.6)	488
Total	158 (15.5)	129 (12.6)	428 (42.0)	305 (29.9)	1,020

cages and the sampling was done between transplanting stages and early reproductive stage of the rice crop.

Statistical analysis

The statistical analyses were done with the use of SPSS software (Version 11.5 for windows, SPSS Inc., Chicago, IL). Relative abundance of mosquito larvae per cage was derived by dividing the number of larvae collected per cage by the number of dips taken on each sampling occasion. Larval counts were log transformed $\log_{10}(x + 1)$ to stabilize the variance and improve normality of distribution. Chi-square test was used to show the differences in the presence or absence of both the *Anopheles* and culicine larvae at the cages at the center and periphery. One-way analysis of variance (ANOVA) was used to compare the differences in larval counts between the periphery and the center of the paddy.

RESULTS

The cages were visited for sampling on 1,114 occasions of which 94 times (8.4%) were found to be dry whereas 1,020 times (91.6%) had water. The cages at the periphery were sampled 532 times, whereas center cages were sampled on 488 occasions. The cages at the center were found dry in 36 occasions (6.9%) and (93.1%). There was a no significant difference between the presence of water in the cages at the periphery and the center ($\chi^2 = 3.14$, $P = 0.076$) during the study period.

The *Anopheles* larvae were found at the cages in the periphery on 30.7% ($n = 84$) of all visits and on the center 39.2% ($n = 74$) of all visits. Table 1 shows the distribution of anopheline and culicine larvae in the cages at the periphery and the center. Both types of mosquito larvae were found absent in the cages at periphery on 69.3% occasions ($n = 190$) and at the center 60.8% ($n = 115$). The cages at the center had anopheline larvae coexisting together with culicines on 78.6% of the occasions ($n = 235$), whereas at the periphery they coexisted on 74.8% of the occasions ($n = 193$). Culicines only were found at the cages at the periphery on 25.2% of the occasions ($n = 65$), whereas they were found at the center on 21.4% of the occasions ($n = 64$). The differences in the presence or absence of both the anopheline and culicine larvae at the cages both at the center and periphery were highly significant ($\chi^2 = 262.24$, $P < 0.001$).

A total of 17,218 mosquito larvae were collected at the periphery and a further 17,570 at the center of the paddy. These comprised 7,461 larvae from the genus *Anopheles* and 27,327 from genus *Culex*. The number of pupae collected at the periphery was 1,004, which was 1.5 times greater than those collected at the center ($n = 650$, $F_{(1,27)} = 3.774$, $P = 0.061$). The relative abundance of early and late instars of *Anopheles* was significantly higher at the center than at the periphery of the paddy ($F_{(1,27)} = 10.472$, $P = 0.003$). Conversely, significantly higher numbers of early instars of genus *Culex* were collected at the periphery than from the center those of late instars did not vary significantly between the 2 paddy locations ($F_{(1,27)} = 0.070$, $P = 0.792$). *Culex* larvae were collected in significantly higher numbers both at the center and at the periphery of the paddy than the *Anopheles* larvae ($F_{(142,971)} = 5.270$, $P < 0.001$). Table 2 shows the larval and pupal densities of mosquito collected during the sampling period. From this table, the densities of larvae and pupae were higher for both anopheline and culicine at the center than at the periphery. The densities of anopheline and culicine larvae at the periphery and center increased between transplanting and tillering but decreased during flowering. The pupal densities for both anopheline and culicine mosquitoes was highest during the tillering stage. There was significant difference for densities of early instars of *Anopheles* ($F_{(65,1048)} = 1.490$, $P = 0.008$) and culicine early instars ($F_{(137,976)} = 1.225$, $P = 0.05$) between the center and periphery, whereas there was no significant difference between the densities of late-stage larvae of *Anopheles* ($F_{(40,1073)} = 1.268$, $P = 0.125$) and *Culex* ($F_{(59,1054)} = 1.488$, $P = 0.11$) between the center and periphery. The densities of pupae were significantly difference between the center and periphery ($F_{(37,1076)} = 1.601$, $P = 0.013$). Further the results show that the larval densities were higher at the transplanting and tillering stage both at the center and at the periphery but were lower at the flowering stage. Two-way ANOVA showed that the location of the cage location was significant ($F_{(1,26)} = 41.453$, $P < 0.001$) and the rice growth stage was also significant ($F_{(2,26)} = 86.983$, $P < 0.001$) for *Anopheles* larval densities and the interactions

Table 2. Densities of mosquito immature stages collected in the cages during the rice growth cycle (standard error).

Cage position	Rice stage	*Anopheles* early instars	*Anopheles* late instars	*Culex* early instars	*Culex* late instars	Pupae
Periphery	Transplanting	57.53 ± 0.12	26.50 ± 0.05	633.27 ± 1.45	98.60 ± 0.45	11.80 ± 0.02
	Tillering	121.48 ± 0.05	38.03 ± 0.03	756.15 ± 0.37	119.24 ± 0.12	50.54 ± 0.03
	Flowering	37.20 ± 0.05	11.56 ± 0.01	31.89 ± 0.06	14.31 ± 0.02	8.45 ± 0.01
	Subtotal	216.21 ± 0.04	76.08 ± 0.02	1,421.30 ± 0.31	232.15 ± 0.09	70.79 ± 0.02
Center	Transplanting	121.30 ± 0.26	61.70 ± 0.11	295.10 ± 0.91	21.10 ± 0.07	18.70 ± 0.03
	Tillering	182.65 ± 0.12	57.08 ± 0.04	735.77 ± 0.27	200.93 ± 0.10	77.52 ± 0.04
	Flowering	51.50 ± 0.05	14.90 ± 0.02	69.40 ± 0.08	45.80 ± 0.08	18.50 ± 0.03
	Subtotal	355.45 ± 0.08	133.68 ± 0.03	1100.27 ± 0.21	267.83 ± 0.06	114.72 ± 0.03

between the cage location and rice stage was also found to be significant ($P < 0.001$).

DISCUSSION

This study sought to understand the distribution of mosquito larvae within the paddy, which would form the basis for larvicidal control products in Mwea Irrigation Scheme. The mosquito larvae were distributed in the paddy both at the periphery and at the center, although the center had more *Anopheles* larvae than the periphery. Because of the unevenness in the leveling of the paddy, water tended to accumulate more at the center of the paddy than the periphery. The random distribution of mosquito larvae both at the periphery and the center indicates that both locations are preferred oviposition sites. Identification of suitable ovipositional sites by mosquitoes is a critical feature of their life history, because it ultimately influences the survivorship of their progeny. Empirical observations show that different mosquito species prefer particular types of ovipositional sites, and it is likely that they are guided by combinations of cues. For example, physical cues such as light intensity, temperature, presence/absence and type of vegetation, wetness, water movement, and soil surface characteristics (Clements 1999) strongly influence where female mosquitoes deposit eggs. The center had more *Anopheles*, whereas the periphery had higher densities of *Culex* larvae. These differences may be due to the differences in abiotic and biotic factors within a paddy, which resulted in the differences in the variation in larvae between the center and the periphery. *Anopheles* and *Culex* larvae occupy water with different physicochemical properties. For example, *An. gambiae* larvae were associated with highly turbid aquatic habitats that were devoid of aquatic vegetation and surface film, and persisted for short periods (Gimnig et al., 2001) whereas culicine larvae are associated with older, stable habitats that have vegetation and algae.

This study found out that *Anopheles* and *Culex* larvae coexisted within the paddy habitats. Most of the habitats sampled were inhabited by the 2 species occurring together. Anopheline and culicine larvae were found only in few occasions existing alone in the cages. Although the data indicate that anopheline and culicine mosquito larvae utilize the same habitat for their development, variation in the time of attaining peak densities is apparent. Current studies (Muturi et al. 2006) show that both anopheline and culicines were collected together as adults in houses within Mwea Irrigation Scheme, suggesting that the production of these mosquitoes occur from similar habitats. At the beginning of the rice growth cycle (transplanting), the anopheline and culicine larval densities were high, and increased to the highest densities during the vegetative stages. During the flowering stage, the densities of both species declined significantly. *Anopheles arabiensis* is the common species in the Mwea Irrigation Scheme (Mutero et al. 2004a, Mwangangi et al. 2006b) and likes to breed in open and sunlit pools (Gillies and Coetzee 1987, Gimnig et al. 2001, Mutero et al. 2004a). This makes the habitats suitable for colonization by *An. arabiensis* immediately after rice transplant. During the vegetative stage of rice growth, both anopheline and culicine mosquitoes coexist in the aquatic habitats. At this time the habitats are more stable and the addition of nitrogenous fertilizers (at day 10 and day 35 posttransplanting) acts as an oviposition attractant for both anopheline and culicine mosquitoes (Victor and Reuben 2000, Mutero et al. 2004b). As the rice increases in height, there is increase in canopy coupled with changes in both abiotic and biotic factors, which results in decrease in oviposition preference by *Anopheles* mosquitoes. Culicine mosquitoes can oviposit in shaded habitats, which are an indication of older and more permanent habitats; consequently these mosquitoes are found in higher densities at the late vegetative and flowering stages of rice development. The coexistence of *Anopheles* and culicine mosquito means that these 2 species can both be targeted together during the mosquito microbial larvicidal program in the Mwea Irrigation Scheme, therefore reducing the malaria transmission and biting nuisance within the villages in the scheme.

The study found out that there was no significant difference in mosquito larval abun-

dance during the tillering stage. The tillering stage has been found to be highly associated with the highest larval densities because of the addition of nitrogenous fertilizers at this stage. The larval composition is same at the center and the edge. For effective control of mosquito larvae, the larvicidal agents should be applied at the entire plot at the tillering stage. This ensures a maximum reduction in the larval densities. The present study further showed that there was a significant increase of larval abundance from transplanting to tillering, but after the tillering stage there was a significant reduction in larval densities (Mulla et al. 1990, Mutero et al. 2000).

Rice fields generally constitute an important source of a number of mosquito-borne diseases (Lacey and Lacey 1990). To enhance agricultural production while at the same time avoiding negative effects on public health, a closer collaborative action between health and agriculture sectors in planning and execution of irrigation schemes is required in order to reduce vector density. To address the problem of mosquito-borne diseases through larval control interventions, different propositions have been made. (Fillinger et al. 2004) suggested the need to consider all potential larval habitats for the larval control intervention, whereas Gu and Novak (2005) argued for the need to identify all potential larval habitats and then direct larval control efforts to the most productive habitats. Although these studies differ in their final approach toward achieving effective larval control interventions, they accentuate the need for proper identification of all aquatic habitats in which mosquitoes thrive as a guide for vector control operations. Mwea is currently targeted for microbial control of immature stages of malaria vectors; there is an opportunity to increase the benefits of the program by targeting all mosquito species present in the area. This will not only reduce the risk of mosquito-borne diseases but will also be appreciated by the community, because biting nuisance will also be reduced. In conclusion, the mosquito larvae were randomly distributed in the whole paddy. For effective control of the mosquito larvae, the larvicide should be applied at both the center and the periphery of the paddy, which would enhance the reduction of both anopheline and culicine larvae. The application should be made at the early vegetative stage (within the 3-wk window of productivity following transplanting of rice, [Mwangangi et al. 2006b]) to ensure maximum mosquito larval reduction. The larvicidal agent should last until the flowering stage. Further, a targeted plan should be used wherein the rice cultivation cycle is planned and rice transplanting is synchronized, thereby minimizing the duration of time the rice fields are under rice cultivation.

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