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CENTRAL KENYA**

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ENVIRONMENTAL COVARIATES OF *ANOPHELES ARABIENSIS* IN A RICE AGROECOSYSTEM IN MWEA, CENTRAL KENYA

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ABSTRACT. Water quality of aquatic habitats is an important determinant of female mosquito oviposition and successful larval development. This study examined the influence of environmental covariates on *Anopheles arabiensis* mosquito abundance in the Mwea Irrigation Scheme, Central Province of Kenya, prior to implementation of a malaria vector control program. Experimental rice plots were used to examine the environmental covariates responsible for regulating abundance and diversity of the aquatic stages of malaria vectors. Mosquito larval sampling and water quality analysis were done weekly from the flooding stage to the rice maturation stage. Sampling for mosquito larvae was conducted using standard dipping technique. During each larval collection, environmental covariates such as pH, temperature, conductivity, salinity, dissolved oxygen, water depth, and rice stage were measured. *Anopheles arabiensis* larval density was highest between 1 wk before transplanting and 4 wk after transplanting with peaks at weeks 0, 3, and 8. The fluctuation in values of the various environmental covariates showed characteristic patterns in different rice growth phases depending on the changes taking place due to the agronomic practices. Using a backward linear regression model, the factors that were found to be associated with abundance of *An. arabiensis* larvae at any of the rice growing phases included the following: dissolved oxygen, pH, turbidity, water depth, rice height, number of rice tillers, salinity, conductivity, and temperature. The environmental covariates associated with abundance of *An. arabiensis* were associated with early vegetative stage of the rice growth. For effective control of developmental stages of mosquito larvae, the application of larvicides should be done at the vegetative stage and the larvicides should persist until the beginning of the reproductive stage of the rice.

KEY WORDS Larval habitat, environmental covariates, *Anopheles arabiensis*, larval density, rice growth stages

INTRODUCTION

For larval control to be an integral part of a vector management program, a sound understanding of the factors responsible for larval activity of principal vectors of malaria is crucial (Molineaux 1997). Aquatic water quality is an important determinant of whether gravid female mosquitoes will oviposit and, consequently, whether the resulting larval stages will develop successfully into adult stage. Knowledge of the influence of habitat factors on larval production is critical for understanding the spatial and

temporal distribution patterns of the anopheline species, and in planning and implementing appropriate larval control strategy. In Kenya and Tanzania, *Anopheles arabiensis* Patton is the predominant malaria vector and the only member of *An. gambiae* s.l. present in inland areas with rice cultivation (Ijumba et al. 1990, Mutero et al. 2000, Mutero et al. 2004a); its peak population coincides with the transplanting of rice (*Oryza sativa* L.) seedlings (Mutero et al. 2000).

The distribution and abundance of mosquito larvae reflect the oviposition preferences of adult females and ability of the immature stages to tolerate the conditions that prevail in the aquatic habitats (Reisen et al. 1981). Water in the rice fields shows a marked variation in both physical and chemical composition during the rice crop cycle (Roger and Kurihara 1988). These changes in water quality have a strong influence on the abundance of mosquitoes in the rice fields. Earlier workers have evaluated the influence of physico-chemical variables on the larval densities on rice agroecosystem. Field operations such as weeding and water management regimens have a transient effect on larval densities (Rajendran 1987). Fertilizer application, especially topdressing with nitrogenous fertilizers, was found to increase mosquito larval populations (Simpson and Roger 1991, Victor and Reuben 2000, Sunish and Reuben 2001, Mutero et al. 2004b). Larval

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densities have also been shown to be influenced by water depth, temperature, pH, ionic composition, and conductivity, which consequently affect the larval development rate (Mogi 1978, Kramer and Garcia 1989). This study examined the influence of environmental covariates on the abundance of *An. arabiensis* mosquitoes in the Mwea Irrigation Scheme (MIS), Central Province of Kenya, prior to implementation of a larval control program.

METHOD & MATERIALS

Study site

The study was done in the MIS in the Kirinyaga district, approximately 100 km north-east of Nairobi, Kenya. This area has previously been described by Ijumba et al. (1990), Asimeng and Mutinga (1993), and Mutero et al. (2004a). The Mwea scheme is in a low-lying area of the Kirinyaga district with an altitude of 1,100 m above sea level and characterized by black cotton soil. The mean annual rainfall is 950 mm, with maximum amount falling in April–May (long rains) and October–November (short rains). The average temperatures are in the range of 16–26.5°C. Relative humidity varies from 52% to 67%.

Rice cultivation

In the MIS tenant farmers practice “planned” rice growing; the beginning of each cropping cycle is scheduled according to a predetermined water distribution plan. Most fields are cultivated once a year (main crop), but farmers often opt for a 2nd (ratoon) crop immediately after the main harvest. The typical cultivation cycle includes land preparation (cultivation/rotavation), nursery development (June–July), a sowing–transplanting phase (August), a vegetative phase (August–October), a flowering phase (October–November), and a maturation phase (November–December). Harvesting is done between mid-December and early January. The 2nd crop is cultivated between January and May. The duration of the rice cycle varies between 120 and 150 days depending on rice variety, and includes a vegetative period when plants are growing, a reproductive phase during which plants stop growing and orient toward the development of the panicles and grains, and a ripening phase, in which plants senesce and their water content drops. Rice plants are usually transplanted at 20–30 days old, and the vegetative phase lasts 45–60 days. The vegetative phase includes seedling transplanting, tillering, and stem elongation stages. The vegetative phase extends from the appearance of the 1st tiller until maximum tiller number is reached. During stem

elongation, the tillers continue to increase in number and height, with increasing ground cover and canopy formation. The reproductive phase lasts 20–30 days and includes the panicle initiation, booting, heading, and flowering stages. Plants are considered to be in the reproductive phase when more than 50% of plants have panicles. Finally, the ripening phase lasts 35–65 days, during which the grains fill and turn yellow and the plants senesce.

During the transplanting day the rice fields in the MIS are usually broadcasted with triple super phosphate (TSP). Ten days and 35 days post-transplanting, the fields are topdressed with sulfate of ammonia.

Mosquito larval sampling

At the experimental plots, a weekly larval sampling was done to generate stage-specific estimates of *Anopheles* larval abundance. This study was carried out between August 2004 and March 2005. The experimental plot was developed at the Mwea Irrigation and Agricultural Development Center experimental station, MIS. The experimental rice plots used for this study have previously been described (Mwangangi et al. 2006b). Briefly, 1 rice test plot (1 acre; 63 m × 63 m) was established in the MIS. Within each 1-acre plot, 10 blocks (50.4 m × 3.15 m), each with 10 subblocks (6.3 m × 3.15 m) were established. Each block was hydrologically isolated using unidirectional inflow and outflow canals to avoid mixing between plots. The plots were exposed to natural colonization of *An. gambiae* complex. The plots were maintained with a water level of approximately 10 cm throughout the rice growing stages. Samples were taken using standard dipping technique once every week. Twenty dips were taken from each subplot.

The mosquito larvae collected were sorted out according to the subfamilies as either anopheline or culicine. The 3rd and 4th anophelines were identified morphologically using taxonomic keys (Gillies and Coetzee 1987). The *An. gambiae* s.l. was further identified to the specific sibling species using a rDNA polymerase chain reaction (PCR) method (Scott et al. 1993).

Measurement of environmental covariates

In the experimental plots, several environmental covariates were measured, including rice height, number of tillers, floating vegetation, turbidity, pH, conductivity, dissolved oxygen, temperature, salinity, total dissolved solids (TDS), nitrates, phosphates, ammonia, and sulfate content. Turbidity was measured by placing water samples in a glass test tube and holding it against a white background; turbidity was

Table 1. Mean *Anopheles arabiensis* larval densities collected at different rice growth cycle (\pm SE)

Rice stage	Early instars	Late instars	Pupae
Land preparation phase	0.99 \pm 0.18	0.19 \pm 0.05	0.01 \pm 0.005
Transplanting phase	1.39 \pm 0.17	0.23 \pm 0.04	0.06 \pm 0.02
Vegetative phase	0.54 \pm 0.09	0.11 \pm 0.02	0.011 \pm 0.004
Reproductive phase	0.31 \pm 0.04	0.05 \pm 0.01	0.01 \pm 0.003
Maturation phase	0.12 \pm 0.03	0.02 \pm 0.01	0.01 \pm 0.01
Total	0.67 \pm 0.06	0.12 \pm 0.01	0.02 \pm 0.004

classified into 3 levels: clear, low, and high. Rice height was measured from the same point every sampling time using a 1-m ruler. Floating vegetation was estimated as percentage covered using a 1-m² grid. The pH, conductivity, dissolved oxygen, and temperature were measured using the handheld YSI 650 Multiparameter Display System (YSI Environmental, YSI Incorporated, Yellow Springs, OH). Salinity and TDS were measured in the field using the handheld YSI EC 300 (YSI Environmental). Nitrates, phosphates, ammonia, and sulfate content were measured using spectrophotometric technique using a portable HACH machine (HACH®, DR/2400 spectrophotometer, Hach Company, Ames, IA). All environmental covariates were measured on site at the time of mosquito larval sampling. Three points were taken in each selected subplot to determine the environmental covariates during the mosquito larval sampling time and the average was recorded to represent the reading for the subplot.

Statistical analysis

The statistical analyses were done using SPSS software (Version 11.5 for Windows, SPSS Inc., Chicago, IL). A 1-way ANOVA was used to compare *An. arabiensis* larval densities among the 5 rice-growing phases and a Tukey honestly significant difference test was used to separate the means. Multiple linear regression analysis by the backward elimination method was used to determine the best predictor variables explaining the densities of *An. arabiensis*. This meant that variables in each equation were removed in each step of the method, if their adjusted partial correlation coefficient was no longer significant ($P > 0.05$). Three separate multiple regression equation (for the early, late, and pupae stages) were obtained for each of the 5 rice-growing phases. Statistical analyses were done after a log transformation, $\log_{10}(n + 1)$, of *An. arabiensis* larval abundance values to normalize the distribution and minimize the standard error.

The larval densities of *An. arabiensis* were defined as the total number of larvae collected in each sampling time divided by the total number of dips (larvae/dips). The *An. arabiensis* larvae collected were age-graded and categorized as

early instars (consisting of instars I and II), late instars (instars III and IV), and pupae and these were considered as dependent variables in the analysis. The environmental covariates measured were taken as independent variables in the model.

RESULTS

The *Anopheles* larval densities increased during transplanting and decreased slightly during the vegetative phase. Larval densities decreased further during the reproductive phase until the maturation phase (Table 1). The 1-way ANOVA comparing *An. arabiensis* larval densities among the rice growing phases showed significant variation ($F_{[4, 359]} = 28.577, P < 0.001$). There was an increase of larval densities between land preparation and the transplanting phase. The larval densities peaked during the transplanting phase and declined in the vegetative phase. This decline in larval densities continued until the maturation stage, which had the lowest abundance.

Identification of the late-stage larvae yielded *An. arabiensis* (76.90%, $n = 1,774$), *Anopheles pharoensis* (20.07%, $n = 463$), *Anopheles rufipes* (1.78%, $n = 41$), *Anopheles funestus* (0.65%, $n = 15$), *Anopheles coustani* (0.48%, $n = 11$) and *Anopheles maculipalpis* (0.13%, $n = 3$). Further analysis of 352 morphologically identifiable *An. gambiae* s.l. larvae by rDNA PCR technique confirmed that all were *An. arabiensis*. The majority of the *An. arabiensis* were collected between land preparation and vegetative phases of rice-growing (78.52%, $n = 1,393$).

There was weekly variation in *An. arabiensis* larval densities from the land preparation phase through the maturation phase (Fig. 1). Larval abundance was highest 1 wk before and 4 wk after transplanting. There was a peak in larval abundance at week 8, but it declined almost immediately the following week, and the decline continued until week 14. Unlike early-stage instars, the densities of late larval instars and pupae were lower but followed similar weekly distribution pattern during land preparation through maturation phases of rice cultivation. Overall, the peaks of larval densities were seen at week 0, week 3, and week 8 of rice cultivation. These peaks were preceded by application of fertilizers: at transplanting TSP was applied;

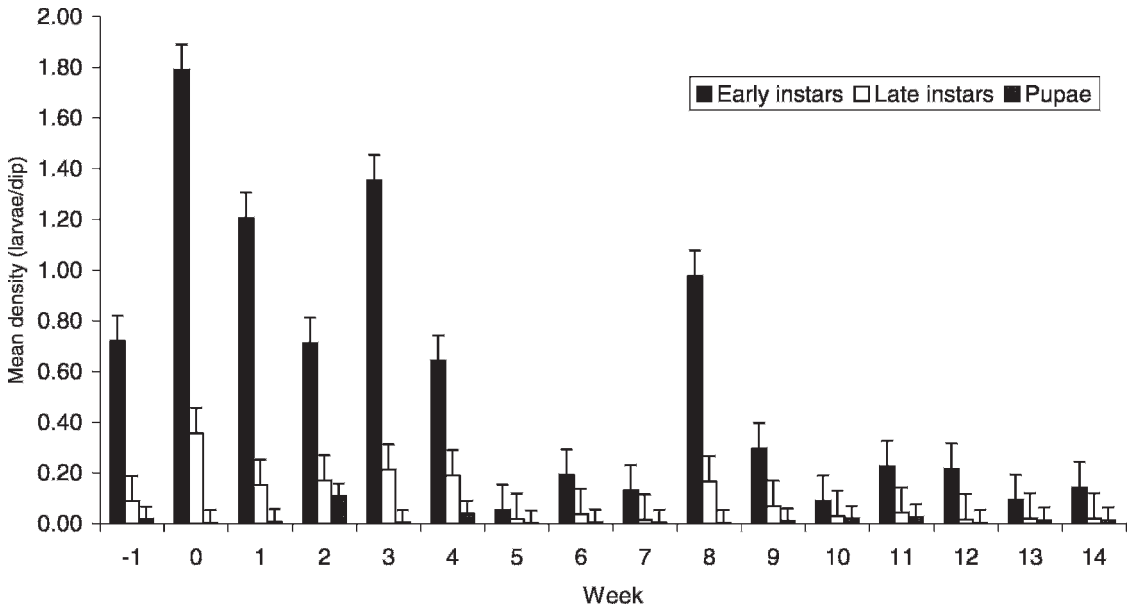


Fig. 1. The *Anopheles arabiensis* larval variation from land preparation to rice maturation stage.

10 days posttransplanting (i.e., week 2) and at week 7 (35 days posttransplanting), sulfate of ammonia was applied.

A familiar pattern in values of the various environmental covariates fluctuated during different rice growth phases (Table 2). Rice height increased steadily from transplanting to maturation stage (from 34.83 cm at transplantation stage to 101.67 cm at maturation stage). Similarly, the number of tillers increased steadily. Water temperature was highest between land preparation and transplanting phases and declined in subsequent phases. Water pH was highest at the transplanting stage and lowest at the maturation stage (pH range: 7.04–8.07). Dissolved oxygen concentration was higher at land preparation and transplanting phases. Salinity was highest between the transplanting phase and the vegetative phase. The nitrate, sulfate, and nitrogen ammonia were highest between the transplanting and vegetative stages but were lowest at the maturation stage.

Several environmental covariates influenced the abundance of *An. arabiensis* immatures at different stages of rice growing cycle. Beta-coefficient values relative to the significant individual environmental covariates for each of the rice growing phases are shown in Table 3. Factors associated with abundance of *An. arabiensis* larvae at any of the rice growing phases included dissolved oxygen, pH, turbidity, water depth, rice height, number of rice tillers, salinity, conductivity, and temperature. Larval abundance during land preparation was positively associated with temperature and pH, whereas at transplanting phase key predictors of late instar and pupae

abundance were water depth, temperature, dissolved oxygen, and turbidity (Table 3). During the vegetative stage, larval abundance was negatively associated with rice height and dissolved oxygen, but positively associated with temperature. During the reproductive stage, *An. arabiensis* larval abundance was negatively associated with rice height and pH, whereas it was positively associated with turbidity and number of rice tillers. During the maturation phase, *An. arabiensis* larval densities were positively associated with number of rice tillers, turbidity, pH, and conductivity, whereas it was negatively associated with rice height, salinity, and dissolved oxygen.

DISCUSSION

This study examined the influence of environmental covariates in the abundance of *An. arabiensis* mosquitoes in MIS, Central Province of Kenya. Larval abundance was found to be highest between the transplanting and vegetative stages. Water temperatures at the experimental plots were highest during these stages of the rice growth cycle. When the rice was at the reproductive stage, water temperatures declined subsequently. A number of workers have documented the effects of temperature on the development and relative abundance of riceland mosquito larvae (Lacey and Lacey 1990). Our results further indicated that water temperature was an important factor for the abundance of *Anopheles* larvae. During the transplanting and vegetative stages, there are many open spaces between the plants, which provide conducive

Table 2. Mean \pm SE of the environmental covariates in rice growing plots

Physicochemical variable	Land preparation	Transplanting	Vegetative	Reproductive	Maturation
Rice height (cm)	0.00 \pm 0.00	34.83 \pm 0.62	55.89 \pm 0.91	96.33 \pm 2.14	101.67 \pm 2.93
Tillers	0.00 \pm 0.00	2.04 \pm 0.14	11.49 \pm 0.47	21.59 \pm 0.73	22.07 \pm 0.96
Temperature ($^{\circ}$ C)	29.30 \pm 0.44	27.13 \pm 0.32	25.95 \pm 0.27	24.56 \pm 0.25	24.79 \pm 0.16
Conductivity (mS/cm)	45.72 \pm 2.23	58.40 \pm 2.97	77.32 \pm 3.99	52.08 \pm 4.42	48.43 \pm 4.00
Salinity (ppt)	30.26 \pm 1.69	40.63 \pm 2.92	57.72 \pm 4.11	35.50 \pm 3.55	32.14 \pm 3.07
Dissolved oxygen concentration (mg/liter)	5.10 \pm 1.19	3.74 \pm 0.68	2.67 \pm 0.25	0.03 \pm 0.00	0.04 \pm 0.01
pH	7.54 \pm 0.03	8.07 \pm 0.09	7.51 \pm 0.03	7.36 \pm 0.08	7.04 \pm 0.04
Nitrate (mg/liter)	13.60 \pm 0.05	25.90 \pm 4.99	12.64 \pm 5.09	5.43 \pm 2.32	3.20 \pm 1.24
Sulfate (mg/liter)	80.00 \pm 5.28	54.40 \pm 11.00	68.22 \pm 28.59	10.00 \pm 5.03	7.53 \pm 3.05
Nitrogen ammonia (mg/liter)	0.28 \pm 0.01	4.10 \pm 0.81	0.14 \pm 0.02	0.12 \pm 0.09	0.08 \pm 0.01

conditions for mosquito larval development. When the rice plants achieved maximum vegetation, water temperatures declined because the shade of the rice canopy blocked direct sunlight. *Anopheles arabiensis* is the most predominant species in the MIS (Mutero et al. 2000, Mwangangi, Muturi, et al. 2006, Mwangangi, Shililu, et al. 2006) and has been previous shown to have

a preference for shallow and exposed ground pools (Gillett and Smith 1972).

Depth was found to be positively associated with *An. arabiensis* larval abundance. Previous studies found water depth to have an influence on larval densities (Chandler and Highton 1976, Rao 1984, Palchick and Washino 1985). Rice fields in the MIS are maintained at 5–10 cm water depth

Table 3. Multiple linear regression for the environmental covariates measured during the rice growth cycle

Rice growing phase	Instars	Variables	Coefficient	<i>t</i>	
Land preparation (<i>n</i> = 70)	Early	None			
	Late	Temperature	0.452	2.944	
	Pupae	pH	0.356	2.691	
Transplanting (<i>n</i> = 70)	All ¹	None			
	Early	None			
	Late	Water depth	0.280	2.363	
		Temperature	0.289	2.303	
		Dissolved oxygen	0.480	2.047	
	Pupae	Turbidity	0.362	2.781	
Vegetative (<i>n</i> = 175)	All ¹	Temperature	0.291	2.451	
	Early	Rice height	-0.303	-2.722	
	Late	Rice height	-0.241	-2.722	
	Pupae	Temperature	0.192	2.320	
		Dissolved oxygen	-0.249	-2.756	
	All ¹	Rice height	-0.304	-2.745	
Reproductive (<i>n</i> = 95)	Early	Rice height	-0.380	-2.890	
		No. of tillers	0.392	3.568	
		Turbidity	0.228	2.146	
	Late	Rice height	-0.314	-2.332	
		pH	-0.290	-2.518	
	Pupae	None			
	All ¹	Rice height	-0.325	-2.384	
		Tillers	0.288	2.528	
	Maturation (<i>n</i> = 30)	Early	Tillers	1.269	4.632
			Turbidity	1.799	3.935
		pH	0.784	3.733	
Late		None			
Pupae		Rice height	-1.010	-3.374	
		Conductivity	23.202	3.866	
		Salinity	-22.662	-3.831	
All ¹		Rice height	-0.816	-5.484	
		No. of tillers	1.521	8.248	
		Water depth	0.523	4.498	
	Turbidity	2.188	7.113		
	Dissolved oxygen	-0.353	-2.324		
	pH	0.635	4.492		

¹ All immature forms combined (early instars, late instars, and pupae).

through out the growth cycle but due to evapotranspiration and direct evaporation, at times the depth of the water declines. Shallow rice fields are an indication of drying of the fields, which the gravid *An. arabiensis* would avoid during oviposition, whereas the higher depths are an indication of stable habitats. The gravid *An. arabiensis* preferred more stable habitats with higher water levels because of the ability of these habitats to persist longer, hence creating better conditions for the survival of larvae. The fluctuation in water depth provided conducive water temperatures, which have a direct influence on the anopheline larval densities.

Water turbidity was found to be an important parameter associated with the abundance of *An. arabiensis* larvae in the habitats. Most larvae were collected from water with either clear or low turbidity. Gimnig et al. (2001) found increasing *An. gambiae* s.l. larvae densities with increasing turbidity. Robert et al. (1998) found a clear-water preference by *An. arabiensis* breeding in wells in urban Dakar. A study by Ye-Ebiyo et al. (2003) found that the production of *An. arabiensis* was favored in moderately turbid water, although excessive turbidity limited the production of larvae. Water that is turbid from particles not edible by *Anopheles* sp. larvae could disfavor the production of larvae, whereas water that is turbid from food particles represents a very suitable habitat. McCrae (1984) found that mosquitoes preferred to oviposit in petri dishes with a dark background. Addition of nitrogenous fertilizers (sulfate of ammonia) was found to lower turbidity of water at the rice paddies. When the topdressing was done, water became clear and larval population increased in these paddies. Application of fertilizer to rice paddies reduced turbidity, an important cue for mosquito oviposition, consequently increasing larval densities. Ovipositing female mosquitoes are known to choose among water bodies based on cues such as temperature, light, water depth, turbidity, and presence of competitors (Lee 1991).

Other important factors for the abundance of anopheline mosquito larvae in the habitats were pH and conductivity. In rice agroecosystems, pH and conductivity have been shown to be important factors affecting larval abundance (Cates 1968, Case and Washino 1975). Fertilizer application increased pH and conductivity, which raised larval abundance due to greater oviposition by the gravid *Anopheles* mosquitoes. Nitrogenous fertilizers have been documented to increase anopheline and culicine larvae densities in rice agroecosystems (Victor and Reuben 2000, Mutero et al. 2004b). In our study pH was key factor associated with an increase in anopheline larval abundance.

In conclusion, *Anopheles* larval abundance was associated with several factors in the rice agro-

ecosystem. These parameters, including temperature, turbidity, depth, pH, and conductivity, were associated with the early vegetative stages of the rice growth cycle, which was associated with larval abundance. For effective control of immature stages of mosquito, larvicide application should be started during the vegetative phase and sustained through the reproductive phase of rice cultivation.

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