Mosquito Species Succession and Physicochemical Factors Affecting Their Abundance in Rice Fields in Mwea, Kenya

EPHANTUS J. MUTURI,[1,2] JOSEPH MWANGANGI,[3,4] JOSEPHAT SHILILU,[3,5] SIMON MURIU,[2]
BENJAMIN JACOB,[1] EPHANTUS KABIRU,[6] WEIDONG GU,[1] CHARLES MBOGO,[4]
JOHN GITHURE,[3] AND ROBERT NOVAK[1]

J. Med. Entomol. 44(2): 336–344 (2007)

ABSTRACT The succession of mosquito species and abiotic factors affecting their distribution and abundance in rice (*Oryza* spp.) fields was investigated over a 16-wk rice growing cycle covering the period between January and May 2006. Fifteen experimental rice plots were sampled for mosquito larvae and characterized based on rice height, number of tillers, floating vegetation cover, water depth, water temperature, turbidity, salinity, pH, dissolved oxygen, total dissolved solids, and conductivity. Microscopic identification of 3,025 larvae yielded nine mosquito species predominated by *Anopheles arabiensis* Patton (45.0%), *Culex quinquefasciatus* Say (35.8%), *Anopheles pharoensis* Theobald (9.0%) and *Ficalbia splendens* Theobald (7.1%). Other species, including *Anopheles rufipes* Gough, *Anopheles coustani* Laveran, *Anonopheles maculipalpis* Giles, *Culex annulioris* Theobald, and *Culex poicilipes* Theobald made up 3.1% of the total collection. *Anopheles gambiae* s.l., *Cx. quinquefasciatus*, and *An. pharoensis* occurred throughout the cycle, but they were more abundant up to 4 wk posttransplanting with peaks after fertilizer application. As rice plants became established, three groups of mosquitoes were recognized: the first groups included *An. rufipes*, *Fl. splendens*, and *Cx. annulioris*, which occurred throughout much of the second half of the rice cycle, whereas the second group included *Cx. poicilipes*, which was found in the middle of the rice cycle. *An. coustani* and *An. maculipalpis* formed the third group occurring toward the end of the cycle. Dissolved oxygen, number of tillers, and rice height were negatively associated with the abundance of *An. arabiensis* and *Cx. quinquefasciatus* larvae. In addition, *Cx. quinquefasciatus* also was associated with water depth (−ve) and turbidity (+ve). Abundance of *An. pharoensis* larvae was significantly associated with water temperature (+ve), the number of tillers (−ve), and rice height (−ve), whereas *Fl. splendens* was significantly associated with the number of tillers (+ve). The results demonstrate a complex nature of the interactions between some of the factors in the ecosystem and mosquito species abundance and calls for time-dependent and species-specific mosquito control operations.

KEY WORDS rice, mosquitoes, succession, physicochemical, Kenya

Development of water projects meant to increase agricultural production, particularly in arid areas, are associated with increased risk of mosquito-borne diseases. Studies from various parts of Africa have reported increased prevalence of Bancroftian filariasis, arboviruses, and malaria as a consequence of water-related projects (Ghebreyesus et al. 1999, Mawuli et al. 1999, Diallo et al. 2000, Miller et al. 2000). Among the irrigated crops, rice (*Oryza* spp.) is considered to pose the greatest danger to health because it is grown in flooded conditions, which are ideal aquatic habitats for diverse mosquito species. Few studies have demonstrated a strong relationship between rice cropping cycle and mosquito species succession in Africa. In Mali, *Anopheles gambiae* s.s. Giles and *Anopheles pharoensis* Theobald dominated the first 6–8 wk of rice cycle followed by a sharp decline thereafter and the dominance of *Anopheles rufipes* Gough and *Anopheles funestus* Giles (Klinkenberg et al. 2003). In the Gambia, Snow (1983) demonstrated succession of different mosquito genera in the rice fields. In this study, *Anopheles gambiae* s.l., *An. rufipes,* and *Culex neavei* Theobald were predominant during the early stages of rice development and *Culex ethiopicus* Edwards and *Culex poicilipes* Theobald around the middle of the rice cycle, whereas *Anopheles ziemanni* Grunberg peaked as the rice matured. *Culex antennatus* Becker, *Mansonia uniformis* Theobald, and *Mansonia africana*

---

[1] Medical Entomology Laboratory, Centre for Ecological Entomology, Illinois Natural History Survey, 1816 South Oak St., Champaign, IL 61820.

[2] Corresponding author, e-mail: emuturi2@uiuc.edu.

[3] Human Health Division, International Centre of Insect Physiology and Ecology, Nairobi, Kenya.

[4] Centre for Geographic Medicine Research-Coast, Kenya Medical Research Institute, Kilifi, Kenya.

[5] Department of Zoology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.

[6] Department of Pathology, Kenyatta University, Nairobi, Kenya.

Theobald occurred throughout the growing season, but they had little relationship with the cycle of rice growth. In Kenya, little is known about succession of mosquito species in rice fields. The only documented study on the subject was conducted three decades ago in Kisumu (Chandler and Highton 1975). This study documented *An. gambiae* s.l. and *An. pharoensis* to occur during the early growing cycle, whereas *Mn. uniformis, Mn. Africana,* and *Mimomyia splendens* Theobald had no relation with rice cycle. Unlike in the Gambia, *An. ziemmani* was abundant during the middle of rice cycle together with *Cx. poicilipes,* whereas *Cx. antennatus* occurred toward the end of the cycle. These findings demonstrate that rice fields have the potential to support a range of vector species capable of transmitting a myriad of mosquito-borne diseases and could impact negatively on health if not properly managed.

Proper understanding of the factors contributing to the distribution and relative abundance of vector species in rice fields is an important prerequisite of vector control operations. However, despite the importance of African rice fields in proliferation of diverse mosquito species, little is known about the factors regulating their relative abundance and temporal distribution. Where studies have been done, the focus has mainly been on malaria vectors at the expenses of other species (Klinkenberg et al. 2003). Moreover, these studies consider only a limited number of factors affecting larval abundance, making it impossible to predict larval abundance, because there are a large number of variables that act interdependently to influence larval abundance.

Each mosquito species has its optimum abiotic and biotic characteristics that act as oviposition cues for gravid female mosquitoes and provide ideal environment for the development of the immatures. These factors exhibit marked variation during the crop cycle and impact tremendously on the relative abundance (Sunish and Reuben 2001) and succession of mosquito species in the course of the growing cycle. An increase in rice height and floating vegetation cover may inhibit oviposition by some species directly through physical obstruction or indirectly through reduced temperature and microbial growth (Ramachandra Rao 1984). The numerous shallow pools created by rice workers during rice transplanting create ideal sites for the breeding of *An. gambiae* s.l. (Chandler and Highton 1976), whereas application of nitrogenous fertilizer increases its larval densities (Mutero et al. 2004b). Rainfall also may dilute the water in rice fields, altering their physicochemical properties, resulting in changes in larval densities and species succession. In Tamil Nadu State, India, paddy height, water temperature, dissolved oxygen, ammonia nitrogen, and nitrate nitrogen were observed to be the best predictors associated with abundance of immature stages of *Cx. vishnui* (Sunish and Reuben 2001). The current study investigated the relationship among rice cropping cycle, mosquito species succession, and the physical chemical factors affecting their abundance in the Mwea rice agroecosystem in Kenya.

Materials and Methods

Study Site. The study was conducted in Mwea division in Kirinyaga District, 100 km northeast of Nairobi. The study area has been described previously (Mutero et al. 2004a,b). 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/yr. The average temperature is 21.3°C (range 16.0–26.5°C), and the relative humidity averages 59.5% (range 52.0–67.0%). 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 ≈13,640 ha. More than 50% of the scheme area is used for irrigated rice cultivation; the remaining area is used for subsistence farming, grazing, and community activities.

Experimental Rice Paddies. An experimental paddy measuring 63 by 63 m was developed at the Mwea Irrigation and Agricultural Development Center in Mwea Irrigation Scheme. Eight paddy blocks each consisting of eight subplots measuring 6.3 by 3.15 m were established. The plots were hydrologically isolated using unidirectional inflow and outflow canals to avoid water mixing between plots. Rice was planted in all the 64 subplots and 15 subplots were randomly selected and monitored for mosquito larvae throughout the rice growing cycle. The remaining plots were used for other ecological studies.

Larval Sampling and Identification. Larval sampling was done once weekly to generate stage-specific estimates of larval densities from 1 wk pretransplanting to 15 wk posttransplanting. Up to 20 dipper samples, depending on the amount water in each subplot, were taken at intervals throughout the subplot by using a standard mosquito dipper (350 ml). If the subplot was covered with floating vegetation, the vegetation was carefully opened up to allow for water pooling before dipping was done. Samples from each subplot were pooled in plastic bags (whirl paks) and transported to the laboratory where they were sorted into different instars of either anopheline or culicine, counted, and recorded. All third and fourth instars were immediately preserved in 95% ethanol and later identified morphologically to species by using taxonomic keys (Hopkins 1952, Gillies and Coetzee 1987). The first and second instars were reared in plastic pans under semifield conditions, and those instars that survived to third instar also were preserved and identified morphologically. The pupae were kept in mosquito emergent cages (Bioquip Products, Inc., Rancho Dominguez, CA), and the resultant emergent mosquitoes were identified morphologically. A subset of the *An. gambiae* s.l. was further identified by rDNA polymerase chain reaction (PCR) technique into sibling species (Scott et al. 1993).

Paddy Characterization. Environmental variables recorded for each subplot during each larval sampling occasion were floating vegetation cover, rice height, number of tillers, water depth, turbidity, salinity, total dissolved solids (TDS), pH, temperature, conductivity, and dissolved oxygen concentration. Rice height and water depth were measured using a metal ruler. Floating vegetation cover was measured as a percentage of the covered area. Turbidity was measured through visual examination of the water against a white background and categorized as clear, low, or high. The pH, conductivity, dissolved oxygen, and temperature were measured using hand-held YSI 650 Multi-Parameter Display System (YSI Inc., Yellow Springs, OH). Salinity and TDS were measured using field hand-held equipment YSI EC 300 (YSI Inc.).

Data Analyses. Data were entered in Microsoft Excel files and analyzed using SPSS version 11.5 statistical package (SPSS Inc., Chicago, IL). The relative abundance of mosquitoes was expressed as the number of mosquito larvae per 20 dips. The degree of association between anopheline and culicine larvae in the paddies was tested by chi-square. Pearson correlation analysis was used to determine the association among the environmental variables. Step-up multiple regression analysis was used to obtain the best predictor variables explaining the abundance of mosquito immatures. Statistical analyses was done after log transformation $\log_{10}(n + 1)$ of larval abundance values to normalize the distribution and minimize the standard error.

Results

Mosquito Species Composition in the Experimental Plots. In total, 240 collections were made from the 15 experimental plots over the 16-wk rice growing cycle. anopheline larvae were found in 196 collections, and 33 of these collections (13.8%) had only anophelines. Culicine larvae were found in 189 collections, and 26 of these collections (10.8%) had only culicines. Both anopheline and culicine larvae were found in 163 collections (67.9%), suggesting that the mosquito larvae from subfamilies anophelinae and culicinae coexist in the majority of rice fields. Chi-square analysis further indicated that anopheline and culicine larvae were more likely to coexist in the same rice field than would be expected by chance alone ($\chi^2 = 12.443, P < 0.01$). The mean number of anopheline larvae collected was 9.74 ± 0.99 (mean ± SE) per 20 dips, whereas that of culicine larvae in the same number of dips was 11.69 ± 1.22.

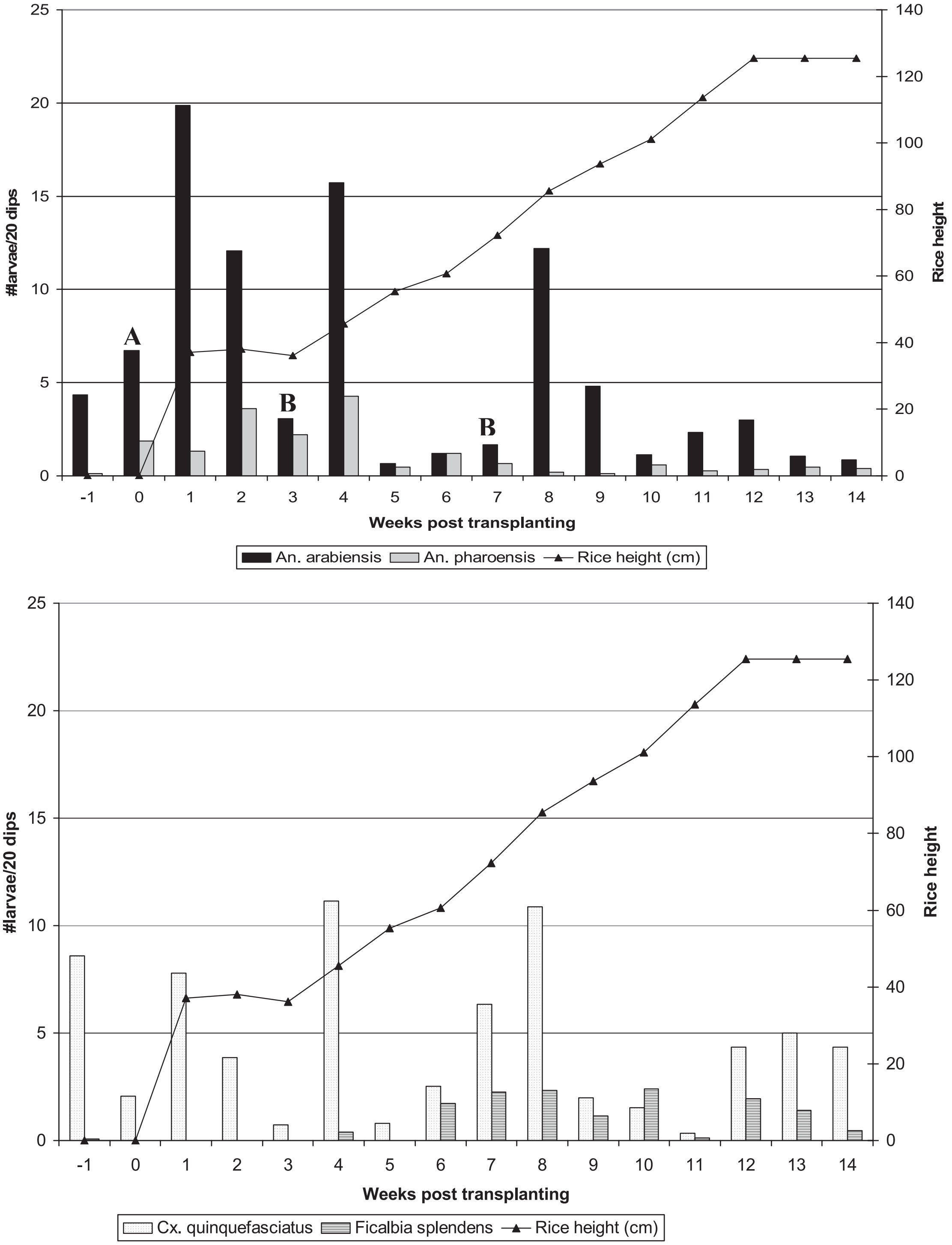
In total, 3,025 larvae were examined microscopically and identified morphologically to species by using taxonomic keys. Samples yielded nine mosquito species predominated by *An. gambiae* s.l. (45.0%), *Cx. quinquefasciatus* (35.8%), *An. pharoensis* (9.0%), and *Ficalbia splendens* (7.07%). Other species included *Cx. annulioris* (1.39%), *An. rufipes* (1.22%), *An. coustani* (0.33%), *An. maculipalpis* (0.1%), and *Cx. poicilipes* (0.07%). Further analysis of 300 *An. gambiae* s.l. larvae by rDNA PCR confirmed *An. arabiensis* as the only sibling species present in these samples.

Succession of Mosquito Species Over Time. The relative abundance of mosquito species over the 16-wk sampling period is represented in Figs. 1 and 2. *An. arabiensis*, *An. pharoensis*, and *Cx. quinquefasciatus* were the predominant species throughout the growing cycle, but their abundance was greater 1 wk before transplanting of rice seedlings up to 4 wk posttransplanting after which their abundance sharply declined with their only other peak occurring at week 8. Three peaks were observed for *An. arabiensis*, *An. pharoensis*, and *Cx. quinquefasciatus*, and these peaks were preceded by fertilizer application the previous week. Sulfate of ammonia (SA) and muriate of potash (MOP) were applied during the rice seedlings transplanting phase, whereas SA also was applied at the third and seventh week posttransplanting. *An. rufipes* and *Fl. splendens* occurred during much of the second half of the rice growth, whereas *Cx. annulioris* occurred at specific times during the middle (5–8 wk posttransplanting) and the late stage (>11 wk posttransplanting) of rice growth. Other species, including *Cx. poicilipes*, *An. maculipalpis*, and *An. coustani* occurred at specific times during either the middle or late stage of the rice growth and in low densities. According to Pearson correlation analysis, *An. arabiensis* and *An. pharoensis* were negatively associated with rice height ($r = -0.194$ and $r = -0.262$, df = 238, $P < 0.01$), whereas *An. maculipalpis*, *Cx. annulioris*, and *Fl. splendens* were positively associated with rice height (r = 0.132, 0.232, and 0.275; df = 238; $P < 0.01$).

Seasonal Fluctuations in Abiotic Factors and Correlation among Environmental Variables. The seasonal fluctuation of abiotic factors during the growing cycle is shown in Table 1. Abiotic factors showed characteristic patterns in different rice growing stages depending on the changes taking place within the ecosystem as a result of agricultural activities. Floating vegetation cover, conductivity and total dissolved solids showed an increasing trend as the rice plants increased in height and vegetative growth from transplanting to harvesting. In contrast, water temperature, water depth, and pH decreased as the rice growth cycle progressed. Salinity and dissolved oxygen were highest during the middle stage of the rice cycle.

Correlation coefficients among the environmental variables are shown in Table 2. More than half of the variables (32 of 55) were significantly correlated and there is a biological reason for many of them. For example, rice height was inversely correlated with water temperature because as rice height increases, the amount of sunlight reaching the water surface is reduced, resulting in lower temperatures. Similarly, salinity was positively correlated with both conductivity and TDS because dissolved salts form part of dissolved solids and in turn affect the conductivity of the water.

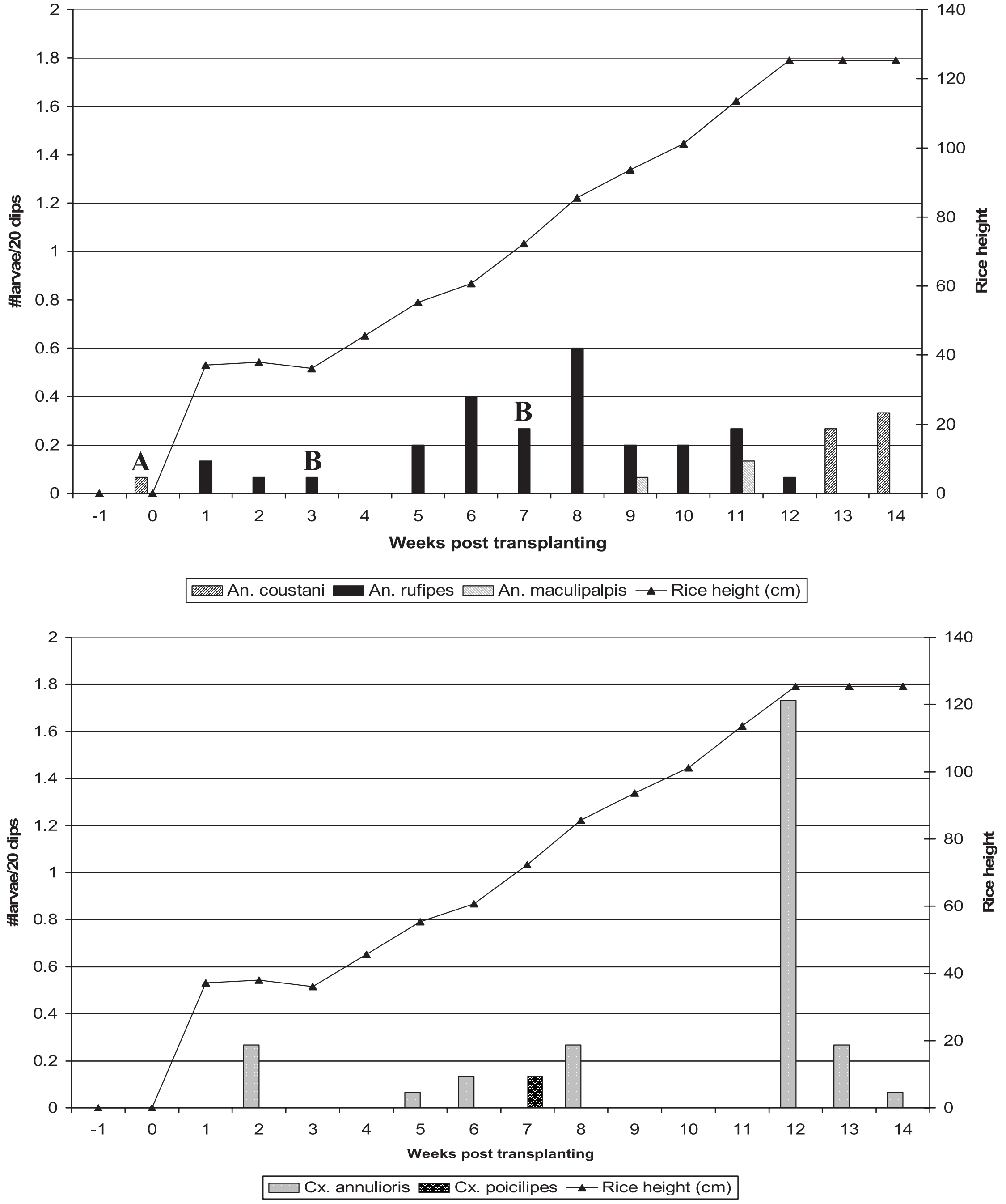
Association between Occurrence of Mosquito Larvae and Environmental Variables. Step-up multiple regression analysis was used to test which of the abiotic factors could best explain the relative abundance of the four most common species: *An. arabiensis*, *Cx. quinquefasciatus*, *An. pharoensis*, and *Fl. splendens*. The



A: Transplanting period: SA and MOP applied; B: SA applied

Fig. 1. Weekly abundance of common mosquito species in relation to rice height.

results of the step-up multiple regression analysis are represented in Tables 3. The variables included in the model varied among species ranging from one variable for *Fl. splendens* to five variables for *Cx. quinquefasciatus.* Of the 11 abiotic variables examined, six variables were found to be significant predictors of at least



A: Transplanting period: SA and MOP applied; B: SA applied

Fig. 2. Weekly abundance of rare mosquito species in relation to rice height.

one of the four common species. These included number of tillers, rice height, dissolved oxygen, water temperature, turbidity, and water depth. The influence of some of these factors varied between species and the model explained up to 42% of the observed variance. Among the anophelines, the number of tillers, rice height, and dissolved oxygen were negatively associated with *An. arabiensis* abundance, whereas the number of tillers (−ve), rice height (−ve), and water temperature (+ve) were significant predictors of *An. pharoensis* larval abundance. For the culicines, *Fl. splendens* was positively associated with the number of

Table 1. Seasonal fluctuation of abiotic factors (mean ± SE; *n* = 239)

Variable	−1–4 wk	5–9 wk	10–14 wk
Floating vegetation cover (%)	24.96 ± 2.96	51.80 ± 3.33	60.33 ± 2.22
Rice ht (cm)	26.14 ± 2.03	73.49 ± 1.95	96.69 ± 2.86
No. of tillers	2.23 ± 0.31	17.24 ± 1.08	22.07 ± 0.60
Water depth (cm)	9.11 ± 0.22	7.49 ± 0.24	7.69 ± 0.37
TDS (ppt)	0.10 ± 0.01	0.18 ± 0.02	0.12 ± 0.01
Temp (°C)	27.36 ± 0.21	27.06 ± 0.27	26.25 ± 0.23
Conductivity (mS/cm)	59.29 ± 4.17	85.27 ± 7.61	61.16 ± 4.27
Salinity (ppt)	41.89 ± 3.85	66.97 ± 8.29	41.22 ± 3.65
Dissolved oxygen (mg/liter)	1.48 ± 0.34	1.63 ± 0.31	0.04 ± 0.00
pH	7.62 ± 0.03	7.53 ± 0.10	7.17 ± 0.03

tillers, whereas *Cx. quinquefasciatus* was positively associated with turbid water and negatively associated with rice height, number of tillers, water depth, and dissolved oxygen.

Discussion

Rice fields in Mwea rice scheme seem to be excellent larval habitats for several mosquito species, particularly *An. arabiensis, Cx. quinquefasciatus, An. pharoensis,* and *Fl. splendens.* The first three species occurred throughout the growing season but in prolific numbers during the first third of the rice cycle. Numbers declined thereafter with the only other peak occurring at the onset of the second half triggered by nitrogenous fertilizer application. Because *An. arabiensis* and *Cx. quinquefasciatus* outnumbered the other species, it seems that at least in the rice fields during the growing season, the two species present the most significant public health problem in the area. *An. arabiensis* is the main vector of malaria in the area and also a potential vector of Bancroftian filariasis and O'nyong-nyong virus, whereas *Cx. quinquefasciatus* is a potential vector of Bancroftian filariasis and several arboviruses (Mutero et al. 2004a, Muturi et al. 2006). During the second half of the rice growth when the abundance of *An. arabiensis, An. pharoensis,* and *Cx. quinquefasciatus* was lower, other species became important. These species could be grouped into three categories. The first category included *Cx. poicilipes,* which occurred at a specific period in the middle stage of rice growth. The second category was composed of *An. coustani* and *An. maculipalpis,* which were closely associated with the late stage. The last category was made up of *Fl. splendens, Cx. annulioris,* and *An. rufipes,* which occurred throughout much of the second half of the rice cycle. Considering *An. arabiensis, An. pharoensis,* and *Cx. quinquefasciatus* as pioneer species, four groups of mosquito species can therefore be recognized. Similar succession of mosquito species in rice fields has been reported previously (Chandler and Highton 1975, Snow 1983, Klinkenberg et al. 2003). The vector status of *An. pharoensis* in malaria transmission is considered secondary in the study area (Ijumba et al. 1990), whereas that of most of the other species collected, including *An. rufipes, An. coustani, An. maculipalpis, Cx. annulioris,* and *Cx. poicilipes* remain uncertain. However, lessons from other parts of Africa indicate that the majority of these species may be important in disease transmission. In the Senegal River Basin where *An. gambiae* s.l. and *Cx. quinquefasciatus* are uncommon, *Cx. poicilipes* and *Mn. uniformis* predominate and have been involved in arboviral outbreaks (Diallo et al. 2005). *An. coustani, An. maculipalpis,* and *An. pharoensis* also have been incriminated as vectors of arboviruses (Logan et al. 1991, Gordon et al. 1992). These findings demonstrate the need to understand the temporal and geographic distribution of species before designation of an integrated mosquito control program.

Step-up multiple regression analysis results demonstrated a strong interaction between some of the abiotic factors and the relative abundance of the four major mosquito species: *An. arabiensis, An. pharoensis, Cx. quinquefasciatus,* and *Fl. splendens.* Some of the six variables examined were significant predictors of different mosquito species explaining in part why these species were able to coexist in the rice fields. For example, rice height, and the number of tillers impacted negatively on *An. arabiensis, An. pharoensis,* and *Cx. quinquefasciatus* explaining why they were more abundance during the early stage of the rice cycle, whereas *Fl. splendens* was positively associated with the number of tillers and hence more common

Table 2. Correlation coefficients between the measured environmental variables in the experimental plots

	Rice ht	No. tillers	Floating	Water depth	Turbidity	pH	Dissolved oxygen (mg)	Salinity	Conductivity	Temp
Rice ht										
No. of tillers	0.787**									
Floating vegetation	0.562**	0.549**								
Water depth	−0.314**	−0.254**	−0.182**							
Turbidity	−0.145*	−0.103	−0.082	0.148*						
pH	−0.325**	−0.269**	−0.29**	0.048	−0.047					
Dissolved oxygen (mg)	−0.16*	−0.197**	0.102	−0.094	−0.137*	0.279**				
Salinity (ppt)	0.091	−0.046	−0.009	−0.122	−0.161*	−0.042	0.188**			
Conductivity	0.128*	−0.045	0.035	−0.122	−0.178**	−0.071	0.241**	0.925**		
Water temp	−0.338**	−0.26**	0.002	0.063	0.049	0.272**	0.129*	−0.049	−0.036	
TDS	0.178**	0.011	0.125	−0.195**	−0.214**	−0.103	0.191**	0.741**	0.809**	−0.041

* Correlation significant at 0.05 level; **, correlation significant at 0.01 level.

Table 3. Multiple step-up regression for the four common mosquito species in relation to paddy characteristics

Species		R^2	Coefficient	SE	Standard coefficient	t	P
	(Constant)	42.3	0.71	0.08		8.87	0.00
	No. tillers		−0.54	0.10	−0.66	−5.15	0.00
	Rice ht		−0.31	0.08	−0.47	−3.67	0.00
An. arabiensis	Dissolved oxygen		−0.29	0.13	−0.17	−2.32	0.02
	(Constant)	28.9	−2.05	0.75		−2.72	0.01
	No. tillers		−0.32	0.06	−0.56	−5.32	0.00
	Rice ht		−0.19	0.05	−0.41	−3.89	0.00
An. pharoensis	Water temp		1.57	0.52	0.19	3.03	0.00
	(Constant)	36.5	0.39	0.25		1.60	0.11
	No. of tillers		−0.20	0.11	−0.18	−1.80	0.07
	Turbidity		0.20	0.05	0.23	3.96	0.00
	Dissolved oxygen		−0.41	0.11	−0.23	−3.89	0.00
	Water depth		−0.50	0.23	−0.13	−2.20	0.03
Cx. quinquefasciatus	Rice ht		−0.20	0.09	−0.22	−2.20	0.03
	(Constant)	22.2	−0.02	0.04		−0.45	0.65
Fl. splendens	No. of tillers		0.20	0.03	0.37	6.19	0.00

during the later stages of rice development. Similarly, both *An. arabiensis* and *Cx. quinquefasciatus* were associated with low levels of dissolved oxygen. Similar findings were observed in South Arcot district in India (Sunish and Reuben 2001) and are to be expected because of the complex nature of the interactions between some of the factors in the ecosystem. In addition, factors out side the rice fields such as vector resting sites and bloodmeal sources also have been reported to affect larval production (Wood et al. 1991, 1992; Minakawa et al. 2002).

The six predictor variables can be categorized in two interdependent groups. The first includes rice height, number of tillers, water temperature, and water depth. The first three variables had a positive relationship, whereas the last two were inversely related to rice height. The increase in rice height and vegetative growth may reduce the amount of sunlight reaching the water surface, resulting in lower temperatures. Reduced temperature causes a decline in microbial growth upon which mosquito larvae depend on (Ramachandra Rao 1984). In addition, emergent vegetation such as rice plant may obstruct some species from ovipositing especially those that require shallow, open sunlit habitats, such as *An. arabiensis* and some culicines (Rajendran and Reuben 1991, Gimnig et al. 2001, Shililu et al. 2003). The rice vegetative growth is characterized by tiller formation accompanied by an increase in the rice height. This period favors proliferation of mosquito species suited to conditions of a well-established rice stand such as *Fl. splendens* in the current study. Hopkins (1952) reported *Fl. splendens* to be strongly associated with the water weed *Pistia stratiotes* L. mainly occurring under the leaves of this plant. The association of this species with rice plants would therefore be expected, because the rice plant provides habitat conditions analogous to those provided by *P. stratiotes.* The second category of interrelated variables included turbidity and dissolved oxygen concentration, which were inversely related. Water turbidity results mainly from the presence of microorganisms and suspended organic and inorganic matter (Hammer 1986), which may in turn affect the content of dissolved oxygen in the water body and consequently the pH (Sunish and Reuben 2001). In most areas of its distribution, *Cx. quinquefasciatus* prefers habitats with turbid water caused by organic matter (Asimeng and Mutinga 1993), as in the current study. However, the mechanism underlying this association is not clearly known. Because the amount of dissolved oxygen decreased as the water turbidity increased, we hypothesize that this change may be responsible for the abundance of *Cx. quinquefasciatus* in highly turbid water. Lower amounts of dissolved oxygen may be an indicator of the amount of decaying organic matter that influences oviposition and/or survivorship of many species. The interesting role of dissolved oxygen as a potential significant variable influencing abundance of *Anopheles* larvae has been reported by several studies (Grillet 2000, Piyaratne et al. 2005) and needs further exploration. Anophelines respire primarily at the water surface, and this raises the question as to whether it is oxygen per se or an associated physicochemical or biotic factor that influences the abundance of this species.

Ammonia nitrogen, nitrate nitrogen, sulfate, and phosphate are also important factors regulating larval abundance in rice fields and fluctuate greatly in the rice field ecosystem after the application of fertilizers incorporated in them (Sunish and Reuben 2001). Unfortunately, due to logistic difficulties, these parameters were not measured in this study despite their importance. Undoubtedly however, application of fertilizers containing these chemicals resulted in significant increase in abundance of *An. arabiensis, An. pharoensis,* and *Cx. quinquefasciatus* a week later. This increase is consistent with previous findings in the same area (Mutero et al. 2004b) and in Tamil Nadu State, India (Sunish and Reuben 2001). Beehler and Mulla (1995) also demonstrated increased oviposition by *Culex stigmatosoma* Dyar, and *Cx. quinquefasciatus* after addition of organic matter in experimental ponds. Nitrogenous fertilizer application accelerates multiplication of microorganisms, which form the main diet for mosquito larvae and also increases pupation rate (Mogi 1978). Ammonia nitrogen also has been shown to be an oviposition attractant (Sunish et al. 1998, Mutero et al. 2004b). However, it takes some days for larvae to hatch and for ammonia nitrogen to decompose into nitrates (Sunish and Reuben 2001). This explains why fertilizer application is not immediately associated with increased larval densities. Further studies should be conducted to investigate the impact of fertilizer application on larval abundance in similar areas.

In summary, this study has demonstrated the importance of rice fields in supporting diverse mosquito species capable of transmitting a myriad of mosquito-borne diseases. The results suggest that a complex of many interacting variables act together to regulate mosquito species composition and abundance in the course of the rice growing cycle. Moreover, some variables seem to have similar influence on more than one species, whereas others are species specific. To address the problem of mosquito-borne diseases in these areas, control programs tailored to suit the ecology of each mosquito species should be devised. Importantly, more detailed studies should be conducted to understand the biotic and abiotic factors regulating mosquito populations in rice fields to ensure development of an ecologically sound mosquito control program.

Acknowledgments

We are grateful to Prof. Christian Baumgartner (International Centre of Insect Physiology and Ecology) and Hans R. Herren for strong support in this project. We acknowledge the data collection provided by Peter Barasa, Enock Mpanga, James Wauna, Peter M. Mutiga, William Waweru, Nelson Maingi, Martin Njigoya, Paul K. Mwangi, Christine W. Maina, Isabel M. Marui, Gladys Karimi, Irene Kamau, Julius Murimi, Susan W. Mugo, Nicholus Gachoki, Charles Kiura, and Naftaly Gichuki. This research was supported by National Institutes of Health, National Institute of Allergy and Infectious Diseases U01A154889 (to R.N.).

References Cited

Asimeng, E., and M. Mutinga. 1993. Effect of rice husbandry on mosquito breeding at Mwea Rice Irrigation Scheme with reference to biocontrol strategies. J. Am. Mosq. Control Assoc. 9: 17–22.

Beehler, J. W., and M. S. Mulla. 1995. Effects of organic encroachment on temporal distribution and abundance of culicine egg rafts. J. Am. Mosq. Control Assoc. 11: 167–117.

Chandler, J. A., and R. B. Highton. 1975. The succession of mosquito species (Diptera, Culicidae) in rice fields in the Kisumu area of Kenya, and their possible control. Bull. Entomol. Res. 65: 295–302.

Chandler, J., and R. Highton. 1976. Breeding of *Anopheles gambiae* Giles (Diptera: Culicidae) in rice fields in the Kisumu area of Kenya. J. Med. Entomol. 13: 211–115.

Diallo, M., L. Lochouarn, L. Ba, K. Sall, A. Modo, L. Girault, and C. Mathiot. 2000. First isolation of the Rift Valley fever virus from *Culex poicilipes* (Diptera: Culicidae) in nature. Am. J. Trop. Med. Hyg. 62: 702–704.

Diallo, M., P. Nabeth, K. Ba, A. A. Sall, Y. Ba, M. Mondo, L. Girault, M. O. Abdalahi, and C. Mathiot. 2005. Mosquito vectors of the 1998–1999 outbreak of Rift Valley Fever and other arboviruses (Bagaza, Sanar, Wesselsbron and West Nile) in Mauritania and Senegal. Med. Vet. Entomol. 19: 119–126.

Ghebreyesus, T. A., M. Haile, K. H. Witten, A. Getachew, A. M. Yohannes, M. Yohannes, H. D. Teklehaimanot, S. W. Lindsay, and P. Byass. 1999. Incidence of malaria among children living near dams in northern Ethiopia: community based incidence survey. Br. Med. J. 319: 663–666.

Gillies, M. T., and M. Coetzee. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afro-tropical region). Johannesberg Public South Afr. Inst. Med. Res. 55: 1–143.

Gimnig, J. E., M. Ombok, K. Luna, and W. A. Hawley. 2001. Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. J. Med. Entomol. 38: 282–288.

Gordon, S., R. Tammariello, K. Lincithicum, D. Dohm, J. Digoutte, and M. Calvo-Wilson. 1992. Arbovirus isolations from mosquitoes collected during 1988 in the Senegal River basin. Am. J. Trop. Med. Hyg. 47: 742–748.

Grillet, M. 2000. Factors associated with distribution of *Anopheles aquasalis* and *Anopheles oswaldoi* (Diptera: Culicidae) in a malarious area, northeastern Venezuela. J. Med. Entomol. 37: 231–238.

Hammer, M. J. 1986. Water and waste water technology. Wiley, New York.

Hopkins, G. 1952. Mosquitoes of the Ethiopian region: larval bionomics of mosquitoes and taxonomy of culicine larvae. Adlard and Son Ltd., London, United Kingdom.

Ijumba, J., R. Mwangi, and J. C. Beier. 1990. Malaria transmission potential of *Anopheles* mosquitoes in the Mwea-Tebere irrigation scheme, Kenya. Med. Vet. Entomol. 4: 425–432.

Klinkenberg, E., W. Takken, F. Huibers, and Y. Toure. 2003. The phenology of malaria mosquitoes in irrigated rice fields in Mali. Acta Trop. 85: 71–82.

Logan, T., K. Lincithicum, P. Thande, J. Wagateh, and C. Roberts. 1991. Mosquito species collected from a marsh in western Kenya during the long rains. J. Am. Mosq. Control Assoc. 7: 395–399.

Mawuli, D., S. K. Dunyo, C. K. Ahorlu, W. Z. Coker, M. A. Appawu, E. M. Pedersen, and P. E. Simonsen. 1999. Bancroftian filariasis in an irrigated project community in southern Ghana. Trop. Med. Int. Health 4: 13–18.

Miller, B. R., R. S. Nasci, S. M. Godsey, M. H. Savage, J. J. Lutwama, R. S. Lanciotti, and P. J. Clarence. 2000. First field evidence for natural vertical transmission of West Nile virus in *Culex univittatus* complex mosquitoes from Rift Valley province, Am. J. Trop. Med. Hyg. 62: 240–246.

Minakawa, N., P. Seda, and Y. Guiyun. 2002. Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. Am. J. Trop. Med. Hyg. 67: 32–38.

Mogi, M. 1978. Population studies on mosquitoes in the rice field area of Nagasaki, Japan, especially on *Culex tritaeniorhynchus.* Trop. Med. 20: 173–263.

Mutero, C., C. Kabutha, V. Kimani, L. Kabuage, G. Gitau, J. Ssennyonga, J. Githure, L. Muthami, A. Kaida, L. Musyoka, et al. 2004a. A transdisciplinary perspective on the links between malaria and agroecosystems in Kenya. Acta Trop. 89: 171–186.

Mutero, C., P. Ng'ang'a, P. Wekoyela, J. Githure, and F. Konradsen. 2004b. Ammonium sulphate fertiliser increases larval populations of *Anopheles arabiensis* and culicine mosquitoes in rice fields. Acta Trop. 89: 187–192.

Muturi, J. E., J. Shililu, J. Benjamin, W. Gu, J. Githure, and R. Novak. 2006. Mosquito species diversity and abundance in relation to land use in a riceland agroecosystem in Mwea, Kenya. J. Vector Ecol. 31: 129–137.

Piyaratne, M. K., M. P. Amerasinghe, P. H. Amerasinghe, and F. Konradsen. 2005. Physico-chemical characteristics of *Anopheles culifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka. J. Vector Borne Dis. 42: 61–67.

Rajendran, R., and R. Reuben. 1991. Evaluation of water fern *Azolla microphylla* for mosquito population management in the rice land agro-ecosystem of south India. Med. Vet. Entomol. 5: 299–310.

Ramachandra Rao, T. 1984. The Anophelines of India revised edition. ICMR, New Delhi, India.

Scott, J. A., W. G. Brogdon, and F. H. Collins. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49: 520–529.

Shililu, J., G. Tewolde, S. Fessahaye. S. Mengistu, H. Fekadu, Z. Mehari, G. Asmelash, D. Sintasath, G. Bretas, C. Mbogo, et al. 2003. Larval habitat diversity and ecology of anopheline larvae in Eritrea. J. Med. Entomol. 40: 921–929.

Snow, W. 1983. Mosquito production and species succession from an area of irrigated rice fields in The Gambia, West Africa. J. Trop. Med. Hyg. 86: 237–45.

Sunish, I. P., D. Raghunatha Rao, and A. Gajanana. 1998. Nitrogenous fertilizers, neem and vectors of Japanese encephalitis virus. Curr. Sci. 75: 1107.

Sunish, I. P., and R. Reuben. 2001. Factors influencing the abundance of Japanese encephalitis vectors in ricefields in India. I. Biotic. Med. Vet. Entomol. 15: 381–392.

Wood, B. L., L. R. Beck, R. K. Washino, K. A. Hibbard, and J. S. Salute. 1992. Estimating high mosquito-producing rice fields using spectral and spatial data. Int. J. Remote Sens. 13: 2813–2826.

Wood, B., R. Washino, L. Beck, K. Hibbard, M. Pitcairn, D. Roberts, E. Rejmankova, J. Paris, C. Hacker, J. Salute, et al. 1991. Distinguishing high and low anopheline-producing rice fields using remote-sensing and GIS technologies. Prev. Vet. Med. 11: 277–288.

Received 1 July 2006; accepted 6 November 2006.