Dynamics of immature stages of *Anopheles arabiensis* and other mosquito species (Diptera: Culicidae) in relation to rice cropping in a rice agro-ecosystem in Kenya

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ABSTRACT: We determined changes in species composition and densities of immature stages of *Anopheles arabiensis* mosquitoes in relation to rice growth cycle in order to generate data for developing larval control strategies in rice ecosystems. Experimental rice paddies (6.3m x 3.15m) exposed to natural colonization of mosquitoes were sampled weekly for two rice growing cycles between February 2004 and March 2005. Overall, 21,325 *Anopheles* larvae were collected, of which 91.9% were 1st and 2nd instars and 8.1% were 3rd and 4th instars. *An. arabiensis* was the predominant species (84.1%) with other species, *An. pharoensis* (13.5%), *An. funestus* (2.1%), *An. coustani* (0.3%), and *An. maculipalpis* (0.1%) accounting for only a small proportion of the anophelines collected. *Culex quinquefasciatus* (65.7%) was the predominant species among the non-anopheline species. Others species collected included: *C. annulioris* (9.9%), *C. poicilipes* (7.3%), *C. tigripes* (7.2%), *C. duttoni* (0.6%), *Aedes aegypti* (5.3%), *Ae. cumminsii* (3.5%), and *Ae. vittatus* (0.7%). The densities of the major anopheline species were closely related to rice stage and condition of the rice field. *An. arabiensis,* the predominant species, was most abundant over a three-week period after transplanting. Low densities of larvae were collected during the late vegetative, reproductive, and ripening phases of rice. An increase in larval density ten days post-transplanting was found to correlate with the application of fertilizer (sulphate of ammonia). Culicine and aedine species densities were significantly higher during the post-harvesting period. Our results suggest that the transplanting stage is favorable for the growth of immature stages of *An. arabiensis* and provides a narrow window for targeted larval intervention in rice. ***Journal of Vector Ecology* 31 (2): 245-251. 2006.**

Keyword Index: Rice growth cycle, *Anopheles* larval species, rice height, tillers, water depth, larval control.

INTRODUCTION

Irrigation development projects worldwide have been associated with negative impacts on human health, particularly in respect to vector-borne diseases, and rice fields in particular constitute an important source of vector mosquitoes (Lacey and Lacey 1990). It is notable that the presence and abundance of mosquito breeding sites associated with rice irrigation often results in a corresponding increase of malaria vectors and water-borne diseases. In the Ahero Rice Irrigation Scheme, western Kenya, a 70-fold increase in the abundance of malaria vectors, mainly *Anopheles arabiensis,* has been recorded compared to nearby non-irrigated areas (Surtees 1970).

Riceland agro-ecosystems present complex systems in which water is present throughout much of the crop cultivation season, hence sustained vector production throughout the year with only limited variability as a result of changes in habitat nature and water management regimen. The physical and chemical properties of rice field water exhibit marked variations during the crop cycle (Roger and Kurihara 1988). These changes have a tremendous impact on the relative abundance of mosquitoes breeding in rice fields and associated habitats. Variations may occur in response to dilution effect by rain, dispersion of the surface soil by cultivation practices, biological phenomena, and fertilizer application (Sunish and Reuben 2001). Agricultural operations like weeding and drying up of the field have a transient effect on the larval population (Rajendran 1987). Broadcasting nitrogenous fertilizers in rice fields has been found to enhance mosquito larval populations (Simpson and Roger 1991, Victor and Reuben 2000). Water source and depth (Collins and Washino 1980), temperature (Mogi 1978), pH, ionic composition, and conductivity (Kramer and Garcia 1989) have been reported to influence rate of larval development and subsequent densities.

Source reduction through management of larval habitats has been the key to malaria eradication efforts in the United States, Italy, and Israel (Kitron and Spielman 1989). The suppression and even eradication of malaria in these regions has been attributed to effective large-scale programs that target the immature vector mosquitoes or reduce the extent of suitable habitats in proximity to vulnerable human populations (Killeen et al. 2002).

Identification of the relationship between mosquito larval abundance and rice growth stages can provide useful information for designing targeted larval interventions in rice ecosystems. In this study we investigated the effect of rice growth cycle on abundance and species composition of mosquito larvae using experimental rice plots.

MATERIALS AND METHODS

Study site

The study was conducted in the Mwea Rice Irrigation Scheme (00°67'S, 37°35'E) which is about 100 km northeast of Nairobi, Kenya. The study area has been previously described (Mukiama and Mwangi 1989, Mutero et al. 2000). The Mwea Rice Irrigation Scheme is located in the west central region of Mwea Division and covers an area of about 13,640 ha with more than 50% of the area under irrigated rice cultivation. The rest of the area is used for subsistence farming. Mwea is a low-lying plain characterized by black cotton soil. The mean annual rainfall is approximately 950 mm with maximum rain falling in April/ May (long rains) and October/ November (short rains). The average maximum temperatures are in the range 16-26.5° C, while relative humidity varies from 52-67%. Mwea division has a population of approximately 150,000 persons in 25,000 households.

Experimental rice paddies

An experimental paddy measuring 1 acre (63 m x 63 m) was developed at the Mwea Irrigation and Agricultural Development Center (MIAD) in the Mwea Irrigation Scheme. Within the acre block, eight plots (50.4 m x 3.15 m) each with eight sub-plots (6.3 m x 3.15 m) were established. The plots were hydrologically isolated using unidirectional inflow and outflow canals to avoid water mixing between plots. The plots were exposed to natural colonization of *Anopheles* mosquitoes. Larval production was followed over two rice growing cycles (Feb 2004 to March 2005).

Larval sampling and habitat characterization

Larval sampling was done once weekly to generate stage-specific estimates of *Anopheles* and culicine larval densities, invertebrate abundance, and diversity. Samples were taken using standard dipping techniques with a plastic dipper (Mosquito Control Service and Supplies, U.S.A.). Twenty dips were taken from each sub-plot and the mosquito larvae collected sorted to the sub-families as either anopheline or culicine. The larvae were grouped according to instars and identified morphologically (Hopkins 1952, Gillies and Coetzee 1987). Water depth and rice height were measured at the same spot on every sampling visit. Rice growth cycle was characterized using standard agronomic categories for Basmati 217 into five categories: 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 post-harvest phase.

Data analysis

Densities of mosquito larvae were expressed as number of larvae per ten dips since the numbers of larvae collected were generally low. Data for the two rice cropping cycles was pooled. Correlation analysis was used to assess the relationship between larval densities and physical characteristics of the larval habitats. Log-transformed values ($\log_{10} n+1$) of larval counts were used in the statistical analysis to normalize the data. Data analysis was performed using the SPSS version 11.5 statistical package.

RESULTS

Species diversity and relative abundance

A total of 21,325 *Anopheles* larvae were collected of which 91.9% (n = 19,604) were early instars and 8.1% (n = 1,721) were late instars. At least 87.3% (n = 17,401) and 12.7% (n = 2,525) of the culicine larvae collected were early and late stage instars, respectively. A total of 513 pupae were collected. Morphological identification of the late stage larval instars yielded five anopheline, five culicine, and

Table 1. Species diversity and relative abundance of late stage instars of immature mosquito stages in experimental rice fields.

***Anopheles* spp**	**Number**	***Culex* spp**	**Number**	***Aedes* spp**	**Number**
An. arabiensis	1,274 (84.1) *	*C. quinquefasciatus*	585 (65.7)	*Ae. aegypti*	47 (5.3)
An. funestus	32 (2.1)	*C. Poicilipes*	65 (7.3)	*Ae. cumminsi*	31 (3.5)
An. pharoensis	204 (13.5)	*C. annulioris*	88 (9.9)	*Ae. vittatus*	6 (0.7)
An. maculipalpis	1 (0.1)	*C. tigripes*	64 (7.2)		
An. coustani	4 (0.3)	*C. duttoni*	5 (0.6)		
Total	**1,515**		**807**		**84**

* Values in parentheses represent percent of total identified.

Table 2. Correlation coefficients between mosquito larval abundance and physical parameters in the experimental rice plots.

Variables	Mean ± SE	*Anopheles* Early instars	*Anopheles* Late instars	*Anopheles* total	Culicine Early instars	Culicine Late instars	Culicine total	Pupae
Rice Height (cm)	73.50 ± 0.760	-0.426**	-0.245**	-0.434**	-0.217	0.041	-0.191*	-0.068*
Number of Tillers (Shoots)	22.18 ±0.250	-0.384**	-0.225**	-0.391**	0.208**	0.037	-0.186**	-0.086*
Water Depth (cm)	8.47 ± 0.087	-0.144**	-0.095**	-0.149**	-0.004	0.106**	0.028	0.017

*Significant at the 0.05 level (2-tailed).
**Significant at the 0.01 level (2-tailed).

three aedine species. *An. arabiensis* was the predominant species and was observed throughout the rice growing season with two peaks, one during the early vegetative stages and the other at the post harvest/ ratoon period when the plots were fallow. *An. arabiensis* represented 84.1% (n = 1,274) of the total late stage anophelines identified. Other species, *An. pharoensis* (13.5%, n = 204), *An. funestus,* (2.1%, n = 32), *An. coustani* (0.3%, n = 4), and *An. maculipalpis* (0.7%, n = 1) were present only in low densities. *C. quinquefasciatus* represented 65.7% (n = 585) of the total 891 late stage culicine and aedine larvae identified. Other species identified included: *C. annulioris* (9.9%), *C. poicilipes* (7.3%), *C. tigripes* (7.2%), *C. duttoni* (0.6%), *Ae. aegypti* (5.3%), (n = 31), *Ae. cumminsii* (3.5%), and (n = 6) *Ae. vittatus* (0.7%). This data reveals a wide range of mosquito species breeding in rice fields in Mwea (Table 1).

Temporal patterns of *Anopheles* larval densities

A bimodal distribution pattern of *Anopheles* larvae was evident over the 17 weeks (120 days) of rice growth (Figure 1). Overall, larval densities of *Anopheles* species increased in early stages of rice vegetative growth (tillering stage) and during late ripening and harvesting stages. During paddy preparation (denoted as week 0) the densities were low but rose in week 1 to reach a peak in week 3 with a mean density of 11.2 anophelines per ten dips. A second peak was recorded in week 17 with a mean density of 8.7 anophelines/ ten dips. A slight increase in density was observed from week four to week six. From week seven to week 14 the density of anopheline larvae was generally low, averaging less than one larva per ten dips. A similar bimodal trend was observed for the non-anopheline mosquito species but with densities being greatest during the late stages of rice growth (peak density 5.6 larvae per dip in week 17). The non-anopheline larval densities remained generally low between week 4 and week 15 (mean density 3.8 larvae/ten dips). The data shows that the distribution of the major anopheline vector species, *An. gambiae s.l.*, followed a pattern closely linked to rice crop phenology and condition of the rice field. Applications of sulphate of ammonia fertilizer (18.5 g/m^2) at day 10 post-transplanting (week 2) tended to promote larval productivity thereby leading to the peak seen in week 3 (11.2 larvae per ten dips). Low larval densities were found during the late vegetative, reproductive, and maturation phases of rice between tillering and harvesting. Figure 2 shows the densities of immature stages of mosquito species at different stages of rice growth. Culicine and aedine species favored the post-harvesting period when the rice paddies were fallow as evidenced by the increase in larval abundance.

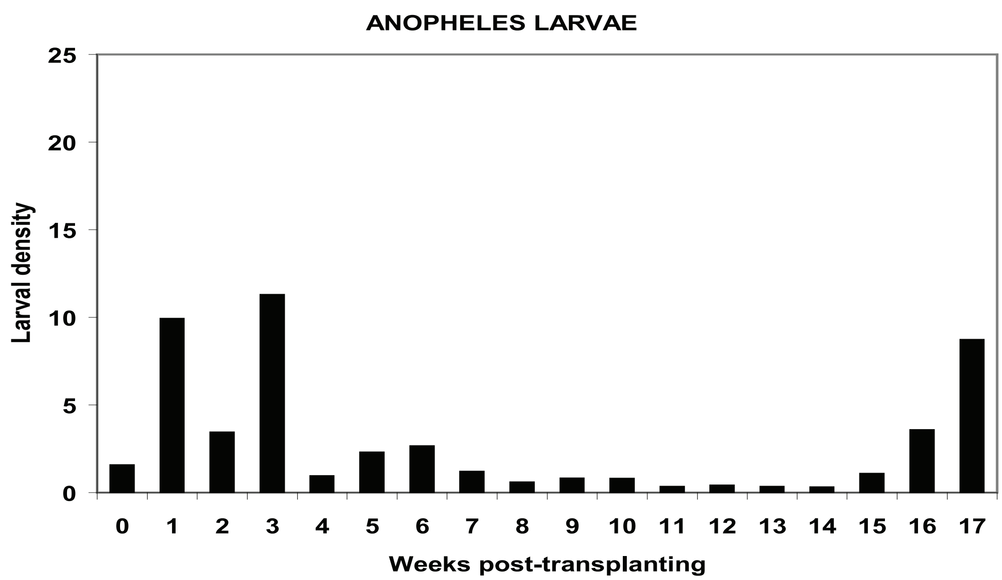
Association between larval abundance and habitat variables

A strong positive association between anopheline larvae and culicine larval densities (r= 0.305, P< 0.01) was observed indicating that prevailing habitat variables tended to affect their density dynamics equally (Table 2). Rice height showed a significant negative association with both *Anopheles* (r= -0.434, *P*< 0.01) and culicine larval densities (r=-0.191, *P*<0.01). The effect, however, was greater among the early immature stages (1st and 2nd instars) during mosquito development. Equally, the number of rice tillers showed significant negative association with *Anopheles* and culicine larval density. However, water depth was only significantly associated with density of *Anopheles* larvae but not with the culicine larvae. This shows that rice height, number of tillers (shoots), and water depth were important in explaining changes in productivity of anopheline and culicine mosquitoes in rice paddies as exemplified by the significant association between the two factors and pupal density in these habitats.

DISCUSSION

This study describes the rice growing cycle and how it influences the mosquito larval diversity and abundance. The critical time for *Anopheles gambiae* production in rice fields is the first three-week period following transplanting of rice seedlings. Most *Anopheles* larvae were collected between

Figure 1. Weekly density fluctuation in mosquito larvae over 2 rice growing seasons in Mwea, Kenya. Larval density is expressed as number of larvae per 10 dips; Weeks 0–1 = Field preparation; Week 1 = Transplanting; Weeks 2 and 3: Early vegetative phase; Weeks 4–8: Late vegetative phase; Week 9–13: Reproductive phase; Weeks 14–17: Ripening and maturation phase.



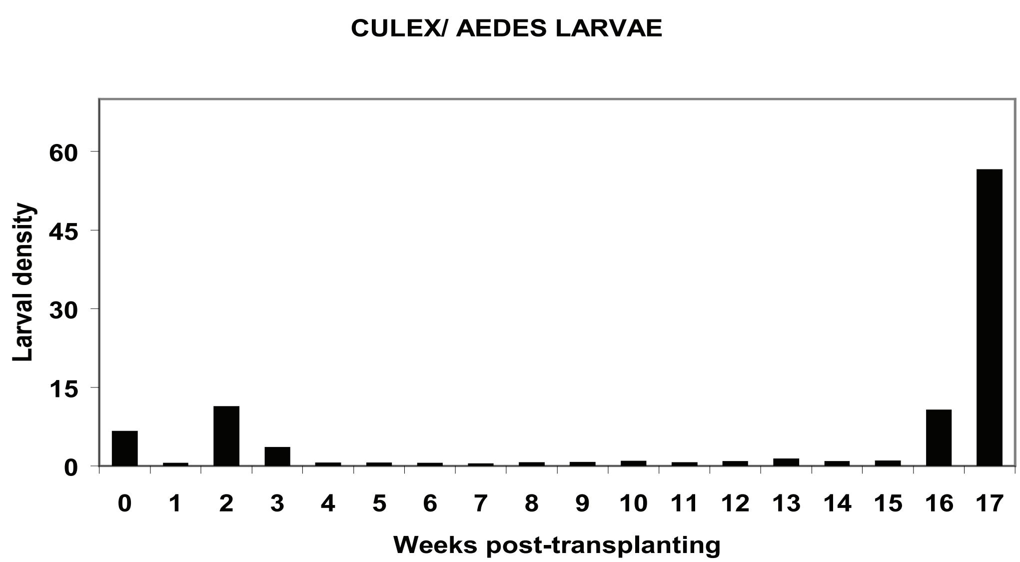
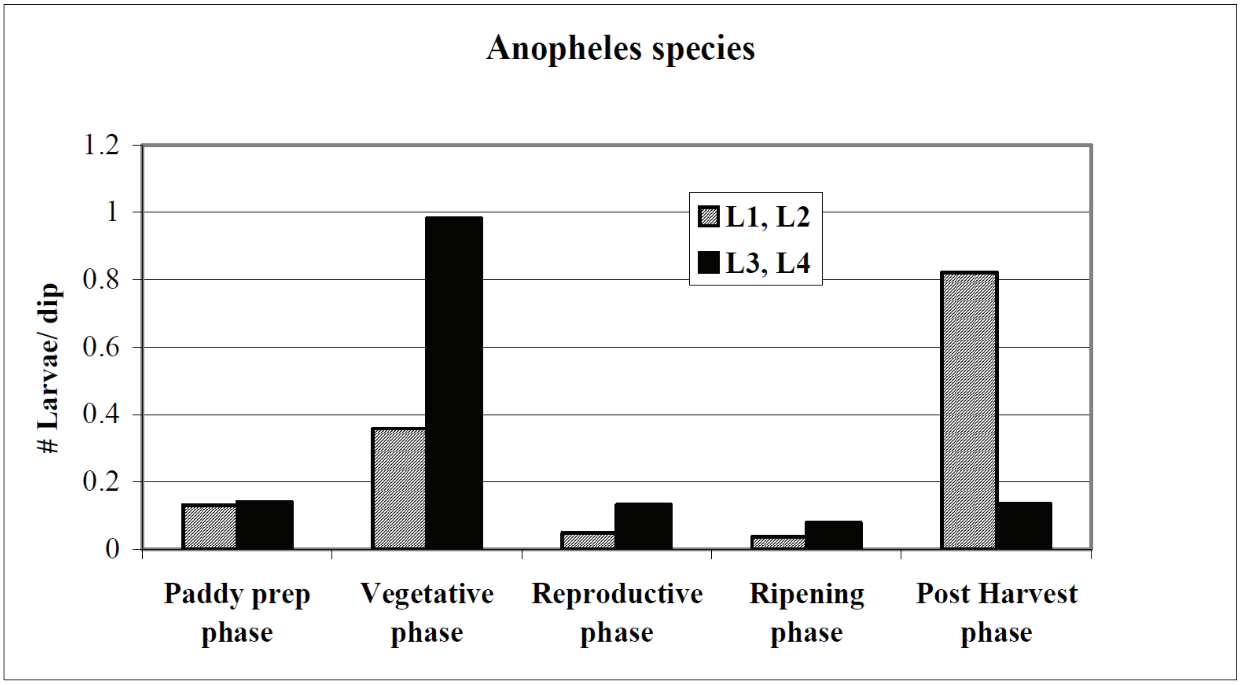
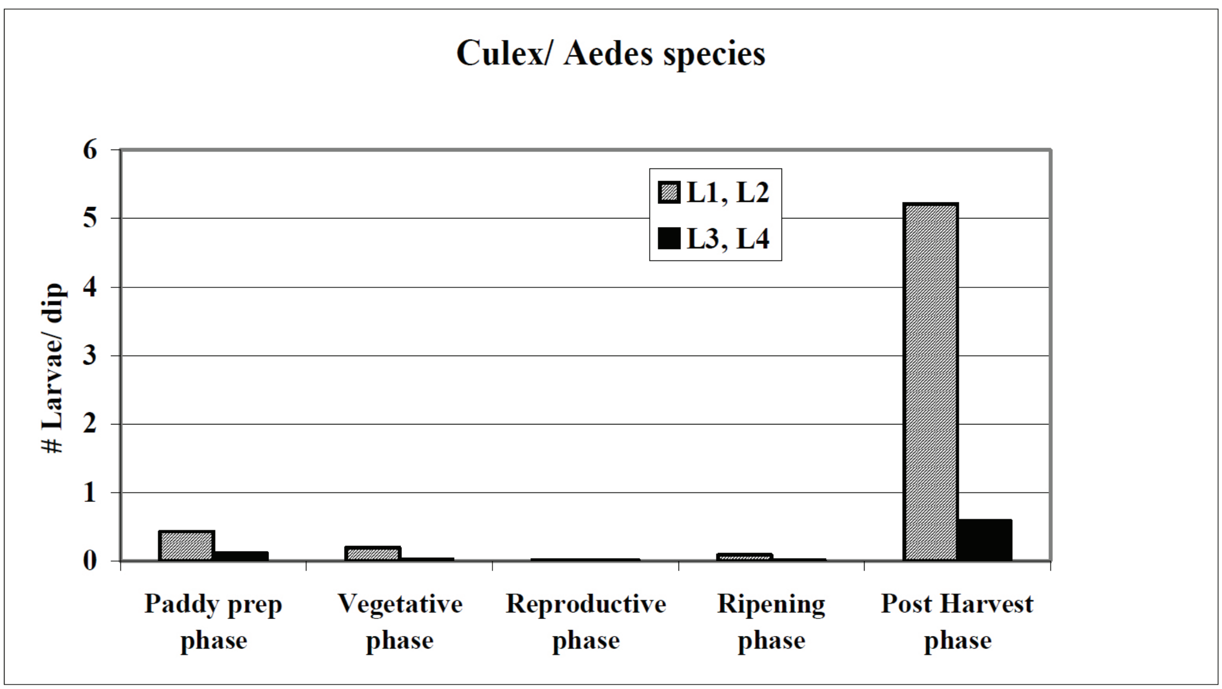


Figure 2. Relative densities immature stages of *Anopheles* and *Culex* mosquitoes at different rice cultivation stages in Mwea, Kenya.





land preparation and tillering stage (early vegetative phase). During this phase of rice development the field is more exposed and the water level is reduced to a minimum (<5 cm) to allow for root establishment. The exposed water surfaces with the widely distributed pockets of water in footprints left behind during transplanting and residual pools in depressions within the rice field provide suitable conditions for *Anopheles* oviposition and subsequent development. This is coupled with low predator population during the early phases of rice development, thus eliminating the role of natural regulation during the first three weeks of rice growth. Mosquito predators have been shown to play an important role in regulating vector populations (Mogi 1993). Basal fertilizer applied during transplanting (DAP at 16.3gm/m^2) provides a further stimulus for oviposition that enhances larval densities. Supplementation of nutrients ten days after transplanting by the addition of fertilizer tends to attract *Anopheles* and culicine mosquitoes to oviposition (Victor and Reuben 2000, Mutero et al. 2004). At transplanting, the short rice seedling and single plant tiller (shoot) provide sufficient penetration of sunlight as evidenced by the significant negative association between larval densities and height from our data. Anopheline larvae are known to breed in open, sunlit habitats (Gillies and Coetzee 1987). Our findings are similar to other studies conducted in the Mwea rice scheme that focused on adult mosquito densities, which have shown that *An. arabiensis* normally have a major adult population peak annually that coincides with the transplanting of rice seedlings during the main rice growing season (Mukiama and Mwangi 1989, Ijumba et al. 1990, Mutero et al. 2000).

A strong positive correlation between anopheline and culicine larval densities was evident in the present study. Although the data indicates that anopheline and culicine mosquito larvae utilize the same habitat for their development, variation in the time of attaining peak densities was apparent. The culicine larval abundance increased significantly from rice flowering to harvesting, a period when anopheline larval production was depressed probably due to changes in water physico-chemical determinants. After transplanting and the application of basal fertilizers, water turbidity was quite low, but it changed with rice growth (Mutero et al. 2004). After harvesting, the water was highly turbid with large amounts of organic matter due to decomposing rice straws, conditions that favored culicine development.

The goal of this study was to evaluate the potential for targeted larval control in rice, and the data generated provides sufficient evidence for time-specific application of larvicides taking advantage of the three-week window of productivity following the transplanting of rice. The targeted plan would be more effective in situations where there is organized rice cultivation and synchronous rice transplanting, thereby minimizing the duration of time the rice fields are under rice cultivation. For effective control of the mosquito/ malaria problem in Mwea Irrigation Scheme, consolidated efforts should therefore be targeted against the pre-adult stage between the transplanting and early tillering stage. At the same time, water management before rice transplanting should be considered as there is strong evidence that continuous flooding reduces survival and productivity (Mutero et al. 2000). Water management through intermittent flooding and draining of the rice plots applied during the first three weeks after transplanting would aid in flushing any mosquito larvae in the rice fields and have an impact on mosquito densities. A more integrated approach to vector control using larval control approaches and strategies to reduce man-vector contact may be a feasible plan to reduce the malaria burden in similar rice agro-ecosystems in sub-Saharan Africa.

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REFERENCES CITED

Collins, F.H. and W.K. Washino. 1980. The effects of irrigation water source and crop rotation on the abundance of *Culex tarsalis* in California rice fields. Proc. Calif. Mosq. Vector Contr. Assoc. 48: 103-108.

Gillies, M.T. and M. Coetzee, 1987. A supplement to anophelinae of Africa south of Sahara (Afro-tropical region). Public. South Africa Instit. Med. Res. 55: 1-143.

Hopkins, G.H.E. 1952. *Mosquitoes of Ethiopian Region. Larval bionomics of mosquitoes and taxonomy of culicine larvae*. 2nd edition. Adlard and Sons Ltd., London.

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

Killeen, G., U. Fillinger, I. Kiche, L.C.Gouagna, and B.G. Knols. 2002. Eradication of *Anopheles* gambiae from Brazil: Lessons for malaria control in Africa. Lancet Infect. Dis. 2: 618-627.

Kitron, U. and A. Spielman 1989. Suppression of transmission of malaria through source reduction: anti-anopheline measures applied in Israel, the United States and Italy. Rev. Infect. Dis. 11: 391-406.

Kramer, V.L. and R. Garcia. 1989. An analysis of factors affecting mosquito abundance in California wild rice fields. Bull. Soc. Vector Ecol. 14: 87-92.

Lacey, L.A. and C.M. Lacey. 1990. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. J. Am. Mosq. Contr. Assoc. 6: 1-93.

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

Mogi, M. 1993. Effect of intermittent irrigation on mosquitoes (Diptera: Culicidae) and larvivorous predators in rice fields. J. Med. Entomol. 30: 309-319.

Mukiama, T.K. and R.W. Mwangi. 1989. Field studies of larval *Anopheles arabiensis* Patton of Mwea Irrigation Scheme, Kenya. Insect Sci. Applic. 10: 55-62.

Mutero, C.M., H. Blank, F. Konradsen, and W. van der Hoek. 2000. Water management for controlling the breeding of *Anopheles* mosquitoes in rice irrigation schemes in Kenya. Acta Trop. 76: 253-263.

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

Rajendran, R. 1987. Mosquito larval density in relation to agricultural practices. *Alternatives to synthetic insecticides in integrated pest management system*. Center for Research in Medical Entomology, Madurai, India.

Roger, P.A. and Y. Kurihara. 1988. Flood water biology of tropical wetland rice fields. *Proceedings of the First International Symposium on Paddy Soil Fertility, 6 - 13 December, 1988.* University of Chiang Mai, Thailand: 275-300.

Simpson, I. and P.A. Roger. 1991. Impact of agrochemicals on mosquito larvae populations in rice fields. Intern. Rice Res. Newsl. 16: 29-30.

Sunish, I. P. and H. Reuben. 2001. Factors influencing the abundance of Japanese encephalitis vectors in the rice fields in India. Med. Vet. Entomol. 15: 381-392.

Surtees, G. 1970. Effects of irrigation on mosquito populations and mosquito-borne disease in man, with particular reference to rice field extension. Int. J. Environ. Stud. 1: 35-42.

Victor, T. J. and R. Reuben. 2000. Effects of organic and inorganic fertilisers on mosquito populations in rice fields of southern India. Med. Vet. Entomol. 14: 361-368.