ECOLOGY OF VECTOR MOSQUITOES IN SRI LANKA – SUGGESTIONS FOR FUTURE MOSQUITO CONTROL IN RICE ECOSYSTEMS

Junko Yasuoka and Richard Levins

Department of Population and International Health, Harvard School of Public Health, Boston, MA, USA

Abstract. Mosquito-borne diseases are a major public health threat in Asia. To explore effective mosquito control strategies in rice ecosystems from the ecological point of view, we carried out ecological analyses of vector mosquitoes in Sri Lanka. During the 18-month study period, 14 *Anopheles*, 11 *Culex*, 5 *Aedes*, 2 *Mansonia*, and 1 *Armigeres* species were collected, most of which are disease vectors for malaria, filariasis, Japanese encephalitis, or dengue in Sri Lanka and elsewhere in Asia. The density and occurrence of *Anopheles* and *Culex* species were the highest in seepage pools and paddy fields, where the majority of niche overlaps between larval mosquito and aquatic insect species were observed. All 7 aquatic insect species, which are larval mosquito predators, overlapped their niche with both *Anopheles* and *Culex* larvae. This suggests that conserving these aquatic insect species could be effective in controlling mosquito vectors in the study site. Correlations between several climatic factors and mosquito density were also analyzed, and weather conditions, including higher temperature, lower relative humidity, and higher wind velocity, were found to affect mosquito oviposition, propagation, and survival. These findings deepen our understanding of mosquito ecology and will strengthen future mosquito control strategies in rice ecosystems in Asia.

INTRODUCTION

Mosquito-borne diseases such as malaria, Japanese encephalitis and dengue are a major public health threat in Asian countries, including Sri Lanka. Mosquito and disease control efforts have not been successful mainly because of continued dependency on chemical spraying, the lack of intersectoral co-operation and interdisciplinary approaches, and the lack of environmentally sound mosquito control activities (Silva, 1997; Konradsen *et al*, 2000). Interest in formulating ecological approaches has been growing over the past three decades particularly because of the limitations of chemical use, including mosquitoes' insecticide resistance, the health risks for human and domestic animals, and disturbances to the natural balance such as predator-prey relationships (Yasuoka *et al*, 2006).

Agricultural practices in rice ecosystems encourage perennial propagation of mosquito vectors by creating different kinds of breeding sites such as paddy fields, irrigation canals, seepage pools, and tanks (reservoirs). Associations between the development of irrigated rice lands and malaria epidemics have been reported in several studies in Sri Lanka (Amerasinghe and Ariyasena, 1990, Amerasinghe *et al,* 1991, 1992; Ramasamy *et al,* 1992, Amerasinghe and Indrajith, 1994), and major epidemics of Japanese encephalitis have also occurred in these rice-growing areas (Peiris *et al,* 1993). Therefore, further

Correspondence: Junko Yasuoka, Department of Population and International Health, Harvard School of Public Health, Building I, Room 1219, 665 Huntington Avenue, Boston, MA 02115, USA.
Tel: +1 617 432 1484; Fax: +1 617 566 0365.
E-mail: jyasuoka@post.harvard.edu

understanding of mosquito ecology in rice ecosystems is crucial for developing future mosquito control strategies.

Several studies in Sri Lanka have identified mosquito breeding sites (Amerasinghe *et al*, 1997, van der Hoek *et al,* 1998), examined correlations between mosquito breeding and physical environmental conditions (Amerasinghe and Munasingha, 1988, van der Hoek *et al,* 1998), analyzed the impact of irrigation development on mosquito ecology (Amerasinghe, 1990; Amerasinghe and Ariyasena, 1991; Amerasinghe *et al,* 1991; Amerasinghe and Indrajith, 1994), and evaluated large-scale water management trials for the elimination of mosquito breeding sites (Konradsen *et al*, 1998, Matsuno *et al,* 1999, van der Hoek *et al*, 2003). However, few studies have observed mosquito ecology in a broader context, including mosquitoes' associations with other species in the local ecosystem.

The objective of the study therefore was to examine the ecology of mosquito vectors and their interactions with environmental, biologic, and climatic factors in the rice ecosystem. This study investigated a wide range of ecological characteristics of mosquitoes, including habitat types in rice ecosystems and associations with predatory insects and climatic conditions. The study explored the relationship between larval mosquitoes and their predators, which only a few previous studies have attempted to address. Suggestions for future mosquito control in rice ecosystems are made based on the results of these ecological analyses.

MATERIALS AND METHODS

Study sites

This 18-month study was conducted from October 1, 2002 to March 31, 2004 in Habaraluwewa (6.39N, 80.91E) in the southern rice-growing region of Sri Lanka. The area is within the watershed of the Uda Walawe reservoir (2,442 km^2), which generates electricity and supplies water for irrigation. The cropping pattern is largely paddy rice and irrigated bananas, with a small amount of other field crops, including sugarcane. There are 2 rice cultivation seasons: Maha, the major cultivation season from late September to early March, which is fed with inter-monsoon rains and with the northeast monsoon, and is well distributed on the island; and Yala, the minor cultivation season from early April to early September, when the southwest monsoon brings rain mostly to the southwest region of Sri Lanka. Habaraluwewa is in a high malaria risk area and from 1989-1994 had the highest level of malaria incidence in the country: the average annual number of microscopically confirmed malaria cases exceeded 40 per 1,000 persons (Konradsen *et al,* 2000).

Entomological survey

Mosquito sample collection was carried out in Habaraluwewa every two weeks from October 1, 2002 - March 31, 2004. The entomological team of the regional malaria office of the Anti-malaria Campaign in Moneragara assisted in mosquito sampling and identification.

Larval mosquito sampling. Larval mosquitoes were sampled every two weeks using standard 0.43-liter (1 pint) dippers. Collection was designed to obtain samples from 5 kinds of representative mosquito breeding habitats. Each collection day, 20 samples, each of which consisted of a mixture of 6 dips, were taken: 4 from 2 paddies, 4 from 2 irrigation canals, 4 from 4 seepage pools, 4 from a tank bed (a reservoir that stored water for irrigation and household use), and 4 from a residential area (*eg* wells, water storage containers and coconut shells). The 3rd and 4th instars were identified to species using taxonomic keys for the Oriental and Southeast Asian Regions (Amerasinghe, 1990; Amerasinghe and Ariyasena, 1990; Amerasinghe, 1992) and counted separately from the 1st and 2nd instars.

Aquatic insect sampling. Carnivorous aquatic insects, which feed on mosquito larvae, were collected from the same samples of mosquito larvae, identified to species, and classified into 7 groups: backswimmers (*Hemiptera: Notonectidae*), dragonfly nymphs (*Odonata: Aeshnidae, Gomphidae, Libellulidae*), water beetles (*Coleoptera: Dytiscidae, Hydrophilidae*), water boatmans (*Hemiptera, Corixidae*), water bugs (*Hemiptera: Belostomatidae, Nepidae, Ranatridae*), water measurers (*Hemiptera: Hydrometridae*), and water scorpions (*Hemiptera: Nepidae*).

Adult mosquito sampling. Adult mosquitoes were sampled every two weeks throughout the study period, using a net trap. The net trap was 3x3x3 m tent with 15 cm wide mosquito nets above the ground, and it was set up approximately 1 km away from the larval collection sites. A cow, which was borrowed from a farmer, was placed in the net trap at 06:15 PM, and trapped mosquitoes were collected by aspiration the following morning at 05:30 AM. Only females were identified to species, using the taxonomic keys described above.

Climatic data. Daily weather data, including rainfall (mm), temperature (degree Celsius), relative humidity (%), wind velocity (km/h), and sunshine hours (h), were obtained during the entire study period from the Sugarcane Research Institute in Uda Walawe, which is located approximately 11 km from the study site.

Statistical analysis

In order to thoroughly examine mosquito ecology in the rice ecosystem, several approaches were taken to examine determinants of mosquito density, including environmental, biological, and climate factors. Quantitative data on each mosquito species at the larval stage are described in terms of density and number of occurrences in 144 samples taken from each habitat during the study period. Percentage occurrence of each species (the number of occurrences divided by total mosquito-positive samples) was also calculated. In this paper, “density” is defined as the number of larval mosquito, aquatic insect, or adult mosquito species collected in a certain number of samples or the net trap. Niche width was calculated for each species, using a formula: niche width = the number of habitat types where a species was collected divided by 5, which is the total number of habitat types. Associations between a specie’s niche width and its larval and adult densities were examined by Spearman’s rank correlation.

The number of adult female mosquitoes collected by a cattle-baited net trap during the study period was also calculated by species. Associations between the densities of each species at larval and adult stages were analyzed by Spearman’s rank correlation in order to examine if larval sampling covered major breeding habitats (*eg,* if a species had low larval density and high adult density, it indicates that larval sampling did not cover its major breeding habitats).

Regarding associations between larval mosquitoes and aquatic insects, niche overlap in all habitat types except residential area was analyzed using the formula: niche overlap = the number of samples with both species A and B divided by the number of samples with at least one of the 2 species. (The residential area was excluded from this analysis due to the absence of samples with aquatic insects.) In addition, densities of the 1st and 2nd instars, the 3rd and 4th instars, and the 3 *Anopheles* and 3 *Culex* species (that were collected most across paddy fields, seepage pools, and the tank bed) were checked for their correlations with 4 species of aquatic insects.

In order to examine the impact of climatic factors on mosquito density, correlations between mosquito density at both the larval and adult stages and weather data were examined using Spearman’s rank correlation. The weather data included rainfall, temperature, relative humidity, wind velocity and sunshine hours, for which average and cumulative variables were calculated, taking an average of the following 2 values: 1) weather data between the last and current sampling dates; and 2) those of the last 2 weeks. The densities of mosquito larvae were aggregated by genera because each species density per collection date was too small to have statistical meaning.

RESULTS

Mosquito species and their disease transmission

In total, 14 *Anopheles*, 11 *Culex*, 5 *Aedes*, 2 *Mansonia*, and 1 *Armigeres* species were collected in Habaraluwewa during the 18-month study period. Most of these species are human disease vectors and have been reported to transmit one or more diseases, including malaria, filariasis, Japanese encephalitis, and dengue, in Sri Lanka and elsewhere in Asia (Lacey and Lacey, 1990; Konradsen *et al,* 2000).

Larval mosquito density and occurrence in different habitat types

Breeding of *Anopheles, Culex*, and *Aedes* species in each habitat, including paddy fields, irrigation canals, seepage pools, a tank bed, and a residential area, was investigated during the whole study period (Table 1). Except in irrigation canals, where only 7.6% of the samples were mosquito positive, the variety of *Anopheles* and *Culex* species collected was almost the same (10 or 11 species in total) in the other 4 habitats. The density and occurrence of *Anopheles* and *Culex* were the highest in seepage pools, followed by paddy fields, the tank bed and the residential area. *Aedes* were found in the residential area only.

In total, 10 *Anopheles* species and 9 *Culex* species were collected in 5 habitat types, while 3 *Aedes* species were collected in the residential areas only. Overall, these species with the 4 highest densities were *Cx. tritaeniorhynchus*,

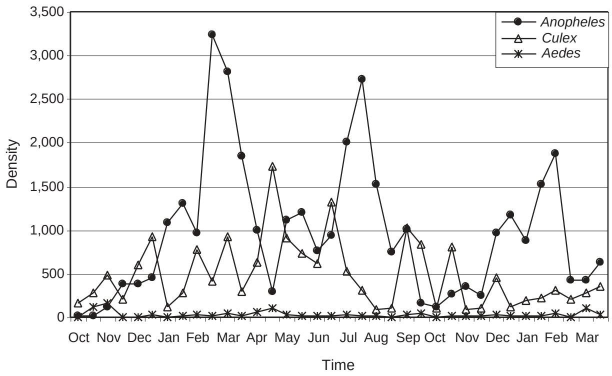

Fig 1–Adult female mosquitoes (cattle-baited net trap).

Ae. albopictus, *An. peditaeniatus*, and *An. vagus*. A strong correlation was found between a specie's niche width and its density at the larval and adult stages (Spearman's r = 0.848, p < 0.0001 at larval stage, and r = 0.641, p = 0.0001 at adult stage).

Larval mosquito densities did not show distinct seasonal patterns and fluctuated irregularly in both paddy fields and seepage pools (data not shown).

Adult mosquito density

At the adult stage, 14 *Anopheles*, 5 *Culex*, and 5 *Aedes* species were collected during the study period (Table 1). Major species included *An. peditaeniatus*, *An. nigerrimus*, *Cx. tritaeniorhynchus*, *An. vagus*, *Cx. pseudovishnui*, *Cx. gelidus*, and *Cx. fuscocephala*. Species composition during the entire study period was highly correlated between the larval and adult stages (Spearman's r = 0.518, p = 0.003).

Adult *Anopheles* density showed distinct patterns according to rice cultivation cycles (Fig 1). The density started to increase from the first water issue days, showed a small peak in 1 to 2 months, hit a big peak in 1 to 2 months after the small peak, and dropped suddenly once the harvest was complete. On the contrary, adult *Culex* did not show such patterns related to cultivation cycles.

Table 1
Density and occurrence of larval mosquitoes and aquatic insects in five habitat types and density of adult mosquitoes.

Species	Paddy field			Irrigation canal			Seepage pool			Tank bed			Residential area[c]			Larvae		Adults
	Density[a]	No. Occurrence	% Occurrence[b]	Density[a]	No. Occurrence	% Occurrence[b]	Density[a]	No. Occurrence	% Occurrence[b]	Density[a]	No. Occurrence	% Occurrence[b]	Density[a]	No. Occurrence	% Occurrence[b]	Niche breadth	Total density[d]	Density (females)
An. aconitus	0	0	0.0	0	0	0.0	1	1	1.0	0	0	0.0	2	1	1.9	0.4	3	764
An. annularis	0	0	0.0	0	0	0.0	10	7	7.1	12	5	14.7	0	0	0.0	0.4	22	102
An. barbirostris	0	0	0.0	0	0	0.0	1	1	1.0	0	0	0.0	1	1	1.9	0.4	2	488
An. culicifacies	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	3
An. jamesii	3	3	3.2	0	0	0.0	1	1	1.0	0	0	0.0	0	0	0.0	0.4	4	316
An. maculatus	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	5
An. nigerrimus	3	2	2.2	1	1	9.1	6	3	3.1	0	0	0.0	0	0	0.0	0.6	10	9,783
An. pallidus	0	0	0.0	0	0	0.0	0	0	0.0	1	1	2.9	0	0	0.0	0.2	1	6
An. peditaeniatus	75	35	37.6	2	2	18.2	94	41	41.8	20	13	38.2	11	5	9.3	1	202	18,922
An. ramsayi	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	4
An. subpictus	0	0	0.0	0	0	0.0	2	1	1.0	0	0	0.0	0	0	0.0	0.2	2	39
An. tessellatus	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	353
An. vagus	46	11	11.8	2	1	9.1	45	10	10.2	8	3	8.8	3	1	1.9	1	104	4,006
An. varuna	0	0	0.0	0	0	0.0	0	0	0.0	3	3	8.8	11	5	9.3	0.4	14	188
Total	127	51		5	4		160	65		44	25		28	13			364	34,979
Cx. bitaeniorhynchus	12	6	6.5	0	0	0.0	16	7	7.1	1	1	2.9	0	0	0.0	0.6	29	175
Cx. fuscanus	6	4	4.3	0	0	0.0	12	9	9.2	10	4	11.8	4	3	5.6	0.8	32	53
Cx. fuscocephala	5	2	2.2	0	0	0.0	37	6	6.1	36	4	11.8	5	1	1.9	0.8	83	2,631
Cx. gelidus	11	7	7.5	0	0	0.0	0	0	0.0	6	2	5.9	0	0	0.0	0.4	17	2,704
Cx. infula	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	2	1	1.9	0.2	2	2
Cx. mimulus	0	0	0.0	3	1	9.1	0	0	0.0	0	0	0.0	0	0	0.0	0.2	3	0
Cx. pseudovishnui	34	10	10.8	0	0	0.0	38	9	9.2	6	4	11.8	0	0	0.0	0.6	78	3,129
Cx. quinquefasciatus	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	32	2	3.7	0.2	32	73
Cx. sitiens	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	94
Cx. tritaeniorhynchus	237	51	54.8	54	9	81.8	261	56	57.1	27	6	17.6	26	4	7.4	1	605	8,434
Cx. vishnui	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	200
Total	305	80		57	10		364	87		86	21		69	11			881	17,495

Ae. albopictus	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	322	36	66.7	0.2	322	24
Ae. pallidostriatus	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	103
Ae. scutellaris	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	99
Ae. uniformis	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	14	1	1.9	0.2	14	812
Ae. vittatus	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	7	3	5.6	0.2	7	26
Total	0	0		0	0		0	0		0	0		343	40			343	1,064
Back swimmer	54	22	26.5	4	3	3.3	51	21	22.1	5	4	6.2	0	0	0.0			
Dragonfly nymph	20	11	13.3	16	11	12.2	27	16	16.8	58	28	43.1	0	0	0.0			
Water beetle	35	14	16.9	56	10	11.1	37	20	21.1	21	7	10.8	0	0	0.0			
Water beetle larva	21	15	18.1	14	7	7.8	21	17	17.9	16	10	15.4	0	0	0.0			
Water boatman	6	3	3.6	0	0	0.0	11	6	6.3	3	2	3.1	0	0	0.0			
Water bug	52	33	39.8	166	76	84.4	80	51	53.7	37	25	38.5	0	0	0.0			
Water bug larva	12	8	9.6	9	7	7.8	20	9	9.5	3	2	3.1	0	0	0.0			
Water measurer	17	10	12.0	21	14	15.6	36	18	18.9	21	16	24.6	0	0	0.0			
Water scorpion	4	3	3.6	0	0	0.0	5	5	5.3	0	0	0.0	0	0	0.0			
Total	221	119		286	128		288	163		164	94		0	0				

[a]Number per 144 samples, [b]Percentage occurrence in mosquito-positive samples, [c]Sampling sites changed every time, [d]Number of larvae per 720 samples

Associations between larval mosquitoes and aquatic insects

Aquatic insects collected with mosquito larvae in 4 habitat types were identified to 7 species (Table 1), and their niche overlaps with larval mosquito species were examined. Water beetles and their larvae overlapped their niche with the most varieties of mosquito species (10 and 8 species, respectively), followed by water bugs and their larvae (11 and 6), dragonfly nymph (10), and water measurer (9) (Table 2). Backswimmer (7), water boatman (4), and water scorpion (4) also had niche overlap with smaller number of mosquito species. A striking finding is that all aquatic insect species shared their niche with the 1st and 2nd instars of both *Anopheles* and *Culex* species.

The correlation between 3 major *Anopheles* (*An. peditaeniatus*, *An. vagus*, and *An. annularis*) and 3 major *Culex* species (*Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, and *Cx. fuscocephala*) and 4 aquatic insect species was evaluated (Table 2). In a predator-prey relationship, a positive correlation may come about in two ways: either the presence of mosquito larvae attracts its enemies in some behavioral way, or they are both attracted to the same micro-habitats. A negative correlation would arise if the predators exterminated their prey in the micro-habitats before sampling. It may also reflect competition among predators.

Correlations, although moderate or weak, were observed as follows: 17 correlations (14 positive and 3 negative) were found in 3 habitat types (paddy field, seepage pool, and tank) between 4 aquatic insect species and mosquito larvae, including 4 species and cumulative densities of the 1st and 2nd instars and the 3rd and 4th instars.

Overall, significant correlations were found between aquatic insects and *Anopheles* larvae in paddy field, seepage pools, and the tank bed: water beetles had 5 positive and 2 negative correlations, water bugs had 3 positive and 1

Table 2
Summary of nich overlap and significant correlations between major larval mosquito and aquatic insect species.

Aquatic insect species	Stage	Niche overlap (# larval mosquito spp)	Larval mosquito spp with significant correlation	Habitat type	Spearman's rho	p-value
Water beetle	Adults	10	*An.* $3^{rd}+4^{th}$ instars	Paddy field	-0.333	0.047
			An. $3^{rd}+4^{th}$ instars	Tank bed	0.366	0.028
			An. vagus	Tank bed	0.343	0.041
			Cx. $1^{st}+2^{nd}$ instars	Seepage pool	0.346	0.039
	Larvae	8	*An.* $1^{st}+2^{nd}$ instars	Tank bed	0.342	0.042
			An. annularis	Tank bed	0.388	0.020
			Cx. pseudovishnui	Seepage pool	-0.387	0.020
Water bug	Adults	11	*An. vagus*	Paddy field	-0.388	0.020
			Cx. $3^{rd}+4^{th}$ instars	Tank bed	0.344	0.040
	Larvae	6	*An. peditaeniatus*	Seepage pool	0.342	0.041
			Cx. pseudovishnui	Seepage pool	0.379	0.023
Dragonfly	Nymphs	10	*An. annularis*	Seepage pool	0.416	0.012
Water measurer	Adults	9	*An.* $1^{st}+2^{nd}$ instars	Tank bed	0.401	0.016
			An. $3^{rd}+4^{th}$ instars	Tank bed	0.425	0.010
			An. peditaeniatus	Tank bed	0.459	0.005

negative correlations, dragonfly nymphs had 1 positive correlation, and water measurers had 3 positive correlations. In other words, *Anopheles* species had 13 correlations (11 positive and 2 negative), and *Culex* had 4 correlations (3 positive and 1 negative) with aquatic insects. As for habitats, 2 negative correlations were found in paddy fields, 4 positive and 1 negative correlations in seepage pools, and 10 positive correlations in the tank bed.

Correlations between mosquito density and climatic factors

Most of the significant correlations were found with adult mosquitoes (Table 3). Rainfall, maximum temperature, and relative humidity had both positive and negative correlations with different species. The majority of the correlations of maximum temperature were negative (with 6 out of 7 species) and those of relative humidity were positive (with 6 out of 8 species). Wind velocity and sunshine hours were only negatively correlated with adult mosquitoes (3 and 1 species, respectively).

DISCUSSION

This study found diverse mosquito species in the rice ecosystem throughout the year, most of which are human disease vectors that transmit malaria, filariasis, Japanese encephalitis, or dengue. Fortunately, very few of the primary malaria vector in Sri Lanka, *An. culicifacies*, were found in the study site, probably due to the lack of their breeding sites such as drying-up rivers with slow-flowing water or with pools of sandy bottoms (Carter and Jacocks, 1929, Gill, 1936, Amerasinghe and Munasingha, 1988, Amerasinghe and Ariyasena, 1990). However, other malaria vectors, including *An. peditaeniatus*, *An. vagus*, and *An. nigerrimus*, were abundant, especially at their adult stages. Four of the five *Culex* spe-

Table 3
Summary of significant correlations between mosquito densities and climatic factors.

Climatic factors (Daily average)	Mosquito species[a] (Larval habitat type)	Spearman's rho	p-value
Cumulative rainfall (5.14 mm)	*An. jamesii*	0.446	0.006
	An. nigerrimus	-0.373	0.025
	Ae. pallidostriatus	-0.344	0.040
	Ae. larvae (Residential area)	0.385	0.021
Average maximum temperature (32.8ºC)	*An. annularis*	-0.345	0.039
	An. culicifacies	-0.346	0.039
	An. jamesii	-0.392	0.018
	An. subpictus	-0.418	0.011
	An. tessellatus	-0.697	<0.0001
	An. vagus	-0.600	0.0001
	Ae. scutellaris	-0.395	0.017
	Cx. tritaeniorhynchus	0.365	0.029
	Cx. larvae (Tank bed)	-0.340	0.042
	Cx. larvae (Residential area)	-0.392	0.018
Average relative humidity (74.6%)	*An. nigerrimus*	-0.387	0.020
	An. peditaeniatus	-0.399	0.016
	An. subpictus	0.394	0.018
	An. tessellatus	0.478	0.003
	An. vagus	0.480	0.003
	An. larvae (Tank bed)	0.471	0.004
	Cx. quinquefasciatus	0.356	0.036
	Ae. albopictus	0.350	0.036
	Ae. scutellaris	0.488	0.003
Average wind velocity (3.0 km/h)	*An. tessellatus*	-0.548	0.0005
	Cx. larvae (Residential area)	-0.387	0.020
	Ae. scutellaris	-0.432	0.009
	Ae. vittatus	-0.392	0.018
Cumulative sunshine hours (6.6 h)	*An. annularis*	-0.454	0.005
	An. larvae (Paddy field)	0.373	0.025

[a]Adult stage if not indictated as "larvae"

cies reported to carry Japanese encephalitis virus in the field in Sri Lanka (Peiris *et al,* 1986; Amerasinghe *et al,* 1989; Peiris *et al,* 1992; Amerasinghe *et al*, 1998) were found in the study site (*Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. fuscocephala*, and *Cx. gelidus*). *Culex quinquefasciatus*, which was abundant in residences, is a major vector of filarialsis throughout tropical region. Mosquito control efforts are therefore greatly needed in order to reduce the risk and burden of mosquito-borne diseases in the region.

Analyses in this study demonstrated the complexity of mosquito ecology in the rice ecosystem and highlighted the necessity of mosquito control strategies that integrate ecological characteristics of local mosquito vectors and their interactions with environmental, biologic, and climatic factors. Those factors include: mosquito habitat types, especially for breeding; associations between mosquito larval and aquatic insect species that coexist in the same habitat types; and climatic factors such as temperature, humidity, and wind velocity.

In order to eliminate mosquito breeding sites, specific targets as well as broad coverage are necessary. Ecological analyses of mosquito breeding found that seepage pools and paddy fields were the two major breeding sites that should be the focus of efforts to control *Anopheles* and *Culex* species. The findings also indicated that species that are able to breed in different habitat types could propagate, survive, and protect themselves better against uncertainties such as sudden environmental or weather changes. In fact, 3 major larval mosquito species (*Cx. tritaeniorhynchus*, *An. peditaeniatus*, and *An. vagus*) were found to breed in all five habitat types and had the widest niche (niche width = 1) among all larval species collected in the village. A specie's niche width was also strongly correlated with its larval and adult densities. Therefore, in order to control major larval mosquito species, it would be ideal to cover all possible habitat types while placing greater importance on their major breeding sites.

Identifying breeding sites for mosquito control requires a combination of field surveys and information from previous studies in the region. In this study, strong correlations were found between species compositions at the larval and adult stages, which indicates that the larval collection sites covered the main breeding sites of the majority of the species. However, the densities of *An. nigerrimus* at the larval and adult stages were highly discrepant: although its larval density was extremely low, the adults were the second most common among all adult species. One possible reason for the discrepancy could be that this species bred in habitat types that were not sampled in this study, such as marshes and streambed pools as reported in previous studies (Amerasinghe and Munasingha, 1988; Amerasinghe *et al,* 1997). Therefore, mosquito control in or around marshes and other possible breeding sites of major vectors, which were not specified by this study, should also be incorporated in any future strategies.

Maximizing predator-prey relationships between aquatic insect species and mosquito larvae that coexist in the same habitat types would also be useful for mosquito control. Niche analyses in this study showed only small niche overlaps between mosquito and aquatic insect species. However, most of the aquatic insect species, especially water beetles, water bugs, and their larvae, shared their niche with a variety of larval mosquito species across all habitat types, except the residential area, where no aquatic insects were found. In addition, all aquatic insect species shared their niche with the 1st and 2nd instars of both *Anopheles* and *Culex* species. Thus, it is very likely that aquatic insects and mosquito larvae competed over food and space. In addition, the predator-prey relationships encouraged by increasing the density of aquatic insects or by aggregating mosquito larvae and aquatic insects in smaller numbers of water bodies could suppress mosquito larval density.

Climatic factors should also be taken into account in order to develop effective mosquito control strategies. Overall, maximum temperature had negative, relative humidity had positive, and wind velocity had negative correlations with larval and adult mosquito species densities in the study site. The negative correlations between temperature and mosquito density could be explained by the fact that the optimum temperature for mosquito survival is in the range of 20-25ºC (Molineaux, 1988, Martens *et al,* 1995), and that the average maximum temperature in the study site was 32.8ºC, which was much higher than their favorable temperature. Positive correlations were found between relative humidity and mosquito density mainly because mosquitoes prefer a relative humidity above 60% and because higher humidity promotes mosquito longevity (Martens *et al,* 1995). Negative correlations between wind velocity and mosquito density reflect the fact that wind tends to suppress mosquito flight (Clements, 1999) and thus could have affected their oviposition. Therefore, lower temperature, higher relative humidity, lower wind velocity, and shorter cumulative sunshine hours could serve as precautionary warnings of increasing mosquito density and could indicate when mosquito control efforts and personal protective measures against mosquito bites are most needed.

One limitation of the study is that larval collection was carried out in only 5 habitat types. As discussed above, the collection covered breeding sites for the majority of species, but not for some species that breed in other habitats, including marshes and small temporary pools. Adult mosquito collection was done only by a cattle-baited net trap. This method might have caught more zoophilic than anthrophilic species.

One of the strengths of the study is the multidimensionality of the analyses. Several correlation and niche analyses were conducted to examine local mosquito ecology from different angles. This is also one of the very few studies that addressed the relationships between larval mosquito and aquatic insect species in different habitat types. The findings from these analyses provided us fuller understanding of the ecology of local mosquitoes and risk factors that encourage mosquito propagation, which could serve as crucial information in developing future mosquito control strategies in the region.

This study also indicated that it is possible for residents in rice ecosystems to monitor mosquito larval density and relevant biologic and climatic factors without extensive instruments. Mosquito larvae and aquatic insects in different habitats could be collected by dipping. Climatic factors could also be measured with simple instruments and used as an indicator for mosquito density change. Regular monitoring of biologic and climatic factors by local residents could be the most timely and sustainable way to follow up mosquito ecology and raise awareness about mosquito control and mosquito-borne disease prevention.

ACKNOWLEDGEMENTS

We are grateful to A Spielman and BH Singer for their helpful comments on the manuscript. We heartily thank RMK Ratnayake and his entomological team at the Anti-malaria Campaign in Moneragala, who made an enormous effort for sample collection.

REFERENCES

Amerasinghe FP, Amerasinghe PH, Indrajith NG, *et al.* Japanese encephalitis activity in Anuradhapura during the 1988/89 northeast monsoonal period. *Proc Sri Lanka Assoc Advance Sci* 1989; 45: 19-20.

Amerasinghe FP, Amerasinghe PH, Peiris JSM, Wirtz R. Anopheline ecology and malaria infection during the irrigation development of an area of the Mahaweli project, Sri Lanka. *Am J Trop Med Hyg* 1991; 45: 226-35.

Amerasinghe FP, Amerasinghe PH, Wirtz R, *et al*. Malaria transmission by *Anopheles subpictus* (Diptera: Culicidae) in a new irrigation project in Sri Lanka. *J Med Entomol* 1992; 29: 577-81.

Amerasinghe FP, Ariyasena TG. Larval survey of surface water-breeding mosquitoes during irrigation development in the Mahaweli project, Sri Lanka. *J Med Entomol* 1990; 27: 789-802.

Amerasinghe FP, Ariyasena TG. Survey of adult mosquitoes (Diptera: Culicidae) during irrigation development in the Mahaweli project, Sri Lanka. *J Med Entomol* 1991; 28: 387-93.

Amerasinghe FP, Indrajith NG. Nocturnal biting rhythms of mosquitoes (Diptera: Culicidae) in Sri Lanka. *Trop Zool* 1995; 8: 43-53.

Amerasinghe FP, Indrajith NG. Postirrigation breeding patterns of surface water mosquitoes in the Mahaweli project, Sri Lanka, and comparisons with preceding developmental phases. *J Med Entomol* 1994; 31: 516-23.

Amerasinghe FP, Konradsen F, Fonseka KT, Amerasinghe PH. Anopheline (Diptera: Culicidae) Breeding in a traditional tank-based village ecosystem in north central Sri Lanka. *J Med Entomol* 1997; 34: 290-7.

Amerasinghe FP, Munasingha NB. A predevelopment mosquito survey in the Mahaweli development project area, Sri Lanka: Immatures. *J Med Entomol* 1988; 25: 286-94.

Amerasinghe FP, Peiris JSM, Karunarantne SHPP, Amerasinghe PH. Epidemiology of the 1987 Japanese encephalitis outbreak in the Anuradhapura area. II. Entomological aspects. *Proc Sri Lanka Med Assoc* 1998; 101: 22-3.

Amerasinghe FP. A guide to the identification of the anopheline mosquitoes (Diptera: Culicidae) of Sri Lanka. I. Adult females. *Ceylon J Sci* (Biol Sci) 1990; 21: 1-16.

Amerasinghe FP. A guide to the identification of the anopheline mosquitoes (Diptera: Culicidae) of Sri Lanka. II. Larvae. *Ceylon J Sci* (Biol Sci) 1992; 22: 1-13.

Carter HF, Jacocks WP. Observations on the transmission of malaria by anopheline mosquitoes in Ceylon. *Ceylon J Sci* 1929; 2: 67-86.

Clements AN. The biology of mosquitoes. Vol 2. Sensory reception and behaviour. New York: CABI Publishing, 1999.

Gill CA. Some points in the epidemiology of malaria arising out of the study of the malaria epidemic in Ceylon in 1934-35. *Trans R Soc Trop Med Hyg* 1936; 29: 426-80.

Gunawardena DM, Wickremasinghe AR, Muthuwatta L, *et al*. Malaria risk factors in an endemic region of Sri Lanka, and the impact and cost implications of risk-factor based interventions. *Am J Trop Med Hyg* 1998; 58: 533-42.

Konradsen F, Matsuno Y, Amerasinghe FP, Amerasinghe PH, van der Hoek W. *Anopheles culicifacies* breeding in Sri Lanka and options for control through water management. *Acta Trop* 1998; 71: 131-8.

Konradsen F, Amerasinghe FP, van der Hoek W, Amerasinghe PH, eds. Malaria in Sri Lanka, current knowledge on transmission and control. Colombo, Sri Lanka: International Water Management Institute, 2000.

Lacey LA, Lacey CM. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. *J Am Mosq Control Assoc* 1990; 6: 1-93.

Martens WJM, Niessen LW, Rotmans J, Jetten TH, McMichael AJ. Potential impact of global climate change on malaria risk. *Environ Health Perspect* 1995; 103: 458-64.

Matsuno Y, Konradsen F, Tasumi M, van der Hoek W, Amerasinghe FP, Amerasinghe PH. Control of malaria mosquito breeding through irrigation water management. *Int J Water Resources Develop* 1999; 15: 93-105.

Molineaux L. The epidemiology of human malaria as an explanation of its distribution, including some implications for its control. In: Wernsdorfer WH, McGregor I, eds. Malaria, principles and practice of malariology, New York: Churchill Livingstone, 1988: 913-98.

Peiris JSM, Perera LP, Arunagiri CK, Amerasinghe FP. Arboviruses in Sri Lanka. Bangkok: Proceedings of the First International Conference on the Impact of Viral Diseases on the Development of Asian Countries 1986: 177.

Peiris JSM, Amerasinghe FP, Amerasinghe PH, Ratnayake CB, Karunaratne SHPP, Tsai TF. Japanese encephalitis in Sri Lanka - the study of an epidemic: vector incrimination, porcine infection and human disease. *Trans R Soc Trop Med Hyg*1992; 86: 307-13.

Peiris JSM, Amerasinghe FP, Arunagiri CK, *et al*. Japanese encephalitis in Sri Lanka: comparison of vector and virus ecology in different agro-climatic areas. *Trans R Soc Trop Med Hyg* 1993; 87: 541-8.

Ramasamy R, De Alwis R, Wijesundere A, Ramasamy MS. Malaria transmission at a new irrigation project in Sri Lanka: the emergence of *Anopheles annularis* as a major vector. *Am J Trop Med Hyg* 1992; 47: 547-53.

Silva KT. “Public health” for whose benefit? Multiple discourses on malaria in Sri Lanka. *Med Anthropol* 1997; 17: 195-214.

van der Hoek W, Amerasinghe FP, Konradsen F, Amerasinghe PH. Characteristics of malaria vector breeding habitats in Sri Lanka: relevance for environmental management. *Southeast Asian J Trop Med Public Health* 1998; 29: 168-72.

van der Hoek W, Konradsen F, Amerasinghe PH, Perera D, Piyaratne MK, Amerasinghe FP. Towards a risk map of malaria for Sri Lanka: the importance of house location of malaria for Sri Lanka: the importance of house location relative to vector breeding sites. *Int J Epidemiol* 2003; 32: 280-5.

Yasuoka J, Levins R, Mangione TW, Spielman A. Community-based rice ecosystem management for suppressing vector anophelines in Sri Lanka. *Trans R Soc Trop Med Hyg* 2006; 100: 995-1006.