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DENSITY AND DIVERSITY OF MOSQUITO LARVAE ASSOCIATED WITH RICE FIELD AND MARSHLAND HABITATS IN TWO CLIMATICALLY DIFFERENT AREAS IN SRI LANKA

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ABSTRACT

Rice field and marshland habitats have significant influence on production of mosquito larval populations including vector mosquitoes and hence the disease transmission. Study revealed that mosquito larval density in rice fields of two climatic regions were significantly different and that of rice fields in semi dry zone are more diverse than those of wet zone (H' semi dry zone = 1.48; H' wet zone = 1.35). Marshland habitats in wet zone were represented mainly by *Culex gelidus*. Both *Culex gelidus* and *Culex tritaeniorhynchus* larvae were highly available in rice fields of two climatic regions. They were mainly associated with high Total Dissolved Solids, (TDS) ≥10.00 mg/L, pH level of 6-8, low Dissolved Oxygen, (DO) 5.0 – 6.0 mg/L, low nitrate level, ≤ 5.0 mg/L and low phosphate level, ≤ 1.0 mg/L. However, they could tolerate a range of Biological Oxygen Demand (BOD) level in water. Mosquito larval density in two habitat types in wet zone was not significantly different. Temporal variation of *C. gelidus* and *C. tritaeniorhynchus* was positively or negatively correlated with cultivation cycle of rice in semi dry zone area. The main goal of this study was to determine the variation of mosquito larval diversity in the rice field and marshy land habitats in two climatically different areas, wet zone and semi-dry zone in Sri Lanka. Sampling of mosquito larvae was done using standard dipping method.

Keywords: *Culex, Armigeres*, rice field habitat, marsh land.

INTRODUCTION

Mosquitoes generally exploit shallow water bodies with higher nutrient and salinity levels and with low dissolved oxygen content. In such habitats they have higher rates of survival due to abundant food sources and low predator populations (Tennessen, 1993; Yadav, 2009). Flood irrigated rice fields serve as a breeding site for potential vector mosquito species resulting negative impacts on human health. There is evidence for direct relationship between irrigation development and increased malaria transmission (Mwangangi *et al*., 2010). Studies carried out by Amerasinghe (1993) and Bambaradeniya (2000) has also stated about vector mosquitoes reported from irrigated rice fields. Mainly larvae of *Culex* and *Anopheles* species are found in rice fields, nursery paddy beds and large stagnant water bodies. They are major vectors of Malaria and Japanese encephalitis. In accordance to that, major epidemics of Japanese encephalitis have occurred in rice growing areas (Peiris *et al*., 1993). Agricultural practices in rice ecosystems encourage perennial propagation of mosquito larvae by creating different kinds of breeding sites such as paddy fields, irrigation canals, seepage pools and reservoirs (Yasuoka and Levins, 2007). They reported that higher diversity of mosquito species in rice field ecosystems throughout the year; most of them are human disease vectors that transmit malaria, filariasis, Japanese encephalitis and dengue. Variation in distribution and abundance of mosquito populations observed in these studies may reflect the oviposition preferences of vector mosquitoes and the factors affecting the larval development. Gravid female mosquitoes select the aquatic habitats for oviposition based on location, the physico-chemical condition of the water body and the presence of potential predators

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(Piyaratne *et al*., 2005). Physico-chemical factors such as salts, dissolved organic and inorganic matter, degree of eutrophication, turbidity, suspended mud, temperature, light and shade and hydrogen ion concentration influence the oviposition, survival and spatio-temporal distribution of mosquitoes (Mogi, 1978; Amerasinghe *et al*., 1995). Climatic changes are also very important factors for the abundance and distribution of mosquitoes. Rain-fall, air and water temperature, light and air humidity are directly affect for their survival (Kettle, 1995). The main objective of this study was to describe mosquito larval habitats and determine the spatial and temporal variation of larval density and diversity in rice fields and marshland habitats in wet zone and semi-dry zone in Sri Lanka. Thus proper understanding of the factors that enhance the mosquito production may provide useful information to mitigate the negative impacts of irrigated rice cultivation on human health.

MATERIALS AND METHODS

Study area and sampling sites: Irrigated and cultivated rice fields (n=10) were selected within an extent of 20km^2 in Kurunegala area of the North Western province in the semi-dry zone (Figure 1) of Sri Lanka. Marshlands were not come across in this extent of area. Five rice fields and five marshlands were selected as sampling sites within a similar extent in Kelaniya area of the Western province in the wet zone (Figure 2) in Sri Lanka.

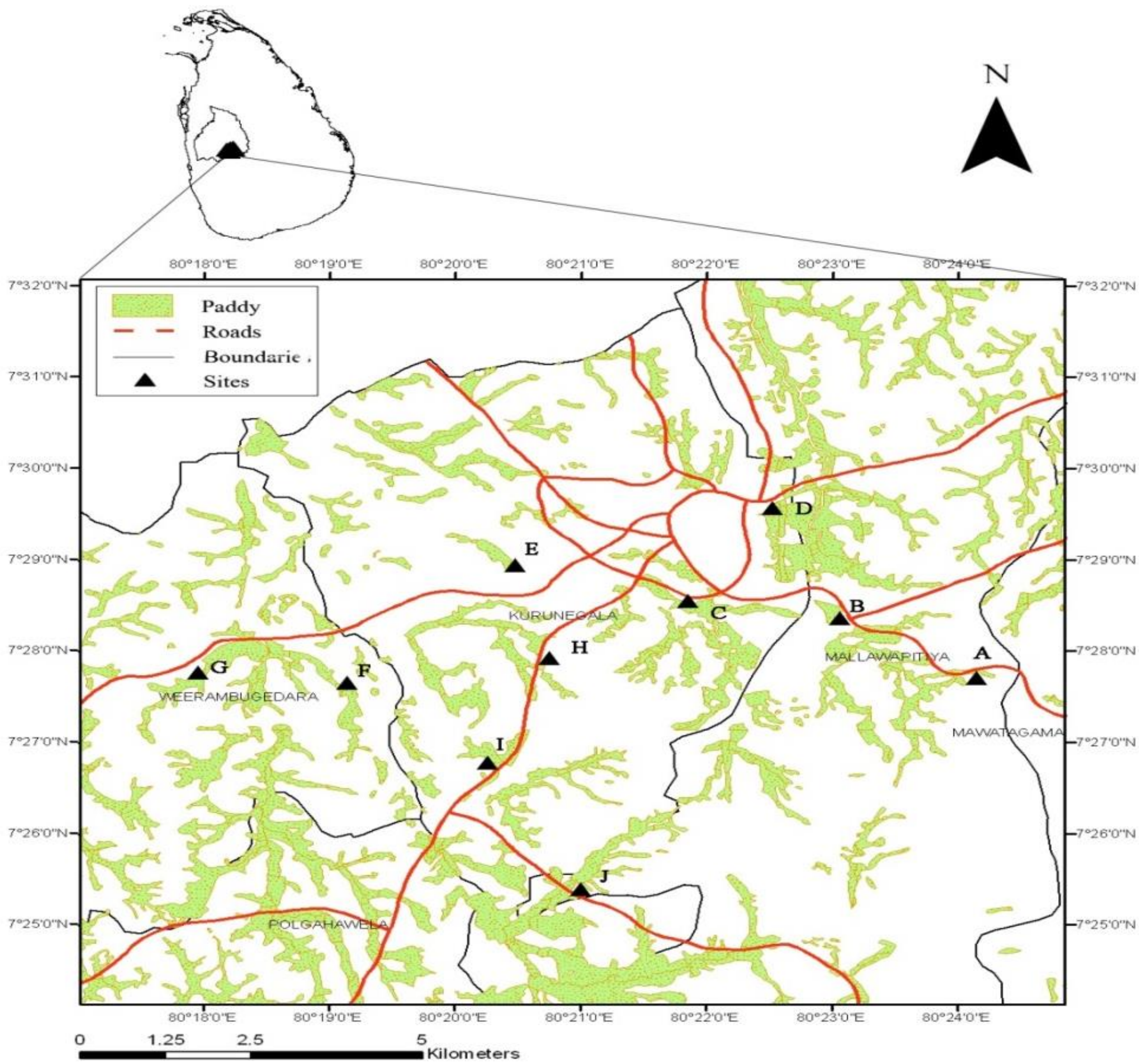

Figure 1. Site map of the mosquito breeding locations found in Kurunegala area in Kurunegala district, North western province of Sri Lanka.

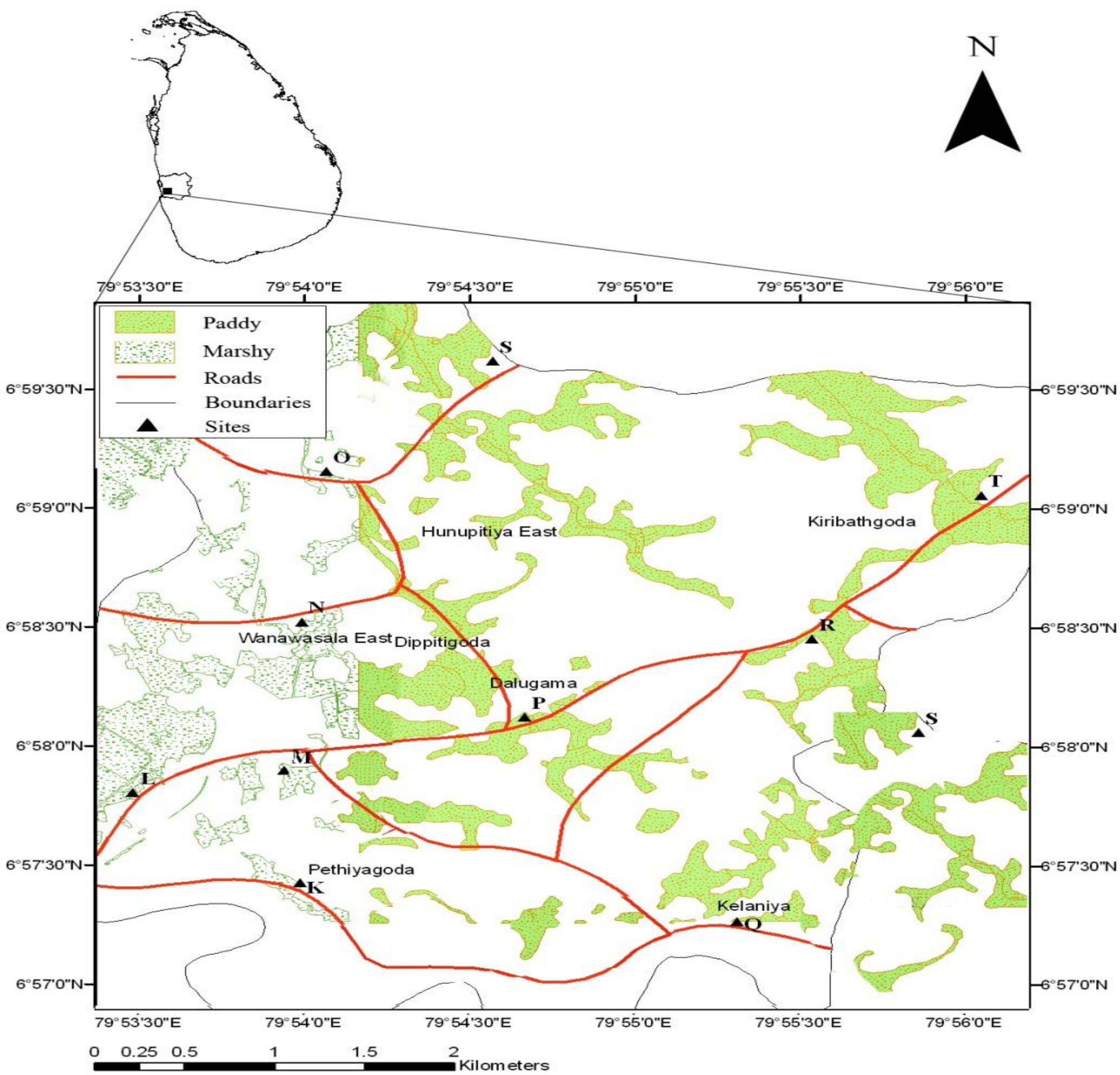

Figure 2. Site map of the mosquito breeding locations found in Kelaniya area in Gampaha district, Western province of Sri Lanka.

Locations of these sites were recorded using a portable global positioning system / GPS (GARMIN-etrex SUMMIT). Climatically Kelaniya area is warm and humid for a major part of the year and it has an average annual air temperature of 27^0 C and an average annual rainfall of 2390 mm. Kurunegala area has a tropical and warm climatic condition throughout the year providing a minimum and maximum annual air temperature of 23^0 C and 32^0 C respectively. The annual average rainfall of this area is about 2000 mm. Kurunegala is a major agro-climatic zone of the country and rice fields are highly abundant. Cultivated rice fields are less abundant in Kelaniya area, while the 1/10th of the land is covered with paddy fields and marshes (www.kelaniya.ds.gov.lk.html, 02.10.1012). Rice fields in Kurunegala area usually cultivated in two rice cultivation seasons in Sri Lanka, which is "Yala' and "Maha' seasons. Maha is the major cultivation season from late September to early March, and Yala is the minor cultivation season from early April to early September. However, the rice fields in Kelaniya area usually practice only in Maha season (September-March) while rest of the year, they remained uncultivated. All the rice fields in Kurunegala area were at ploughing/sowing stage at the time of commencing this study. Rice fields in Kelaniya comprised of mud flats and remained uncultivated owing to the fact that the study period coincided with non-planting season ("Yala") of the year. Marshlands in Kelaniya area were comprised of a range of naturally occurring aquatic vegetation. Approximately 250 ml water samples (n=3) were collected from each site using a metal larval scooper (width 11.5 cm, height 5.5 cm). Samples were collected into transparent plastic containers (diameter 11.5 cm, height 7.5 cm) and mosquito larvae were counted *in situ*. Sampling was continued biweekly intervals from March 2012 to July 2012. Containers were carefully brought into the laboratory after each sampling. Minimum five individuals of 4th instar larvae were separated using a pasture pipette and preserved in 70% ethanol for the larval identification. The remaining larval samples were covered by small mesh sized nylon net (mesh size; 1mm) and they were reared in the laboratory until the emergence of adult mosquitoes. Mosquito larvae were given fish meal over the rearing period. Both mosquito larvae and female adults were identified up to nearest possible taxonomic level, using standard mosquito keys (Chelliah, 1984; Reuben *et al*, 1994; Amarasinghe, 1995).

Physicochemical parameters: Water pH and total dissolved solid content was measured *in situ* using membrane pH meter (model: PH 315i/SET) and conductivity meter (model: Cond 340i/SET) respectively. Dissolved oxygen (DO) content in the water was measured by two methods, using DO meter (model: oxi 315i/SET) *in situ* and using Winkler method in the laboratory. Three day biological oxygen demand (BOD3) and nitrate and phosphate level in water were also measured. Average monthly rainfall, relative humidity and minimum and maximum air temperature of Kurunagala and Kelaniya relevant to the study period were obtained from the Meteorological Department in Colombo.

Statistical analysis: The statistical data analysis was performed using MINITAB 14 version and PRIMER 5 version. The density of mosquito larvae was expressed as the number of mosquito larvae and pupae per one dip (one scooper). The mosquito density data of rice fields of two climatically different areas were analyzed using Two Way ANOVA (response = larval density, factors = climatic area and month). Mosquito density data of rice fields and marshy lands in Kelaniya area were analyzed using Two Way ANOVA (response = larval density, factors = habitat and month). The diversity of mosquito species in two climatically different areas (Kelaniya and Kurunegala) and in two habitat types in Kelaniya area (rice fields and marshy lands) were analyzed using Shannon Weiner Diversity Index. The cluster analysis was carried out to determine the difference or the similarity of density of the recorded mosquito species at different sampling sites in Kelaniya area. The analysis of similarity (ANOSIM) test was used to determine the variation between different sampling sites in Kelaniya area. Principal Component Analysis (PCA) was used to identify the total variation in different sampling sites based on the species composition and density.

RESULTS AND DISCUSSION

Density and diversity of mosquito species associated with rice fields: There were 8 mosquito species belonging to 4 genera namely *Aedes* (one sp.), *Anopheles* (one sp.), *Culex* (five spp.) and *Lutzia* (one sp.) with percentage numbers of 1.3%, 0.5%, 97.5% and 0.7% respectively recorded from Kelaniya. Seven mosquito species belonging to four genera that include *Aedes* (one sp.), *Anopheles* (one sp.), *Culex* (four spp.) and *Mansonia* (one sp.) with percentage numbers of 17%, 0.7%, 81.7% and 0.7% respectively recorded from Kurunegala (Table 1).

Table 1. Mosquito larval species composition (numbers and percentages) collected from rice fields in Kelaniya and Kurunegala areas.

Mosquito species	Kelaniya	Kurunegala
C. gelidus	396 (37%)	150 (19.5%)
C. tritaeniorhynchus	378 (35.3%)	331(43%)
C. fuscocephala	215 (20.1%)	46 (6%)
C. pseudovishnui	45 (4.2%)	101(13.1%)
A. pipersalatus	0	131 (17%)
A. linnaetopenis	14 (1.3%)	0
C. quinquefasciatus	10 (0.9%)	0
L. fuscana	8 (0.7%)	0
Mansonia sp.	0	5 (0.7%)
Anopheles sp.1	5 (0.5%)	0
Anopheles sp. 2	0	5 (0.7%)

Among the *Culex* species *C. fuscocephala*, *C. gelidus*, *C. pseudovishnui* and *C. tritaeniorhynchus* which are potential Japanese encephalitis vectors were recorded from both locations. Insignificant number (percentage< 1.0%) of *Lutzia fuscana*, *Aedes linnaetopennis* and *Anopheles* sp.1, are non-vector mosquitoes and *Culex quinquefasciatus* the sole vector of urban filariasis were reported only from rice fields of Kelaniya area. About 10-20% of *Aedes pipersalatus* a non-vector mosquito and insignificant number of *Mansonia* sp., the vector of *brugian filariasis* and the dog heart worm (*Dirofilaria repens*), were only reported from rice fields of Kurunegala area. Larval densities in two climatically different areas are significantly different (F = 4.75, df = 1, P = 0.031 < 0.05). Comparing the mosquito larval densities, Kurunegala area is more diverse than Kelaniya area (Shannon wiener diversity index of Kurunegala = 1.48, H1 of Kelaniya = 1.35).

Density of mosquitoes associated with marshlands: The genera and species composition of mosquitoes reported from marsh lands shown in Table 2. There were eight mosquito species belonging to three genera namely *Culex* (six spp.), *Mansonia* (one sp.) and *Mimomyia* (one sp.) with percentage numbers of 95.4%, 4.9% and 0.7% respectively were recorded. *C. gelidus* and *C. tritaeniorhynchus* mosquitoes, which are major vectors of Japanese encephalitis, were recorded in significantly high numbers. 10-50% of *C. quinquefasciatus,* the vector of urban filariasis, *C. fuscocephala*, a vector of Japanese encephalitis, and *Mansonia,* the vector of *brugian filariasis*, were recorded from marshlands. Insignificant number of *C. bitaeniorhynchus, C. pseudovishnui* and *Mimomyia* sp. were recorded from marshlands of Kelaniya area and among them *C. bitaeniorhynchus* and *Mimomyia* sp. are non-vector mosquitoes while the *C. pseudovishnui* involve in transmitting Japanese encephalitis.

Table 2. Mosquito species composition (numbers and percentages) recorded from marshlands in Kelaniya area.

Mosquito species	Larvae number	Larvae percentages
C. gelidus	314.0	54.5%
C. tritaeniorhynchus	162.0	28.1%
C. quinquefasciatus	36.0	6.3%
Mansonia sp.	28.0	4.9%
C. fuscocephala	23.0	4.0%
C. bitaeniorhynchus	6.0	1.0%
Mimomyia sp.	4.0	0.7%
C. pseudovishnui	3.0	0.5%

Temporal variation of mosquitoes in rice fields and marshlands: Variation of the total larval density of mosquitoes in rice fields located in Kelaniya and Kurunegala and in marshlands located in Kelaniya shows a similar trend during the study period. The density of *C. tritaeniorhynchus* which is the primary vector of Japanese encephalitis was shown an increasing trend over the time in rice fields in both areas (Table 3 and Table 4). The density of *C. gelidus* was increased in June and decreased considerably in July in rice fields in Kelaniya but this species disappeared from May in Kurunegala area. Density variation of these two mosquito species over the rice cultivation cycle is shown in Figure 3. This shows that there is a negative correlation between the *C. tritaeniorhynchus* and *C. gelidus* (Pearson correlation = -0.974 and P < 0.05). One-way ANOVA and Tukey's pair wise test revealed that, monthly mean density of *C. gelidus,* was significantly different from that of *C. quinquefasciatus, L. fuscana* and *Anopheles* sp. 2 found in rice fields in Kelaniya area (F = 4.23, df = 7, P < 0.05). Monthly mean density of *C. tritaeniorhynchus,* was significantly different from that of *C. fuscocephala, Mansonia* and *Anopheles* sp. 1 found in rice fields of Kurunegala area (F = 3.38, df = 6, P < 0.05).

Temporal variation of the density of *Culex tritaeniorhynchus* in marshlands of Kelaniya area was shown an increasing trend while the density of *Culex gelidus* was shown an exceptional increase in June (Table 5). *Culex gelidus* is the most common mosquito in marshlands in Kelaniya area during the study period. *Culex tritaeniorhynchus* were recorded in insignificant densities in early months and was shown a considerable increase in July. *Culex fuscocephala* and *Culex quinquefasciatus* which are important vector mosquitoes were shown moderate densities, whereas insignificant densities of *Mansonia, Culex pseudovishnui, Culex bitaeniorhynchus* and *Mimomyia* species were recorded in marshlands in Kelaniya area. According to the analysis of One-way ANOVA and Tukey's pair wise test, larval density of *Culex gelidus* was significantly different from other species except *Culex tritaeniorhynchus* ($F_{7,32}$= 3.43, P < 0.05) in marshlands in Kelaniya area.

Table 3. The density of mosquito larvae and pupae recorded from rice fields of Kurunegala area.

Species	Number of larvae and pupae / dip					Mean ± SE
	March	April	May	June	July	
C. tritaeniorhynchus	0.6	0.3	3.0	3.4	3.7	2.20 ± 0.73^{a}
C. gelidus	2.7	2.3	0	0	0	1.00 ± 0.62^{ab}
A. pipersalatus	0	0	0.6	1.8	2.0	0.88 ± 0.43^{ab}
C. pseudovishnui	0	0	0.7	1.6	1.1	0.68 ± 0.31^{ab}
C. fuscocephala	0.4	0.3	0.2	0.3	0.3	0.30 ± 0.03^{b}
Mansonia sp.	0	0	0.1	0	0	0.02 ± 0.02^{b}
Anopheles sp.1	0	0	0	0	0.2	0.04 ± 0.04^{b}

(Same superscript in the table are not significantly different P>0.05).

Table 4. The density of mosquito larvae and pupae recorded from rice fields of Kelaniya area.

Species	Number of larvae and pupae / dip					Mean ± SE
	March	April	May	June	July	
C. gelidus	3.3	1.5	1.6	6.7	0.1	2.64 ± 1.13^{a}
C. tritaeniorhynchus	1.0	1.0	0.8	4.1	5.8	2.54 ± 1.02^{ab}
C. fuscocephala	1.3	0.6	1.3	2.5	1.5	1.44 ± 0.31^{ab}
C. pseudovishnui	0	0.2	0.3	1.0	0	0.30 ± 0.18^{ab}
Aedes linnaetopennis	0.3	0.2	0	0	0	0.10 ± 0.06^{ab}
C. quinquefasciatus	0.3	0	0	0	0	0.06 ± 0.06^{b}
Lutzia fuscana	0	0	0.1	0.2	0	0.06 ± 0.04^{b}
Anopheles sp.2	0	0	0	0	0.2	0.04 ± 0.04^{b}

(Same superscript in the table are not significantly different P>0.05).

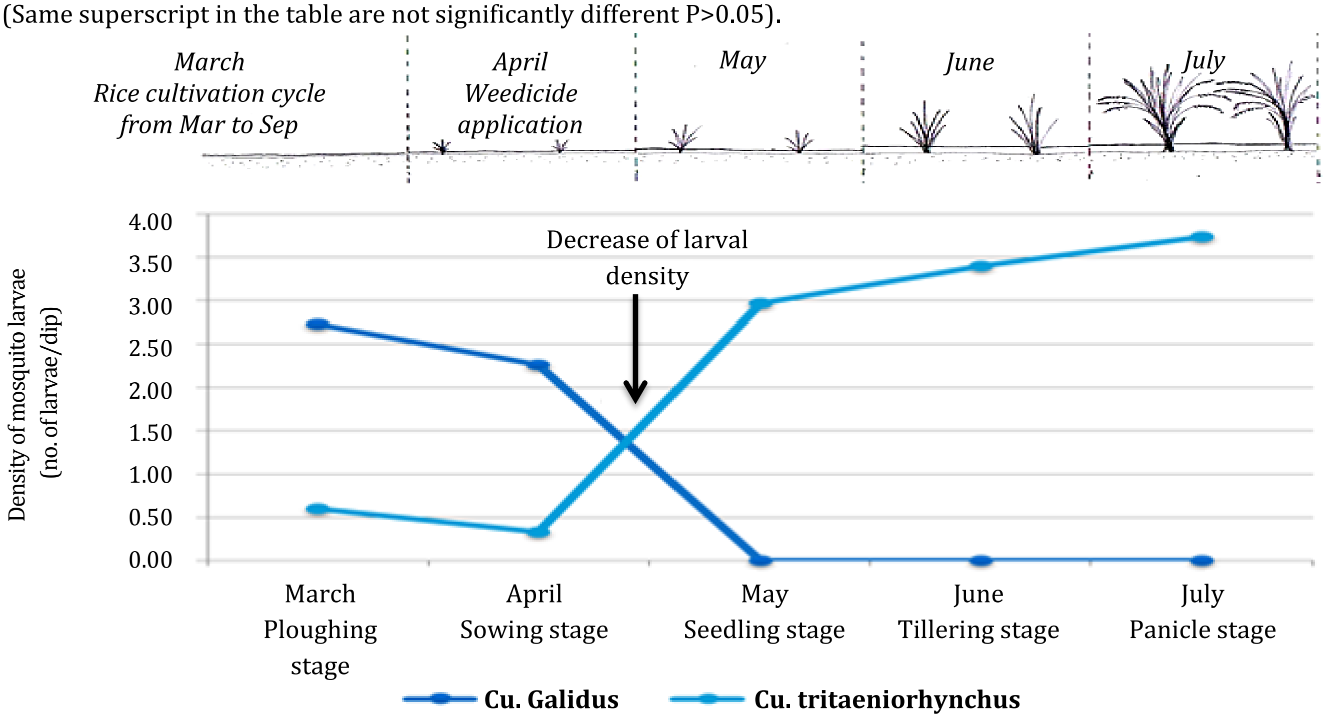

Figure 3. Larval density variation of *Culex tritaeniorhynchus* and *Culex gelidus* in rice fields of Kurunegala area over the study period. (A) Rice cultivation cycle from March to July in Kurunegala. (a) In March rice fields were ploughed and irrigated. Hence the water was found with the inundation of old stubble. (b) Seedlings were appeared. Weedicides were applied in this period. (c, d) Water level was increased during this period. (e) Panicles were arisen in early July and water level was decreased.

Table 5. The density (Number of larvae and pupae / dip) of mosquito larvae recorded from marshlands of Kelaniya area.

Species	March	April	May	June	July	Mean±SE
Culex gelidus	1.9	1.5	1.9	4.9	0.2	2.09±0.77[b]
Culex tritaeniorhynchus	0.6	0.4	0.0	0.1	4.3	1.08±0.81[ab]
Culex fuscocephala	0.3	0.3	0.1	0.0	0.0	0.15±0.71[a]
Culex quinquefasciatus	0.2	0.2	0.8	0.0	0.0	0.20±0.15[a]
Mansonia sp.	0.2	0.1	0.3	0.3	0.0	0.19±0.06[a]
Culex bitaeniorhynchus	0.2	0.0	0.0	0.0	0.0	0.04±0.04[a]
Mimomyia sp.	0.0	0.0	0.0	0.0	0.1	0.03±0.02[a]
Culex pseudovishnui	0.0	0.0	0.0	0.0	0.1	0.02±0.02[a]

Variation of density and diversity of mosquito species associated with rice fields and marshy lands in same climatic zone: Rice fields are the most diverse habitats from two selected habitat types in Kelaniya area; Shannon wiener diversity index/ H^1 = 1.35. Shannon wiener diversity index/ H^1 of marshy lands is 1.25. The mean larval density of rice field habitat is not significantly different from that of marshy land habitat ($F_{1,10}$ = 3.09, P = 0.109). The 10 sites in Kelaniya area could be divided into three clusters in 45% similarity level (Figure 4). Site R is represented by a separated cluster meanwhile site M and site S are represented by another cluster. In 45% similarity level all other sites are represented by a one cluster. According to the cluster analysis there are four major clusters could be divided in 50% similarity level. In this level K, L, N and O sites are represented by one separated cluster. All these sites are marshy lands and similar species composition could be observed in these sites. Site R is clustered separately as a unique cluster at almost 25% similarity level from other clusters (Figure 4), due to unique species composition observed in that site. Although 4 clusters could be observed, ANOSIM revealed that clusters are not significantly different from each other. Therefore, there was no significant difference in distribution of mosquito species between selected sites of same habitat type or between two habitat types Principle Component Analysis (PCA) shows that the cumulative percentage variation is high as 100% in 11 mosquito species. The total variation of mosquito density could be explained by both PC1 and PC2 axis (Figure 5). According to PC1 axis *Culex bitaeniorhynchus*, *Mansonia* sp. and *Mimomyia* sp. have obtained the highest negative values and lowest score values. So their larval density is higher in site K and site N, which are marshy lands. In site O, PC1value and the score value is higher. In site O *Culex tritaeniorhynchus*, *Culex fuscocephala* and *Culex pseudovishnui* have high larval density. In PC2, the highest values are given by *Aedes linnaetopennis* and *Anopheles* sp. According to the results density of *Aedes linnaetopennis* is highest in site R.

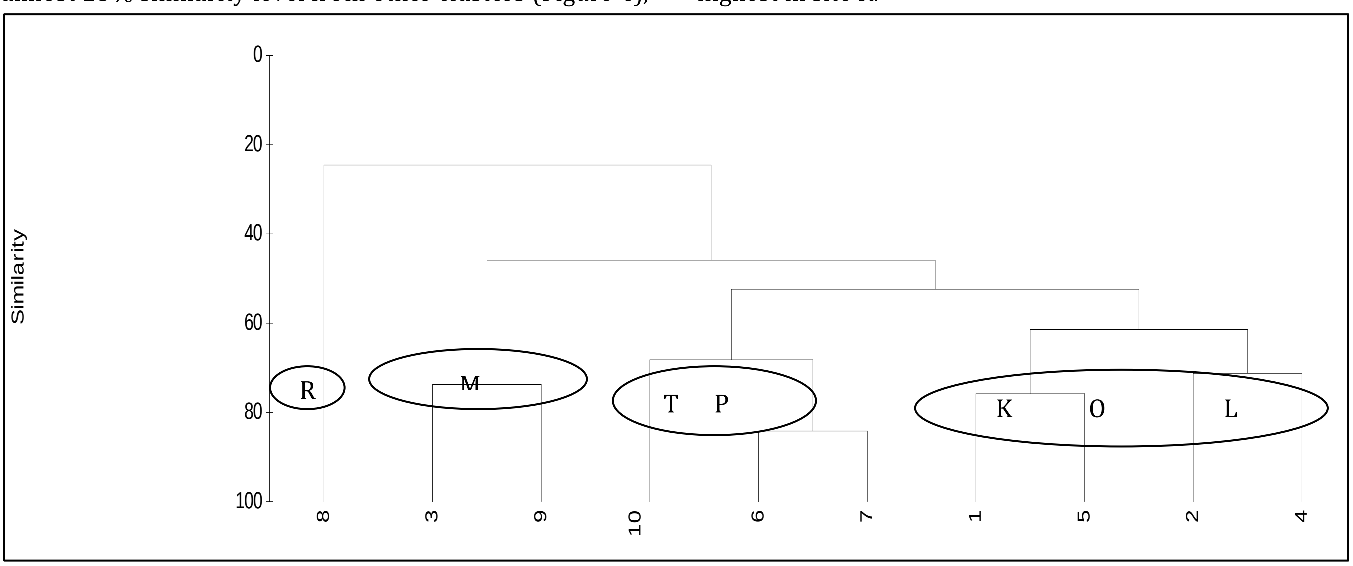

Figure 4. Dendogram showing the clustering of breeding sites respect to density (No of larvae and pupae/dip) of different mosquito species found in Kelaniya area. (K, L, M, N and O are marshy lands and P, Q, R, S and T are rice field habitat.

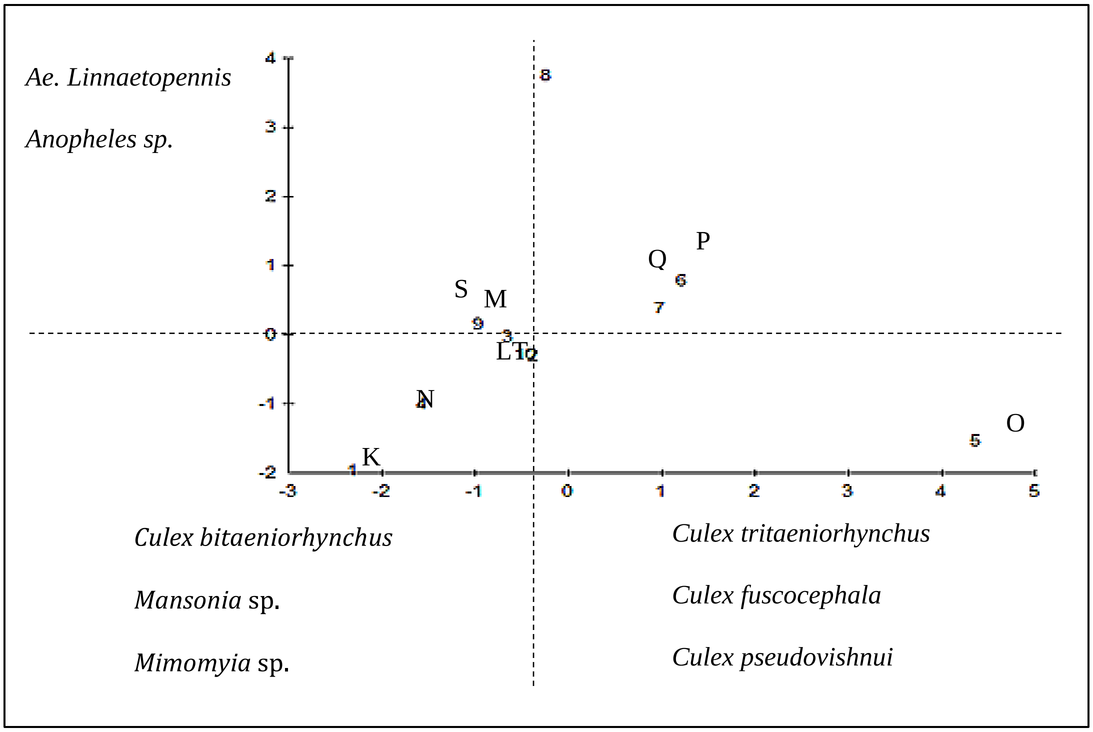

Figure 5. Principle Component Analysis of the mosquito larval density in ten selected sites in Kelaniya area. (Site K, L, M, N and O are marshy lands and site P, Q, R, S and T are rice fields).

Principal Component Analysis

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	3.45	31.3	31.3
2	2.47	22.5	53.8
3	2.17	19.8	73.6
4	1.06	9.7	83.2
5	0.88	8.0	91.2
6	0.44	4.0	95.2
7	0.41	3.7	98.9
8	0.11	1.0	99.9
9	0.02	0.1	100.0
10	0.00	0.0	100.0
11	0.00	0.0	100.0

Eigenvectors

(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3
C. quinquefasciatus	-0.105	-0.004	0.555
C. gelidus	-0.160	-0.004	0.550
C. tritaeniorhynchus	0.497	-0.192	-0.026
C. bitaeniorhynchus	-0.295	-0.328	-0.357
C. fuscocephala	0.478	-0.194	-0.107
Mansonia sp.	-0.251	-0.282	-0.254
A. linnaetopennis	-0.024	0.534	-0.321
C. pseudovishnui	0.497	-0.180	-0.039
L. fuscana	0.124	0.111	-0.032
Mimomyia sp.	-0.268	-0.247	-0.264
Anopheles sp.	0.049	0.575	-0.244

Principal Component Scores

Sample	SCORE 1	SCORE 2	SCORE 3	SCORE 4
1	-2.313	-1.966	-1.855	0.241
2	-0.375	-0.296	0.251	-0.043
3	-0.645	-0.021	2.598	0.507
4	-1.563	-1.000	-1.004	-0.198
5	4.362	-1.522	-0.403	0.937
7	0.985	0.383	-0.168	0.113
8	-0.238	3.758	-1.426	1.002
9	-0.972	0.155	2.446	0.100
10	-0.456	-0.274	-0.241	0.043

Physico-chemical parameters: All mosquito larvae inhabiting in marshlands were recorded in higher TDS values above 10.00 mg/L, whereas larvae were distributed in a wide range of TDS levels in water in rice fields. Majority of larvae associated with marshlands (83%) were in alkaline water and majority of larvae associated with rice fields (70%) were recorded in acidic water. Majority of mosquito larvae (about 60%) inhabiting both marshlands and rice fields were recorded with 5-6 mg/L dissolved oxygen levels. In marshlands larvae were recorded in a wide range of DO levels, whereas no larvae were recorded from below 4.00mg/L DO in rice fields. More than 60% of larvae in marshlands and 80% of larvae in rice fields were recorded from 5-10 mg/L BOD levels; Considerable density of larvae was recorded from BOD levels higher than 10.00mg/L in marshlands, whereas no larvae were recorded in higher BOD values in rice fields. When considering the nitrate level in water majority (about 60%) of mosquito larvae were recorded from 1-5 mg/L level in rice fields and marshland habitats. In marshlands mosquito larvae were distributed in a wide range of nitrate levels. But in rice fields less variation of nitrate levels could be observed and about 45% of larvae were recorded from nitrate levels less than 1.00mg/L. Phosphate levels in water in rice fields and marshlands were less than nitrate levels and about 70% of mosquito larvae in both habitats were recorded in less than 1.00mg/L phosphate levels. This parameter also varied in a wide range in marshlands whereas mosquito larvae inhabited in rice fields were recorded in a narrow range of phosphate levels in water. *Culex gelidus* and *Culex tritaeniorhynchus* larvae were mainly associated with high TDS, low pH and moderate levels of DO and BOD in water. Further mosquito larvae were more available in water with very low levels of nitrate and phosphate. Variation of the physico-chemical parameters was associated with the climatic factors.

DISCUSSION

Aside from the nuisance and serious bites caused by mosquitoes, their ability of transmitting disease causing pathogens and parasites is a major problem to humans and livestock. The growing demand for effective mosquito larval control measures require a better understanding of varying larval population densities in different habitats and their associated factors. This study provides important information on vector mosquitoes in rice field and marshland habitats and effect of associated factors. Further this study also revealed that the effect of regular chemicals applied in cultivated rice fields on the diversity of the ecosystem. Therefore data generated by this type of studies can be utilized when controlling strategies are implemented.

This study was designed to test two main hypotheses made on the variation of mosquito density within two climatic zones and in two habitat types within a same climatic zone. Study was planned to determine the variation of density and diversity of mosquitoes inhabiting rice fields within two climatic zones; Kelaniya area in wet zone and Kurunegala area in semi-dry zone in Sri Lanka and to determine the density variation of mosquitoes inhabiting marshlands and rice fields. Further the temporal variation of mosquito larval density in rice fields and marshlands in selected areas was studied. Mainly the results demonstrate that *Culex* larval abundance varies spatially and temporally and is strongly influenced by environmental characteristics, including the surrounding vegetation structure, aquatic fauna and aquatic chemistry.

This study was found diverse mosquito species in rice field and marshland habitats within five month time period. Total of 12 mosquito species belong to six genera could be found during the study from marshlands and rice field habitats and from that, seven mosquito species in three genera are responsible for disease transmitting, whereas other five species are non-harmful. Most predominant mosquito species inhabited in rice fields and marshlands during the study period were *Culex gelidus*, *Culex tritaeniorhynchus, Culex fuscocephala* and *Culex pseudovishnui* which are major Japanese encephalitis vectors in Sri Lanka (Peiris *et al.,* 1992; Amerasinghe *et al*., 1998). Other than that *Culex bitaeniorhynchus* was also recorded from marshlands which were considered as a vector of Japanese encephalitis (Reuben *et al*., 1994). Other than Japanese encephalitis vectors, *Culex quinquefasciatus*; primary vector of bancroftian filarasis in Sri Lanka and *Mansonia*, the vector of brugian filariasis were found during the study (Herath, 2005). Non-harmful species recorded from rice field and marshland habitats were *Aedes pipersalatus, Aedes linnaetopennis, Lutzia fuscana, Mimomyia* sp. and *Anopheles* sp. A total number of 769 mosquitoes were recorded from rice fields of Kurunegala area and 1071 mosquitoes were recorded from rice fields in Kelaniya area during the study period. This difference is mainly due to the difference in cultivation pattern of rice fields in Kelaniya and Kurunegala. Further the higher temperature of Kurunegala area than Kelaniya had led to decrease the larval number. Mwangangi *et al*. (2008) mentioned that warm water reduce the mosquito larval development. Rice fields in Kurunegala area are cultivated in both major cultivation seasons "Yala" and "Maha", whereas rice fields in Kelaniya area are cultivated only in "Maha" season which is from late September to early March. During the study period from March to July these rice fields were not cultivated. Since they were not cultivated application of chemicals or fertilizers was not occurred in rice fields in Kelaniya area. Fertilizer and chemical application taken place in rice fields in Kurunegala area has led to decrease the total number of mosquitoes in rice fields of Kurunegala area than in Kelaniya area. Also unmanaged urbanization in Kelaniya area may have caused to increase the mosquito density. In all selected rice fields in Kurunegala area in semi-dry zone mosquito species were recorded in a similar composition. No significant difference could be observed within sites ($P > 0.05$). That may be due to the similar environmental conditions observed in all sites which were cultivated in same pattern with irrigation and applying weedicides and fertilizers. From rice fields in Kurunegala area seven mosquito species belonging to four genera were recorded from which 81% was *Culex* mosquitoes. Comparing to that eight mosquito species belonging to four genera were recorded from rice fields in Kelaniya area from which 97.5% were *Culex* mosquitoes.About 50% of *Culex* mosquitoes in rice fields of Kurunegala area were *Culex tritaeniorhynchus* whereas other *Culex* species such as *C. gelidus*, *C. fuscocephala* and *C. pseudovishnui* were recorded in low numbers. In Kelaniya area *Culex gelidus, Culex tritaeniorhynchus* and *Culex fuscocephala* were recorded in similar numbers, hence one species was not prominent in rice fields of Kelaniya area. The total number of *Culex gelidus* recorded from rice fields in Kelaniya area (396) was higher than the number recorded from Kurunegala (150), whereas *Culex tritaeniorhynchus* was recorded in similar numbers (378 in Kurunegala and 331 in Kelaniya). This is accordance to Murty *et al.* (2010) who stated that *Culex gelidus* mainly recorded from urban areas. Interesting fact is that mosquitoes belonging to Genus *Culex* were recorded in significant numbers from rice fields in both Kurunegala and Kelaniya. All four *Culex* species mentioned above are, ground water breeders, with *Culex tritaeniorhynchus* closely associated with rice fields (Amarasinghe and Ariyasena, 1990). Majority of these recorded *Culex* species are found to be associated with rice field habitats and they are vectors of Japanese encephalitis. *Mansonia, Anopheles, Lutzia* and *Aedes* species were recorded in insignificant numbers from rice fields in Kurunegala and Kelaniya. This may be due to the competition of other prominent species in the same habitat. Similar study done by Yasuoka and Levins (2007) stated that, *Anopheles, Culex, Aedes, Mansonia* and *Armigeres* species could found in irrigated rice lands in Habaraluwewa in the Southern rice growing region of Sri Lanka and density of *Anopheles* and *Culex* were highest in seepage pools and paddy fields in this study. Larval densities of different mosquito species recorded from rice fields of Kurunegala and Kelaniya areas were significantly different and the mosquito diversity was higher in rice fields in Kurunegala area than in Kelaniya area (H^1Kurunegala = 1.48 and H^1Kelaniya= 1.35). This is due to the climatic difference in two areas and the level of disturbance. Disturbances such as application of chemicals made by human may led to destroy the larvae in the habitat and after some time when the magnitude of the disturbance was decreased it is a good habitat with less competition for many species. Total number of 576 mosquitoes was recorded from marshlands in Kelaniya area was less than the number recorded from rice field habitats in Kelaniya area. This variation was due to the difference in physico-chemical and biological parameters associated with marshland and rice field habitats which is discussed at the end of this section. Also water volume of marshlands may be affected to reduce the mosquito larval populations. The selected marshlands were with higher water volume than rice fields. Eight mosquito species belonging to three genera were recorded from marshlands from which 95% were belonging to Genus *Culex*. As Bondurant (2010) stated *Culex* species are the most common mosquitoes in marshes and wetlands. From the eight species recorded from marshlands in Kelaniya area six species were *Culex* mosquitoes which are *Culex gelidus, Culex tritaeniorhynchus, Culex fuscocephala, Culex quinquefasciatus, Culex pseudovishnui* and *Culex bitaeniorhynchus*. All those species are human disease vectors. More than 50% from the recorded mosquitoes is *Culex gelidus* in marshlands. Comparatively higher number of *Mansonia* larvae was recorded from marshlands than rice fields. This is due to the high vegetation cover with aquatic plants such as *Eichornia* and *Pistia.* Because *Mansonia* larvae obtain oxygen from aerenchyma cells of aquatic plants by inserting its serrated siphon. Mosquito larvae of Genus *Mimomyia* were recorded only from marshlands. According to the mosquito densities obtained from all habitat types in Kurunegala and Kelaniya, *Culex tritaeniorhynchus* was available in all sites over the study period in a considerable density. Similar study of Yasuoka and Levins (2007) stated that, *Culex tritaeniorhynchus* had the widest niche in rice field ecosystems in their study. Considering the temporal variation in rice field and marshland habitats in Kelaniya and rice fields in Kurunegala, an increasing trend of mosquito larval density was observed over the study period. However mosquito larval density in marshlands was low in April and highest in June whereas the larval density in rice fields in Kelaniya was highest in June and drastically decreased in July. In June larval density in both habitats in Kelaniya was increased due to the heavy rain occurred in May. Due to the heavy rains occurred in May, larval habitats were flooded and many of the larvae were displaced. Larval habitats gradually returned to its original condition with the time and larval density was increased in this situation. In rice fields of Kurunegala area larval density was lowest in April due to the application of weedicides at the seedling stage and then the larval density was shown an increasing trend with the growth of rice plants. Density variation of *Culex gelidus* and *Culex tritaeniorhynchus* in rice fields in Kurunegala area was shown a negative correlation over the study period ($P < 0.05$). *Culex gelidus* were recorded in high density in March and decreased in April and then drastically decreased in May to zero density, whereas *Culex tritaeniorhynchus* were recorded in low densities in March and April and then increased to higher densities in May, June and July. This density variation was associated with the rice cultivation cycle. In March rice fields were ploughed and irrigated to start the cultivation cycle and the water was highly turbid and foul smelling with mud and inundated stubble. In these conditions densities of *Culex gelidus* were higher and *Culex tritaeniorhunchus* were lower. Sowing of seeds was done in late March and with the emerging of seedlings the water level was increased. With the growth of rice plants density of *Culex tritaeniorhynchus* was increased into higher densities as recorded in July. Density increment of *Culex tritaeniorhynchus* may be due to the effect of rice plant canopy. Takagi *et al.* (1995) revealed that different species react differently to the changing conditions of the habitat as rice plant grows and canopy closes. Similarly several studies were done in Sri Lanka to determine the association between the development of irrigated rice lands and malaria epidemics (Amerasinghe and Ariyasena, 1990; Ramasamy *et al*., 1992; Amerasinghe and Indrajith, 1994). However the reason for this association between *Culex tritaeniorhynchus* and the growth of rice plant is not exactly discovered. A dendogram was prepared to represent the selected mosquito breeding site as clusters according to their similarity levels obtained by the species composition. According to the dendogram only one rice field was separated from rest of the rice fields as an individual cluster in 25% similarity level. Therefore, this site was 75% differ from other sites with its species composition. *Aedes linnaetopennis* was recorded in considerable densities from this site and this species was not recorded from other rice field or marshlands. Three Marshlands in Kelaniya area were clustered as one separate cluster. However one marshland was clustered with a rice field mainly due to the higher densities of *Culex gelidus* recorded from these two sites. Although the sites were clustered separately ANOSIM revealed that the sites are not significantly different. This indicates that the habitat characteristics of two habitats; marshlands and rice fields, in Kelaniya area are similar. This is mainly due to the uncultivation of selected rice fields in Kelaniya area which has led to increase the vegetation cover in rice fields as in marshlands. As mentioned in above paragraph the marshland and rice field sites in Kelaniya area were clustered according to their species compositions. With a Principal Component Analysis the most affected factors for the clustering can be identified. According to the PCA done for these sites, the separation of one rice field was clearly indicated with high PC2 value obtained due to the higher density of *Aedes linnaetopennis*. All these studies revealed the complexity of mosquito ecology in rice fields and marshlands and highlighted the necessity of mosquito control in these habitats. As revealed by the results, rice fields are the major habitats of *Culex* mosquitoes and effective control measures should be applied to control the increasing density of mosquitoes and to prevent the disease epidemics.

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