THE ABUNDANCE OF MOSQUITO LARVAE IN RELATION TO ASSOCIATED MICROBIOTA AND PHYSICOCHEMICAL PARAMETERS OF MARSHLAND HABITATS IN KELANIYA IN GAMPAHA DISTRICT OF SRI LANKA

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ABSTRACT

The abundance of mosquito larvae in relation to associated microbiota and physicochemical parameters of water in marshes from selected habitats in Kelaniya divisional secretariat area, of the Gampaha district, Sri Lanka were studied. *Culex gelidus* was found to be the dominant mosquito species found in marshes followed by *Culex whitmorei*, *Culex tritaeniorhynchus*, *Culex quinquefasciatus*, *Culex fuscocephala*, and *Anopheles peytoni*. Fourteen species/taxa of microbiota were identified. There was a significant positive correlation between the abundance of *Cx. gelidus* larvae and level of organic pollution in marshes as measured by the five-day Biological Oxygen Demand. A positive correlation for the presence of *Cx. fuscocephala* and *Cx. gelidus* in the marshland was also observed. Nevertheless, abundance of *Cx. gelidus* was negatively correlated with *Cx. quinquefasciatus* and *Cx. whitmorei*. *The presence of Culex gelidus* and *Cx. tritaeniorhynchus* were positively correlated with *Daphnia magna* indicating their co-existence in the same habitat requirement. *Culex gelidus* was negatively affected by the epibiont, *Vorticella microstoma*, when the latter organisms were present in higher densities. In contrast, the presence of *Cx. whitmorei* was positively influenced by the *Vorticella microstoma, Difflugia corona*, nauplius larva, and *Keratella valga*. There was a negative correlation between *Cx. whitmorei* and *Monostyla bulla* (Rotifera; Monogononta) and between *the abundance of Cx. fuscocephala* and *Lecane luna* in the same marshland. This study concludes that mosquito larvae and some microbiota are interdependent in marshlands. The level of abundance of mosquito larvae and microbiota varied with the level of water pollution. *Cx. gelidus* larvae are biologically affected by *Vorticella microstoma*. Many microbiota species compete with mosquito larvae for the same food items.

Keywords: *Annona glabra*, *Culex*, *Daphnia*, *Vorticella*, marshes

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INTRODUCTION

Culex species are the most common mosquitoes in marshes and wetlands in Sri Lanka (Amarasinghe and Weerakkodi, 2014). Mosquito larvae depend on a variety of organic detritus, small organisms that include bacteria, fungi, protists, algae, and micro invertebrates, and suspended material in their aquatic habitats as food items. Mosquito larvae collect these food items by filtering, scraping, gathering, preying, and shredding. Water column microorganisms are, therefore, important for the growth and development of mosquito larvae and animal detritus are more important than plant detritus for the growth of container-dwelling mosquitoes (Yee *et al*., 2007).

Physicochemical factors affect the survival, oviposition, and distribution of mosquitoes. Female mosquitoes select breeding habitats for oviposition by considering the location of the site, physicochemical condition of the waterbody, and presence of potential predators (Piyarathna *et al*., 2005). Salts, dissolved organic and inorganic matter, turbidity, degree of eutrophication, presence of suspended mud, presence or absence of plants, light and shade, temperature, and rainfall are considered as physical factors (Muturi *et al*., 2008; Becker *et al*., 2010). Turbidity is caused by non-dissolved substance in water, and it is an important indicator of larval abundance. It is reported that habitats with clear or low turbidity cause to increase in the *Anopheles* larvae (Muwangani *et al*., 2008).

Culex mosquito larvae prefer habitats with high Biological Oxygen Demand (BOD). Orthophosphate, ammonia, nitrate, sulphate, silica, and dissolved solids are positively correlated with mosquito abundance while chloride and dissolved oxygen appear to be inversely correlated (Muturi *et al*., 2008; Yadav, 2009). The ionic content of water mainly affects the toxicity of water and causes adverse effects on aquatic organisms (Yadav, 2009; Garba and Olayemi, 2015). Mosquito larval abundance depends on biological factors like vegetation type and the proportion of its coverage. The presence of vegetation and floating plants provide optimal breeding conditions by acting as food sources as well as shelter from predators. Vegetation also creates stagnant conditions by decreasing water

movement and accumulation of submerged dead stems and dense mats of floating vegetation are helpful for mosquito breeding. Mosquito larvae, predators, and micro invertebrates co-exist in the aquatic water body, and some microbiota act as food for mosquito larvae.

The presence of competitors and predators reduces the survival of mosquito larvae by competition for the same food resources or preying on mosquito larvae (Amarasinghe and Weerakkodi, 2014; Bambaradeniya and Amerasinghe, 2003). Das (2001) has reported Lambornella stegomyiae infection on Aedes albopictus in a sample collected from an earthen pot in Kuala Lumpur. *Chilodonella uncinata* is an endoparasitic ciliate that was found in infected larval head capsules, antennae, body cavity, anal gills, and siphons of culicine and anopheline larvae breed in paddy fields, irrigation channels, marshy areas, wells, ponds and pools in North India. Therefore, high mortalities of *Cx. tritaeniorhynchus* and *Cx. gelidus* were reported in larvae collected from paddy fields and marshy areas due to transovarial transmission of *Chilodonella uncinata* (Das, 2001).

Amarasinghe and Rathnayake (2014) reported multiplication of *Tetrahymena* parasites within the haemocoele of the mosquito larvae causng abnormally transparent or whitish or opaque larvae on heavy infestation. *Zoothamnium* spp and *Vorticella* spp cause the killing effect on the mosquito larvae due to the dense association of these species. Although these microbiota present in the larval body are unable to cause diseases in mosquito larvae they reduce the movement of mosquito larvae by tightly attaching them to the host cuticle. Amarasinghe and Rathnayake, (2014) reported that *Vorticella* spp. were externally attached to the mosquito larvae, and formed a fuzzy coating around the body.

Cyclopoid copepods cause to reduce the survival of mosquito larvae by feeding on young first and second instars of mosquito larvae. If the larval mosquito density is very high, they tend to feed on part of the body of mosquito larvae causing a reduction of the abundance of larvae up to 30%-40% per day (Veronesi *et al*., 2015). *Acanthocyclops vernalis*, *Diacyclops navus*, *Macrocyclops albidus*, *Megacyclops latipes*, *Mesocyclops edax*, *Mesocyclops ruttneri*, and *Mesocyclops longisetus* were recorded as effective predators on mosquito larvae (Marten *et al*., 1994). However, there is a lack of research on mosquito

abundance and their interaction with microbiota. The objective of the present study was to determine the abundance of mosquito species in marshlands in the wet zone of Sri Lanka and to determine the relationship among mosquito larvae, associated microbiota, and physicochemical parameters.

METHODOLOGY

Description of the study area and sampling sites: Kelaniya Medical Officer of Health (MOH) area of the Kelaniya District Secretariat (KDS), Gampaha district of the Western province of Sri Lanka is divided into 37 Public Health Inspector (PHI) Divisions. Marshlands occupy 1.74 km^2 of the total land area of 20 km^2 of the Kelaniya MOH area and receive an average annual air temperature of 27 ^{0}C and an average annual rainfall of 2404 mm (WWW.Kelaniya.ds.gor.lk.html). Eight marshlands were selected randomly (Figure 1; Plate 1-Plate 8) for mosquito larvae and microbiota sampling and identification.

Mosquito larvae and microbiota sampling: From each marshland, six water samples each of 250 mL were collected into six transparent polypropylene containers (diameter 11.5cm, height 7.5cm) using a metal scooper (diameter 11.5cm, height 5.5cm) fixed in a long handle. Three of these water samples were immediately preserved in Rose Bengal stain (5% formalin with 0.04% Rose Bengal stain) solution for microbiota identification (Clesceri *et al*., 1998). The remaining three samples were kept as non-preserved. All samples were labeled and transported carefully into the laboratory for further processing. The number of mosquito larvae per 250 mL was recorded *ex situ*. Sampling was carried out once a month for a period from March 2015 to August 2015.

Physicochemical parameters of the habitats: Water pH was measured using membrane pH meter (model: EZDO-MP103). Conductivity and Dissolved Oxygen (DO) were measured using digital multi-parameter apparatus (model: HACH-HQ40d) in a manner that ¼ of the probe is dipped in the water *in situ*. Three water samples were collected from each sampling site into 250 mL dark Stoppered bottles to determine the five-day Biological Oxygen Demand (BOD_5). BOD_5 was determined as described by APHA, 1998.

Identification of mosquitoes: In the laboratory, larvae in non-preserved samples were fed with commercial fish food daily. Five fourth-instar were separated using a pasture pipette into a glass vial containing 70% ethanol. Preserved larvae were mounted on a glass slide and observed under the stereo microscope. The adult mosquitoes were observed under a stereo microscope x10 magnification. They were identified using standard mosquito identification keys (Chelliah, 1984: Reuben *et al*., 1994; Amerasinghe, 1995).

Identification of microbiota: One mL aliquot each from the preserved sample (n = 10) was examined under a compound microscope (×100 magnification) (OLYMPUS x C21) using a Sedgwick Rafter (S-R) cell (50 mm length, 20 mm width, and 1 mm deep) and HYDRO-BIOS phytoplankton chamber (dimensions, 33 × 33 mm; thickness, 1 mL) for quantifying the microbiota. Microbiota species/taxa were recorded, and identification was done to taxa/species level using temporary slide mounts observed under (×400 magnification) using standard identification keys (Abeywickrama and Abeywickrama, 1979; Corliss, 1979; Lobo and Leighton, 1986; Fernando and Weerawardhena, 2002).

Statistical analysis: Statistical analysis was performed using MINITAB 14 version (APA, 2004). The density of mosquito larvae and microbiota was expressed as the number of larvae or pupae per dip (one scooper) and number per mL respectively. Pearson correlation test was performed to determine the relationship between mosquito larvae and microbiota present in each site at $p \leq 0.05$ = * and $p \leq 0.01$ = **. Two-way ANOVA (response = larval density, factor = location) was done to determine spatial variation of density of mosquitoes. Variation of physicochemical parameters between the habitats was determined by Analysis of Variance ANOVA (response = parameter, factor = site).

Population dynamics of mosquito reported in the sampling sites were expressed according to the formula, C= (*n*/N) x 100% where C is distribution, *n* is the number of positive sites of the species, and N is the number of all sites. Distribution classes accepted by Dzieczkowski (1972), C1: sporadic appearance (constancy 0–20%); C2: infrequent (20.1–40%); C3: moderate (40.1–60%); C4: frequent (60.1–80%); C5: constant (80.1–

100%), were adopted. Mosquito larval density was expressed as a percent of numbers of the species in the whole sample according to the formula, D= (I/L) x 100%, where, D is density, *I* is the number of mosquitoes of the given species, and L is the number of all larvae. Density classes were accepted following Banaszak and Winiewski (1999), satellite species (D < 1%); subdominant species (1 < D < 5%); dominant species (D > 5%).

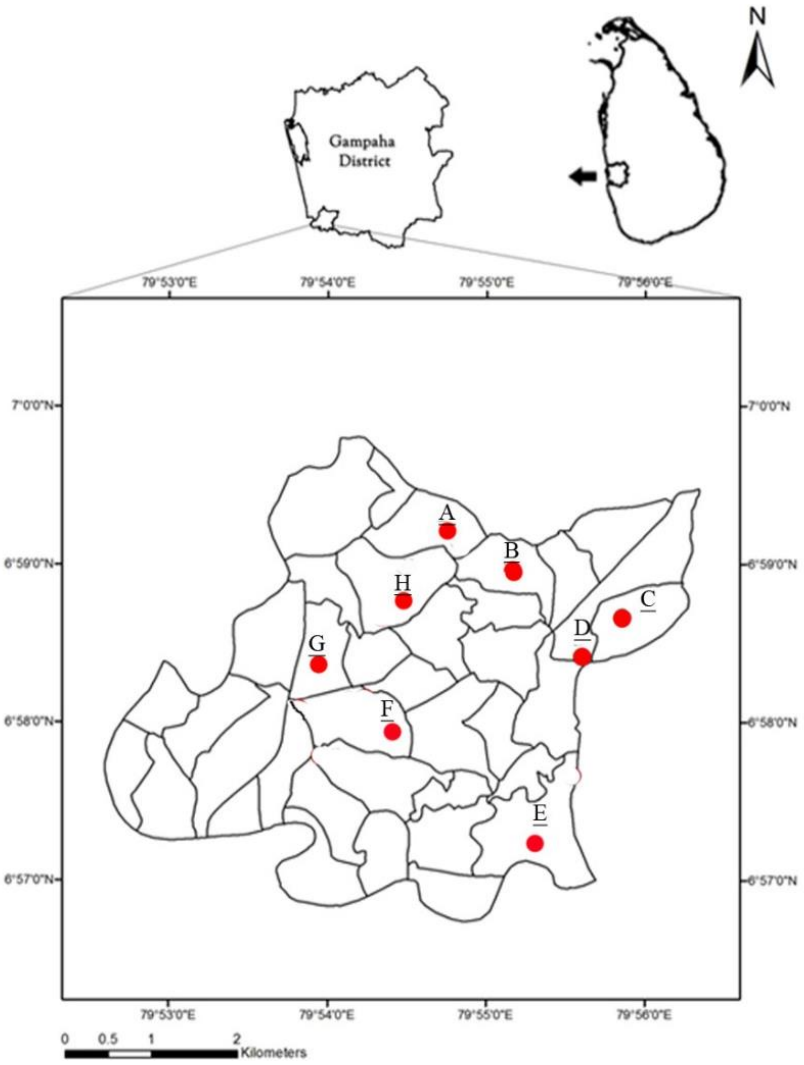

Figure 1: Site map of the mosquito larvae and microbiota sampling locations in Kelaniya MOH in Gampaha district, of Sri Lanka. (A-Nahena, B-Eriyawetiya, C-Kendahena, D-Thalawathuhenpita West, E- Kelaniya, F-Wedamulla, G-Himbutuwelgoda, H-Hunupitiya)

Plate 1. A marshland with *Eichhornia crassipes* and *Pistia* plants

Plate 2. A marshland with *Pistia, Alocasia* and *Eichhornia crassipes*

Plate 3. A marshland with *Alocasia*

Plate 4. A marshland with *Alocasia, Eichhornia crassipes* and weed grass

Plate 5. A marshland *Alocasia* plant

Plate 6. A marshland with weed

Plate 7**.** A marshland with *Annona glabra* and weed grasses.

Plate 8. A marshland with *Annona glabra*. Water surface fully covered with *A. glabra* seeds.

Population dynamics of mosquito larvae encountered in the sampling sites were expressed according to the formula, C= (*n*/N) x 100% where C is distribution, *n* is the number of positive sites of the species, and N is the number of all sites. Distribution classes accepted by Dzieczkowski (1972), C1: sporadic appearance (constancy 0–20%); C2: infrequent (20.1–40%); C3: moderate (40.1–60%); C4: frequent (60.1–80%); C5: constant (80.1–100%), were adopted. Mosquito larval density was expressed as a percent of numbers of the species in the whole sample according to the formula, D= (I/L) x 100%, where, D is density, *I* is the number of mosquitoes of the given species, and L is the number of all larvae. Density classes were accepted following Banaszak and Winiewski (1999), satellite species (D < 1%); subdominant species (1 < D < 5%); dominant species (D > 5%).

RESULTS

Six mosquito species were identified from the selected marshlands. Identified mosquito species and their densities in each of the sampling sites are given in Table 1. In this study, *Culex gelidus* (Dzieczkowski,, 1972) was reported as the dominant and constant species while *Cx. quinquefasciatus, Cx. fuscocephala* and *Cx. whitemorei* were infrequent species; *Cx. tritaeniorhynchus*, and *Anopheles peytoni* were sporadic species. Site H did not record any mosquito larvae throughout the sampling process. Fourteen microbiota/taxa were identified from the sampling locations. The mibrobiota species/taxa identified and their densities in each of the sampling sites are shown in Table 2. *Vorticella microstoma* was found to be present in the highest number in all sites except for D and F followed by *Difflugia corona*, Nauplius larva and *Cyclops strenuus*, and *Daphnia magna*.

Table 1: Species and Mean Mosquito larval density per 250 mL water

Mosquito species	Sampling site							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Culex gelidus	29	19	25	144	37	154	22	0
Culex tritaeniorhynchus	3	0	0	0	5	9	0	0
Culex quinquefasciatus	0	0	0	0	0	0	45	0
Culex. Whitemorei	42	29	0	0	50	6	0	0
Culex fuscocephala	7	21	0	0	0	0	0	0
Anopheles peytoni	0	0	0	0	4	0	0	0

Table 2: Microbiota density per mL of water in different sites

Species/Taxa	Sampling site							
	A	B	C	D	E	F	G	H
Difflugia corona	35	55	49	52	59	64	58	50
Vorticella microstoma	87	100	85	105	89	103	107	73
Cyclops strenuus	21	28	20	18	27	28	26	14
Nauplius larva	33	33	33	38	55	53	23	11
Daphnia magna	23	38	20	22	40	29	16	0
Monostyla bulla	20	27	14	0	1	6	15	17
Paramecium bursaria	5	15	0	1	0	0	0	0
Brachionus forcifula	0	0	1	22	0	0	0	2
Lecane lunaris	6	10	0	1	0	1	0	0
Diaphanosoma brachyurum racyurum	0	0	14	0	0	7	0	0
Keratella valga	0	0	2	0	7	4	0	4
Arcella arenaria	5	0	28	0	16	0	0	4
Collotheca spp.	12	0	10	5	0	3	0	7
Acanthocystis aculeata	4	16	28	32	13	10	6	5

Spatial variation of physicochemical parameters

Physicochemical parameters with respect to the sampling sites are given in Table 3. Dissolved Oxygen level (DO), pH and BOD level which are indicatives of the level of pollution of the habitats are significantly varied between sites ($F_{7, 136} = 2.88$, $F_{7, 136} = 9.20$ and $F_{7, 136} = 3.38$, at $P \leq 0.05$ respectively (One-way ANOVA and Tukey's pairwise tests).

Spatial variation for mosquito larvae and microbiota

Results of the Pearson correlation analysis conducted for density values of the microbiota species and mosquito species larvae for each site is given from Table 4 to Table 10. There was a significant positive correlation between *Cx. gelidus* and *Cx. whitmorei,* (0.701) and between *Cx. whitmorei* and *Monostyla bulla* (0.608) in Site A (Table 4). Table 5 shows that *Cx. fuscocephala* is positively correlated with *Cx. gelidus* in site B whereas there is a negative correlation between *Monostyla bulla* and *Cx. whitmorei* and between *Lacane luna* and *Cx. fuscocephala*. Table 6 shows that there is a significant positive

Table 3: Level of physicochemical parameters at sampling

Data are presented as mean ± SE (n = 3) and range. Means indicated by same superscript letters in a row are not significantly different for each site (ANOVA, Tukey's test, $P \leq 0.05$).

Parameter	Sampling site							
	A	B	C	D	E	F	G	H
DO (mg/L)	2.97 ± 0.44^{a}	2.16 ± 0.19^{ab}	2.43 ± 0.15^{ab}	3.04 ± 0.15^{a}	2.65 ± 0.44^{ab}	1.53 ± 0.06^{b}	2.08 ± 0.36^{a}	2.51 ± 0.37^{ab}
	(1.42 - 6.97)	(0.75 - 3.53)	(1.82 - 3.32	(1.84 - 4.19)	(1.02 - 6.54)	(1.15 - 1.98)	(1.75 - 5.2)	(1.25 - 5.78)
pH	6.64 ± 0.04^{cd}	6.72 ± 0.07^{bcd}	6.75 ± 0.05^{abc}	6.65 ± 0.06^{cd}	6.61 ± 0.04^{cd}	6.54 ± 0.04^{a}	6.94 ± 0.04^{a}	6.90 ± 0.03^{ab}
	(6.45 - 6.95)	(6.29 - 7.40)	(6.45 - 6.96)	(6.18 - 6.94)	(6.42 - 6.94)	(6.11 - 6.73)	(6.94 - 7.14)	(6.73 - 7.10)
BOD (mg/L)	1.57 ± 0.44^{ab}	1.00 ± 0.17^{ab}	2.01 ± 0.16^{a}	1.73 ± 0.19^{a}	2.09 ± 0.39^{a}	0.27 ± 0.07^{b}	1.53 ± 0.41^{ab}	1.32 ± 0.34^{ab}
	(0.17 - 5.56)	(-0.41 - 2.35)	(1.14 - 2.89)	(0.58 - 3.00)	(0.29 - 5.55)	(-0.38 - 0.83)	(-2.70 - 4.95)	(0.15 - 4.21)
Conductivity (µs/cm)	336.39 ± 3.21^{cd}	360.2 ± 15.5^{c}	247.62 ± 9.02^{e}	220.28 ± 5.56^{e}	303.39 ± 4.94^{d}	372.11 ± 1.65^{c}	663.4 ± 27.6^{a}	489.83 ± 7.49^{b}
	(318.00 - 353.00)	17.00 - 503.00)	194.00 - 292.00)	187.00 - 257.00)	272.00 - 340.00)	360.00 - 383.00)	(542.00 - 873.00)	(520.50 - 543.00)

Table 4: Correlation coefficient between density values of microbiota species and mosquito larvae in site

Species	*Cx. gelidus*	*Cx. itaeniorhynchus*	*Cx. whitmorei*	*Cx. fuscocephala*
Cx. gelidus	-	-	-	-
Cx. tritaeniorhynchus	0.401	-	-	-
Cx. whitmorei	**0.701***	0.064	-	-
Cx. fuscocephala	-0.392	0.611	0.027	-
Difflugia corona	-0.026	-0.393	-0.032	-0.254
Vorticella microstoma	0.078	-0.207	0.064	-0.282
Cyclops strenuus	0.103	0.073	0.21	-0.373
Nauplius larva	0.042	0.146	-0.127	0.009
Daphnia magna	-0.071	0.21	0.177	-0.382
Monostyla bulla	0.36	-0.321	**0.608***	-0.257
Paramecium bursaria	0.068	-0.207	0.167	-0.239
Lecane lunaris	-0.296	0.582	-0.08	-0.161
Arcella arenaria	0.14	0.199	-0.082	-0.165
Collotheca spp.	0.007	-0.124	-0.25	0.523
Acanthocystis aculeata	-0.023	0.582	-0.201	-0.161

correlation between *Daphnia magna* and *Cx. gelidus* in site 'C'. However, other microbiota species were not shown any significant correlation with the mosquito species larvae in this site (Table 6). Table 7 shows that there is not any significant correlation between microbiota and mosquito species larvae in site D. *Cx. whitmorei*, *Vorticella microstoma* and *Acanthocystis aculeata* show a significant negative correlation with *Cx. gelidus* in site E (Table 8). In contrast, *Difflugia corona*, *Vorticella microstoma*, Nauplii and *Keratella valga* show significant positive correlation with *Cx. whitmorei*. Table 9 shows that *Cx. tritaeniorhynchus* show significant positive correlation with *Daphnia magna* in site 'F' while others are not shown any significant correlation. There is a significant negative correlation between *Cx. quinquefasciatus* and *Cx. gelidus* in site 'G' (Table 10) while *Daphnia magna* is positively correlated with *Cx. gelidus* in the same site.

Table 5: Correlation coefficient between density values of microbiota species and mosquito larvae in site B

Species	*Cx. gelidus*	*Cx. whitmorei*	*Cx. fuscocephala*
Cx. gelidus	-	-	-
Cx. whitmorei	-0.487	-	-
Cx. fuscocephala	0.274	0.051	-
Difflugia corona	0.242	-0.446	0.392
Vorticella microstoma	-0.337	0.23	0.339
Cyclops strenuus	0.026	0.038	-0.183
Nauplius larva	0.390	-0.148	0.415
Daphnia magna	0.098	-0.192	0.493
Monostyla bulla	0.025	**-0.504***	0.239
Paramecium bursaria	-0.042	0.255	-0.759
Lecane lunaris	0.073	-0.004	**-0.508***
Acanthocystis aculeata	0.058	-0.265	0.151

Table 6: Correlation coefficient between density values of microbiota species and mosquito larvae in site C

Species	*Cx. gelidus*
Cx. gelidus	-
Difflugia corona	0.101
Vorticella microstoma	-0.065
Cyclops strenuous	0.165
Nauplius larva	-0.168
Daphnia magna	**0.472***
Monostyla bulla	0.114
Diaphanosoma brachyurum	-0.139
Keratella valga	-0.451
Arcella arenaria	0.141
Collotheca spp.	-0.241
Acanthocystis aculeata	0.248

Table 7: Correlation coefficient between density values of microbiota species and mosquito larvae in site D

Species	*Cx. gelidus*
Cx. gelidus	-
Difflugia corona	0.181
Vorticella microstoma	-0.307
Cyclops strenuous	0.224
Nauplius larva	0.045
Daphnia magna	0.350
Paramecium bursaria	0.072
Brachionus forcifula	0.290
Lecane lunaris	0.072
Collotheca spp.	-0.255
Acanthocystis aculeata	-0.182

Table 8: Correlation coefficient between density values of microbiota species and mosquito larvae in site E

Species	*Cx. gelidus*	*Cx. tritaeniorhynchus*	*Cx. whitmorei*	*An. peytoni*
Cx. gelidus	-	-	-	
Cx. tritaeniorhynchus	-0.456	-	-	-
Cx. whitmorei	**-0.550***	0.025	-	-
*An. peyton*i	0.195	0.595	-0.294	-
Difflugia corona	-0.323	- 0.195	**0.680***	-0.105
Vorticella microstoma	**-0.664****	0.087	**0.473***	-0.116
Cyclops strenuus	-0.423	-0.04	0.278	-0.185
Nauplius larva	-0.385	-0.197	**0.58***	-0.155
Daphnia magna	-0.321	-0.142	0.423	0.087
Monostyla bulla	-0.008	-0.092	-0.202	-0.086
Keratella valga	-0.445	0.503	**0.540***	-0.182
Arcella arenaria	0.225	0.160	-0.213	0.053
Acanthocystis aculeata	**-0.532***	-0.169	0.816	0.539

Table 9: Correlation coefficient between density values of microbiota species and mosquito larvae in site F

Species	*Cx. gelidus*	*Cx. tritaeniorhynchus*	*Cx. whitmorei*
Cx. gelidus	-	-	-
Cx. tritaeniorhynchus	0.079	-	-
Cx. whitmorei	-0.286	0.534	-
Difflugia corona	-0.073	0.679	-0.231
Vorticella microstoma	-0.212	-0.36	0.306
Cyclops strenuus	0.134	-0.205	0.217
Nauplii	0.103	0.211	0.448
Daphnia magna	0.134	**0.744***	0.623
Monostyla bulla	0.021	0.445	0.348
Lacane luna	0.607	-0.093	-0.099
Diaphanosoma spp.	0.308	-0.22	0.539
Keratella valga	0.146	-0.16	-0.170
Collotheca spp.	0.720	0.000	-0.136
Acanthocystis aculeata	0.091	0.964	-0.155

Table 10: Correlation coefficient between density values of microbiota species and mosquito larvae in site G

Species	*Cx. gelidus*	*Cx. quinquefasciatus*
Cx. gelidus	-	-
Cx. quinquefasciatus	**-0.646***	-
Difflugia corona	-0.264	0.105
Vorticella microstoma	0.021	0.171
Cyclops strenuus	0.286	-0.241
Nauplius larva	0.219	-0.345
Daphnia magna	**0.473***	-0.402
Monostyla bulla	0.15	0.112
Acanthocystis aculeata	0.619	-0.166

DISCUSSION

Culex gelidus is the major mosquito species found during this study. This species serves as the vector for Japanese encephalitis (Reuben *et al*, 1994). Yadav (2009) stated that the presence of vegetation and floating plants provide an optimal breeding condition for *Cx. gelidus* and *Cx. whitmorei* by acting as food sources and shelter from predators. Vegetation also creates stagnant conditions by decreasing water movements. There was a dense coverage of vegetation such as *Alocasia*, *Eichhornia*, *Pistia* and *Typha* in many marshlands which may have favored *Cx. gelidus* and *Cx. whitmorei* population. *Cx. gelidus* can breed in water with a high concentration of organic matter.

Muturi *et al*. (2008) stated that when *Cx. gelidus* is prominent in some habitats, there is little content of *Cx. whitmorei* and vice versa. Similarly, during the present study *Cx. whitmorei* was the prominent mosquito species found in sites A and E where, *Cx. gelidus* abundance was relatively low. In contrast *Cx. whitmorei* was relatively low compared to very high numbers of *Cx. gelidus* in site F. Muturi *et al*. (2007) stated when *Cx. gelidus* and *Cx. whitmorei* present in same habitat one species always become dominant. The alternative pattern of abundance of these two species may be due to the competition of the two species for their habitat or food (Muturi *et al*., 2008).

Culex quinquefasciatus breeds in organically polluted water with the highest BOD (Muturi *et al*., 2007). Similar observations were made in this study too where a higher density of *Cx. quinquefasciatus* was recorded from site G which had comparatively low dissolved oxygen level (2.08 mg/L) and high BOD (1.53 mg/L). Mosquitoes were not recorded from site H throughout the study. This marshland was covered with *Annona glabra*. Leaves and seeds extraction of *Annona* species were found to be potential as larvicidal and anti-mosquito agents (Magadula *et al*., 2009; George and Vincent, 2005).

The abundance of mosquito larvae is also affected by abiotic factors such as DO, BOD, and pH and the biotic factors such as presence of other aquatic microbiota. Microbiota may become beneficial for the mosquito larvae by acting as food items and some species may be harmful by acting as parasites or pathogens. *Vorticella microstoma* was found in all the sites in a higher percentage than the other species. This species is considered the most prevalent microbiota in marshlands in Sri Lanka (Amarasinghe and Rathnayake, 2014). *Coleps hirtus, Chaetonotus, Ichthydium, Diurella stylata, Lecane* spp. and *Rotaria* spp. have not shown considerable effects on the presence of the mosquito larvae. They are filter feeders and have free-living association with the mosquito larvae (Amarasinghe and Rathnayake, 2014). Moreover, Elono *et al.* (2010) reported that the abundance of *Aedes* spp. mainly negatively correlated with the abundance of food competitors present in the same habitat, such as *Ceriodaphnia* spp, *Chydorus* spp, *Daphnia magna, Simocephalus* spp, *Calanoida,* and larvae of Chironomidae (Elono *et al*., 2010). Biota species richness was relatively higher in site A compared to other sites. At the same time density value of *Cx. gelidus* and *Cx. whitmorei* showed a significant positive correlation in this site. This explains as when there is abundance of food resources, competition between mosquito species is reduced.

There was a significant positive correlation between *Cx. whitmorei* and *Monostyla bulla* (Family Lecanidae) in site A. However, observation revealed that these two species are not interdependent, but both species depend on certain physicochemical parameter levels of the habitat such as DO, BOD and pH. Mule and Kharade (2007) stated that the growth of zooplankton depends on the temperature, DO, pH and organic matter content of the water. Therefore, zooplankton act as an indicator of the level of pollution. When the pollution level is high, the abundance of microbiota is reduced. There was a negative relationship between the abundance of *Cx. whitmorei* and *Monostyla bulla* in Site B, a habitat with polluted water with higher BOD levels. There was not any relationship between these two species in sites

E and F. This explains that *Monostyla bulla* is a pollutant tolerant species. *Culex fuscocephala* and *Cx. gelidus* in site B also showed a significant positive correlation indicating their tolerance for polluted water. *Culex fuscocephala* and *Monostyla bulla* were also given a significant positive correlation in this site. A significant positive correlation was observed between *Daphnia magna* and *Cx. gelidus* in sites C and G. *Daphnia magna* also showed a significant positive correlation with *C. tritaeniorhynchus* in site F. *Difflugia corona, Vorticella microstoma,* Nauplius larva and *Keratella valga* were shown a positive correlation with *Cx. whitmorei* in site E indicatng that these species are not interdependent *i.e.*, predator-prey effect or parasite-host relationship. *Difflugia corona, Vorticella microstoma,* Nauplius larva and *Keratella valga* are free-living species only sensitive to the pollution level. *Vorticella microstoma* and *Acanthocystis aculeata* in the same habitats showed a negative correlation with *Cx. gelidus*. However, this correlation is very low ($P<0.01$). When there is a high abundance of *Vorticella microstoma*, they are usually attached to the cuticular surfaces of the thorax and the siphon areas of the mosquito larval body. This restricts the mobility of the mosquito larvae and therefore it may affect the growth and molting of the affected larvae. The dense association of *Vorticella microstoma* causes to death of moribund larvae (Laird, 1956). *Cx. gelidus* and *Cx. quinquefasciatus* found in site G showed a negative correlation. This may be due to the competition between two species for the available resources.

Physicochemical parameters also affected the abundance of mosquito larvae. In this study mosquito larvae were recorded in DO value ranging from 0 to 7 mg/L. Amarasinghe and Dalpadado (2014) stated that mosquitoes can tolerate 0-8 mg/L DO levels and *Cx. quinquefasciatus* can tolerate DO range between 0-4 mg/L. In this study, a higher density of *Culex quinquefasciatus* was recorded in site G which had DO level of 2.08 mg/L with a high BOD level. Large size species of cyclopoids copepods prey on first instar mosquito larvae

and they are effective for biological control of mosquitoes than other predatory invertebrates (Marten at el., 1994). Even though cyclopoids copepods were found in some sites during the study they did not show any significant correlation with density values of mosquito larvae present in these sites.

CONCLUSIONS

Mosquito species recorded from marshes in the Kelaniya MOH area in Sri Lanka were *Culex gelidus, Cx. tritaeniorhynchus, Cx. fuscocephala, Cx. quinquefasciatus, Cx. whitmorei* and *Anopheles peytoni*. Abundance of *Culex gelidus* and *Cx. whitmorei* negatively correlates when they occupy the same habitat with limiting resource of microbiota, nevertheless, they were positively correlated in presence of dense microbiota. The abundance of *Cx. gelidus* larvae was reduced in the presence of a higher density of *Vorticella microstoma*. Higher BOD levels reduced the abundance of microbiota. *Difflugia corona,* Nauplius larva, *Keratella valga, Cyclops strenuus*, *Monostyla bulla, Acanthocystis aculeata,* and *Daphnia magna* act as free-living organisms.

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