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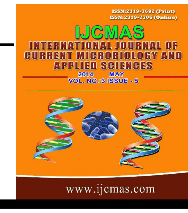

Original Research Article

Prevalence of micro fauna associated with different mosquito breeding habitats in a selected area of Sri Lanka

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A B S T R A C T

The present study was to find out dispersal variability of micro fauna in different mosquito breeding habitats in a selected area in Sri Lanka and their negative effects on mosquito larvae so that such organism could be considered as beneficial for mosquito controlling programs. Paddy fields, water accumulated bamboo tree and tree holes, ponds, abandoned wells, abandoned paddy fields, irrigation canals, marshy lands, cultivated lands, Ma oya/ stream (bed pool) were chosen to collect water samples for micro fauna and mosquito larvae. *Zoothamnium* sp. was found as the most abundant micro fauna in different types of mosquito breeding habitats. Micro fauna abundance is significantly different between species/taxa. *Culex tritaeniorhynchus* was found to be the most abundant mosquito species in the study area. *Tripteroides* sp. were found as a tree hole breeding mosquito and *Aedes albopictus* was found in ponds, abandoned wells and bed pools of Ma-oya.Species/taxa of micro fauna coexist with mosquito species in different habitats but, none of the micro fauna species have a significant effect on the abundance of mosquito larvae. *Zoothamnium* sp. had a weak negative effect on mosquito larvae.

Keywords

Mosquito abundance, breeding habitats, micro fauna

Introduction

Mosquito larvae and their predators or pathogens expected to co-exist in every breeding habitat of mosquito ranging from small temporary sites to large permanent sites. Micro invertebrates are more common, prevalent and numerous than macro invertebrates in aquatic habitats. Micro invertebrates are important as food sources for macro invertebrates and fish (Gilbert and Burns, 1999; Hampton and Gilbert, 2001; Hampton *et al*., 2000). Many also prey on insect larvae (Marten, 1990). So in order to formulate an effective less environmentally hazardous vector control method, knowledge of microfauna associated in mosquito larval habitats and the density of available micro fauna in any mosquito inhabiting area is important. Kramer (1964) reported flagellates are rather common intestinal parasites of adult's mosquitoes. Das (2003) reported pathogens can be used to control mosquitoes during its larval stage also reported several species of protozoans and nematodes can infect mosquito larvae. Platzer (1980) reported the nematode *Romanomermis culicivorax* having the ability to infect mosquito larvae. In mixed populations anophelinae was more susceptible to infection than culicinae.

The infection of endoparasitic ciliated protozoa *Lambornella stegomyiae* has been reportedin *Aedes albopictus* in a sample collected from an earthen pond of Kuala Lumpur (Das, 2003). Another ciliated parasite *Lambornella clarki* was isolated from a tree-hole breeding mosquito larvae, *Aedes sirensis,* in California, USA.In north East India, anopheline larvae (*Anopheles barbirostris, Anopheles hyrcanus* group and *Anopheles philippinensis*) breed in peridomestic ditches were found, infected with *Lambornella* species which is another ciliated parasite. *Tetrahymena pyriformis,* was observed in the body cavity and anal gills of bamboo-breeding *Armigeres* species from a forest near to Kuala Lampur (Das, 2003).

According to Das (2003) another endoparasitic ciliate, *Chilodonella uncinata* have been isolated from infected larval head capsule, 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. High mortalities have been reported in *Culex tritaeniorhynchus* and *Culex gelidus* species collected from paddy fields and marshy areasdue to the natural infections of *Chilodonella uncinata.* It was reported that *Chilodonella uncinata* having the ability for transovarian transmission through its mosquito host (Das, 2003).

Corliss (1961) stated that *Tetrahymena* parasites multiply within the haemocoele of the host and as a result, heavily infested larvae are abnormally transparent or whitish or opaque. According to the author, these protozoans probably enter via the oral route and invade the haemocoele through the gut wall. Parasitized larvae usually succumb to the infection. Also this author reported all species known from *Tetrahymena* are facultative parasites.

Host-parasite interactions between *Lambornella clarki* (Ciliophora: Tetrahymenidae) and its natural host, *Aedes sierrensis* (Diptera: Culicidae), were studied in newly flooded tree holes in northern California between 1986 and 1989 (Washburn *et al*., 1991). They observed first instar host larvae hatched within 1 to 4 hour of flooding, while free-living trophonts of *L. clarki* appeared between 7 and 24 hr. As early as 24 hour after flooding, ciliates initiated the first parasite cycle by forming cuticular cysts on first instar larvae. Washburn *et al*. (1991) recorded when host populations developed with sufficient food, mortality from parasites was additive and reduced the number of emerging mosquitoes.

Based on the literature cited, the objective of the present study was carried out to find out dispersal variability of micro fauna in different mosquito breeding habitats in Sri Lanka and their negative effects on mosquito larvae.

Materials and Methods

Study area and sampling sites

Mawanella (7°14'55.00"N of latitude and 80°26'42.42"E of longitude) from Kegalle District,Sabaragamuwa province was selected as study area. Among the breeding habitats of mosquitoes, maximum of ten permanent breeding habitats were selected as the sampling sites; Paddy fields, bamboo tree holes, ponds, abandoned wells, tree holes, abandoned paddy fields, irrigation canals, marshy lands, cultivated lands, Ma oya/ stream (bed pool). Locations of these sites were recorded using a portable global positioning system/ GPS (GARMIN- etrex SUMMIT).

Approximately 200 ml of water samplesfrom individual habitats (n=3)with or without mosquito larvae were collectedbimonthlyusing a long handled metal larval scooper, the standard dipping method for mosquito larval sampling (Mwangangi *et al.*, 2008). When the habitat is too small to collect required volume of water, a pipette was used. Samples were collected into two transparent plastic containers (diameter 10.5 cm, height 9.5 cm) and the number of larval mosquitoes was counted *insitu*. Content of one container was preserved using 5% formaline. Containers then carefully brought into the laboratory for further observations.

Identification of existing micro fauna in water samples

At the first day of collecting samples, dead or moribund larvae in unpreserved water samples were examined under compound microscope (OLYMPUS CX21) for detection of any associated micro fauna that can be lethal for mosquito larvae. Preserved water samples were subjected to centrifuge and decant the supernent. 1 ml (n=5) of retained water sample was observed under compound microscope for identification of associated live species of micro fauna using a Sedgwick rafter cell. The species present were identified and the number was counted. For enumeration of smaller protozoans (<50 µm), two 5 to 10 µ1 aliquots from each 1 m1 formalin-preserved aliquot per sample were counted on duplicate 0.1 mm^3 blocks on a hemocytometer at 400x (Collins &Lyne, 1976).

Live mosquito larvae were kept under laboratory conditions for a period of 07-09 days until the emergence of adults. Emerged adults were collected into polythene bags and killed by inserting a cotton wool swab soaked in ethyl acetate. Specimens were observed under the low power stereo microscope (MEIJI EMup Z) and each mosquito was identified up to nearest possible taxonomic level, using standard mosquito keys (Chelliah, 1984; Reuben *et al*, 1994).

Data analysis

The statistical data analysis was performed using MINITAB 14 version and PRIMER 5 version. The abundance of micro fauna was expressed as the average number per 200 ml of water. The relative abundance of micro fauna and mosquito species in different breeding habitats was analyzed using cluster analysis and Multidimensional Scaling (MDS ordination). This involves the data transformation of Log(X+1) to reduce the biasness. The variation between different habitat types was tested using similarity (ANOSIM) test. The correlation between mosquito larvae and micro fauna species was analyzed using Pearson's correlation in MINITAB 14 version.

Results and Discussion

Prevalence of micro fauna in mosquito habitats: Seven species of micro fauna namely, *Coleps hirtus*, *Zoothamnium* sp., *Vorticella* sp., *Chaetonotus* sp., *Ichthydium*sp., *Lecane* sp. and *Rotaria* sp. were recorded from Mawanella area (Table 1).

Table.1 Prevalence of micro fauna in different breeding habitats per200ml

Species/taxa	Marshy land	Cultivated paddy field	Irrigation canal	Pond	Bamboo tree	Abandoned well	Tree hole	Abandoned paddy fields	Cultivated land	Ma-oya
Colepshirtus	954	32	676	25	8	398	49	603	398	0
Zoothamnium	32360	14600	178	20600	616	39810	1400	24400	1000	10250
Vorticella	50	49	1202	151	100	631	50	1300	100	50
Chaetonotus	60	123	72	49	123	83	3	60	20	0
Ichthydium	33	98	154	71	3	102	0	10	0	0
Lecane	72	32	7	132	25	63	2	0	2	3
Rotaria	0	9	4	0	41	0	0	0	0	0

Table.2 Mean number of mosquito genera/ species collected from different breeding habitats

Mosquito species	Marshy land	Paddy field	Irrigation canal	Pond	Bamboo tree	Abandoned well	Tree hole	Abandoned paddy field	Cultivated land	Ma-oya
Culex tritaeniorhynchus	27	203	57	6	0	10	0	51	12	0
Cule fuscocephala	14	148	24	0	0	0	0	23	12	3
Culex quinquefasciatus	0	38	4	0	0	0	0	12	0	0
Culex pseudovishnui	0	3	0	0	0	0	0	0	0	0
Tripteroides spp.	0	0	0	0	412	0	6	0	0	0
Aedes albopictus	3	0	0	80	0	72	0	0	0	41
Anopheles vergus	0	0	1	0	0	0	0	0	0	0
Total mosquito larvae	44	392	86	86	412	82	6	86	24	44

Results revealed that the prevalence of micro fauna between different mosquito breeding habitats was significantly different (Two-Way ANOVA, F = 3.36, df = 9, P = 0.002 < 0.05). Results also revealed that there is a significant difference between micro fauna species or taxa in a given habitat (Two-Way ANOVA, F = 25.21, df = 6, P = 0.000 < 0.05).Abundance of *Zoothamnium* species was higher than that of other species. The second most abundant species was *Vorticella*. Compared to *Zoothamnium* sp., rest of all the other micro fauna were in significantly less number. During the study period total number of 1273 of mosquito larvae belonging to seven species was recorded from different habitats (Table 2).

Cluster analysis

Mosquito breeding habitats could be divided into three clusters at the 30% of the similarity level based on prevalence of mosquitoes in each habitat type (Figure 1). It shows that bamboo tree holes and tree hole habitats are clustered together, pond, abandoned well and stream (Ma oya) breeding habitats were clustered together as a separate one. Other breeding habitats such as cultivated paddy field, abandoned paddy field, irrigation canal and marshy lands are clustered together as a single habitat at 30% of similarity level.

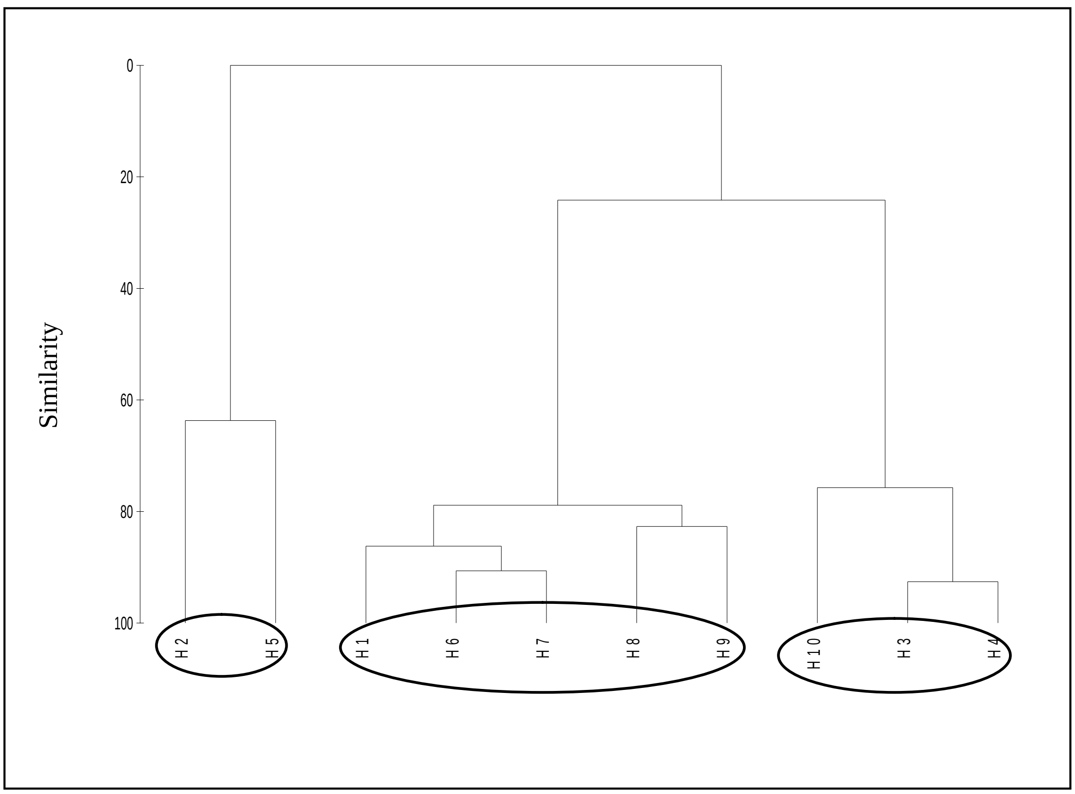

Figure.1 Dendrogram showing the clustering of breeding habitatsaccording to the prevalence of mosquito species. (H1-Paddy fields, H2-Bamboo tree, H3-Pond, H4-Abandoned well, H5-Tree hole, H6-Abandoned paddy field, H7-Irrigation canal, H8-Marshy land, H9-Cultivated land, H10-Ma oya)

Figure.2 shows the two dimensional view of the MDS ordination of different breeding habitats of mosquitoes. According to the MDS plot bamboo tree habitats and tree hole habitats are clustered together (H_2 and H_5), pond, abandoned well and Ma-oya habitats are clustered together and all the other habitats are clustered in one cluster. [According to the ANOSIM cluster 3 and cluster 1 are significantly different from each other (P = 0.048 < 0.05) and Cluster 3 and cluster 2 are significantly different from each other (P = 0.018 < 0.05). But cluster 2 and cluster 1 are not significantly different from each other (P = 0.10 > 0.05)].

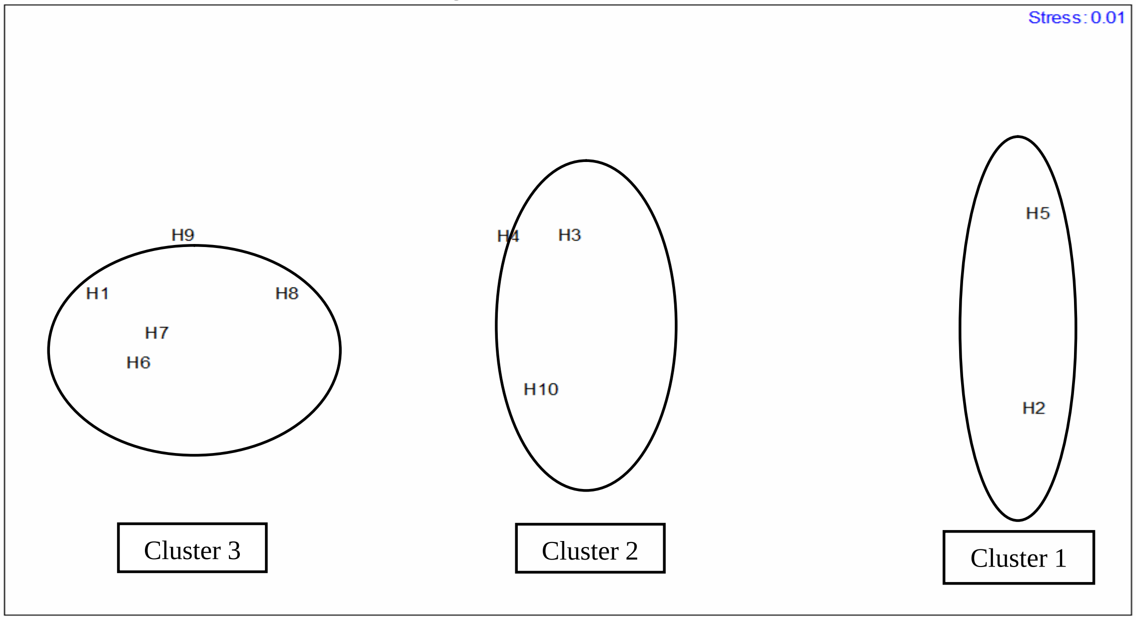

Figure.2 Two dimensional MDS plot showing the clustering of breeding habitats based on the prevalence of mosquito species**.**

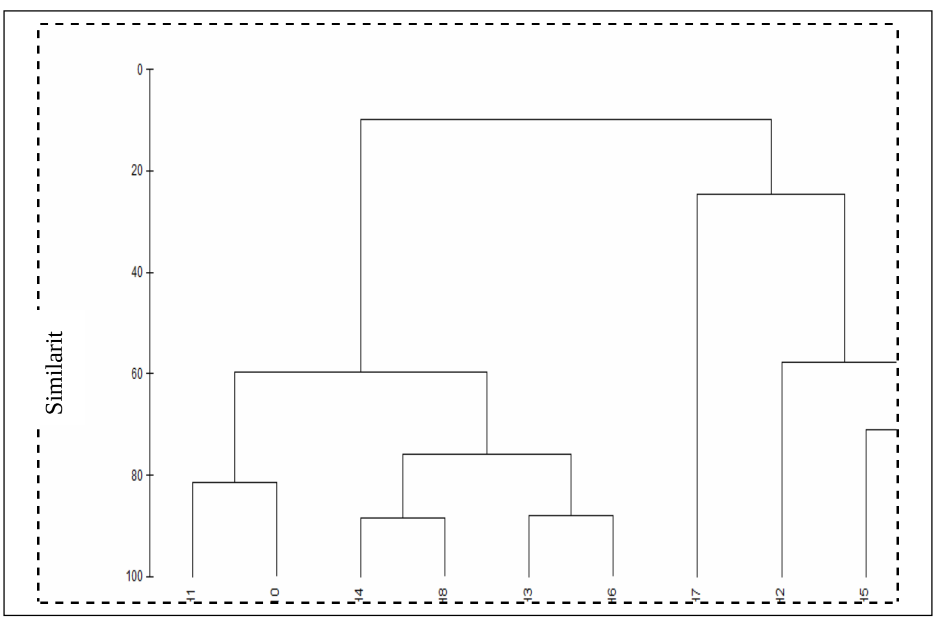

Figure.3 Dendrogram showing the clustering of breeding habitats based on to the prevalence of micro fauna in each habitat type. (H1-Paddy fields, H2- Bamboo tree, H3-Pond, H4-abandoned well, H5-Tree hole, H6-Abandoned paddy field, H7-Irrigation canal, H8-Marshy land, H9-Cultivated land, H10-Ma oya).

According to the figure 3 bamboo tree habitats, tree holes and cultivated lands are clustered together in 30% similarity level. Paddy fields, marshy lands, ponds, abandoned well, abandoned paddy fields and stream (Ma oya) breeding habitats have been clustered together in 30% of similarity level while irrigation canals are clustered separately as a single habitat. Figure 4 shows the two dimensional view of the MDS ordination of different habitats of micro fauna. According to the MDS plot bamboo tree, tree hole and cultivated lands are clustered together (H_2, $H_5$and H_9). Irrigation canals are clustered separately as a single habitat. Remaining habitats have been clustered separately. According to the ANOSIM cluster 3 and cluster 2 are significantly different from each other (P = 0.012< 0.05) and Cluster 3 and 1 are not significantly different from each other (P = 0.143 < 0.05). Cluster 2 and cluster 1 are also not significantly different from each other (P = 0.25 > 0.05).

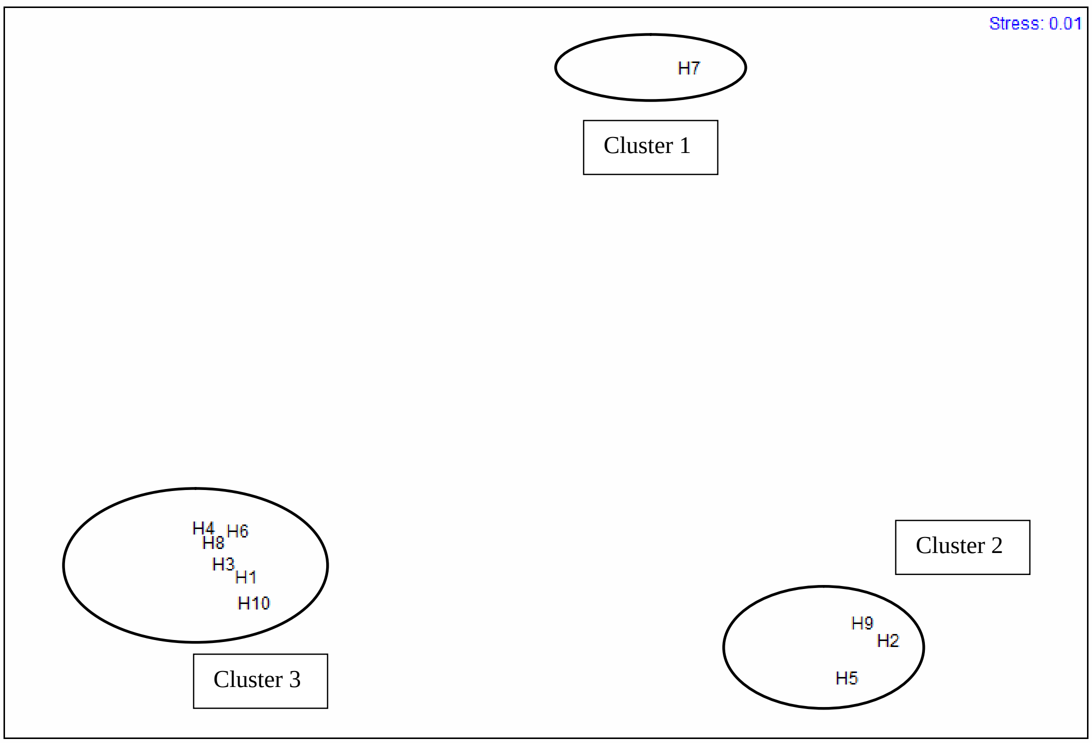

Figure.4 Two dimensional MDS plot showing the clustering of breeding habitats based on to the prevalence of micro fauna in each habitat type.

Both micro fauna and mosquito species clustered in three clusters. In both clusters bamboo tree and tree holes were clustered together. In addition, cultivated land in micro fauna clustering cultivated lands clustered together with bamboo tree and tree hole. In mosquito species clustering, cultivated lands clustered together with paddy fields and other habitats but not with bamboo trees. In bamboo trees and paddy fields highly abundant mosquito species was *Tripteroides* sp. and in cultivated lands abundant species were *Culex tritaeniorhynchus* and *Culex fuscocephala*. When consider micro fauna, all seven species of micro fauna were present in bamboo trees, only five species of micro fauna were present in both tree holes and cultivated lands. In both type of habitats *Rotaria* sp. and *Ichthydium* sp. are absent. Likewise, *Zoothamnium* are not highly abundant in these three kinds of habitats. In all the three habitats *Zoothamnium* sp. was not highly abundant. So bamboo tees, tree holes and cultivated lands have been clustered together.

In mosquito species clustering, irrigation canals have been clustered with paddy fields, cultivated lands and others. But according to the micro fauna abundance, irrigation canals have been clustered separately. In irrigation canals highly abundant mosquito species were *Culex ritaeniorhynchus* and *Culex fuscocephala*. All the species of micro fauna have been reported here but highly abundant species was *Vorticella* sp. *Zoothamnium* sp. was less abundant in irrigation canals. So irrigation canals have been clustered separately.

Other six type of habitats have been clustered together because in all type of habitat *Zoothamnium* are the highly abundant species. Even though they are clustered together here according to the micro fauna abundance, Ma-oya sites, ponds and wells have been clustered separately according to the mosquito species. In these three types of habitats highly abundant mosquito species was *Aedes albopictus*. But in paddy fields, abandoned paddy fields and marshy lands highly abundaned mosquito species was *Culex tritaeniorhynchus*. So according to the results it can be concluded when *Aedes albopictu s*and *Culex tritaeniorhynchus* are highly available, *Zoothamnium* species are highly abundant. But when other mosquito species are highly abundant *Zoothamnium* species population is low. Even though according to the obtained results it is concluded an exact relationship cannot be explained here.

Correlation between mosquitoes and different micro fauna species

Values obtained for the Pearson correlationfor different micro fauna species with mosquito larvae are given in the table 3.

Correlation analysis shows that none of the micro fauna species having a significant relationship with mosquito larvae (Table 3). But only *Zoothamnium* sp. having a weak negative relationship which is having an evidence of a relationship ($P = 0.000 < 0.05$). The other species of micro fauna are not having considerable relationship with mosquito larvae.

Table.3 Correlation analysis between microfauna species/taxa and mosquito larvae

Micro fauna species	Pearson correlation	P- value
Colepshirtus	-0.207	0.038
Zoothamnium	-0.451	0.000
Vorticella	-0.118	0.241
Chaetonotus	0.119	0.239
Ichthydium	-0.026	0.798
Lecane	-0.074	0.461
Rotaria	0.200	0.045

This study revealed that the most prevalent micro fauna species found in mosquito breeding habitats in Sri Lanka is *Zoothamnium* sp. (Phy. Ciliophora). Other important species/taxa were *Vorticella* spp.*Colepshirtus* (Phy. Ciliophora), *Chaetonotus* (Phy.Gastrotricha) and *Ichthydium* (Phy.Gastrotricha), and *Lecane*, *Rotaria* spp. and *Diurella stylata* of Phylum Rotifera. *Zoothamnium* sp., *Vorticella* sp. and Rotifers are found to be important as epibionts with mosquito larvae. *Zoothamnium* sp. Are thought to be ectocommensals and they preferentially attached to a wide variety of metazoans species such as coelenterates, annelid worms, insect larvae, tadpoles, mollusks, fish and crustaceans (Esch *et al*., 1976; Henebry and Ridgeway, 1979). This is proven by the observations made during this study asabove epibionts was almost always present attached to the cuticle of mosquito larvae.

Dead mosquito larvae were found with a dense association of *Zoothamnium* sp. and *Vorticella* sp. These organisms were highly abundant attaching to the cuticular surfaces of thorax and the siphon areas of the larval body. Active larvae were with less abundance of these epibionts but, moribund larvae too were found with dense association of above epibionts. These epibionts have disadvantageous on the basibiont or the organism that provide the substrate to attack. They restrict the mobility of the mosquito larvae and it may affect growth and molting of the larvae. Also, it may adversely affect to the functioning of many organs like eyes, gills and appendages. It may cause an increase of the risk of predation and also compete for available nutrients with the larvae (Wahl, 1989; Threlkeld *et al*., 1993,Becker and Wahl, 1989).According to Laird (1956), 22% of mosquito larval collection made over two-year period included specimens infested with epibiotic ciliates alone. Further this author reports, an unidentifiable species of the genus *Zoothamnium* was noted on the abdomen of a mounted fourth instar larva of *Culex annulirostris*.Even though such micro fauna are not having the ability of causing diseases on mosquito larvae they can reduce the movement of larvae severely by attaching on their cuticle. When they present heavily attached to mosquito larvae, the organisms able to cause external infections. While *Vorticella* spp. present externally attached to larvae, they formed a fuzzy coating around the larvae (Schober, 1967). Although they are frequently encountered in nature, they can cause mortality in mosquito larvae either by physically blocking the larvae from feeding or by overwhelming the larva and reduce the normal movement of the larvae. Readily infested larvae will have trouble reaching the surface to breathe or their siphons may be blocked by attached protists(Larson, 1967). Laird (1956) reported that*Zoothamnium* sp. found from *Culex annulirostris*, while *Vorticella campanula* was found from *C. annulirostris*, *C. pullus*, and *C. pipiens*. Further this author reports that use of these organisms to control of mosquitoes in brackish or fresh fish water is a disadvantage, but they can be used in neglected water ponds to control larvae. Even though they are having mosquito controlling ability to some extent, introduction of them into aquatic habitats is dangerous because mainly they affect for fish and shrimps to causing external fouling. But density of these organisms can be increased in order to control mosquito breeding habitats such as abandoned water bodies,water accumulated tree holes, bamboo trees which are not having direct connections with aquatic.

Other micro fauna such as *Coleps hirtus*, *Chaetonotus*, *Ichthydium*, *Diurella stylata*, *Lecane* sp. and *Rotaria* sp. Found during the study period are not having considerable effect on mosquito larvae. They are having free living association in aquatic habitats and most of them rely on filter feeding. *Coleps hirtus* feed on dead or living algae, flagellates, rotifers, or other protozoa (Rudberg and Sand, 2000). Gastrotrichs are mainly feed on various bacteria, micro algae and protozoans. As a group rotifers are generalized suspension feeders (Herdendorf *et al.*, 2000). These organisms are commonly found within aquatic habitats where mosquitoes are present or not and they are less important as predators of mosquito larvae.

Cluster analysis done for both micro fauna and mosquito species reveals that bamboo trees and tree holes have been clustered together according to the mosquito species. In both type of habitats most common mosquito species was *Tripteroides* sp. which is a tree hole breeder. Tree holes, bamboo trees and abundant paddy fields were clustered together depending on the micro fauna species and most abundant micro fauna species that cause these three types of habitats together is the less abundance of *Zoothamium* sp. In mosquito species clustering Ma oya bed pools, ponds and wells were clustered together separated from paddy fields according to the availability of *Aedes albopictus* mosquito species. But in these three types of habitats and paddy fields, abandoned paddy fields and marshy lands most occurred micro fauna species was *Zoothamnium*. Also in paddy fields, marshy lands and abandoned paddy fields highly abundant mosquito species was *Culex tritaeniorhynchus*. Also in paddy fields, marshy lands and abandoned paddy fields, other abundant mosquito was *Culex* species. Also in these habitats approximately maximum six species of micro fauna were recorded.

According to the values of Pearson correlation, there was no any of the significant positive or negative relationship between micro fauna and mosquito larvae. But only *Zoothamnium* had a weak negative relationship with mosquito larvae. Pearson correlation value for the relationship between mosquito larvae and *Zoothamnium* was -0.451 indicating that there is a negative relationship but, it is not significant. However, the P value for this relationship was 0.00 which indicate that the correlation is significantly different from 0.05. Even though, the P value is significant from 0.05, Pearson correlation value is not strong enough to express that there is a negative relationship between mosquito larvae and micro fauna.

More than 50% of mosquito species reported in this study was *Culex tritaeniorhynchus* that is responsible for Japanese encephalitis. *Culex quinquefasciatus* was found from the habitats where water is retained for longer period of time. According to Singha *et al.* (2011) *Culex quenquefasciates* preferred a wide range of habitats mostly characterized by colored foul water with high nutrient values and low dissolved oxygen such as pumping irrigation wells, canals, rain pools, rice paddy fields, agricultural trenches, vegetable farms etc. (Matthys*et al.*, 2006; Castro *et al.*, 2010). *Aedes albopictus* which is one main species responsible for dengue,reported from pond and abandoned wells. *Anopheles vergus* is the only one species found from genus *Anophele.* This species is considered as non-vector mosquito (Balasubramaniun and Nikhil, 2013).

Tripteroides mosquitoes were recorded from water accumulated bamboo tree and tree holes. No species of *Tripteroides* are known to be involved in the transmission of the pathogenic agents and only a few species in certain locations appear to be pests of humans. Genus *Tripteroides* include domestic and forest species and larvae inhabit in small collections of tree holes, bamboo, coconut shells, husks, pitcher plants, etc and many other artificial habitats. Larvae usually lie upside down on the bottom of the cavity and they feed on arthropods or their remains. The adults appear to be active during the daytime. A few species attack and bite humans, but nothing is known about the feeding habits of most species. Among the Oriental species only *Tripteroide saranoides* is known to bite humans. Some species have become pests in northwestern Australia where they invade houses. (http://www. wrbu. org/ generapages/ tripteroides.htm).

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