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# Occurrence of major and potential malaria vector immature stages in different breeding habitats and associated biotic and abiotic characters in the district of Trincomalee Sri Lanka

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## ABSTRACT

*Background & objectives:* Understanding the effect of biotic and abiotic factors on the biology and ecology of immature stages of anopheline larvae is very important in controlling malaria vector mosquitoes. Therefore, this study was focused on the monitoring of ecological factors affecting the distribution, dynamics, and density of malaria vector mosquitoes in the District of Trincomalee, Sri Lanka.

*Methods:* Permanent and temporary breeding habitats were identified and selected from five possible malaria sensitive sites in the district of Trincomalee. *Anopheles* larvae and macro-invertebrates were collected using standard methods for 16 months (from October 2013 to January 2015) and they were identified microscopically. Eight physico-chemical parameters of the breeding habitats were measured.

*Results:* Overall, a total of 4815 anopheline larvae belonging to 13 species were collected from 3,12,764 dips from 18 permanent and temporary breeding habitats. The abundance of anopheline larvae showed a significant positive correlation ($p$ <0.05) with physico-chemical parameters in breeding habitats, such as temperature, dissolved oxygen, and turbidity. A total of 35 macro-invertebrate taxa were collected from the anopheline mosquito breeding habitats.

*Interpretation & conclusion:* This study represents the first systematic update of water quality parameters, macro-invertebrate communities associated with *Anopheles* mosquito oviposition sites in the District of Trincomalee, Sri Lanka. Rainfall intensity and wind speed are critical meteorological factors for the distribution and abundance of malaria vectors. Knowledge generated on the ecology of *Anopheles* mosquitoes will help to eliminate malaria vectors in the country.

**Key words** Macro-invertebrates; malaria vectors; physico-chemical parameters; Sri Lanka

## INTRODUCTION

Almost half of the world's population is living in malaria-endemic countries, populated by some 3.2 billion people[1]. It is characterized by high sickness and relatively low death rates. In Sri Lanka, the global malaria eradication programme (GMEP) was commenced during the 1960s, which failed, causing the return of malaria to cost the nation early for several decades thereafter. The country has reduced annual parasite incidence (API) of malaria[2] to <1 through a valiant effort from 1991 to 2011. There has been no endemic case of malaria reported in the country since October 2012. Although there has been no indigenous malaria case in the country, travellers of Sri Lankan origin who had contracted malaria overseas along with Pakistani and Indian origin have contributed nearly 60% to the imported malaria cases[3] in 2013.

Dry zones of the country are considered as endemic malarious areas. Even though the number of annual malaria incidences are low in those areas, densities of anopheline mosquitoes including major vector, *Anopheles culicifacies* (Diptera: Culicidae) as well as the subsidiary vectors, *An. subpictus*, *An. annularis*, *An. tessellatus*, and *An. nigerrimus* are high[4–6]. Therefore, vector control strategies should be strengthened to generate information that is needed for evidence-based vector controlling to prevent re-emergence of malaria in Sri Lanka.

The use of insecticides for malaria vector control is increasingly failing in some regions because of the development of insecticide resistance in the mosquito vectors[7–8]. For these reasons, new strategies of malaria control are required to target the vectors. As vector control has been recognized as the most effective way of malaria control through the interruption of parasite transmission, numerous studies focus on the aspects of the vector for achieving this goal. Although the abundance, dynamics, and fitness of the adult mosquitoes are determined by the

immature stages, most studies focus on the adult stage of malaria vectors, and surprisingly comparatively fewer studies are directed towards understanding the biology and ecology of the aquatic immature stages.

As the mosquito immature stages are confined to their aquatic habitats, they are vulnerably exposed to various biotic and abiotic factors in their short life-span. These factors have effect on the life history traits of the immatures, such as their growth, development and survival, which affect habitat productivity and hence, the transmission of malaria. Consequently, the current study was focused on understanding the effects of abiotic factors including physico-chemical parameters of the breeding habitats, and climate and biotic factors on larval density and survival of immature stages of malaria vectors in the district of Trincomalee Sri Lanka which is considered as an endemic malarious area in the dry zone of the country.

## MATERIAL & METHODS

### *Study areas*

The study was carried out in the District of Trincomalee (08° 35′ N, 81° 05′E) which is located in the eastern province of Sri Lanka (Fig. 1), covering an area of 2727 km$^2$ with inland water coverage of 198 km$^2$. The district consists mainly of an undulating plain and the long coastal belt (130 miles) with a peculiar topography forming 10 bays and 15 lagoons[9]. The average temperature ranges from 24.1–33°C with 1649 mm rainfall, annually.

Five possible malaria sensitive areas (sentinel sites), namely Gomarankadawala, Ichchalampaththu, Mullipothana, Padavisiripura, and Thoppur in the District of Trincomalee were selected for the study. A sentinel site was defined as an area, where malaria transmission risk is present over a period of time or/and, where the increased potential for vector breeding is well-established. A high-risk area may be a previously malaria-risk area or an epidemic-prone area. Factors such as environmental conditions, availability of breeding sites, and an established agricultural community were also considered in selecting the study areas in addition to the criteria described above. Four localities situated in each malaria sentinel site were selected as study sites. A total of 20 localities in five sentinel sites were selected in the district.

### *Larval surveillance*

Larval surveillance in each study site was conducted from October 2013 to January 2015. Previously known high-potential *Anopheles* natural larval habitats were

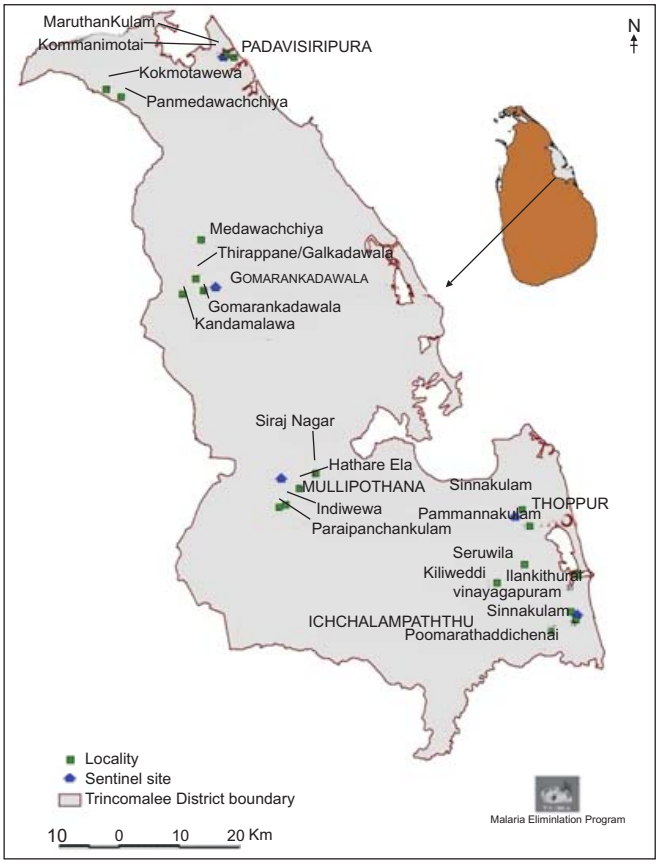


*Fig 1:* Map showing the entomological surveillance sites in the District of Trincomalee, Sri Lanka.

sampled for preliminary analysis. The spatial locations of each breeding site were marked by devising a hand-held GPS receiver (eTrex Gwko$^{TM}$ 201,301). The breeding sites were categorized into major types based on the nature of the habitat.

The number of sampling sites was determined depending upon the size of the breeding places, and a triplicate of samplings per site was performed. From each breeding site, approximately 100 dips were collected from five spots (20 dips per spot) depending on the size of the breeding site using a dipper (250 ml). The number of dips was determined by considering the size of the breeding site (*i.e.* approximately 4 dips for 1 m$^2$ size breeding site). The number of dips and anopheline positive dips were recorded. Among the positive dips, the number of different larval stages was recorded (I–II and III–IV). Two larval surveillances were made in a single locality per week usually from 0800 to 1100 hrs.

### *Physico-chemical parameters of water in anopheline breeding places*

Physico-chemical parameters in each breeding habitat such as temperature (digital meter EUTECH Dowp

300/02K), dissolved oxygen (DO) (digital meter EUTECH Dowp 300/02K), pH (portable meter, Hach SenSION TM), conductivity, total dissolved solids (TDS), salinity (Hach SenSION TM multi-probe meter) and turbidity (Hach 2100Q) were measured *in situ* using digital meters. Water samples were collected close to the bottom of each breeding habitat in sterile bottles to analyze total hardness in the laboratory.

*Collection of the aquatic macro-invertebrate community from anopheline mosquito breeding habitats*

As biological parameters, aquatic macro-invertebrates were sampled using different methods. A rectangular frame net (30 × 20 cm) with a mesh size of 250 µm was used to sample swimming macro-invertebrates. In total, three figure 8 movements were performed to cover uniform sampling in each breeding habitat. Contents collected in the net were emptied on a white sorting tray to enhance visibility and counting. The sampled organisms were kept in vials containing 75% ethanol for later identification and enumeration.

Aquatic benthic community (*i.e.* bottom, mid-water, surface, and near debris) in each sampling instance, three sediment samples (6 cm diameter and 15 cm depth each) were collected using a 6 cm diameter plastic soil corer. These samples were wet sieved *in situ* through a 0.5 mm mesh and the fauna and the residues retained were preserved in a solution of Rose Bengal containing 5% formaldehyde.

*Analysis of biological samples*

Collected III and IV instar larvae were identified under a light microscope (Olympus Optical Co. Ltd., Tokyo) with 10x objective. The I and II instar larvae were reared until III and IV instar larvae which were then identified using standard keys[10]. Collected pupa from each breeding habitat was reared until the adults emerged. Emerged adults were identified to the species level using an achromatic magnifying lens (10×) following the taxonomic keys prepared by Carter[11] and Amarasinghe[12]. Later, in the laboratory, the macro-invertebrate fauna in the samples was identified to the nearest possible taxonomic category under a dissecting microscope (Labomed, Labo America, Inc. USA 7GA9) following the standard identification keys[13–14].

*Analysis of abiotic samples*

The total hardness of the water samples was measured within 1–3 days after the sample collection using ethylenediaminetetraacetic acid (EDTA) titrimetric method[15].

*Collection of meteorological data*

Meteorological data for the period of September 2013 to March 2015 were obtained from the Department of Meteorology, Colombo 7, Sri Lanka. The total annual rainfall, air temperature, wind speed, and relative humidity (RH) were taken for the District of Trincomalee on monthly basis.

*Statistical analysis*

The species richness and the Shannon Wiener (SW) species diversity index (H′) for the sentinel sites and breeding habitats were calculated separately using MINITAB ver. 14 for windows. The variation of each of the physico-chemical parameters between the breeding habitats was analyzed using a two-way analysis of variance (ANOVA). When a significant result was noted for each physico-chemical factor, one-way ANOVA was carried out with respect to that factor, and Kruskal-Wallis comparison tests were carried between breeding habitats. Pearson's correlation coefficient (r) analysis was used to investigate the relationship among physico-chemical factors with larval mosquito densities, the relationship among macro-invertebrates with larval densities, the relationship among meteorological factors with larval mosquito densities, and adult mosquito densities.

*Ethical statement*

Ethical clearance to conduct the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka.

## RESULTS

*Habitat diversity of the immatures*

Breeding of anopheline mosquitoes was recorded from 18 breeding habitat categories which are permanent (agriculture well, built well, canal with vegetation, cemented tank, earth well, irrigation field canal, irrigation main canal, marshy land, paddy field, pond, river margin, and tank margin) and temporary (animal footprints, burrow pits, quarry pits, rock pools, tyre marks and wastewater collections) in nature. The majority of individuals were noted from tank margins (25.87%) and lowest (0.457%) from tyre marks (Table 1).

A total of 4815 anopheline larvae belonging to 13 anopheline species were collected from 3,12,764 larval dips (Table 2). *Anopheles subpictus* (n = 1631; 33.87%) was identified as the main breeder in almost all the habitats followed by *An. peditaeniatus* (n = 1032; 21.43%), *An. nigerrimus* (n = 787; 16.34%) and *An. barbirostris* (n = 465; 9.65%). The highest larval density was identi-

Table 1. Abundance of anopheline mosquitoes in different breeding habitats in the District of Trincomalee

| Breeding habitats | *An. culicifacies* | *An. subpictus* | *An. pedietaeniatus* | *An. palidus* | *An. vagus* | *An. varuna* | *An. barbirostris* | *An. annularis* | *An. aconitus* | *An. jamesii* | *An. nigerrimus* | *An. barbumbrosus* | *An. pseudojamesi* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Permanent breeding habitats* | | | | | | | | | | | | | |
| Agricultural well | – | 1.163 | – | – | – | – | – | 0.706 | – | – | – | – | – |
| Built well | 0.145 | 0.976 | 0.498 | 0.187 | 0.145 | 0.062 | 0.395 | 1.225 | 0.125 | 0.976 | 0.644 | 0.166 | – |
| Canal with vegetation | 0.395 | 0.561 | 0.789 | – | 0.104 | 0.125 | 0.540 | – | – | – | – | – | – |
| Earth well | – | 1.267 | 0.685 | – | 0.062 | – | – | 0.976 | – | – | 0.810 | – | – |
| Irrigation field canal | – | 0.789 | 0.810 | 0.145 | 0.270 | 0.104 | 0.582 | – | 0.021 | – | 0.789 | – | – |
| Irrigation main canal | 0.125 | 0.602 | 0.332 | 0.312 | 0.249 | 0.125 | 0.478 | – | 0.083 | – | 1.080 | – | – |
| Marshy land | 0.104 | 1.433 | 1.205 | 0.125 | 0.042 | 0.145 | 0.270 | – | 0.062 | 0.291 | 1.059 | – | – |
| Paddy field | – | 4.818 | 1.225 | 0.353 | 0.436 | 0.228 | 0.478 | – | – | 0.353 | 1.329 | 0.332 | 0.540 |
| Pond | 0.187 | 4.943 | 5.275 | 0.374 | 0.104 | 0.228 | 1.994 | – | – | – | 3.053 | 0.644 | – |
| River margin | – | 0.706 | 0.395 | 0.062 | 0.125 | – | 0.623 | – | – | – | 1.163 | – | – |
| Tank margin | 0.291 | 8.370 | 7.103 | 0.582 | 0.498 | 0.374 | 2.264 | – | 0.291 | 0.602 | 4.216 | 0.935 | 0.353 |
| *Temporary breeding habitats* | | | | | | | | | | | | | |
| Animal footprint | – | 0.187 | 0.478 | – | – | – | 0.166 | – | – | – | 0.166 | 0.249 | – |
| Burrow pit | 0.166 | 2.015 | 1.018 | 0.249 | 0.249 | 0.145 | 0.644 | – | 0.042 | – | 0.768 | 0.436 | – |
| Quarry pit | – | 1.973 | 0.187 | 0.125 | 0.083 | 0.083 | 0.228 | – | 0.042 | – | 0.353 | – | – |
| Rainwater pool | – | 1.142 | 0.187 | 0.062 | – | 0.083 | 0.312 | – | – | – | – | – | – |
| Rockpool | – | 0.249 | 0.332 | – | 0.021 | – | 0.312 | 0.478 | – | – | – | – | – |
| Tyre mark | – | 0.457 | – | 0.021 | – | – | – | – | – | – | – | – | – |
| Wastewater collection | 0.125 | 2.222 | 0.914 | – | 0.104 | – | 0.374 | – | – | – | 0.914 | – | – |

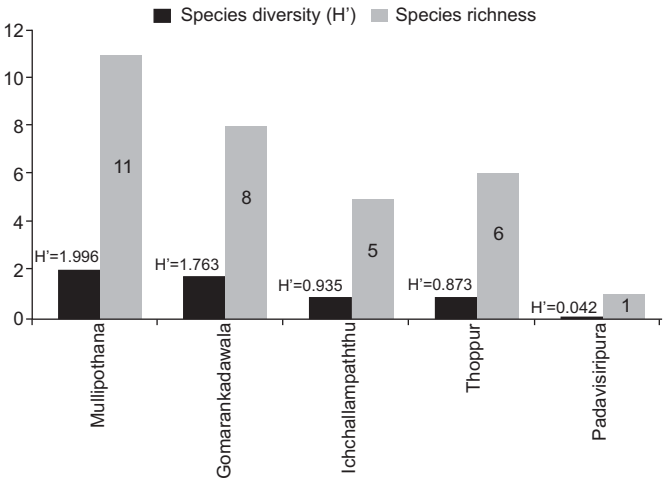


*Fig 2:* Variation of SW species diversity index (H′) and species richness of anopheline mosquito larvae from five sentinel sites in the District of Trincomalee. Numbers within columns indicate the number of species (species richness).

fied from Gomarankadawala (39.08%), and the lowest from the Padavisiripura (1.84%). The SW species diversity index and species richness were calculated for five examined sentinel sites. The highest and lowest species diversity and species richness indices were recorded at Mullipothana (H′ = 1.996) and Padavisiripura (Fig. 2) sentinel sites in the District of Trincomalee, respectively.

*Water quality parameters*

The study investigated what water quality variables impact anopheline mosquito larval diversity and abundance in the study area and whether they differ between breeding habitats. To investigate impacts on anopheline larval diversity and abundance, physico-chemical variables of potential influence were included in the analyses. A total of 4200 samples (3580 in the permanent and 620 in the temporary breeding habitats of anopheline presence) were analyzed for eight physico-chemical parameters (Table 3).

The highest water temperature was recorded for wastewater collections, and the agriculture wells showed the lowest temperature. There was a significant positive relationship between temperature and anopheline larval density ($p$ <0.05, $R^2$ = 68.5%). The highest and lowest mean DO values were detected from marshy lands and wastewater collections, respectively. However, the abundance of anopheline larvae was positively correlated ($p$ <0.05, $R^2$ = 75.8%) with DO in all the breeding habitats. The highest pH levels were recorded from the tank margins, and earth wells showed the least pH values. Anopheline larval density did not correlate significantly

Table 2. Collected anopheline larvae from larval surveillance in the District of Trincomalee

| *Anopheles* species | Number of mosquitoes collected ( Density of mosquito collections per 100 dips) | | | | | |
|---|---|---|---|---|---|---|
| | Padavisiripura[a] | Gomarankadawala[b] | Thoppur[c] | Mullipothana[d] | Ichchlampaththu[e] | Total[f] |
| *An. culicifacies* | – | – | 13 (0.013) | 47 (0.036) | 14 (0.004) | 74 (0.018) |
| *An. subpictus* | 89 (0.251) | – | 1 324 (1.043) | 76 (0.171) | 142 (0.045) | 1 631 (0.521) |
| *An. varuna* | – | – | 28 (0.029) | 40 (0.090) | 14 (0.004) | 82 (0.026) |
| *An. annularis* | – | 154 (0.306) | – | 9 (0.020) | – | 163 (0.052) |
| *An. nigerrimus* | – | 665 (1.321) | 34 (0.036) | 88 (0.198) | – | 787 (0.251) |
| *An. vagus* | – | – | – | 4 (0.009) | 116 (0.037) | 120 (0.038) |
| *An. pallidus* | – | 123 (0.244) | – | 2 (0.004) | – | 125 (0.039) |
| *An. peditaeniatus* | – | 574 (1.140) | 196 (0.207) | 93 (0.210) | 169 (0.054) | 1 032 (0.329) |
| *An. jamesii* | – | 39 (0.077) | – | 89 (0.201) | – | 128 (0.040) |
| *An. barbirostris* | – | 162 (0.321) | 74 (0.077) | 229 (0.517) | – | 465 (0.148) |
| *An. barbumbrosus* | – | 133 (0.264) | – | – | – | 133 (0.042) |
| *An. pseudojamesi* | | – | – | 43 (0.097) | – | 43 (0.0137) |
| *An. aconitus* | | 32 (0.063) | – | – | – | 32 (0.010) |
| Total | 89 (0.251) | 1 882 (3.739) | 1 669 (1.768) | 720 (1.616) | 455 (0.496) | 4815 (1.537) |

Total number of dips per site–35,368[a]; 50,326[b]; 94,369[c]; 44,231[d]; 88,452[e]; and 3,12,764[f].

Table 3. Summary of the analysis of physicochemical parameters in breeding habitats in all the study sites in the District of Trincomalee

| Breeding places | Temperature (°C) | DO (mg/l) | pH (25°C) | Conductivity (µs/cm) | Salinity (mg/l) | TDS (mg/l) | Turbidity (NTU) | Hardness (mg/l) |
|---|---|---|---|---|---|---|---|---|
| *Permanent breeding habitats* | | | | | | | | |
| Agricultural well | 29.67 ± 0.24 (29.55–31.25) | 3.68 ± 0.66 (2.37–4.67) | 7.44 ± 0.05 (7.05–8.26) | 2038 ± 64.55 (689–6421) | 1003 ± 42.38 (269–3134) | 1698 ± 55.12 (336–4234) | 12.10 ± 0.45 (1.54–23.37) | 163.80 ± 0.68 (105–298) |
| Built well | 30.38 ± 0.06 (28.34–32.34) | 4.85 ± 0.45 (2.87–6.87) | 7.14 ± 0.05 (6.59–8.06) | 2974 ± 36.57 (469–6584) | 1123 ± 33.68 (267–3678) | 2658 ± 48.60 (322–4421) | 8.24 ± 0.26 (0.20–18.37) | 139.68 ± 1.22 (98.34–287) |
| Canal with vegetation | 30.54 ± 0.37 (27.39–33.62) | 5.24 ± 0.67 (3.43–7.39) | 7.35 ± 0.03 (6.43–7.96) | 349 ± 23.67 (112–537) | 136 ± 29.34 (56.98–234) | 234 ± 27.35 (79.35–436) | 120.34 ± 7.58 (2.68–486.31) | 45.68 ± 2.54 (21.34–97.64) |
| Earth well | 30.38 ± 0.25 (28.03–31.38) | 4.55 ± 0.23 (4.67–6.48) | 7.09 ± 0.05 (6.98–8.06) | 1874 ± 20.37 (493–4983) | 984 ± 30.54 (208–2975) | 1543 ± 29.37 (305–3767) | 8.34 ± 0.54 (0.24–19.54) | 142.11 ± 0.57 (90.25–204) |
| Irrigation field canal | 31.04 ± 0.24 (29.34–33.16) | 4.25 ± 0.28 (3.68–5.29) | 7.26 ± 0.08 (6.38–8.67) | 167.21 ± 12.68 (89.64–342) | 68.87 ± 15.34 (42.34–135) | 124 ± 14.37 (74.35–234) | 89.38 ± 6.79 (7.69–241.24) | 42.69 ± 0.60 (20.12–87.34) |
| Irrigation main canal | 31.35 ± 0.25 (29.34–33.21) | 4.34 ± 0.06 (3.39–5.39) | 7.53 ± 0.03 (6.41–7.63) | 296.23 ± 14.12 (129.33–684) | 124 ± 25.34 (64.35–421) | 204 ± 23.54 (96.34–548) | 77.54 ± 4.66 (5.68–287.24) | 55.68 ± 1.35 (18.34–87.36 |
| Marshy land | 31.37 ± 0.34 (29.36–32.34) | 6.34 ± 0.72 (3.25–7.68) | 7.21 ± 0.02 (7.01–7.97) | 142.3 ± 12.37 (96.34–341) | 97.37 ± 18.34 (36.78–145) | 123 ± 23.54 (72.34–213) | 45.68 ± 1.23 (7.58–159.34) | 33.64 ± 0.45 (16.67–69.34) |
| Paddy field | 31.35 ± 0.06 (29.84–34.35) | 4.64 ± 0.08 (3.08–6.67) | 7.58 ± 0.08 (6.68–8.69) | 263.21 ± 14.68 (98.64–315 ) | 116.63 ± 16.34 (61.34–165) | 206 ± 14.32 (79.34–267) | 368.54 ± 24.15 (19.54–684) | 42.31 ± 0.55 (19.64–80.34) |
| Pond | 30.34 ± 0.68 (29.35 –33.98) | 5.31 ± 0.16 (4.32–6.84) | 7.06 ± 0.51 (6.38–8.49) | 458 ± 8.15 (134.21–638) | 215 ± 10.34 (63.58–412) | 314 ± 12.87 98.37–348) | 48.37 ± 4.22 (4.84–284.22) | 74.35 ± 4.31 (24.38–123) |
| River margin | 31.24 ± 0.08 (30.34–33.64) | 3.56 ± 0.16 (3.02–4.73) | 7.35 ± 0.01 (6.59–7.89) | 1345 ± 14.39 (698–2698) | 542 ± 10.35 (298–1342) | 896 ± 9.56 (466–1985) | 34.20 ± 6.56 (15.34–59.40) | 65.37 ± 1.01 (27.66–107) |
| Tank margin | 31.76 ± 0.14 (28.53–33.75) | 5.92 ± 0.07 (3.71–7.84) | 7.93 ± 0.06 (6.34–8.64) | 739 ± 38.67 (159.37 –1826) | 467 ± 33.37 (43.56–967) | 603 ± 43.67 (71.26–1634) | 78.21 ± 10.87 (5.98 –245.37) | 80.34 ±5.34 (29.34 –154) |
| *Temporary breeding habitats* | | | | | | | | |
| Animal footprint | 31.35 ± 0.31 (29.34–34.23) | 3.11 ± 0.01 (0.89–3.98) | 7.55 ± 0.03 (7.31–7.92) | 274 ± 2.38 (123.24–458) | 141.21 ± 2.68 (89.64–197.25) | 198.34 ± 2.38 (99.64–318) | 468.35 ± 36.46 (45.32–964) | 34.01 ± 0.04 (15.67–62.80) |
| Burrow pit | 31.38 ± 0.09 (29.98–33.05) | 4.57 ± 0.53 (3.77–6.84) | 7.22 ± 0.04 (6.68–8.34) | 366 ± 33.21 (123.35–654) | 122 ± 42.33 (92.35–388) | 266 ± 25.91 (112.34–468) | 234.87 ± 15.55 (7.58–450) | 56.68 ± 0.85 (18.69–97.64) |
| Quarry pit | 31.38 ± 0.15 (30.41–33.19) | 4.37 ± 0.42 (2.64–6.38) | 7.62 ± 0.03 (7.09–7.88) | 254 ± 11.57 (148.54–478) | 148.67 ± 9.89 (96.24–266) | 193.55 ± 10.68 (116.57–328) | 19.27 ± 2.39 (7.12–39.24) | 52.34 ± 0.40 (23.44–92.30) |
| Rain water pool | 31.10 ± 0.03 (30.31–33.22) | 4.58 ± 0.05 (2.35–5.48) | 7.22 ± 0.03 (7.08–7.92) | 132.24 ± 1.67 (98.34–323) | 86.31 ± 2.12 (45.31–168.32) | 98.31 ± 3.44 (87.21–248) | 168.37 ± 16.33 (8.64–296) | 54.38 ± 0.08 (24.64–80.34) |
| Rockpool | 31.38 ± 0.35 (29.38–33.68) | 4.88 ± 0.25 (3.01–5.37) | 7.59 ± 0.08 (7.08 –7.97) | 263 ± 3.37 (196.32–435) | 123.25 ± 5.67 (113.32 –233) | 196.32 ± 5.34 (186.24–377) | 27.34 ± 0.81 (5.88–98.34) | 45.36 ± 0.06 (16.37–87.40) |
| Tyre mark | 31.37 ± 0.35 (29.38–34.34) | 1.68 ± 0.01 (0.56–2.46) | 7.41 ± 0.02 (7.03 –7.86) | 238 ± 12.35 (158.42–545) | 116.57 ± 10.37 (53.52–225) | 196.34 ± 7.25 (185.37–332) | 59.67 ± 7.55 (6.67–141.50) | 35.67 ± 0.07 (14.67–58.22) |
| Wastewater collection | 32.34 ± 0.18 (29.64–34.36) | 2.15 ± 0.03 (0.49–4.02) | 7.52 ± 0.04 (7.02–8.02) | 1325 ± 69.54 (524–3567) | 689 ± 54.15 (296–1988) | 986 ± 45.71 (325–2823) | 266.48 ± 23.59 (7.85–598.34) | 87.34 ± 0.74 (41.54–124.5) |

Values are mean ± SE; Range in parentheses calculated using display descriptive statistic by Kruskall-Wallis ANOVA followed by Tukey's nonparametric multiple comparison.

($p$ >0.05) with the pH in the breeding habitats during the study period. The highest and least values of conductivity, salinity, TDS, and hardness were recorded in built wells, and tyre marks, respectively. No correlations were noted from the anopheline larval density with the values of conductivity, salinity, TDS, and hardness. The highest mean value of turbidity was recorded from wastewater collections and the least from the built wells. The abundance of anopheline larvae was positively correlated ($p$ <0.05, $R^2$ = 94.1%) with the values of turbidity.

*Macro-invertebrates*

During the study period, 35 different macro-invertebrate taxa were recorded under 15 orders representing six classes among the 2512 individuals belonging to three Phyla (41% Arthropoda, 30.77% Annelida, and 50% Mollusca) from the same breeding habitats where anopheline larvae were present. The list of organisms noted from the present study is given in Table 4. The species diversity and richness were significantly higher in tank margins and burrow pits while wastewater collec-

Table 4. Macro-invertebrate composition and abundance in the District of Trincomalee during the study period

| Organism | Canal with vegetation | Irrigation main canal | Irrigation field | Marshy land | Paddy field | Pond | River margin | Tank margin | Burrow pit | Quarry pit | Waste-water collection |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Phylum Annelida*** | | | | | | | | | | | |
| *Class oligochaeta* | | | | | | | | | | | |
| Order Plesiopora | | | | | | | | | | | |
| *Aeolosoma* sp. | – | 20 | 6 | 3 | 12 | – | – | 15 | 15 | 13 | – |
| *Aulophorus* sp. | 12 | 14 | 1 | – | – | – | – | 8 | 3 | – | – |
| Order Haplotaxida | | | | | | | | | | | |
| *Chaetogaste*r sp. | 2 | 14 | 3 | 7 | 6 | 12 | – | 16 | 14 | 2 | – |
| *Dero* sp. | – | 16 | 17 | 6 | 10 | 4 | 3 | 10 | 14 | 15 | – |
| OrderTubificida | | | | | | | | | | | |
| *Tubifex* sp. | 23 | 20 | 8 | 8 | 12 | 22 | 28 | 89 | 40 | 12 | 198 |
| *Class Hirudinea* | | | | | | | | | | | |
| Order Arhynchobdellae | | | | | | | | | | | |
| *Hirud*o sp. | 1 | – | 2 | – | – | 2 | 2 | 13 | – | – | – |
| ***Phylum Mollusca*** | | | | | | | | | | | |
| *Class Gastropoda* | | | | | | | | | | | |
| Order Mesogastopoda | | | | | | | | | | | |
| *Feunus* sp. | 3 | 4 | 2 | – | 4 | – | 6 | 8 | 2 | – | – |
| *Melanoides* sp. | 12 | 6 | 1 | 1 | 1 | 3 | – | 18 | 7 | 7 | – |
| Order Basommatophora | | | | | | | | | | | |
| *Paludomus* sp. | 26 | – | – | 1 | 2 | 15 | 6 | 14 | 11 | 2 | – |
| *Bithynia* sp. | 12 | 14 | – | 12 | 49 | 19 | 3 | 61 | 56 | 5 | – |
| *Bellamya ceylonica* | 14 | 10 | 6 | 3 | 1 | – | – | 78 | 41 | 9 | – |
| *Pila* sp. | 9 | – | 3 | – | 1 | 3 | 5 | 9 | 7 | 2 | 7 |
| *Lymnaea* sp. | – | 4 | – | – | – | – | – | – | – | – | – |
| *Gyralus* sp. | | 2 | | | 3 | | | 3 | 2 | 1 | |
| *Indoplanorbis* sp. | 14 | 6 | – | 2 | 4 | 3 | 7 | 9 | 3 | 5 | – |
| *Class Bivalvia* | | | | | | | | | | | |
| Order Unionoida | | | | | | | | | | | |
| *Lamellidens* sp. | – | – | – | – | – | – | – | 21 | 1 | – | – |
| *Phylum Arthropoda* | | | | | | | | | | | |
| *Class Malacostraca* | | | | | | | | | | | |
| Order Isopoda | | | | | | | | | | | |
| *Alitropus* sp. | – | 4 | – | – | – | – | – | 3 | 1 | – | – |
| Order Decapoda | | | | | | | | | | | |
| *Caridina* sp. | 74 | 10 | – | – | 2 | 28 | 17 | 125 | 8 | – | – |
| *Paratelphusa* sp. | 12 | 6 | 2 | 2 | 9 | 4 | 8 | 12 | 9 | 3 | – |
| *Class Insecta* | | | | | | | | | | | |
| Order Hemiptera | | | | | | | | | | | |
| *Laccotrephes* sp. | – | 2 | 2 | – | 3 | 2 | – | 15 | 5 | 2 | – |
| *Diaphorocoris* sp. | – | 2 | 1 | 1 | – | – | – | 3 | 3 | 2 | – |
| *Tiphotrephes* sp. | | | | | | | | | | | |
| *Rhagovelia* sp. | – | – | 1 | – | 6 | – | – | 3 | 2 | – | – |
| Order Coleoptera | | | | | | | | | | | |
| *Cybister* sp. | | – | – | – | – | – | – | 3 | 3 | – | – |
| *Laccophilus* sp. | – | – | – | – | 2 | 2 | – | 10 | 24 | – | – |
| *Canthydrus* sp. | – | – | – | – | – | 1 | – | – | – | – | – |

*contd...*

Table 4. (*continued*)

| Organism | Canal with vegetation | Irrigation main canal | Irrigation field | Marshy land | Paddy field | Pond | River margin | Tank margin | Burrow pit | Quarry pit | Waste-water collection |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Order Odonata | | | | | | | | | | | |
| Dragonfly larva | 19 | 10 | 8 | 5 | 30 | 14 | – | 30 | 14 | 6 | – |
| Damselfly larva | 14 | 4 | 3 | 3 | 2 | 2 | – | 5 | 2 | 3 | – |
| Order Plecoptera | | | | | | | | | | | |
| Mayfly larva | 19 | 4 | 3 | 4 | 5 | – | – | 6 | 8 | 6 | – |
| Order Trichoptera | | | | | | | | | | | |
| Caddisfly larva | – | 4 | 2 | 1 | 14 | 6 | – | 9 | 15 | – | – |
| Order Diptera | | | | | | | | | | | |
| Horsefly larva | – | 2 | – | – | – | 2 | – | 1 | 1 | – | – |
| Tabanid larva | 4 | 4 | | 1 | | 2 | | 4 | 8 | 1 | |
| ***Halipid larva*** | | | | | | | | | | | |
| Blackfly larva | – | 2 | – | – | – | 2 | – | 1 | – | 1 | – |
| Chironomid larva | 19 | 6 | 7 | 9 | 7 | 23 | 9 | 17 | 72 | 7 | 117 |
| Total number of Taxa | 19 | 25 | 19 | 17 | 22 | 21 | 11 | 31 | 28 | 19 | 3 |
| Total number of individuals | 289 | 19 | 78 | 69 | 18 | 17 | 94 | 86 | 39 | 104 | 137 |

Table 5. Pearson's correlation coefficient (R) of anopheline mosquitoes with climatic variables in the Trincomalee District

| Climatic variables | Anopheline (Larvae) | | 95% CI | |
|---|---|---|---|---|
| | R | *p*-value | Lower | Upper |
| Total rainfall (mm) | –0.490 | 0.851 | 30 | 195 |
| Total rainfall with one month time lag (mm) | 0.698* | 0.003 | 30 | 195 |
| Total rainfall with two month time lag (mm) | 0.122 | 0.664 | 108.500 | 177.400 |
| Wind speed (km/h) | 0.586$^{+}$ | 0.013 | 5.700 | 13 |
| RH minimum (%) | 0.053 | 0.840 | 63.810 | 86.810 |
| RH maximum (%) | 0.041 | 0.875 | 79.780 | 90.270 |
| Temperature minimum (°C) | 0.225 | 0.386 | 23.848 | 25.694 |
| Temperature maximum (°C) | –0.275 | 0.285 | 28.907 | 36.181 |
| Temperature mean (°C) | –0.294 | 0.252 | 25.652 | 30.217 |

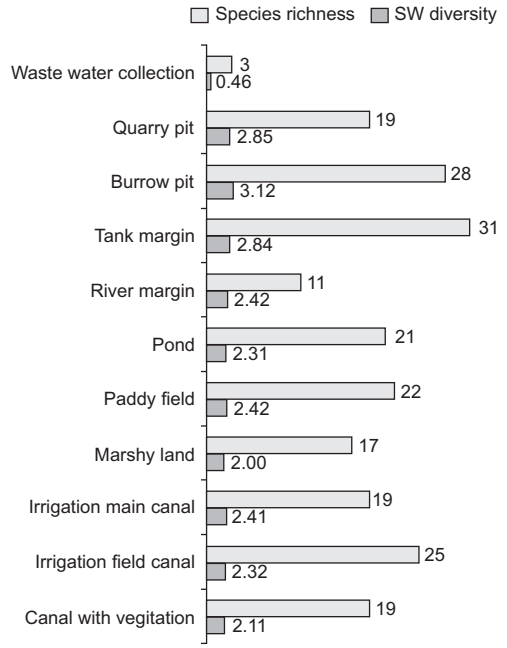


*Fig 3:* Variation of SW species diversity index and species richness of macro-invertebrates in different breeding habitats in the District of Trincomalee. Numbers near to columns indicate SW species diversity index (H′) and species richness.

tions had the lowest (Fig. 3). However, the presence of anopheline mosquito larvae was not significantly correlated ($p$ >0.05) with the prevalence of aquatic macro-invertebrate predators of the Orders Decapoda, Coleoptera, Hemiptera, or Odonata.

*Climatic factors affecting the mosquito abundance*

The highest rainfall was recorded from September 2014 to January 2015. The abundance of anopheline mosquito larvae was positively correlated ($p$ <0.01) with one-month lag rainfall (Table 5). There was no correlation between rainfall of the current month and the two-month lag period with anopheline larval density. There was a strong positive correlation between the abundance of larval density with the average wind speed ($p$ <0.01). Further, there was no significant association of anopheline larval density with the mean air temperature, minimum air temperature, maximum air temperature, and RH.

## DISCUSSION

The present study focused on the distribution of

anopheline larval mosquito species, their breeding habitat diversity, and biotic/abiotic factors affecting their survival in selected areas in the District of Trincomalee, Eastern part of Sri Lanka. During the study, the predominant mosquito species were *An. subpictus* followed by *An. peditaeniatus*, *An. nigerrimus*, and *An. barbirostris*. Other minor species such as *An. annularis*, *An. jamesii*, *An. pallidus*, *An. vagus*, *An. barbumbrosus*, *An. varuna*, *An. pseudojamesi*, and *An. aconitus* were also reported. *Anopheles culicifacies* was recorded only from Mullipothana, Thoppur, and Ichchallampaththu sentinel sites with low densities. Although abundance of the main vector *An. culicifacies* was low, the secondary vector *An. subpictus* was observed in high numbers in different geographic areas in the district. Larval surveillance confirmed that high densities of anopheline mosquito larvae were recorded from the district due to the presence of a variety of permanent and temporary breeding habitats throughout the year. The study has further confirmed that most of the anopheline breeding habitats have been originated from anthropogenic influence such as irrigation activities, urbanization, resettlements, and development projects and these activities alter biotic and abiotic factors in breeding habitats.

The productivity of mosquito breeding habitats is governed by the quality of water in habitats and consequently fluctuates the adult densities capable of malaria transmission[16]. The current study investigated the effect of eight physico-chemical parameters, namely water temperature, DO, pH, conductivity, salinity, TDS, turbidity, and hardness on anopheline larval densities. The results indicated that the abundance of anopheline mosquito larvae significantly correlated ($p$ <0.05) with abiotic parameters such as temperature, DO, and turbidity. The pH, salinity, conductivity, TDS, and hardness did not show any significant relationship with the densities of anopheline larvae. Earlier studies have also confirmed that some of the physico-chemical parameters such as temperature, transparency, TDS, DO, conductivity, salinity, nutrient level, and pH correlated strongly with anopheline larval abundance[17–18].

During the study period, the highest water temperature was recorded from the wastewater collections. The reason may be the presence of a very thin water layer in observed wastewater collections. Besides, these habitats were fully exposed to direct sunlight during the day times. Therefore, sunlight loads more solar radiation and heating of the water column, resulting in a higher temperature in the water layer. The minimum value of the DO level was 0.49 mg/l in wastewater collections during the study period in anopheline mosquito breeding habitats. The DO level of the water bodies which contained below 3 mg/l was categorized as third class[4, 19]. Anopheline mosquitoes, including *An. culicifacies*, can breed in such kind of wastewater bodies with low levels (<3 mg/l) of DO concentration. Recently, Gunathilaka *et al*[4, 6] also revealed that the *An. culicifacies* and other potential malaria vectors breed in drains containing wastewater, in Sri Lanka. According to the investigations of the present study, turbidity is another important physico-chemical parameter. Turbidity can alter the efficiency of light penetration through a water body and it affects algal and aquatic plant growth[20–21] which provides sufficient food for larval development. Therefore, adult mosquitoes prefer to lay eggs in turbid and dark water collections to protect their eggs and immature from predators.

Although salinity, conductivity, TDS, and hardness did not correlate significantly with the anopheline mosquito larval densities; *An. subpictus* was found breeding in some breeding habitats with high salinity. Sometimes it exceeds the salinity threshold of 200–300 mg/l. Hence, it has been suggested that *An. subpictus* species can breed in fresh, brackish, and nearly saline water bodies in Sri Lanka. Earlier studies conducted in Sri Lanka have observed that *An. subpictus* is associated with freshwater to high salinity water[22]. Major anopheline mosquito breeding habitats show a pH range from 7.02–8.12. In this study, the pH values of water in breeding habitats did not show any significant relationship. This may be due to the fact that the pH values did not indicate a significant fluctuation during the study period. Sunish and Reuben[23] have reported the relationship of 13 abiotic variables with the abundance of *Cx. vishnui* subgroup immatures in transplanted rice fields covering three different crop seasons. According to their investigation, the application of synthetic nitrogenous fertilizers to the rice fields increases the concentration of nitrogen level in the rice field water, subsequently increasing the density of mosquito larvae.

Adult female mosquitoes prefer laying their eggs in breeding habitats that have favourable abiotic as well as biotic factors for the survival of their offspring[24]. Aquatic macro-invertebrates play a major role in mosquito breeding habitats as predators and competitors for mosquito larvae. As a result, in order to understand species interactions such as competition and predation with mosquito larvae, macro-invertebrate species diversity and species richness in breeding habitats were calculated in this study. A total number of 35 taxa were associated with anopheline mosquito breeding habitats in the study district. The results indicate that high species richness and abundance were for the classes Insecta, Gastropoda, and Oligochaeta. Among them, *Tubifex* sp, *Bithynia* sp, *Paludomus* sp,

*Bellamya ceylonica*, *Caridina* sp., dragonfly larvae, and chironomid larvae were the dominating macro-invertebrates. The highest species richness and diversity were observed in tank margins, ponds, canals with vegetation, irrigation canals, and burrow pits. Those breeding habitats happen to contain lots of emerging, sub emerged, and floating vegetation cover, providing food and sheltering places to aquatic invertebrates.

The relationships between anopheline larvae and aquatic macro-invertebrates in anopheline mosquito breeding habitats have not been studied in Sri Lanka yet. Therefore, this is the first investigation of aquatic macro-invertebrate communities associated with anopheline mosquito breeding habitats. Similarly, Bond *et al*[24] have first updated the diversity and geographic distribution of the mosquitoes and aquatic insects in Mexico. They have evaluated the diversity of aquatic insects associated with immature mosquitoes along the Pacific coast of Mexico.

The present study demonstrated that the densities of anopheline mosquito larvae were not significantly correlated with the abundance of aquatic macro-invertebrate predators of the Order Decapoda, Coleoptera, Hemiptera, or Odonata. However, aquatic predators can influence the mosquito population by direct predation or affect the rate of development of immature stages. Earlier studies indicated that 98% of anopheline larvae were killed by predators such as dragonflies, backswimmers (Notonectidae), and predatory aquatic beetles (*Dineutus*–Gyrinidae)[25–26]. During the field survey of this study, a number of predatory aquatic macro-invertebrates were in low densities. This is mostly due to the altered frequency of man-made breeding habitats depending on the purpose. Therefore, predator species of aquatic macro-invertebrates cannot complete their life cycle because they have a much longer generation time than the mosquitoes.

Along with the predation, interspecific competitions of mosquito larvae with aquatic macro-invertebrates for the same resources are very important in mosquito breeding habitats. They compete for food resources, space, and other limiting factors[27]. In addition, community interaction is crucial for mosquito larvae to survive until adulthood. Rasheed and Sakthidas[28] have illustrated that helodid beetles are detritus shredders and the function of these beetles in this system are speculated as 'key stone' decomposers while it determines resource availability and community structure.

Macro-invertebrates are very good biological indicator organisms to evaluate the quality of water[29.] In the present study, it was found that the abundance of both chironomids and tubificids increased in the wastewater bodies. The findings on the tubificids and chironomids are in agreement with those made by Martins *et al*[30] and Newburn and Krane[31] where chironomids and tubificids were found to inhabit organically polluted waters and both these species are tolerant of anoxic conditions. Therefore, both tubificids and chironomids appear to be excellent biological indicator organisms in the low level of DO concentration. It is important to note that the anopheline mosquito can breed in this kind of breeding habitats associated with tubificids and chironomids.

Climate has directly and indirectly affected the distribution densities and behaviour of vectors and pathogens. Therefore, climate change is a major risk factor for vector-borne diseases. Air temperature, rainfall, and relative humidity (RH) affect malaria transmission and climate can predict the natural distribution of malaria[32]. In this study, it was found that the density of anopheline mosquitoes was affected not only by the rainfall and air temperature but also by the wind speed. Similarly, Moore[33] has shown that vector-borne diseases such as malaria are significantly associated with heavy rain, hot and dry season, and humidity in sub-Saharan Africa.

Heavy rain can flush vector breeding habitats by flooding, leading to a decrease in vector population[34]. In India, malaria cases were negatively associated with rainfall intensity with the current month[34]. Imbahale *et al*[35] and Grace[36] have shown that rainfall is positively correlated with relative malaria risk. However, in the present study, anopheline larval densities were significantly negatively correlated ($p < 0.05$) with rainfall with a one-month time lag period. Post heavy rainfall period breeding habitats may increase due to the creation of new habitats and expansion of breeding habitats. In contrast, anopheline adult mosquito density was positively correlated with rainfall having a one-month lag[5].

High wind current may run-off the adult mosquitoes as well as reduce the flight activity of the mosquitoes[37]. Therefore, the wind speed was negatively correlated ($p < 0.05$) with the density of anopheline larvae. Wind speed is a very important climatic factor for malaria transmission. This means that generally, low wind speed is suitable for malaria vector to fly[38]. The survival rate of adult mosquitoes depends on the air temperature. Similarly, air temperature directly affects the abundance and behaviour of mosquitoes[39–40]. In addition, mosquito breeding habitats can be dry during the hot climatic period. During the study period, RH did not fluctuate significantly. Therefore, RH did not show any significant correlation with anopheline larval densities. However, humidity can influence vector density and survival rate and RH is one of the most important climatic factors for vector-borne diseases[41].

The present study gains vast knowledge on anopheline mosquito larval distribution across the district, in terms of breeding habitat diversity, their physico-chemical and biological parameters. The environment can be changed due to nutrient, temperature, precipitation, salinity, or vegetation changes. There is a strong need for studies on the adaptability of different anopheline species to new environmental conditions. The majority of these studies would be best executed as manipulative field or semi-field experiments and should be focused on changing breeding habitat diversity, characteristics of species preference according to the biological and physico-chemical factors. Therefore, it will be easy for the responsible personnel and institute to perform entomological investigations and to implement vector control strategies to prevent the re-emergence of malaria in the country.

## CONCLUSION

The study findings confirm that the biotic and abiotic factors can directly or indirectly affect the development, growth, and survival rates of the aquatic stages of anopheline mosquitoes in an important but complex way. This will be a baseline study providing insights into the development of integrated larval controlling strategies suitable for the larval breeding habitats present in the respective study sites. This helps in estimating when a larval habitat is most productive, and when it should be targeted for maximum reduction in adult populations and thereby impart a positive effect on malaria vector reduction in Sri Lanka.

*Conflict of interest*

The authors declare that they have no conflicting interest.

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