Research Articles

J Vect Borne Dis 42, June 2005, pp 39–44

Some characteristics of the larval breeding sites of *Anopheles culicifacies* species B and E in Sri Lanka

S.N. Surendran[a] & R. Ramasamy[b]

[a]*Department of Zoology, Faculty of Science, University of Jaffna, Jaffna;* [b]*National Science Foundation, Maitland Place, Colombo 7, Sri Lanka*

Background & objectives : Anopheles culicifacies Giles, the major malaria vector in Sri Lanka, exists as a species complex comprising two sympatric sibling species—species B and E. Species E is reported to be the major vector of *Plasmodium vivax* and *P. falciparum* parasites in Sri Lanka, whilst species B is a poor or nonvector as in India. Knowledge of the breeding habits of the two sibling species can help in designing optimal vector control strategies. Hence, a survey was conducted in Sri Lanka to study the preferential breeding habitats of *An. culicifacies* species B and E.

Methods: Immature forms of *An. culicifacies* were collected from identified breeding sites in malarious districts. Collected larvae were typed for their sibling species status based on mitotic Y-chromosome structure. Data was analysed using Statistical Package for Social Science version 10.0.

Results: An. culicifacies immature forms were found in 23 collection sites. Among these samples 19 were found to have species E and four to have species B. All species B larvae were collected from Tonigala village in the Puttalam district. None of the 23 sites was found to have both species B and E. Species E, the major vector of malaria, appears to breed in variety of breeding sites which can be of an indication of its adaptive variation to exploit breeding sites with varying limnological characteristics.

Interpretation & conclusion: The present findings have to be taken into account when formulating more effective larval control measures. They also show the need for a detailed study of possible different preferences for larval breeding sites between species B and E.

Key words *An. culicifacies* – breeding habitats – larval control – malaria – species B & E – Sri Lanka

Anopheles culicifacies Giles (Diptera : Culicidae), the major vector of malaria in Sri Lanka, is present in the island as a species complex comprising two sympatric sibling species termed B and E[1]. A similar situation exists in the nearby Rameswaram Island, Tamil Nadu, India[2]. Species B and E in Sri Lanka are reported to differ in their vector potential[3], resistance to insecticides[4] and longevity[5].

Human intervention in the form of hydroelectric dams, irrigation projects, new settlements and open pit mining, has altered natural ecosystems in many countries and paved the way for the emergence of different malaria vectors and their propagation.

In Sri Lanka new irrigation projects aiming at agricultural and industrial developments offered conducive environments for the emergence of different *Anopheles* species as vectors in addition to *An. culicifacies*, the major vector of malaria in the country. In the Mahawali systems B and C, *An. annularis* and in system C, *An. subpictus*[6,7] were reported to be carriers of *Plasmodium vivax* and *P. falciparum*. The emergence of different species as vectors accelerates the

momentum of disease transmission. Therefore, vector ecological studies have to be undertaken in order to locate risk areas for malaria and to formulate an appropriate strategy for vector control. Since there was no published information on the larval breeding sites of *An. culicifacies* species B and E, a survey was performed in Sri Lanka to obtain such information, and the results are presented here for the first time.

Material & Methods

Collection and analysis of larval and water samples: An. culicifacies larvae and water samples were collected from randomly selected breeding sites in the localities, which are associated with agricultural and human settlement systems of Pelawatta, Sellagadiragama and Mauara (in the District Moneragala); Elivitiya, Mouragala and Tonigala (in the District Puttalam); Korabowa and Uyankala (in the District Kurunagala); Puliyamkulam and Palayutru (in the District Trincomalee) covering the dry and intermediate rainfall zones of the island (Fig. 1). Malaria is endemic in the two zones. Sampling was done along the major riverine system of Dedhuru Oya in the Puttalam district; Manik Ganga and branches of Kuda Oya in the Moneragala district and Kimbulvana Oya and Dahamal Oya in the Kurunagala district. Stagnant water bodies such as quarries in the Puttalam district and irrigation channels and domestic wells in the Trincomalee district were also sampled.

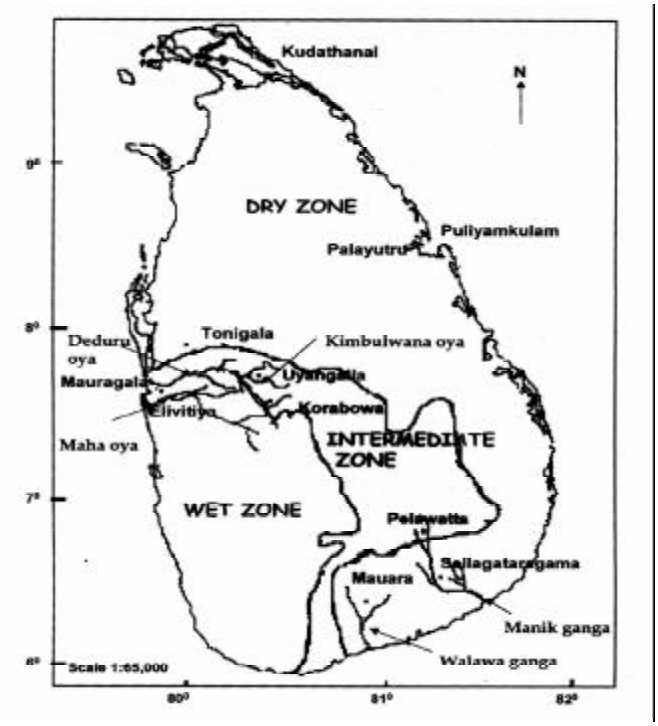

Fig. 1: Larval collection sites associated with major riverine systems

Larvae were collected by dipping 350 ml capacity dippers at the rate of 10 dips/m^2 of breeding habitat surface. Vials were labeled with relevant information such as date, site and number of larvae collected. Collections were carried out from January to November 2001.

Water samples were collected in polyethylene containers from 0830–1030 hrs from the breeding sites where *An. culicifacies s.l.* larvae were present. Collected samples were kept in rigiform containers and brought to the Institute of Fundamental Studies (IFS) better state laboratory as early as possible and stored at 4°C until the completion of analyses. All analyses were completed within two days using previously described protocols[8,9]. The measured physicochemical parameters and the methods used are given in Table 1.

Larval identification: Collected larvae were placed individually in a depression microscopic slide with a minimum amount of water and identified under a light microscope (Olympus Optical Co. Ltd., Tokyo) with an objective (x l0). I and II instar larvae were reared to reach III and IV instar larvae which were then identified using a standard key[10].

Karyotypic characterisation for species identification: Collected larvae were brought to the laboratory and reared as described previously[1]. All identified *An. culicifacies* male larvae in III and early IV instar were used for the Y-chromosome identification as previously described from all collected samples[1].

Analysis of data: Data obtained from analysed water samples were computed and analysed using Statistical

Table 1. Measured physico-chemical parameters and the methodology employed

Parameters	Methods employed
Temperature (°C)	Thermometric
Dissolved oxygen (ppm)	Titrimetric
pH	Potentiometric
Conductivity (?s/cm)	Electrical conductometric
Total dissolved solids (mg/l)	Spectrophotometric
Alkalinity (ppm)	Titrimetric
Ammonia nitrogen (ppm)	Spectrophotometric
Nitrate nitrogen (ppm)	Spectrophotometric
Calcium (ppm)	Atomic absorption Spectrophotometric
Magnesium (ppm)	Atomic absorption Spectrophotometric
Chloride (ppm)	Titrimetric
Total phosphate (ppm)	Spectrophotometric

Package for the Social Sciences (SPSS, version 10.0). Descriptive analysis was done to obtain the means and standard deviations of the obtained data and principal component analysis was done for data reduction.

Results

Only 23 samples out of 82 collected from different types of potential breeding sites contained *An. culicifacies s.l.* larvae. These were analysed for species composition and physico-chemical parameters. A total of 1318 *An. culicifacies* species E larvae were collected from 19 breeding sites in Moneragala, Puttalam, Kurunagala and Trincomalee districts (Table 2a). None of the larval samples collected from the breeding sites in localities in these districts was found to have species B larvae. A total of 177 species B larvae were collected from four breeding sites only in the Tonigala locality in the Puttalam district (Table 2b). In Tonigala, a locality associated with paddy cultivation, species B and E were found to breed in rock pools and sand pools. The rock pools of species B and E in this locality were found to be approximately l km apart. None of the 23 sites containing *An. culicifacies* larvae had both species B and E larvae. Mean (range) values of tested water quality parameters are given in Table 3.

Statistical analysis using data reduction by factor analysis followed by multiple regression of significant factors with abundance of species E showed that the abundance of species E was positively correlated with concentration of dissolved oxygen (DO). The regression model for the relationship between the larval density and the concentration of dissolved oxygen is represented as 56.058 ± 12.18 DO, $p = 0.03$. As only four samples were found to be with species B in Tonigala, the above analysis was not performed for species B.

Discussion

The results showed that *An. culicifacies* species E exploits a wide range of breeding habitats with different limnological characteristics. This probably reflects broad environmental adaptability on the part of species E. The presence of species E in rock pools in quarries with turbid water in Tonigala, a rural village in the Puttalam district, is an indication that physical quality of water may not play a significant role in the propagation of immature stages of *An. culicifacies* species E. It had earlier been considered that the Sri Lankan population of *An. culicifacies* is very sensitive to minute qualitative change in water, preferring to breed in clear, open stagnant water pools[11,12]. However, as previously reported for *An. culicifacies s.l.* and *An. vagus*[13,14], the present investigation also showed a positive association between the larval density of species E and the concentration of dissolved oxygen.

Quarries created for domestic or economic purposes were breeding sites for species E in Tonigala. Therefore, it is essential to take suitable public health measures to avoid water storage in quarries during rainy

Table 2a&b. Larval density of *An. culicifacies* collected from breeding sites found with species E and B

Date of collection	Locality (District)	Type of breeding habitat	Larval density (10 dips/m^2)
		(a) Species E	
10/01/2001	Mauragala (Puttalam)	Sand pool	48
14/01/2001	Palayutru (Trincomalee)	Unbound well	141
14/01/2001	Puliyamkulam (Trincomalee)	Bound well	42
25/01/2001	Tonigala (Puttalam)	Rock pool	54
08/02/2001	Palayutru (Trincomalee)	Irrigation channel	76
20/02/2001	Pelawatta (Moneragala)	Rock pool	49
13/03/2001	Mauara (Moneragala)	Sand pool	78
13/07/2001	Uyangala (Kurunagala)	Sand pool	89
13/09/2001	Uyangala (Kurunagala)	River margins	64
20/09/2001	Korabowa (Kurunagala)	Sand pool	62
11/10/2001	Elivitiya (Puttalam)	Sand pool	158
11/10/2001	Tonigala (Puttalam)	Quarries	63
20/10/2001	Pelawatta (Moneragala)	Sand pool	64
27/10/2001	Elivitiya (Puttalam)	Sand pool	72
27/10/2001	Tonigala (Puttalam)	Rock pool	29
27/10/2001	Tonigala (Puttalam)	Rock pool	56
09/11/2001	Sellagadiragama (Moneragala)	Sand pool	88
16/11/2001	Tonigala (Puttalam)	Quarries	31
16/11/2001	Tonigala (Puttalam)	Quarries	54
Total			1318
		(b) Species B	
06/03/2001	Tonigala (Puttalam)	Rock pool	37
07/09/2001	Tonigala (Puttalam)	Sand pool	58
27/10/2001	Tonigala (Puttalam)	Sand pool	39
27/10/2001	Tonigala (Puttalam)	Rock pool	43
Total			177

seasons. It is noteworthy in this regard that species E is capable of supporting the extrinsic cycle of both *P. vivax* and *P. falciparum* in Sri Lanka[3].

The results also indicate that species E breeds in rock pools and sand pools along the river margins in sugar cane cultivation site (Pelawatta in the Moneragala district) and agricultural sites of Moneragala district (Mauara and Sellagadiragama), Puttalam district (Mauragala and Elivitiya) and Kurunagala district (Uyangala and Korabowa). In the Trincomalee district high larval density of species E was found in open

Table 3. Range of measured physico-chemical parameters of the larval breeding sites

Analysed parameters	N	Minimum	Maximum	Mean	Std. deviation
Temperature (°C)	23	27	31	28.7	1.2
Dissolved oxygen (ppm)	23	2.6	7.1	5.17	1.2
pH	23	6.8	8.8	7.7	0.4
Conductivity (? s/cm)	23	120	1384	715.7	319.9
Total dissolved solids (mg/l)	23	1.8	4.86	3.5	0.8
Alkalinity (ppm)	23	134.2	701.7	374.5	121.8
Ammonia nitrogen (ppm)	23	1.46	19.58	9.1	4.7
Nitrate nitrogen (ppm)	23	0.05	0.86	0.3	0.2
Calcium (ppm)	23	116.35	1075.85	323.8	243.4
Magnesium (ppm)	23	923.15	3816.53	1855.8	739.8
Chloride (ppm)	23	9.45	178.4	61.8	52.2
Total phosphate (ppm)	20	0.01	0.45	0.1	0.1

N— No. of samples analysed.

bound well and unbound well used for domestic purposes, and in an irrigation channel in Palayutru and Puliyamkulam localities which are situated in close proximity to the major town and with human settlements. Species E larvae were also found at a depth of 9–10 m in domestic well water in Puliyamkulam locality, which consists of many human dwellings. This property of *An. culicifacies* species E shows the potential for malaria spreading from the traditionally endemic rural areas to towns and suburbs of cities such as Trincomalee, where wells remain common. In Rameswaram Island, Tamil Nadu, India, *An. culicifacies s.l.* breeds in pits used for coconut and casurina plantation and domestic wells[15]. Although species B larvae in Tonigala, Sri Lanka was found in rock pools and sand pools, the sample size is too small to describe its breeding preferences.

Although during the present survey only species E larvae were collected from breeding sites in Puliyamkulam in the Trincomalee district and Pelawatta in the Moneragala district, a previous study reported the prevalence of adult females of species B and E in these localities[1]. The breeding sites of species B in these localities remained to be identified.

The observation that none of the larval samples examined was found to contain both species B and E suggests that the females of the two species may differ in their micro-environmental egg-laying preferences. This may be considered as an adaptive ecological feature to minimise interspecific competition. However, it may be concluded that the larvae collected during this study could through a stochastic process, be derived from a single female, and therefore the findings may not readily be attributable to different micro-environmental egg-laying preferences between the two species. With the limited data available on species B, it is difficult to draw a conclusion of differential breeding preferences of these two species. However, based on laboratory experiments interspecific competition between larvae of *An. arabiensis* and *An. gambiae s.s.* in the *An. gambiae* complex was reported. The prevalence of mixed-species *(An. arabiensis* and *An. gambiae s.s.)* found to have a detrimental effect on larvae of *An. arabiensis* causing high mortality[16]. Therefore, mixed-larval populations of two sibling species of a species complex can possibly be disadvantageous for one species when the two species have the same preference for egg-laying sites.

Species B and E differing in vector potentiality and in their egg laying preferences can affect the efficacy of vector control measures formulated without knowing the occurrence of the two populations in different habitats. Therefore, a detailed study to investigate the possible different preferences of *An. culicifacies* species B and E for breeding sites and habitats is justified.

Acknowledgement

Financial assistance from National Science Foundation and assistance in larval collection by members of entomology team in respective districts are gratefully acknowledged.

References

1. Surendran SN, Abhayawardana TA, de Silva BGDNK, Ramasamy MS, Ramasamy R. *Anopheles culicifacies* Y-chromosome dimorphism indicates the presence of sibling species (B and E) with different malaria vector potential in Sri Lanka. *Med Vet Entomol* 2000; *14:* 437–40.
2. Kar I, Subbarao SK, Eapen A, Ravindran J, Satyanarayana TS, Raghavendra K, Nanda N, Sharma VP. Evidence for a new vector species E within the *Anopheles culicifacies* complex (Diptera: Culicidae). *J Med Entomol* 1999; *36:* 595–600.
3. Surendran SN, de Silva BGDNK, Srikrishnaraj KA, Ramasamy MS, Ramasamy R. Establishment of species E, not B as the major vector of malaria in the *Anopheles culicifacies* complex in the country. *Proc Sri Lanka Assoc Advmt Sci* 2003; *59:* 18.
4. Surendran SN, de Silva BGDNK, Ramasamy MS, Ramasamy R. Differential susceptibility to malathion by two members (B and E) of the *Anopheles culicifacies* (Diptera: Culicidae) species complex in Sri Lanka. *Proc Sri Lanka Assoc Advmt Sci* 2002; *58:* 153.
5. Surendran SN, de Silva BGDNK, Ramasamy MS, Ramasamy R. Comparative fecundity and age composition of two sibling species of *Anopheles culicifacies* (Diptera: Culicidae) complex in Sri Lanka. *Proc Sri Lanka Assoc Advmt Sci* 2002; *58:* 154.
6. Ramasamy R, de Alwis R, Wijesundere A, Ramasamy MS. Malaria transmission at a new irrigation project in Sri Lanka: the emergence of *Anopheles annularis* as a major vector. *Am J Trop Med Hyg* 1992; *47:* 547–53.
7. Amerasinghe PH, Amerasinghe FP, Wirtz RA, Indrajith NG, Somapala W, Preira LR, Rathnayake AMC. Malaria transmission by *Anopheles subpictus* Grassi in a new irrigation project in Sri Lanka. *J Med Entomol* 1992; *29:* 577–81.
8. Silva EIL, Namaratne SY, Werasooriya SVR, Manuweera L. *Water analysis: user friendly field/laboratory manual*. Institute of Fundamental Studies, Sri Lanka.
9. *Standard methods for the examination of water and waste water.* In : Clesceri LS, Greenberg AE, Trussell RR editors. XVII edn. Washington DC: American Public Health Association 1989.
10. Amerasinghe FP. A guide to the identification of the anopheline mosquitoes (Diptera: Culicidae) of Sri Lanka–II larvae. *Cey J Sci* 1992; *22:* 1–13.
11. Carter HF. Further observations on the transmission of malaria by anopheline mosquitoes in Ceylon. *Cey J Sci* 1930; *2:* 159–76.
12. Wickramasinghe MB. Malaria and its control in Sri Lanka. *Cey Med J* 1981; *26:* 107–15.
13. Amerasinghe FP, Indrajith NG, Ariyasena TG. Physico-chemical characteristics of mosquito breeding habitats in an irrigation development area in Sri Lanka. *Cey J Sci* 1995; *24*(2): 13–29.
14. Piyaratne MK, Amerasinghe FP, Amerasinghe PH, Konradsen F. Physico-chemical. characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding habitats in a dry zone stream in Sri Lanka. *Proc Sri Lanka Assoc Advmt Sci* 2000; *56:* 184.
15. Sabesan S, Krishnamoorthy K, Jambulingam PK, Rajendran G, Kumar PN, Rajagopalan PK. Breeding habitats of *Anopheles culicifacies* in Rameswaram Island. *Indian J Med Res* 1986; *84:* 44–52.
16. Schneider P, Takken W, McCall PJ. Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *An. gambiae*. *Med Vet Entomol* 2000; *14*(2): 165–70.

Corresponding author: Mr. S.N. Surendran, Department of Zoology, University of Jaffna, Jaffna, Sri Lanka.
e-mail: noble@jfn.ac.lk