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Physico-chemical characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka

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Background & objectives: Selected physico-chemical characteristics of flowing and pooled water in a stream that generated two malaria vectors, *Anopheles culicifacies s.l.* Giles and *Anopheles varuna* Iyengar, were investigated during August–September 1997 and July 1998 at the Upper Yan Oya watershed in north-central Sri Lanka.

Methods: The physico-chemical parameters measured were: temperature, dissolved oxygen, pH, conductivity, total dissolved solids, alkalinity, ammonia nitrogen, nitrate nitrogen, calcium, magnesium, carbondioxide, ferrous iron, phosphate, colour and turbidity. In total, 75.5% of 151 samples analysed were mosquito-positive. Logistic regression was used for statistical analysis.

Results: Among physico-chemical parameters, *An. culicifacies* (the major malaria vector in the country) was positively related only to temperature, and *An. varuna* (a secondary malaria vector) to calcium. Among habitat characteristics, *An. culicifacies* was associated with light and vegetation, and negatively associated with the presence of potential predators. *An. varuna* was positively associated with other aquatic fauna.

Interpretation & conclusion: Surprisingly this detailed study did not find an association between *An. culicifacies* and dissolved oxygen as previously found in the few studies that have looked at physico-chemical characteristics of malaria vector breeding habitats in south Asia. This study, along with existing information from other studies indicate that most of the physico-chemical parameters measured under natural conditions within the same habitat type is insufficient to explain the distribution of vectors within such habitats. However, it seems likely that both *An. culicifacies* and *An. varuna* follow a strategy whereby ovipositing females scatter their eggs over most of or all of a highly temporary and only transiently available stream bed pool habitat, in order to optimise breeding success.

Key words Mosquito breeding – Sri Lanka – water quality

A major reason for the study of mosquito larval ecology is to glean information on factors that may determine oviposition, survival, and the spatial and temporal distribution of important disease vector species. Breeding water quality is an important determinant of whether female mosquitoes will lay their eggs, and whether the resulting immature stages will successfully complete their development to the adult stage. This is, however, a somewhat neglected area of research in relation to vectors in general, and malaria vectors in particular. In Sri Lanka, the primary vector of malaria, *Anopheles culicifacies s.l.* (Diptera: Culicidae) is known to breed in natural streams and rivers, especially when pools are created during dry periods, or rainfall creates fresh pools on previously dry stream and river-beds[1–5].

While there is an extensive body of literature on various aspects of mosquito breeding in the country, there is only a single previously published investigation of the relationship between water quality and vector breeding[6]. Similarly, in the south Asian region, there is a large body of information on *Anopheles* larval ecology but relatively few studies on the characteristics of breeding water, examples being Reisen *et al*[7]; and Russell and Rao[8].

A study on anopheline larval ecology in an ancient cascade tank irrigation system in the malarious north-central province of Sri Lanka showed that the major malaria vector, *An. culicifacies,* and a secondary vector, *An. varuna*[9], bred primarily in a natural stream that also doubled as an irrigation conveyance canal[5]. The water quality parameters of this waterway were, therefore, investigated, and associations with the abundance of the previously mentioned vector species examined. The results are reported herein.

Material & Methods

Study area: The study was done in the Yan Oya stream situated in the Upper Yan Oya watershed area close to Huruluwewa in the Anuradhapura district of north-central Sri Lanka. Detailed descriptions of the study area have been published previously[5]. Briefly, the Upper Yan Oya area is a narrow elongated watershed consisting of degraded secondary forest, ancient villages and components of an ancient cascade tank irrigation system. The total area of the watershed is about 41,950 ha. The major waterway in the Huruluwewa watershed area consists of two parts: a constructed feeder canal from the upstream Bowatenna reservoir to Sigiri Oya and a natural waterway (Yan Oya stream) from Sigiri Oya to the large downstream Huruluwewa irrigation reservoir (7,500 ha). The Yan Oya is the only major waterway in the Upper Yan Oya watershed. Natural and man-made obstacles (such as fallen trees, logs, sand mining pits and temporary earth dams) block the water flow and create pools in which mosquitoes breed.

Larval mosquito sampling: Larval mosquitoes occurring in surface water in the Yan Oya stream were collected at 2-week intervals during August–September 1997 and July 1998 by the dipping technique (using standard 0.35 litres mosquito dippers). Three sampling sites located near the villages of Asirigama (site-1), Mahameegaswewa (site-2) and Siyambaladamana (site-3) were used. Six dips per sq m of breeding habitat surface area were taken from small pools (< 10 m^2 area). For larger pools and the flowing stream, dipping was done within 10 × 0.5 m (i.e. 5 m^2) areas along the margin edges. Larvae and pupae were preserved using 4% formaldehyde solution and identified to species. Identifications were confirmed by rearing selected immatures to the adult stage. The taxonomic keys of Amerasinghe[10-12] were used in identification.

Physico-chemical parameters: Water samples positive for *An. culicifacies* and *An. varuna* larvae were first determined by larval dipping. Randomly selected positive samples as well as negative samples were evaluated for water quality parameters. On each visit, initially collected immatures were identified in the field and collection places were marked. Later in the day (i.e. between 1000 and 1800 hrs) the same places were visited to carry out the physico-chemical tests.

Initially gross physical and biological characteristics were evaluated visually. Light conditions were scored as fully exposed to sunlight or partially/totally shaded; water conditions as clear or turbid/foul; the bottom as sandy or mud/rock; macrofauna present in the selected samples were noted, together with potential mosquito immature predators such as odonates, carnivorous hemipterans and fish. Vegetation was assessed as absent or present in the form of dead leaves, exposed roots, algae marginal grasses and herbs.

All physico-chemical parameters were tested on site at the time of collection. Temperature, pH, conductivity, total dissolved solids (TDS), and dissolved oxygen (DO) were measured using the M90 multi-probe meter (Mettler-Toledo AG, CH 8603, Schwerzenbach, Switzerland). Alkalinity, ammonia nitrogen, nitrate nitrogen, calcium, magnesium, ferrous iron, carbondioxide, colour, phosphate and turbidity were measured using LaMotte water analysis test kits (Lamotte Chemical Products Company, Chester town, Maryland, USA).

Quantitation was based on colorimetric comparison with standards of known value, or titration of the sample with solutions of known value. Details of the chemical basis for quantitation are provided in Renn[13]. Briefly, carbondioxide was determined by sodium hydroxide-phenolphthalein indicator titration; ammonia nitrogen by nesslerisation; nitrate nitrogen by reduction to nitrite and diazotisation; phosphates by the reduction of vanadomolybo-phosphoric acid to molybdenum blue; sulphates by precipitation in the form of barium sulphate; sulphides by conversion of *p*-aminodimethylaniline to methylene blue; chlorides by silver nitrate potassium chromate indicator titration; silica by conversion to molybdosilicic acid; turbidity by comparison with a standardised suspension of Fullers' Earth; phenolphthalein and total alkalinity by phenolphthalein and bromocresol green-methyl red indicator titration; calcium hardness by buffering with sodium hydroxide, and titrating murexide indicator with ethylene diamine tetra acetic acid-di sodium salt; and total hardness by buffering with borate buffer and titrating calmagite indicator with ethylene diamine tetra acetic acid-di sodium salt.

Chemical constituents were measured in parts per million (ppm), with total alkalinity and hardness expressed in terms of ppm $CaCO_3$. Turbidity was measured in Jackson turbidity units (JTU)[13]. Six of the total 21 parameters measured in 1997 (i.e. sulphide, sulphate, chloride, chlorine, fluoride and silica) returned zero values, and were not further tested in 1998.

Statistical analysis: Differences between physico-chemical parameters among the three sites and two habitat types (stream and streambed pools) were examined using analysis of variance (ANOVA). Associations between vector species presence/absence and biological parameters of the habitats were examined by simple logistic regression. Relationships between mosquito abundance and physico-chemical variables in breeding water were initially examined by a multivariate factor analysis in order to overcome the effects of inter-correlations among physico-chemical variables, but problems of normality within the vector datasets (which could not be corrected by data transformations) seriously biased the results. For the physico-chemical data, simple correlations between vector abundance and individual parameters were first checked, and only significant associations further examined by multiple logistic regression, setting parameter values as "high" or "low" based on the median value of each set. Results are reported as odds ratios (OR) and their 95% confidence intervals (CI).

Results

An. culicifacies and/or *An. varuna* were present in 98 of 131 (74.8%) streambed pools and 16 of 20 (80%) flowing stream samples. The other samples (i.e. 33 from pools and four from the flowing stream) were mosquito negative. Streambed pools contained 96% of collected *An. culicifacies* larvae within 37% of positive samples, while the flowing stream yielded 4% of larvae from 17% of positive samples. For *An. varuna,* streambed pools produced 89% of collected larvae from 63% of positive samples, while the flowing stream yielded 11% of larvae from 83% of positive samples.

In total, 151 samples (131 streambed pools and 20 from flowing stream) were analysed (Table 1). The mosquito breeding water in the stream was low in am-

Table 1. Number of immature *An. culicifacies* and *An. varuna* collected at different sites and habitats

Sites	Habitats							
	Site-1		Site-2		Site-3		Total	
	STR	SBP	STR	SBP	STR	SBP	STR	SBP
No. of samples	8	59	8	42	4	30	20	131
No. of *An. culicifacies*	2	195	14	116	0	68	16	379
No. of *An. varuna*	59	354	5	229	16	62	80	645

STR—Flowing stream; SBP—Streambed pools.

monia nitrogen, nitrate nitrogen and phosphate. Alkalinity and hardness content were relatively high. There was no significant difference (ANOVA, $p > 0.05$) among the three surveyed sites in respect of the 15 physico-chemical parameters tested and, therefore, the consolidated results across sites are presented in Table 2. However, conductivity, alkalinity, calcium, TDS, and phosphate differed between streambed pools and the flowing stream (ANOVA, $p < 0.05$).

Only results from 131 water samples drawn from streambed pools were used for further analysis. Spearman correlation analysis (two-tailed) of the 15 parameters and vector abundance revealed that *An.*

Table 2. Mean ± SD (Range) of physico-chemical parameters in *Anopheles* breeding water in the Yan Oya stream

Parameter	Habitat			
	Flowing stream (n = 20)		Streambed pools (n = 131)	
Temperature (°C)	29 ± 1.6	(26–32)	30.3 ± 2.3	(25–38)
Conductivity (µS/cm)	534.7 ± 83a	(427–778)	828.8 ± 557.3b	(2–1921)
Alkalinity (ppm)	198 ± 57a	(28– 264)	687.4 ± 285.3b	(84–1400)
Ammonia (ppm)	0 ± 0	(0–0)	1.1 ± 1.3	(0–8)
Calcium (ppm)	100 ± 29.2a	(50–160)	326.3 ± 173.8b	(65–929)
Carbondioxide (ppm)	7.4 ± 4.1	(0–15)	13.3 ± 13.2	(0–88)
Colour (ppm)	1.2 ± 2.5	(0– 8)	4.7 ± 3.8	(0–24)
TDS (S)	265.3 ± 41.1a	(207–390)	407.4 ± 275.9b	(1– 963)
pH	7.7 ± 0.3	(7–8)	7.9 ± 0.4	(6– 9.4)
Dissolved oxygen (mg/l)	3.5 ± 1.5	(0.9–6.7)	2 ± 2.3	(0– 20)
Magnesium (ppm)	511.9 ± 549.4	(84–1692)	1238.5 ± 592.3	(0–3096)
Ferrous (ppm)	0.7 ± 0.5	(0–2)	8 ± 41.7	(0– 400)
Nitrate (ppm)	0.07 ± 0.12	(0–0.5)	0.11 ± 0.07	(0–0.4)
Phosphate (ppm)	0.03 ± 0.06a	(0–0.2)	0.22 ± 0.16b	(0– 0.5)
Turbidity (JTU)	19.5 ± 20.7	(5–90)	26.6 ± 31.2	(2–200)

Row means followed by different letters differ at $p < 0.05$ level of significance (ANOVA).

culicifacies abundance was weakly but significantly positively associated with temperature ($r = 0.29$; $p = 0.001$), pH ($r = 0.19$; $p = 0.025$) and DO ($r = 0.25$; $p = 0.004$), and *An. varuna* positively associated with calcium ($r = 0.43$; $p < 0.001$) and CO_2 ($r = 0.21$; $p = 0.018$). Follow-up logistic regression analysis established only a positive association between *An. culicifacies* presence and temperature, with more occurrences from low temperature samples than high (OR = 2.74; CI = 1.29–5.86). In the case of *An. varuna* too, more occurrences were recorded from low than high calcium habitats (OR = 5.28; CI = 2.47–11.30). Thus, in each species, seen through simple correlation, the trend of greater abundance related to temperature (*An. culicifacies*) and calcium (*An. varuna*), respectively, was compounded by an opposite trend related to occurrence. Among habitat characteristics, *An. culicifacies* was positively associated with light (OR = 2.38; CI = 1.08–5.35) and vegetation (OR = 4.36; CI = 1.24–15.4), and negatively associated with the presence of potential predators (OR = 0.33; CI = 0.12–0.93). *An. varuna* was positively associated with other aquatic fauna (OR = 2.77; CI = 1.12–6.84).

Discussion

There are three previous published studies on the association between water quality and mosquito abundance on the Indian subcontinent: Russell and Rao[8] in India, Reisen *et al*[7] in Pakistan, and Amerasinghe *et al*[6] in Sri Lanka. All bring out the importance of DO for *An. culicifacies s.l.* In Reisen *et al*'s study[7], the significant factor in multivariate factor analysis included DO and pH as the major variables, while in Amerasinghe *et al*'s study[6], the significant factor included DO, temperature, phosphate (positively associated with abundance) and carbondioxide (negatively associated). Surprisingly this study did not find an association between *An. culicifacies* and DO. The role of DO as a potentially significant variable as found in several studies is interesting and needs further exploration. Mosquito larvae are air-breathing, and this raises the question as to whether it is oxygen *per se*, or an associated physico-chemical or biotic factor that influences the occurrence and abundance of this species— this study indicates that it may not be the oxygen by itself that determines abundance.

This study found through logistic regression *An. culicifacies* abundance to be significantly associated with temperature with a higher occurrence from low temperature samples supporting earlier findings in Sri Lanka by Amerasinghe *et al*[6]. Also, temperature has been previously shown to be an important determinant of *An. culicifacies* breeding success in India, with the range 28–32^{o}C providing the optimum conditions for egg, larval and pupal development[14].

In this study *An. culicifacies* habitats were associated with light and vegetation and *An. varuna* associated with other aquatic fauna in line with previous entomological studies in the same study area[15]. However, in the previous study by Van der Hoek *et al*[15] the biological and physical characteristics could not well explain the preference for certain habitats by potential vectors of malaria. Generally when reviewing the literature for *An. culicifacies* from south Asia it seems to be able to exploit specific habitat type — river-bed pools, with very different biological, chemical and physical characteristics. Based upon this information it seems likely that both *An. culicifacies* and *An. varuna* follow a strategy whereby ovipositing females scatter their eggs over most of or all of a highly temporary and only transiently available streambed pool habitat, in order to optimise breeding success. Thus, larvae may occur even in less suitable habitats, but the abundance will be greater in those habitats where the conditions are optimum for larval growth.

The Indian subcontinent is located in the middle of the geographic range of *An. culicifacies*, which extends from Eritrea to Vietnam[16]. There is no information on water quality parameter ranges for this species from other areas of its geographic range. Similarly, for *An. varuna*, whose range extends from India to Thailand[16]. In addition, *An. culicifacies* exists as a species complex with five known siblings (A–E) within the Indian subcontinent. India is host to all of these, while sibling species B and E are known from Sri Lanka, of which E is the more efficient vector[17]. None of the studies to date have investigated breeding water quality parameters in relation to sibling species occurrence and abundance and work is needed to explore potential differences in habitat requirements.

Relating water quality parameters with preferences for potential disease vectors will not be easy, as information on surface water quality in general is not in an easily accessible published form in Sri Lanka. Available published data on selected chemical parameters of some major freshwater lakes and rivers in Sri Lanka show pH to range from 4.4–8.4, chloride from 0.7–132 ppm, silica from 1.0–10.6 ppm, sulphate from 0.2–10.5 ppm, DO from 4.3–13.2 ppm, ammonia from 0.04–6.07, nitrate from 0.02–4.3 ppm, and phosphate from 0.08–1.5 ppm[18,19]. The present and previous[6] studies contribute baseline water quality data for two streams in the north-central and eastern regions of the country, respectively.

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