

## PHYSICO-CHEMICAL CHARACTERISTICS OF MOSQUITO BREEDING HABITATS IN AN IRRIGATION DEVELOPMENT AREA IN SRI LANKA

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### ABSTRACT

Selected physico-chemical characteristics of mosquito breeding habitats were studied in an area of System C of the Mahaweli Development Project, Sri Lanka, during January-December 1986 when the area was undergoing rapid irrigation development. The analysis of 280 mosquito-occupied surface water samples produced mean (range) values of 14 parameters as follows: temperature = 28.0 (22.0 - 39.0) °C; pH = 7.8 (6.5 - 10.0); dissolved oxygen = 4.7 (0.0 - 26.0) ppm; carbon dioxide = 11.4 (0.0 - 65.0) ppm; ammonia nitrogen = 0.6 (0.0 - 24.0) ppm; nitrate nitrogen = 0.03 (0.0 - 0.5) ppm; phosphate = 0.7 (0.0 - 8.0) ppm; silica = 14.7 (0.1 - 40.0) ppm; sulphide = 0.007 (0.0 - 1.0) ppm; sulphate = 2.5 (0 - 200) ppm; chloride = 49.7 (7 - 400) ppm; turbidity = 171.3 (0 - 6400) ppm; total alkalinity = 195.9 (24 - 672) ppm; and total hardness = 135.7 (24 - 608) ppm. There were significant differences in physico-chemical conditions between different habitat types such as temporary ground pools, riverbed pools, streambed pools, flowing streams, marshes and canal turnouts. Significant associations between mosquito abundance and specific physico-chemical parameters were recorded for *Anopheles culicifacies*, *An. nigerrimus*, *An. subpictus*, *An. vagus*, *Culex mimulus*, *Cx. pseudovishnui*, *Mansonia annulifera*, *Mimomyia chamberlaini* and *Mi. hybrida*. The study provided baseline data against which irrigation-related changes in water quality in general, and mosquito breeding-habitat characteristics in particular, could be evaluated in the future.

### INTRODUCTION

The occurrence and abundance of mosquito immatures in different breeding habitats reflects the oviposition preferences of females as well as the ability of the immatures to survive in the conditions under which they find themselves. Changes in the physico-chemical and biotic characteristics of surface water habitats may create conditions either favourable or unfavourable to the breeding success of mosquitoes, depending on the ranges of tolerance of different species. This can have implications for vector-borne diseases, because habitat changes that favour potential vector species can ultimately lead to increased rates of parasite or pathogen transmission.

Large-scale environmental modification concomitant with processes such as forest clearing, irrigation development and human resettlement inevitably result in changes in surface water quality, and affect the survival of mosquito species breeding in surface water habitats. The Accelerated Mahaweli Development Project of Sri Lanka, initiated in 1978, envisaged the development of 165,000 ha of land and the resettlement of 1 million humans within a 10 year time span. A study on the ecological effects of such a large-scale, rapid environmental change on mosquitoes and mosquito-borne diseases was initiated in 1984 in an area of the Project, with a view to documenting changes in mosquito species composition, breeding habits, vector status, and the incidence of human mosquito-borne disease. Reports on several aspects of

mosquito bionomics and disease relations arising out of this study already have been published (see Amerasinghe & Munasingha 1988a,b; Amerasinghe & Ariyasena 1990, 1991; Amerasinghe & Indrajith 1994; Amerasinghe *et al.* 1991, 1992; Peiris *et al.* 1993).

As part of this multidisciplinary research effort, we surveyed the physico-chemical conditions of natural surface water collections in the study area during the construction phase of the Development Project, immediately after forest clearing. The present paper reports the outcome of this investigation, which was done to document the conditions under which the immature stages of surface water breeding mosquito species existed in the drastically modified environment, and to establish a baseline against which surface water quality in general and mosquito breeding water conditions in particular, could be assessed in the years after the establishment of the irrigated rice ecosystem for which the area was being developed.

## MATERIALS AND METHODS

The study area was located in Block 6 of Zone 4 within System C of the Mahaweli Development Project in the Eastern Province dry zone of Sri Lanka. The originally forested 4000 ha. area was developed into an irrigated riceland during 1985-1987. Detailed descriptions of the topography, vegetation, climate and the sequence of developmental events leading upto the establishment of an irrigated rice ecosystem have been published previously (Amerasinghe & Munasingha 1988a; Amerasinghe & Ariyasena 1990; Amerasinghe & Indrajith 1994). The study reported in the present paper was done in January-December 1986, during the phase of infrastructure construction following forest clearing.

### Mosquito sampling

Details of mosquito collections in surface water breeding habitats in the study area have been reported previously (Amerasinghe & Ariyasena 1990). Briefly, larval and pupal stages of mosquitoes were collected by dipping (using standard pint [0.5 l] dippers) at the rate of 6 dips per m<sup>2</sup> of breeding habitat surface area. For large habitats (streams, marshes) dipping was done within 5 m<sup>2</sup> areas along the edges. Immatures of root-respiring species attached to floating waterplants (*Pistia*, *Salvinia*) were collected by vigorously washing the plants that were taken up during routine dipping. Species identifications were based on immature chaetotaxy and associated adult morphology (see Amerasinghe & Munasingha 1988b; Amerasinghe & Ariyasena 1990).

### Physico-chemical variables

Randomly selected habitats that proved to be positive for mosquito immatures were further evaluated for physico-chemical characteristics. A water sample was collected from each breeding site concurrently with the collection of mosquito immatures, between 09:00-12:00 hr on each sampling day. Temperature (°C) and hydrogen ion concentration (pH) were determined on-site at the time of collection, the latter using BDH pH-paper. Other parameters were analyzed in a field laboratory within 2-3 hr. of collection. A previous study in Pakistan demonstrated no significant differences in physico-chemical parameters of mosquito breeding water even 12 hr. after collection (Reisen *et al.* 1981). Thus, it is assumed that significant physico-chemical changes would not have occurred within the short time lag between collection and analysis in the present study.

Apart from temperature and pH, other measured parameters were dissolved oxygen, carbon dioxide, ammonia nitrogen, nitrate nitrogen, phosphate, sulphate, sulphide, chloride, silica, turbidity, phenolphthalein alkalinity, total alkalinity, calcium hardness and total hardness. Water analysis test kits were obtained from LaMotte Chemical Products Co., Maryland, USA. Quantitation was based on one of two chemical test methods: colorimetric comparison with standards of known value, or titration of the sample with solutions of known value. The test kits provided comparator and axial reader equipment with which to make quantitative determinations. Details of the chemical basis for quantitation are provided in Renn (1970). Briefly, dissolved oxygen was measured using the azide modification of the Winkler method; carbon dioxide by sodium hydroxide-phenolphthalein indicator titration; ammonia nitrogen by nesslerization; nitrate nitrogen by reduction to nitrite and diazotization; 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 standardized 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<sub>3</sub>. Turbidity was measured in Jackson turbidity units (JTU) (Renn 1970).

#### Statistical analysis

Mosquito species abundance (expressed as mean number of immatures/dip/sample  $\pm$ se) in different breeding habitat types was examined by Kruskal-Wallis analysis of variance (ANOVA) followed by Turkey's multiple comparisons test (Zar 1984). Relationships between abundance and physico-chemical variables in breeding water were examined by correlation and regression analysis. Initial correlation matrix analysis showed that 14 major physico-chemical parameters measured (ie. the independent variables) were highly inter-correlated. Thus, a multivariate factor analysis (SAS Institute 1988) was done in order to objectively screen for relationships between groups of physico-chemical variables. Factors with eigen values  $>1$  were further evaluated for relationships with mosquito abundance by stepwise multiple regression, with each factor represented by the physico-chemical variable that provided the highest factor-loading contribution to that factor. Where a significant ( $P < 0.05$ ) association was obtained, relationships between mosquito abundance and the constituent physico-chemical variables with the relevant factors were characterized by Pearson's correlation coefficient (Zar 1984). Only results for statistically significant ( $P < 0.05$ ) correlations are presented in the text.

## RESULTS

In total, 280 water samples drawn from temporary ground pools, riverbed pools, streambed pools, flowing streams, marshes and newly built canal control structures (turnouts) were analyzed for 16 physico-chemical parameters. Of these, 14 are reported on in detail. Phenolphthalein alkalinity was recorded only in canal turnouts, where it constituted only 4.2% of total alkalinity. Thus, only results relating to total alkalinity are further reported. Calcium hardness contributed to 27-65% (mean 34%) of total hardness in ground pools, 17-81% (mean 56%) in riverbed pools, 3-75% (mean 33%) in streambed pools, 8-89% (mean 38%) in streams, 19-62% (mean 24%) in marshes and 7-94% (mean 53%) in canal turnouts. The balance in each case represented the contribution of magnesium hardness to the total. Only details for total hardness are further reported in this paper.

In general, mosquito breeding water was low in ammonia, nitrate, phosphate and sulphide content and highly variable in relation to the other tested chemical parameters (Table 1). Several parameters varied significantly among different mosquito breeding habitats. Temperature was highest in ground and riverbed pools that were usually fully exposed to the sun. Hydrogen ion concentration (pH), dissolved oxygen and phosphate levels were highest in riverbed pools; carbon dioxide, silica and total alkalinity in streambed pools; nitrate in canal turnouts; chloride in streambed pools and marshes; and total hardness in streambed pools and samples from flowing streams. Ammonia, sulphide and sulphate levels did not vary significantly among different habitats (Table 1).

Thirty eight mosquito species were collected from the breeding habitats surveyed. *Aedes* (6 species), *Anopheles* (14), *Culex* (11), *Mansonia* (3), *Mimomyia* (2), *Ficalbia* (1), and *Uranotaenia* (1) were the genera represented. Detailed results are provided only for 14 species, each of which occurred in  $>5\%$  of the total samples taken. *Anopheles barbirostris* van der Wulp and *An. barbumbrosus* Strickland & Choudhury were significantly more abundant in streambed pools than other habitats. *Anopheles culicifacies* Giles was most abundant in riverbed pools, *An. nigerrimus* Giles in marshes, *An. subpictus* Grassi in canal turnouts, *An. vagus* Doenitz in temporary ground pools, and *An. varuna* Iyengar in flowing streams (Table II). *Culex mimulus* Edwards was most abundant in streambed pools and temporary ground pools, and *Cx. pseudovishnui* Colless in the marsh habitat. *Mansonia annulifera* (Theobald) and *Mimomyia hybrida* (Leicester) occurred exclusively in marshes, and *Mi. chamberlaini* (Ludlow) primarily in this habitat. Significant habitat-related differences in abundance were not seen in *An. jamesii* Theobald and *Cx.*

*bitaeniorhynchus* Giles, but the former was collected mainly from marshes and the latter from riverbed pools (Table II).

Tables III and IV provide details of physico-chemical conditions of the breeding water in which individual species were collected. On average, *An. barbirostris* occurred in samples with a comparatively high carbon dioxide, silica, chloride, sulphate, alkalinity and hardness content, and low oxygen, ammonia, nitrate, phosphate, and turbidity levels. Similar trends were seen in *An. barbumbrosus*, *An. nigerrimus* and *An. jamesii*, except that the first species occurred in water with a comparatively lower sulphate content, and the latter two species in samples with a lower silica content. *Anopheles subpictus* and *An. vagus* were common in turbid water, the latter in samples with higher phosphate, sulphate and total hardness levels than the former. *Anopheles culicifacies* and *Cx. bitaeniorhynchus* occurred in samples with a higher dissolved oxygen, ammonia and phosphate content, and lower carbon dioxide level than most of the other species (Table III). This was most likely a function of their heavy breeding in riverbed pools, in preference to other available habitats (Tables I and II).

*Culex mimulus* showed trends similar to *An. barbirostris*, and *Cx. pseudovishnui* to *An. nigerrimus*. These similarities in physico-chemical conditions could have been related to their major breeding habitats: streambed pools for the first pair, and the marsh habitat for the second. *Mansonia annulifera*, *Mi. hybrida* and *Mi. chamberlaini* showed basically similar trends, occurring in water with comparatively low oxygen and silica levels, and high carbon dioxide and chloride content (Table IV). This was undoubtedly related to the conditions in their primary habitat, marshes (Table I).

Multivariate analysis of the 14 physico-chemical variables resulted in the definition of 5 factors with eigen values >1. Based on the factor loading values obtained, the 14 variables segregated as follows: Factor 1 included dissolved oxygen as the major factor-loading contributor, together with carbon dioxide, phosphate, chloride and temperature; Factor 2 contained total alkalinity (major contributor), total hardness, sulphate and pH; Factor 3 contained nitrate (major contributor), ammonia and turbidity; Factors 4 and 5 contained a single variable each, ie. sulphide and silica, respectively. The analysis established that variables included within a particular factor were significantly more strongly correlated with each other than with other variables.

The results of stepwise multiple regression of mosquito species abundance (dependant variable) against the five Factors (independent variables), followed by correlation analysis of the relationships between abundance and the physico-chemical variables included within significant factors are presented in Table V. The abundance of *An. culicifacies*, *An. nigerrimus* and *An. vagus* was associated with Factor 1 variables. *Anopheles culicifacies* correlated positively with dissolved oxygen, phosphate and temperature, and negatively with carbon dioxide. Similarly, *An. vagus* was positively correlated with the first three variables, but uncorrelated with the last. *Anopheles nigerrimus* was negatively correlated with dissolved oxygen and temperature. The association of *An. jamesii* and *Cx. pseudovishnui* with Factor 2 variables was due to a positive correlation with total alkalinity in both species. *Anopheles subpictus* and *Cx. mimulus* were significantly associated with Factors 3 and 5, respectively, with the former species positively correlated with nitrate and the latter species with silica (Table V).

Three species showed significant associations with multiple factors (Table V). Both *Ma. annulifera* and *Mi. hybrida* (Factors 1 and 5) correlated negatively with oxygen and silica, and positively with chloride. *Mimomyia chamberlaini* (Factors 1,2,3, and 5) was correlated negatively with oxygen and silica, and positively with chloride, total alkalinity, ammonia, and nitrate. Statistically significant associations with multivariate factors were not obtained for *An. barbumbrosus*, *An. barbirostris*, *An. varuna* and *Cx. bitaeniorhynchus*.

## DISCUSSION

Natural surface waters in the forest land that was newly cleared for irrigation development in our study area in Mahaweli system C had a high alkalinity and hardness content. Alkalinity was likely associated with the high carbon dioxide content of the water in most habitats. Average hardness in all habitats was higher than 120 ppm, which qualifies as hard water under international health standards for drinking water (WHO 1984). Hardness was due primarily to carbonates, since sulphate levels (and also sulphides, which may index reduced sulphates) were extremely low. Salinity (indexed by chloride content) was low, and exceeded the taste threshold of 200-300 ppm (WHO 1984) in only a few riverbed pool samples. Silica levels were moderate and well within the range (0-75 ppm) for natural waters (Renn 1970).

On average, turbidity was higher than the WHO guideline value of 5 JTU in all surface water habitats except natural streams; this is not surprising because the water samples were taken from an area that was undergoing large-scale land re-structuring at the time. There was little evidence of organic pollution, as indexed by low ammonia, nitrate and phosphate levels. Surprisingly, this was true even of the long established marsh habitat that was previously very obviously polluted by wild buffalo and elephant excrement. The clearing of surrounding forests was undoubtedly responsible for the virtual cessation of large wild mammal activity within the marshes at the time this study was done. Apart from hardness and turbidity, all other measured physico-chemical parameters were, on average, within international health and aesthetic standards for drinking water (WHO 1984). This is relevant to human health, because construction workers and early settlers in the area used water from the Mahaweli river, streams, marshes and even large rainwater pools, prior to the construction of the canal system and tube wells.

General information on surface water quality in Sri Lanka is scattered, unpublished or in unprocessed form (NARESA 1991). Available published data on selected chemical parameters of some major freshwater lakes and rivers in Sri Lanka shows pH to range from 4.4 - 8.4, turbidity from 7 - 170 ppm, chloride from 0.7 - 132.2 ppm, silica from 1.0 - 10.6 ppm, sulphate from 0.2 - 10.5 ppm, dissolved oxygen 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 (Costa 1980; NARESA 1991).

There are no previously published reports on the physico-chemical characteristics of mosquito breeding habitats in Sri Lanka. Data in Reisen *et al.* (1981) provide a comparison with mosquito breeding water conditions in a long-established rice cultivation area around Lahore, Pakistan. Samples in that study were taken from habitats such as temporary ground pools, large ponds, ricefields, borrow pits, ditches, streams, seepage canals, and cement catch basins at tube-well taps. The major differences in chemical characteristics were the higher dissolved oxygen, ammonia, phosphate and total alkalinity content (approximately double the overall mean values recorded in our study) and very much higher sulphate (>35 times) and nitrate (>100 times) content in the water samples from Pakistan (Reisen *et al.* 1981). Phosphate, nitrate and ammonia levels, in particular, indexed the extent of organic pollution, attributed mainly to contamination from human and domestic animal faecal matter (Reisen *et al.* 1981). Latrine use is a well-established practice in Sri Lanka, and Mahaweli settlers were provided material assistance to build pit latrines upon settlement. Thus, human wastes are unlikely to significantly affect surface water quality. However, domestic animal wastes and agrochemicals are likely to raise surface water ammonia, nitrate, phosphate and sulphate levels in the Mahaweli development areas in the future.

Studies on the Indian subcontinent provide comparative information on associations between larval abundance and breeding water physico-chemical characteristics in the case of some of the mosquito species investigated in the present work. As in Sri Lanka, studies from India and Pakistan have shown the abundance of *An. culicifacies* to be positively associated with dissolved oxygen (Reisen *et al.* 1981; Russel & Ramachandra Rao 1942). Relationships with carbon dioxide, phosphate and temperature recorded in our study are a likely reflection of inter-correlation with oxygen, since all segregated within the same multivariate factor. The predominance of *An. culicifacies* in riverbed pools which were generally associated with high oxygen, phosphate and temperature, and low carbon dioxide levels is probably the major factor contributing to these associations in our study. In India, larval abundance was significantly negatively correlated with amorphous organic matter and total plankton content (Russel & Ramachandra Rao 1942). Temperature was the most important determinant among physical parameters, with the range 28 - 32 °C providing the most suitable conditions for the development of eggs, larvae and pupae (Pal 1945): *Anopheles culicifacies* is the primary vector of human malaria in Sri Lanka. Only sibling species B of *An. culicifacies* occurs on the island (Abhayawardana *et al.* 1993), unlike on the Indian subcontinent where siblings A, B, C, and D occur (Subbarao 1988). The identity of the siblings involved in the investigations from India and Pakistan reported herein is unknown.

Among the other anophelines, *An. subpictus* abundance has been shown to be positively correlated to turbidity and suspended solids and may also have some association with total alkalinity and nitrates (Reisen *et al.* 1981). In our study, the species was positively correlated only with nitrates, though it commonly occurred in water samples with a high turbidity content. Ramachandra Rao (1984), reported that the species can tolerate high salinity and occurs in brackish waters. The last observation obviously related to the brackish-water breeding sibling species B of *An. subpictus* (Suguna 1982). Both siblings A and B occur in Sri Lanka (Abhayawardana *et al.* 1993), but the species occurring in our study area was the freshwater breeding sibling species A, determined on the basis of egg and adult morphology (Suguna 1982;

Reuben & Suguna 1983). This species has been shown to be a secondary vector of malaria in Sri Lanka (Amerasinghe *et al.* 1992; Mendis *et al.* 1992). Unlike in our study, Reisen *et al.* (1981) did not record significant correlations between *An. nigerrimus* abundance and physico-chemical conditions in breeding water. Means and ranges of parameters such as pH, dissolved oxygen, total alkalinity and turbidity under which this species occurred were comparable in the two studies, whilst levels of phosphate, ammonia, nitrate and sulphate were higher in Pakistan, consistent with the more polluted water conditions found in that study. Comparable information on the physico-chemical conditions of breeding water are not available for other anopheline species investigated in our study. However, *An. vagus* has been reported to breed more intensely in muddy (turbid) than clear waters, and *An. barbirostris* in habitats rich in vegetation and organic matter (Ramachandra Rao 1984).

Among culicine species, *Cx. pseudovishnui* abundance has been reported to correlate positively with temperature and negatively with pH, phosphate and sulphate (Reisen *et al.* 1981). In our study, this species was positively correlated with total alkalinity, but not with any of the above parameters. Reisen *et al.* (1981) found no correlations between physico-chemical conditions and *Cx. bitaeniorhynchus* abundance, but reported an association with filamentous green algae such as *Spirogyra*. In our study too, *Cx. bitaeniorhynchus* was observed to co-occur with *Spirogyra*, particularly in the riverbed pool habitat.

Forest clearing and land restructuring for the establishment of a new irrigation system in Mahaweli System C resulted in the commencement of a new floral and faunal ecological cycle. Surface water collections that were previously shaded and presumably contained a high organic content due to decaying vegetation, were replaced by exposed habitats largely devoid of vegetation. The physico-chemical conditions in mosquito breeding habitats during our study represented a transitional phase between a previously human-unoccupied forest ecosystem that existed for centuries and the human-inhabited irrigation system that replaced it. Surveys conducted in our study area have shown important changes in breeding habitat types and mosquito species composition concomitant with human settlement and irrigation development (Amerasinghe and Ariyasena 1990; Amerasinghe and Indrajith 1994). The present results define the physico-chemical conditions under which the aquatic stages of different mosquito species occurred in the early period of Project implementation in an area of Mahaweli system C. They also provide baseline data on surface water quality against which to evaluate long-term changes that may occur as a result of water table changes, agrochemical use, animal husbandry practices and other human activities, in this newly developed irrigated riceland.

#### ACKNOWLEDGEMENTS

We thank U.G.Nimal, S.G. Wickremasinghe, H.M. Weerasekera, K.A.W. Samarakoon and M.Kumarasinghe for field assistance and S. Samitha of the Department of Crop Science, Faculty of Agriculture, University of Peradeniya for assistance with the statistics. The Mahaweli Authority of Sri Lanka is thanked for permission to conduct investigations in Mahaweli System C. Funding was provided by the Board on Science and Technology for International Development (BOSTID) of the U.S. National Academy of Sciences, by way of a grant from USAID.

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Table 1

Physico-chemical characteristics (mean  $\pm$  se, range) of habitats sampled

	Ground Pool	Riverbed Pool	Streambed Pool	Stream	Marsh	Canal Turnout	ALL HABITATS
Number of Samples	20	45	29	63	76	47	280
Temperature ( $^{\circ}$ C)	34.5 $\pm$ 1.0a (25.0 - 39.0)	29.8 $\pm$ 0.3a (24.0 - 33.0)	25.6 $\pm$ 0.5d (22.0 - 35.0)	27.2 $\pm$ 0.2bc (24.0 - 34.0)	27.2 $\pm$ 0.2bc (24.0 - 36.0)	27.5 $\pm$ 0.3b (24.0 - 33.0)	28.0 $\pm$ 0.2 (22.0 - 39.0)
Ph	7.5 $\pm$ 0.08cd (7.0 - 8.5)	8.5 $\pm$ 0.09a (7.0 - 9.5)	7.7 $\pm$ 0.07bc (7.0 - 8.5)	7.7 $\pm$ 0.05bc (6.0 - 8.5)	7.4 $\pm$ 0.05d (6.5 - 9.5)	8.1 $\pm$ 0.12ab (7.5 - 10.0)	7.8 $\pm$ 0.04 (6.5 - 10.0)
Oxygen (ppm)	5.7 $\pm$ 0.6ab (1.3 - 10.4)	10.4 $\pm$ 0.9a (0.0 - 26.6)	3.0 $\pm$ 0.4cd (0.0 - 7.4)	5.0 $\pm$ 0.2b (0.0 - 8.0)	1.8 $\pm$ 0.2d (0.0 - 10.0)	4.3 $\pm$ 0.3bc (0.0 - 7.6)	4.7 $\pm$ 0.2 (0.0 - 26.6)
Carbon Dioxide (ppm)	14.0 $\pm$ 2.5b (0.0 - 32.0)	3.6 $\pm$ 0.8d (0.0 - 25.0)	23.2 $\pm$ 2.7a (2.8 - 65.0)	11.4 $\pm$ 0.9bc (3.0 - 46.0)	13.3 $\pm$ 0.8b (0.0 - 32.0)	7.7 $\pm$ 0.9c (0.0 - 31.0)	11.4 $\pm$ 0.6 (0.0 - 65.0)
Ammonia Nitrogen (ppm)	0.2 $\pm$ 0.1 (0.0 - 1.5)	1.6 $\pm$ 0.5 (0.0 - 16)	0.1 $\pm$ 0.08 (0.0 - 1.5)	0.1 $\pm$ 0.05 (0.0 - 2.5)	0.5 $\pm$ 0.3 (0.0 - 24)	0.8 $\pm$ 0.2 (0.0 - 3.5)	0.6 $\pm$ 0.1 (0.0 - 24.0)
Nitrate (ppm)	0.03 $\pm$ 0.02b (0.0 - 0.2)	0.03 $\pm$ 0.01b (0.0 - 0.2)	0.003 $\pm$ 0.003b (0.0 - 0.1)	0.02 $\pm$ 0.008b (0.0 - 0.2)	0.02 $\pm$ 0.008b (0.0 - 0.5)	0.09 $\pm$ 0.02a (0.0 - 0.5)	0.03 $\pm$ 0.005 (0.0 - 0.5)
Phosphate (ppm)	0.2 $\pm$ 0.06b (0.0 - 0.7)	2.7 $\pm$ 0.5a (0.0 - 8.0)	0.2 $\pm$ 0.09b (0.0 - 2.0)	0.3 $\pm$ 0.06b (0.0 - 2.0)	0.3 $\pm$ 0.07b (0.0 - 3.2)	0.5 $\pm$ 0.05b (0.0 - 1.2)	0.7 $\pm$ 0.1 (0.0 - 8.0)
Silica (ppm)	19.2 $\pm$ 2.9b (2.0 - 38.0)	12.4 $\pm$ 1.7cd (0.1 - 40.0)	29.8 $\pm$ 1.9a (8.0 - 40.0)	15.4 $\pm$ 1.4bc (1.5 - 36.0)	7.0 $\pm$ 0.8d (0.5 - 40.0)	17.0 $\pm$ 1.6bc (0.5 - 40.0)	14.7 $\pm$ 0.7 (0.1 - 40.0)

Mosquito Breeding Habitats

	Ground Pool	Riverbed Pool	Streambed Pool	Stream	Marsh	Canal Turnout	ALL HABITATS
Number of Samples	20	45	29	63	76	47	280
Sulphide (ppm)	0.05 ± 0.2 (0.0 - 1.0)	0 0	0 0	0 0	0 0	0 0	0.007 ± 0.005 (0.0 - 1.0)
Sulphate (ppm)	0 0	1.1 ± 0.7 (0 - 20)	10.3 ± 7.6 (0 - 200)	0 0	1.6 ± 0.9 (0 - 60)	5.1 ± 3.0 (0 - 100)	2.5 ± 0.9 (0 - 200)
Chloride (ppm)	25.8 ± 2.9b (16 - 72)	29.2 ± 1.3b (20 - 56)	80.4 ± 16.7a (7 - 400)	37.3 ± 2.6b (12 - 120)	81.8 ± 4.4a (16 - 176)	26.5 ± 2.2b (12 - 104)	49.7 ± 2.7 (7 - 400)
Turbidity (JTU)	60.0 ± 60.0b (0 - 1200)	48.9 ± 32.2b (0 - 1400)	10.3 ± 5.8b (0 - 100)	0b 0	2.6 ± 2.6b (0 - 200)	937.2 ± 176.9a (0 - 6400)	171.3 ± 36.6 (0 - 6400)
Total Alkalinity (ppm)	169.9 ± 14.6b (92 - 360)	154.8 ± 6.4b (72 - 270)	265.7 ± 37.1a (28 - 752)	216.7 ± 12.8ab (4 - 440)	213.1 ± 15.7ab (24 - 672)	191.5 ± 11.3b (84 - 576)	195.5 ± 7.1 (24 - 672)
Total Hardness (ppm)	171.9 ± 20.8ab (76 - 360)	130.6 ± 10.5bc (64 - 460)	272.3 ± 33.9a (24 - 596)	214.9 ± 19.8a (28 - 608)	169.3 ± 9.3ab (38 - 308)	124.8 ± 15.1c (28 - 440)	135.7 ± 7.6 (24 - 608)

ppm = Parts per million; JTU - Jackson turbidity units; alkalinity and hardness are expressed as ppm CaCO<sub>3</sub>

Values are given to the nearest significant decimal. Row means with dissimilar letters are significantly different (P < 0.05) based on Kruskal-Wallis ANOVA followed by Turkey's non-parametric multiple comparisons test. Absence of letters denotes non-significance of ANOVA

Table II

Abundance (mean immature/dip/sample  $\pm$  se) of mosquito species in habitats measured for physico-chemical variables.

Number of Samples	Ground Pool	Riverbed Pool	Streambed Pool	Stream	Marsh	Canal Turnout
	20	45	29	63	76	47
<i>An. barbirostris</i> (n= 127)	0.03 $\pm$ 0.02ab	0.004 $\pm$ 0.004b	0.1 $\pm$ 0.04a	0.01 $\pm$ 0.003ab	0.009 $\pm$ 0.003ab	0.003 $\pm$ 0.001ab
<i>An. barbumbrosus</i> (n = 163)	0.07 $\pm$ 0.05b	0b	0.1 $\pm$ 0.06a	0.007 $\pm$ 0.002b	0.002 $\pm$ 0.001b	0b
<i>An. culicifacies</i> (n= 754)	0b	2.2 $\pm$ 0.4a	0.01 $\pm$ 0.01b	0b	0b	0.002 $\pm$ 0.001b
<i>An. jamesii</i> (n = 95)	0	0	0	0.001 $\pm$ 0.001	0.04 $\pm$ 0.02	0.002 $\pm$ 0.001
<i>An. nigerrimus</i> (n = 102)	0b	0b	0.02 $\pm$ 0.02ab	0.003 $\pm$ 0.002b	0.04 $\pm$ 0.01a	0b
<i>An. subpictus</i> (n = 668)	0.06 $\pm$ 0.03ab	0b	0b	0.06 $\pm$ 0.03ab	0.01 $\pm$ 0.009b	0.29 $\pm$ 0.12a
<i>An. vagus</i> (n = 1308)	1.5 $\pm$ 0.6a	1.2 $\pm$ 0.6b	0.03 $\pm$ 0.02c	0c	0.02 $\pm$ 0.02c	0.22 $\pm$ 0.06b
<i>An. varuna</i> (n = 84)	0b	0b	0.02 $\pm$ 0.008ab	0.03 $\pm$ 0.006a	0.001 $\pm$ 0.001b	0.001 $\pm$ 0.001b
<i>Cx. bitaeniorhynchus</i> (n = 88)	0	0.1 $\pm$ 0.04	0.01 $\pm$ 0.01	0	0.009 $\pm$ 0.004	0
<i>Cx. mimulus</i> (n = 1000)	0.9 $\pm$ 0.3a	0.3 $\pm$ 0.3b	1.4 $\pm$ 0.5a	0.001 $\pm$ 0.001b	0.002 $\pm$ 0.001b	0.03 $\pm$ 0.02b
<i>Cx. pseudovishnui</i> (n = 1301)	0.02 $\pm$ 0.01ab	0b	0.001 $\pm$ 0.001b	0.001 $\pm$ 0.001b	0.6 $\pm$ 0.3a	0.001 $\pm$ 0.001b
<i>Ma. annulifera</i> (n = 219)	0b	0b	0b	0b	0.1 $\pm$ 0.3a	0b
<i>Mi. hybrida</i> (n = 239)	0b	0b	0b	0b	0.1 $\pm$ 0.03a	0b
<i>Mi. chamberlaini</i> (n = 135)	0b	0b	0b	0.001 $\pm$ 0.001b	0.07 $\pm$ 0.02a	0b

Values are given to the nearest significant decimal. Row means with dissimilar letters are significantly different ( $P < 0.05$ ) based on Kruskal-Wallis ANOVA followed by Turkey's non-parametric multiple comparisons test. Absence of letters denotes non-significance of ANOVA.

**Table III**  
Means ( $\pm$  se) and ranges of physico-chemical conditions under which different anopheline species occurred in breeding habitats

	<i>An. barbirostris</i>	<i>An. barbumbrosus</i>	<i>An. culicifacies</i>	<i>An. jamesii</i>	<i>An. nigerrimus</i>	<i>An. subpictus</i>	<i>An. vagus</i>	<i>An. varuna</i>
Temperature	27.2 $\pm$ 0.5 (22.0 - 38.0)	27.3 $\pm$ 0.7 (22.0 - 39.0)	29.5 $\pm$ 0.3 (23.0 - 33.0)	26.7 $\pm$ 0.4 (24.0 - 30.0)	26.3 $\pm$ 0.3 (23.0 - 30.0)	28.2 $\pm$ 0.6 (24.0 - 37.0)	29.9 $\pm$ 0.5 (24.0 - 39.0)	26.6 $\pm$ 0.3 (23.0 - 30.0)
Ph	7.8 $\pm$ 0.08 (6.5 - 10.0)	7.7 $\pm$ 0.08 (6.5 - 8.5)	8.5 $\pm$ 0.09 (7.5 - 9.5)	7.6 $\pm$ 0.1 (7.0 - 9.5)	7.5 $\pm$ 0.08 (6.5 - 9.5)	7.9 $\pm$ 0.08 (7.5 - 9.5)	7.9 $\pm$ 0.07 (7.0 - 9.5)	7.7 $\pm$ 0.04 (7.0 - 8.0)
Oxygen (ppm)	3.8 $\pm$ 0.4 (0.0 - 9.8)	4.1 $\pm$ 0.4 (0.0 - 9.8)	9.6 $\pm$ 0.8 (0.0 - 26.6)	2.5 $\pm$ 0.4 (0.0 - 6.6)	2.0 $\pm$ 0.3 (0.0 - 7.4)	4.9 $\pm$ 0.3 (0.8 - 7.6)	5.9 $\pm$ 0.6 (0.0 - 26.6)	4.8 $\pm$ 0.3 (1.4 - 8.0)
Carbon dioxide (ppm)	14.3 $\pm$ 1.6 (0.0 - 50.0)	18.3 $\pm$ 2.5 (2.8 - 65.0)	4.8 $\pm$ 1.0 (0.0 - 31.0)	10.1 $\pm$ 1.0 (0.0 - 17.0)	12.9 $\pm$ 1.8 (0.0 - 50.0)	5.9 $\pm$ 0.7 (0.0 - 16.0)	8.8 $\pm$ 1.1 (0.0 - 40.0)	13.7 $\pm$ 1.9 (3.0 - 65.0)
Ammonia N <sub>2</sub> (ppm)	0.2 $\pm$ 0.1 (0.0 - 3.5)	0.04 $\pm$ 0.04 (0.0 - 1.5)	1.5 $\pm$ 0.5 (0.0 - 16.0)	0.09 $\pm$ 0.09 (0.0 - 2.0)	0 0	0.3 $\pm$ 0.1 (0.0 - 2.0)	0.8 $\pm$ 0.3 (0.0 - 16.0)	0.1 $\pm$ 0.07 (0.0 - 2.5)
Nitrate N <sub>2</sub> (ppm)	0.01 $\pm$ 0.007 (0.0 - 0.2)	0.003 $\pm$ 0.003 (0.0 - 0.1)	0.04 $\pm$ 0.01 (0.0 - 0.3)	0.01 $\pm$ 0.01 (0.0 - 0.3)	0 0	0.08 $\pm$ 0.02 (0.0 - 0.3)	0.06 $\pm$ 0.01 (0.0 - 0.5)	0.04 $\pm$ 0.01 (0.0 - 0.3)
Phosphate (ppm)	0.2 $\pm$ 0.04 (0.0 - 1.2)	0.09 $\pm$ 0.02 (0.0 - 0.5)	2.5 $\pm$ 0.5 (0.0 - 8.0)	0.1 $\pm$ 0.04 (0.0 - 0.8)	0.1 $\pm$ 0.04 (0.0 - 8.0)	0.3 $\pm$ 0.1 (0.0 - 3.2)	1.0 $\pm$ 0.2 (0.0 - 8.0)	0.4 $\pm$ 0.09 (0.0 - 2.0)
Silica (ppm)	17.3 $\pm$ 1.7 (1.4 - 40.0)	23.6 $\pm$ 1.9 (1.0 - 40.0)	12.4 $\pm$ 1.5 (0.1 - 40.0)	7.4 $\pm$ 1.3 (0.5 - 24.0)	10.9 $\pm$ 1.9 (1.0 - 40.0)	13.5 $\pm$ 1.8 (2.0 - 36.0)	17.5 $\pm$ 1.5 (0.5 - 40.0)	15.4 $\pm$ 1.8 (0.5 - 36.0)
Sulphide (ppm)	0.02 $\pm$ 0.02 (0.0 - 0.1)	0.03 $\pm$ 0.03 (0.0 - 1.0)	0 0	0 0	0 0	0 0	0.01 $\pm$ 0.01 (0.0 - 1.0)	0 0
Sulphate (ppm)	8.0 $\pm$ 4.8 (0 - 200)	0 0	3.0 $\pm$ 2.1 (0 - 100)	0 0	0.6 $\pm$ 0.6 (0 - 20)	0.7 $\pm$ 0.7 (0 - 20)	5.2 $\pm$ 3.3 (0 - 200)	0.5 $\pm$ 0.5 (0.0 - 20.0)

	<i>An. barbirostris</i>	<i>An. barbumbrosus</i>	<i>An. culicifacies</i>	<i>An. jamesii</i>	<i>An. nigerrimus</i>	<i>An. subpictus</i>	<i>An. vagus</i>	<i>An. varuna</i>
Chloride (ppm)	47.3 ± 5.7 (16 - 280)	45.3 ± 7.4 (7 - 284)	31.1 ± 2.4 (16 - 124)	79.3 ± 10.2 (16 - 176)	59.8 ± 5.8 (16 - 176)	30.0 ± 5.9 (16 - 168)	33.5 ± 3.3 (12 - 168)	40.0 ± 3.3 (16 - 120)
Turbidity (JTU)	30.0 ± 17.9 (0 - 800)	0 0	81.0 ± 35.8 (0 - 1400)	39.8 ± 36.4 (0 - 800)	39.8 ± 36.4 (0 - 800)	861.3 ± 231.8 (0 - 6400)	412.9 ± 123.4 (0 - 6400)	15.0 ± 11.1 (0 - 400)
Total Alkalinity (ppm)	246.5 ± 18.2 (24 - 620)	230.3 ± 24.4 (36 - 752)	169.8 ± 12.9 (72 - 564)	240.9 ± 38.0 (120 - 672)	240.9 ± 37.9 (120 - 672)	199.6 ± 20.8 (100 - 640)	202.4 ± 15.1 (52 - 640)	216.2 ± 12.6 (40 - 360)
Total Hardness (ppm)	229.2 ± 23.7 (28 - 596)	217.0 ± 22.3 (26 - 472)	141.2 ± 13.2 (64 - 480)	180.0 ± 21.2 (64 - 308)	180.0 ± 21.2 (64 - 308)	111.6 ± 9.7 (60 - 204)	155.6 ± 14.3 (20 - 596)	228.7 ± 22.4 (26 - 608)

Note: Means and standard errors are reported to the first significant decimal.

**Table IV**  
**Mean  $\pm$  se (range) of physico-chemical conditions under which different culicine species occurred in breeding habitats**

	<i>Cx.</i> <i>bitaeniorhynchus</i>	<i>Cx.</i> <i>mimulus</i>	<i>Cx.</i> <i>pseudovishnui</i>	<i>Ma.</i> <i>annulifera</i>	<i>Mi.</i> <i>chamberlaini</i>	<i>Mi.</i> <i>hybrida</i>
Temperature (°C)	28.5 $\pm$ 0.6 (24.0 - 34.0)	28.3 $\pm$ 0.6 (22.0 - 38.0)	27.6 $\pm$ 0.5 (24.0 - 39.0)	28.0 $\pm$ 0.4 (25.0 - 36.0)	27.1 $\pm$ 0.4 (24.0 - 36.0)	27.6 $\pm$ 0.3 (25.0 - 30.0)
Ph	8.2 $\pm$ 0.2 (7.0 - 9.5)	7.7 $\pm$ 0.1 (6.0 - 10.0)	7.6 $\pm$ 0.07 (6.5 - 9.0)	7.5 $\pm$ 0.07 (6.5 - 8.5)	7.5 $\pm$ 0.07 (7.0 - 8.5)	7.4 $\pm$ 0.06 (6.5 - 8.0)
Oxygen (ppm)	6.9 $\pm$ 1.2 (0.0 - 18.40)	3.8 $\pm$ 0.4 (0.0 - 16.8)	2.8 $\pm$ 0.3 (0.0 - 9.8)	1.9 $\pm$ 0.3 (0.0 - 10.0)	1.8 $\pm$ 0.2 (0.0 - 5.4)	1.8 $\pm$ 0.2 (0.0 - 3.8)
Carbon dioxide (ppm)	5.2 $\pm$ 1.3 (0.0 - 18.0)	16.3 $\pm$ 1.7 (0.0 - 48.0)	14.3 $\pm$ 1.6 (0.0 - 48.0)	14.3 $\pm$ 1.0 (0.0 - 25.0)	14.2 $\pm$ 1.3 (0.0 - 31.0)	14.2 $\pm$ 1.3 (3.0 - 24.0)
Ammonia N <sub>2</sub> (ppm)	1.8 $\pm$ 1.1 (0.0 - 24.0)	0.9 $\pm$ 0.4 (0.0 - 16.0)	0.6 $\pm$ 0.6 (0.0 - 24.0)	0.2 $\pm$ 0.09 (0.0 - 2.5)	1.3 $\pm$ 0.9 (0.0 - 24.0)	0.4 $\pm$ 0.3 (0.0 - 7.0)
Nitrate N <sub>2</sub> (ppm)	0.04 $\pm$ 0.02 (0.0 - 0.5)	0.03 $\pm$ 0.01 (0.0 - 0.5)	0.02 $\pm$ 0.01 (0.0 - 0.5)	0.006 $\pm$ 0.006 (0.0 - 0.2)	0.03 $\pm$ 0.02 (0.0 - 0.5)	0.007 $\pm$ 0.007 (0.0 - 0.2)
Phosphate (ppm)	1.7 $\pm$ 0.6 (0.0 - 8.0)	0.4 $\pm$ 0.2 (0.0 - 8.0)	0.3 $\pm$ 0.08 (0.0 - 3.2)	0.3 $\pm$ 0.1 (0.0 - 3.2)	0.3 $\pm$ 0.1 (0.0 - 3.2)	0.3 $\pm$ 0.08 (0.0 - 1.6)
Silica (ppm)	8.3 $\pm$ 1.8 (0.5 - 28.0)	23.7 $\pm$ 1.9 (0.5 - 40.0)	11.7 $\pm$ 1.8 (0.5 - 40.0)	6.3 $\pm$ 1.0 (0.5 - 32.0)	6.6 $\pm$ 1.2 (1.0 - 32.0)	6.4 $\pm$ 1.0 (2.5 - 20.0)
Sulphide (ppm)	0 0	0.02 $\pm$ 0.02 (0.0 - 1.0)	0 0	0 0	0 0	0 0
Sulphate (ppm)	3.9 $\pm$ 2.7 (0 - 60)	9.4 $\pm$ 4.9 (0 - 200)	0.9 $\pm$ 0.6 (0 - 20)	0.6 $\pm$ 0.4 (0 - 10)	1.0 $\pm$ 0.6 (0 - 10)	0.4 $\pm$ 0.4 (0 - 10)

	<i>Cx.</i> <i>bitaeniorhynchus</i>	<i>Cx.</i> <i>mimulus</i>	<i>Cx.</i> <i>pseudovishnui</i>	<i>Ma.</i> <i>annulifera</i>	<i>Mi.</i> <i>chamberlaini</i>	<i>Mi.</i> <i>hybrida</i>
Chloride (ppm)	61.1 ± 13.1 (20 - 280)	52.6 ± 9.0 (7 - 400)	71.4 ± 6.4 (16 - 168)	103.3 ± 8.1 (32 - 284)	82.6 ± 6.5 (16 - 168)	98.1 ± 5.2 (36 - 140)
Turbidity (JTU)	65.2 ± 60.8 (0 - 1400)	25.5 ± 11.2 (0 - 400)	0.7 ± 6.3 (0 - 200)	6.3 ± 6.3 (0 - 200)	0 0	0 0
Total Alkalinity (ppm)	223.1 ± 31.4 (88 - 640)	202.4 ± 20.9 (4 - 620)	234.3 ± 24.4 (4 - 640)	191.2 ± 24.4 (92 - 566)	228.8 ± 28.2 (26 - 640)	187.9 ± 6.9 (132 - 280)
Total Hardness (ppm)	168.1 ± 19.5 (68 - 456)	200.5 ± 22.4 (20 - 596)	163.2 ± 15.1 (38 - 388)	198.4 ± 20.6 (80 - 472)	179.5 ± 11.3 (32 - 212)	194.8 ± 6.9 (132 - 228)

Note: Means and standard errors are reported to the first significant decimal.

**Table V**  
**Relationships between mosquito abundance and physico-chemical variables in breeding water<sup>a</sup>**

Species	Stepwise multiple regression ANOVA				Correlation analysis	
	Factor	F	df	P	Variable	r
<i>An. culicifacies</i>	1	24.58	1,278	< 0.001	Oxygen	0.29
					Phosphate	0.18
					Temperature	0.14
					Carbon dioxide	-0.14
<i>An. nigerrimus</i>	1	5.68	1,278	0.02	Oxygen	-0.14
					Temperature	-0.17
<i>An. vagus</i>	1	4.49	1,278	0.04	Oxygen	0.13
					Phosphate	0.17
					Temperature	0.19
<i>An. jamesii</i>	2	14.18	1,278	<0.001	Total Alkalinity	0.22
<i>Cx. pseudovishnui</i>	2	38.59	1,278	<0.001	Total Hardness	0.35
<i>An. subpictus</i>	3	9.03	1,278	0.003	Nitrate	0.18
<i>Cx. mimulus</i>	5	5.12	1,278	0.02	Silica	0.13
<i>Ma. annulifera</i>	1,5	6.92	2,277	0.009	Oxygen	-0.16
					Chloride	0.25
					Silica	-0.12
<i>Mi. hybrida</i>	1,5	8.19	2,277	< 0.001	Oxygen	-0.16
					Chloride	0.24
					Silica	-0.16

Species	Stepwise multiple regression ANOVA				Correlation analysis	
	Factor	F	df	P	Variable	r
<i>Mi. chamberlaini</i>	1,2,3,5	9.18	4,275	< 0.001	Oxygen	-0.13
					Chloride	0.14
					Total Alkalinity	0.19
					Ammonia	0.38
					Nitrate	0.16
					Silica	-0.16

<sup>a</sup> Stepwise multiple regression analysis of mosquito abundance (dependant variable) against Factors 1-5 defined by multivariate analysis. Only regression ANOVA data relating to significant factors ( $P < 0.05$ ) are included. Relationships between abundance and physico-chemical variables within significant factors are further analyzed by Pearson's correlation coefficient ( $r$ ). Only significant correlations ( $P < 0.05$ ) are reported.