78

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ECOLOGY OF FOUR POTENTIAL *CULEX* VECTORS (DIPTERA: CULICIDAE) OF JAPANESE ENCEPHALITIS IN KANDY, SRI LANKA.

by

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ABSTRACT

A field study on the four major potential vectors of Japanese encephalitis in Sri Lanka, i.e., *Culex tritaeniorhynchus* Giles, *Cx. gelidus* Theobald, *Cx. pseudovishnui* Colless and *Cx. fuscocephala* Theobald, was done from September 1986 - December 1987 at 4 study sites around the city of Kandy, in the Central Province of Sri Lanka. A total of 73,968 mosquitoes belonging to 10 genera and 45 species were trapped by CDC light traps operated on a weekly basis in a piggery at each site. Nearly 88% of light-trapped mosquitoes belonged to the above four species. Two population peaks were observed in May - June and October - December, coinciding with the periodicity of monsoonal rains and rice cultivation cycles. Time segregated light trap catches showed that the four species were active throughout the night, with dusk and dawn peaks in *Cx. pseudovishnui* and *Cx. gelidus* respectively.

INTRODUCTION

Japanese encephalitis (JE) is a major public health problem in the Oriental and Southeast Asian regions, with a distribution ranging from Japan to India (Umenai et al. 1985). The disease has probably been endemic in Sri Lanka for decades, but has become a serous public health problem relatively recently, following epidemics in 1985-86 and 1987-88 in the low country dry zone areas of the north-central and northwestern Provinces of the country.

The principal pan-asian vector of JE is widely accepted as being *Culex tritaeniorhynchus* Giles. However, species such as *Cx. vishnui* Theobald, *Cx. gelidus* Theobald and *Cx. fuscocephala* Theobald, have been incriminated as vectors in different areas of the region (Gould *et al.*, 1974; Olson *et al.*, 1985; Dhanda and Kaul, 1980; Hu and Grayston, 1962). In Sri Lanka, *Culex tritaeniorhynchus*, *Cx. gelidus* and *Cx. fuscocephala* have been incriminated as major vectors of epidemic JE (Peiris *et al.* 1992). Virus isolation-based evidence also has implicated *Cx. pseudovishnui* Colless in JE transmission in the country (Peiris *et al.* 1987).

This paper presents the results of a study on the abundance, seasonality and nocturnal periodicity of these 4 mosquito species at sites around the city of Kandy, an area where JE transmission, as evidenced by porcine seropositivity (Peiris *et al.* 1987) occurs in nature, though outbreaks of human disease have not been recorded to date.

MATERIALS AND METHODS

The study was done during September 1986 - December 1987 at sites around the city of Kandy, situated in the Central Province (upland, wet zone) of Sri Lanka (Lat. 5°55'-9°51', Long. 79°41'-81°53'), at a mean elevation of 530m. above sea level. Mean daily temperature ranged from 13.0 - 35.9°C and mean annual rainfall from 1900 - 2500 mm. Monthly rainfall data for Kandy during the study period are presented in Figure 1A.

Mosquitoes were collected from 4 study sites: (I) Pilimatalawa, a low-lying village habitat dominated by rice fields and home gardens situated on the western outskirts of the city; (II) Udaperadeniya, a hilly village area of mainly wooded slopes and some terraced rice fields, also on the western flank of the city; (III) Ampitiya, a hilly semi-urbanized area with a moderate extent of rice fields situated on the south-eastern flank of the city; (IV) Bahirawakanda, a semi-urbanized area of grassy slopes within the municipal limits of the city.

Collections were done at weekly intervals, using 2 CDC light traps suspended 1-2 m. above ground level and operated overnight between 1800-0600 hr. within a piggery at each site. Each piggery contained 10-20 pigs. Trapped mosquitoes were taken live to the laboratory, anaesthetized using chloroform and identified to species and sex using standard taxonomic keys and descriptions (see references in Amerasinghe and Ariyasena, 1990). Female trophic status was recorded for the four *Culex* species studied. To observe nocturnal periodicity, hourly segregated collections were done at site I (Pilimatalawa) using a single trap on 10 nights during a population peak in November - December 1987.

Mosquito density indices are reported in terms of prevalence (percentage of each species of the total collected) and abundance (mean number of females per trap-night ± standard deviation (SD). Since there was heterogeneity of variance among means at different sites (Bartlett's test, $p < 0.05$), site comparisons of mean monthly abundance were done by non-parametric methods using Kruskall-Wallis Analysis of Variance (ANOVA) followed by non-parametric Tukey-type multiple comparisons testing (Zar, 1984). The relationship between monthly rainfall and mean monthly mosquito abundance (lagged by 1 month to allow for immature development) was examined by Spearmen's Rank Correlation (Zar, 1984).

RESULTS

A total of 73,968 mosquitoes representing 10 genera and 44 species were taken from 416 trap-nights of collection. The majority (81.2% of specimens representing 8 genera and 38 species) were trapped at site I, followed in decreasing order by sites II (12.2%, 9 genera, 36 species), III (5.1%, 9 genera, 35 species) and IV (0.8%, 6 genera, 18 species). Mean mosquito catch rates per trap-night at sites I - IV were 500.4, 75.9, 36.1 and 15.4, respectively. The all-site mean catch rate was 117.8 per trap night.

Culex tritaeniorhynchus, *Cx. gelidus*, *Cx. pseudovishnui* and *Cx. fuscocephala* collectively accounted for 87.6% of the total catch and were the four most prevalent species at every site. More than 99% of trapped individuals were females; the majority (95.4%) were unfed or freshly engorged, but small proportions of partially gravid (4.3%) or fully gravid (0.3%) individuals also were represented.

Table 1. Abundance of 4 Culex species at different sites in Kandy.

Species	Site I	Site II	Site III	Site IV
Cx. fuscocephala	118.7 ± 144.9a	3.5 ± 7.3b	4.3 ± 5.6b	3.9 ± 8.7b
Cx. gelidus	50.2 ± 58.8a	7.2 ± 14.2b	2.4 ± 3.1b	1.4 ± 2.8b
Cx. pseudovishnui	134.3 ± 151.0a	29.7 ± 36.6b	10.4 ± 11.3b	6.6 ± 11.3b
Cx. tritaeniorhynchus	80.7 ± 105.8a	3.6 ± 5.7b	3.5 ± 4.1b	1.5 ± 2.1b

a Mean females per trap-night ± SD
Statistical analysis by Kruskall-Wallis ANOVA and non-parametric Tukey-type multiple-comparisons testing. Row means followed by different letters are significant at the $p < 0.001$ level.

All 4 species were significantly more abundant at site I than at the other three sites (Table 1). Patterns of seasonal abundance were similar in all species at all sites (data not shown); the pattern at site I is here presented, since this was the most productive site for all 4 species (Figure 1B). Two clear population peaks were evident, the first in May - June and the second in October - December. These coincided with the periodicities of the southwest and northeast monsoons, respectively, and the main rice cultivation cycles in the area. Periods of drought in February and July of 1987 were reflected in markedly reduced abundance during February - March and August - September of that year. Abundance was significantly positively correlated with rainfall in all species, the values being: *Cx. tritaeniorhynchus* ($r = 0.51$, df = 14, $p = 0.04$), *Cx. pseudovishnui* ($r = 0.66$, df = 14, $P = 0.006$), *Cx. gelidus* ($r = 0.61$, df = 14, $p = 0.01$), and *Cx. fuscocephala* ($r = 0.56$, df = 14, $p = 0.02$).

Time segregated collections at site I showed that all 4 species were active throughout the night (Figure 2) with some evidence of an evening post-crepuscular peak in *Cx. pseudovishnui*, a late night peak in *Cx. tritaeniorhynchus*, and a pre-dawn peak in *Cx. gelidus*. No clear peaks were evident in *Cx. fuscocephala*. Light traps would not have been effective in attracting mosquitoes during pre-sunset and post-dawn hours when the natural light intensity was high. Thus we can draw no conclusions regarding mosquito activity during these periods.

FIGURE 1

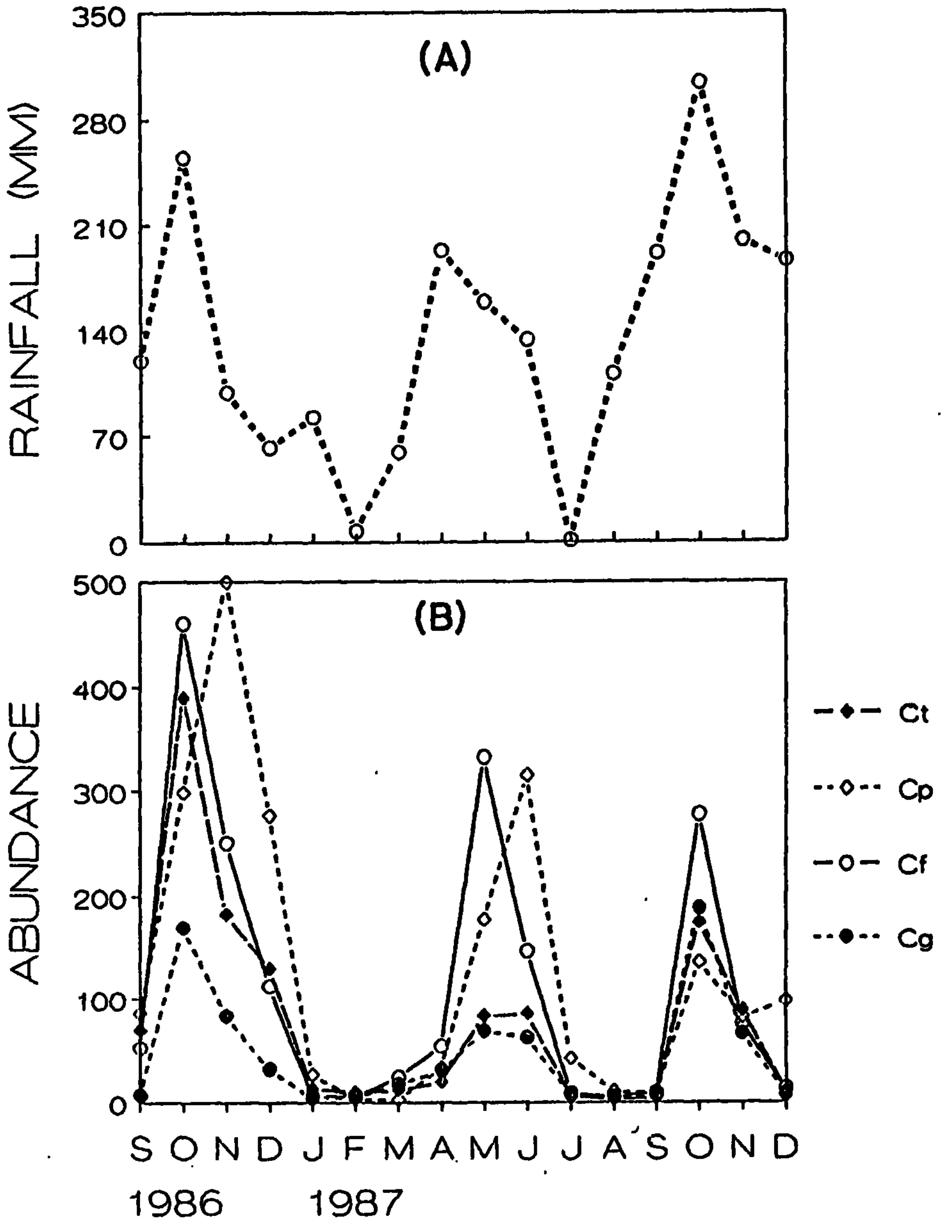

Fig. 1. (A) Monthly rainfall at Kandy, recorded at the Agricultural Research station, Gannoruwa; (B) Mean monthly abundance (females per trap night) of *Cx. fuscocephala* (Cf), *Cx. gelidus* (Cg), *Cx. pseudovishnui* (Cp), and *Cx. tritaeniorhynchus* (Ct), at site I (Pilimatalawa) during September 1986 - December 1987.

FIGURE 2

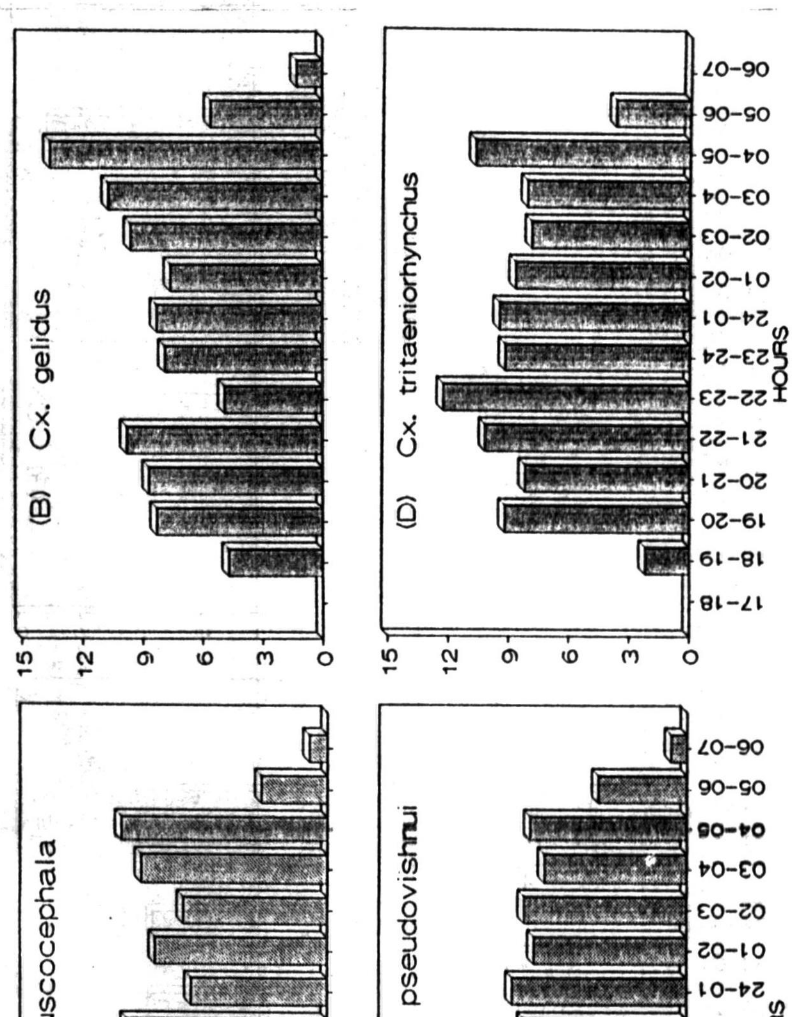

Fig. 2. Nocturnal periodicity of (A) *Cx. fuscocephala*, (B) *Cx. gelidus*, (C) *Cx. pseudovishnui* and (D) *Cx. tritaeniorhynchus* collected in CDC light traps at site I (Pilimatalawa) during November - Decmber 1986.

DISCUSSION

Light traps are not unbiased tools in sampling mosquitoes because different species exhibit different degrees of phototaxy (Service 1976). However, CDC traps have been widely used to monitor populations of *Culex* vectors of JE (Cates *et al.*, 1969; Ree *et al.*, 1969; Simasathien *et al.*, 1972; Self *et al.*, 1973; Ksiazek *et al.*, 1980; Olson *et al.*, 1985). In Sri Lanka, too, these traps have been successfully used to monitor potential JE vectors in an epidemic area (Peiris *et al.* 1992, 1993).

Culex pseudovishnui was clearly the most abundant of the potential JE vectors in Kandy. This is the only species that has been implicated in natural transmission of the virus in the Kandy area (Peiris *et al.* 1987). Elsewhere in Sri Lanka, *Cx. tritaeniorhynchus*, *Cx. gelidus* and *Cx. fuscocephala* have been implicated as major epidemic vectors (Peiris *et al.* 1992).

All 4 species are ground water breeders, with *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* being closely associated with rice fields (Reuben 1971c; Amerasinghe and Ariyasena 1990). *Culex gelidus* and *Cx. fuscocephala* breed in a variety of temporary and permanent ground water habitats (Sirivanakarn, 1976, Herath *et al.* 1986; Amerasinghe and Munashingha, 1988; Amerasinghe and Ariyasena, 1990); the former, in particular, favours polluted habitats such as husk pits (Lawrence and Samarawickrema, 1966; Samarawickrema *et al.* 1982) which are not common in the Kandy area. Ground water habitats were plentiful at the low lying areas around site I (Pilimatalawa) where all 4 species where the most abundant. Such sites where less plentiful at sites II (Udaperadeniya) and III (Ampitiya) situated on hillier terrain. Site IV was steeply hilly and breeding sites were scarce in its immediate vicinity. Thus, the significant site-related differences in abundance of the 4 species sites were probably related to the availability of breeding habitats.

The seasonal peaks in the abundance of these species corresponded to periods of monsoonal rains and rice cultivation cycles. Similar patterns have been recorded elsewhere in the region as well. Reuben (1971a) and Gould *et al.* (1974) have shown the importance of rainfall in initiating breeding, while Heathcote (1970) and Hill (1970) have emphasized the role of breeding habitats created by rice field flooding, independent of rainfall patterns. In Kandy, only rain-fed rice cultivation was practiced; thus mosquito population peaks were associated with breeding in both riceland habitats and a variety of rain-fed pools.

All 4 species have been observed previously to be active throughout the night, but with varying patterns of peaks in different geographical areas of the SE Asian region (Hu and Grayston, 1962; Reuben, 1971b; Reisen *et al.*, 1976; Reisen and Aslamkhan, 1978). Thus, the risk of being bitten by virus infected females exists throughout the nocturnal period, unlike in some mosquito vectors that show narrowly defined biting periodicities.

Considered cumulatively, the abundance of these 4 *Culex* species in Kandy was approximately 15 times lower than during the same period at Anuradhapura (North Central Province), where epidemic JE occurs, and approximately 5 times higher than in the highlands (Nuwara Eliya District) where the disease has not been reported to date (Peiris *et al.* 1993). Porcine seropositivity (which is a sensitive index of the level of JE virus activity in nature) in Kandy has increased rapidly from a very low level (4%) in 1984 to 64% in 1986 (Peiris *et al.* 1987), indicating increased virus transmission in nature during this period. While human outbreaks have so far been limited to the low country dry zone of the island, mid-elevation areas such as Kandy pose a distinct risk for JE should vector abundance reach levels comparable with those in the dry zone.

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