

## SURVEILLANCE OF MOSQUITOES AND ARBOVIRUS INFECTION AT THE ROSS RIVER DAM (STAGE 1), AUSTRALIA

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**ABSTRACT.** This paper describes the temporal and spatial abundance of the mosquito fauna of the Ross River Dam (Stage 1) in northern Queensland, Australia. *Culex annulirostris*, *Anopheles annulipes* s.l., *Mansonia uniformis*, *Mansonia septempunctata*, and the nondam breeding *Aedes vigilax* were the major species collected by dry ice-supplemented light traps set at various distances from the edge of the reservoir. To estimate the level of arbovirus activity in these different zones, sentinel chicken flocks were bled 4 times a year and their antibody conversion rates determined by the hemagglutination-inhibition test. Although mosquito abundance at sites close to the reservoir were 1.5-6.1 times higher than at the more distant sites, arbovirus conversion rates, particularly to the alphaviruses Ross River and Sindbis, varied according to zone and year, suggesting that risk of infection was no greater around the dam than elsewhere.

### INTRODUCTION

In a previous article (Kay et al. 1990), we pointed out that Australia is fortunate in lacking widespread and serious vector-borne diseases, although the alphaviruses Ross River (RR) and Barmah Forest (BF), and flaviviruses Murray Valley encephalitis (MVE), Kunjin (KUN), Kokobera (KOK), and dengue (DEN) cause clinical infection. In that paper we also described the Ross River Dam (19°26'S, 146°45'E) near Townsville, north Queensland, and indicated that mosquito and arbovirus activity was high in its vicinity. Our present submission analyzes those data further by examining the spatial and temporal abundance of the mosquitoes, especially those of public health importance, in relation to arbovirus activity at different distances away from the dam.

In relation to surveillance methodologies, our basic hypothesis relates to mosquito abundance as a reliable indicator of arbovirus transmission. Bunnag et al. (1979) found that anophelines and malaria prevalence increased with the construction of the Srinagarind Dam in western Thailand. In his concise review, Eldridge (1987) pointed out that measures of mosquito abundance alone generally have been poor indicators of arbovirus epidemics, but nevertheless, in California, Reeves

et al. (1990) were able to relate light trap returns to the activity of western equine encephalomyelitis and St. Louis encephalitis viruses. Trap counts of *Culex tarsalis* Coq. were then used as a basis for implementing control.

As the costs of surveillance systems increase with their complexity, our basic approach towards designing such a system was first through detailed understanding of spatial and temporal trends of vector populations and second, through association of those populations to arbovirus transmission. The potential elucidation of a simplistic management tool based solely on abundance has advantages over those which out of necessity include other epidemiological considerations.

### MATERIALS AND METHODS

**Study area:** The Ross River Dam (Stage 1) was constructed in 1973 with an augmented storage capacity of 109,000 megaliters and at full supply level inundates approximately 3,000 ha. Details of the dam have been given by Kay et al. (1990). In 1987-88, proposed works for enlargement to Stage 2a were completed, which increased the storage capacity to 236,000 megaliters.

Weekly recordings of the level of water at the spillway, and monthly calculations of the amount of water stored in the dam, were maintained by the Townsville City Council, and meteorological data were recorded at James Cook University, 2 km away from the dam.

**Mosquito sampling:** Adult mosquitoes were sampled with Encephalitis Virus Surveillance (EVS) light traps baited with dry ice (Rohe and Fall 1979), modified by the addition of a photosensitive switching device to turn the traps on and off at dusk and dawn. A baffle was installed between the fan unit and the collection bag to prevent the escape of collected mosquitoes when

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the light trap switched off. Traps were suspended 1 m above the ground in shaded areas and operated for 2 consecutive nights per week from January 1984 to April 1985. From May 1985 to September 1985, trapping frequency was reduced to one night per week for 3 wk and 2 nights per week for the other week.

Seven EVS trap sites for mosquito sampling were selected to represent 3 distinct zones:

- 1) Zone 1, adjacent to the Stage 1 shoreline (3 trap sites). Two sites were chosen along the northern shoreline (Big Bay, Ti-Tree Bay) and one on the eastern shoreline (Round Island). The 3 sites were vegetatively similar, being dominated by open eucalypt woodlands with ti-trees (*Melaleuca* species) also common in the wetter soils, at the wetter end of slopes (Round Island), and in broad drainage depressions.
- 2) Zone 2, about 2 km from the shoreline (2 trap sites, at Oak Valley and Toonpan). These were sited close to the proposed shoreline of Stage 2 of the dam, thus providing baseline data of future monitoring. Both comprised open woodland, interspersed with the introduced shrub *Zizyphus mauritania*, and with open areas cleared of vegetation for rural residential development (Oak Valley) or grazing (Toonpan).
- 3) Zone 3, about 4 km from the shoreline (2 trap sites, Kelso and Stanley). These 2 localities will still be at some distance from the dam after the Stage 2 expansion. As with the Zone 2 sites, Kelso and Stanley had been cleared for residential development and for grazing, respectively. Trapping did not begin until April 1984 at Stanley.

*Sentinel chicken flocks and virus surveillance:* Seven flocks each of 10–30 chickens were maintained within Zone 1 (Big Bay, Round Island), Zone 2 (Oak Valley, Toonpan), and Zone 3 (Kelso, Stanley) with a supplementary flock being held at Pallarenda (an area 15 km to the northeast bordering coastal saltmarsh, and here labelled as Zone 4). Each November, prior to the wet season, which was recognized as the probable period of greatest arbovirus activity, antibody-positive chickens were discarded and flocks replenished with nonimmunized birds. Chickens were bled from the wing vein 3 or 4 times per year: usually in mid-wet season (February–March), and at the end of the wet season (April–May) to define this activity and once thereafter until November. A flock was maintained at each of the 6 sites from November 1983 to June 1987. Sampling at the 7th site, Pallarenda, was terminated in February 1986.

Sera were dispatched to the Laboratory of Mi-

crobiology and Pathology, Queensland Health, Brisbane, for testing by the hemagglutination-inhibition (HI) test, using a modified Clarke and Casals (1958) procedure. Ether was deleted from the extraction procedure and acetone-extracted goose erythrocyte adsorbed sera was used.

Ross River, Sindbis, Murray Valley encephalitis, and Getah hemagglutination antigens were prepared in suckling mice using sucrose acetone extraction procedures. Flavivirus antigens were prepared initially using the above procedure, but were later prepared in C6/36 cell culture fluid which was precipitated with polyethylene glycol and resuspended in borate saline buffer (pH 9.0).

Virus was identified when titers were at least 4-fold higher than other group reactors, or when it was the sole reaction.

*Analysis:* All mosquito counts were transformed to  $\log(\text{count} + 1)$  to stabilize the variance in formal analyses, and all statistical analyses were carried out using SAS Institute (1988) procedures. Three main sets of analyses were carried out to examine the behavior of mosquito populations.

- 1) To compare the species composition of the total catch at each trap location, cluster analyses were used, with the data transformed to standard scores with mean 0 and variance 1. The results presented use average linkage cluster analysis, but the same clustering sequences were obtained using alternative procedures (centroid, flexible beta, complete linkage).
- 2) To examine the extent to which mosquito abundance at different sites covaried through the sample period, Pearson's correlation coefficients were calculated for each pair of sites, using as data points the 115–149 separate collections made at each site. These correlation coefficients were calculated for the 4 most common species breeding at the reservoir, and also for *Aedes vigilax* (Skuse), which does not breed at the reservoir. Because *Ae. vigilax* is a coastal saltmarsh species that hatches synchronously in response to tidal cycles, all individuals of this species that dispersed to the dam environs were probably derived from a common source. We would therefore expect that its abundance would vary in similar ways at all sites, and hence that correlations among all trapping sites would be uniformly high. Similarly, freshwater-breeding species derived from sites subject to the same environmental influences should similarly show high correlations. Conversely, a low correlation for a particular pair of sites may indicate that mosquito abundance is determined by different factors at each site.

3) To examine the seasonal pattern of abundance, and the effects of zone and location within zone, on the abundance of particular taxa and on the total catch, mixed model analyses of variance were performed (using the general linear model procedure to allow for the unbalanced design). To accommodate the data set to the computing capacity available, the sampling period was divided into 2 monthly intervals, and trap-nights within an interval were treated as replicates. The analytical design therefore involved 2 crossed factors, Zone and Month (both fixed factors), with Locations (random factor) nested within Zones, and a variable number of observations for each factor combination. This analysis was applied to the total mosquito counts, and also to the 5 most abundant taxa. Four of these (*Culex annulirostris* Skuse, *Anopheles annulipes* s.l. Walker, *Mansonia uniformis* Theobald, and *Mansonia septempunctata* Theobald) were known to breed in the reservoir. The 5th, *Ae. vigilax*, does not breed in the reservoir, as noted earlier.

The sentinel chicken data were analyzed by logistic regression using GLIM 3.77 (Baker and Nelder 1978) to compare the proportion of the chicken flocks that developed antibodies to alphaviruses and flaviviruses among years and among sites.

## RESULTS

*Weather and water levels throughout the study:* In 1984, wet season rains were restricted to January and February (ca. 1,000 mm) but in 1985, the wet season failed. After February 1984 when the capacity was 101,000 megaliters, the volume steadily decreased to 30,500 megaliters at the termination of the study in September 1985. Minimum and maximum monthly temperatures varied from July (ca. 13–24°C) to January (ca. 24–32°C).

*Species composition and total catch of mosquitoes:* The number of species captured (Table 1) did not differ greatly between zones; if anything, locations in Zone 2 were slightly more species-rich than locations in the other 2 zones. Traps in Zones 1 and 2 caught more (average 80 and 55, respectively) individuals than traps in Zone 3. There are also smaller differences between locations within each zone: within Zone 3, fewer mosquitoes were caught at Stanley than at Kelso; and in Zone 2, fewer were caught at Oak Valley than at Toonpan.

Mosquitoes were categorized into 2 groups: those known to breed at the dam, and those not

recorded as larvae at the dam (Rae 1983<sup>5</sup>). Because some species known to breed in the reservoir also breed in other freshwater bodies within all 3 zones, the extent to which the community structure of the dam-breeding species follows a zonal pattern gives some evaluation of the extent to which mosquitoes derived from the dam dominate the region's mosquito fauna, and also gives an estimate of which zones are subject to the reservoir's influence. The non-dam-breeding species provide a form of "control," because there is no reason to expect them to follow a zonal pattern.

Clustering only on the dam-breeding species (Fig. 1) separates the sites into 2 major groupings: the 5 locations in Zones 1 and 2, vs. the 2 locations in Zone 3. Within the first group, locations do not completely separate by zone: the species composition of Round Island (Zone 1) was more similar to the Zone 2 sites than it was to the other Zone 1 sites. The main reason for this was its relatively low numbers of the 2 *Mansonia* species compared to the other 2 Zone 1 sites (see Table 1). This analysis suggests that the effects of the dam on mosquito abundance and species composition extend for at least 2 km from the shoreline, and that mosquitoes from the dam probably do dominate the mosquito fauna within this area.

Clustering on species that do not breed in the reservoir gave a very different pattern (Fig. 1). Toonpan was clearly the most dissimilar to the other 6 locations, mainly because of its very high abundance of a number of the *Aedes* species (Table 1). Although Big Bay and Ti-Tree Bay proved to be the most similar locations (note that they are also the 2 closest together), site groupings did not correspond in any way to the zones.

*Temporal correlations among sites in species abundances:* Examination of the patterns of correlation for particular species in cases among sites (Table 2) strongly reinforces the conclusions above. We would, in general, expect to see positive correlations among sites, since they share a common seasonal pattern. But we would expect to see stronger correlations where the sites receive mosquitoes from a common source: that is, for dam-breeding species, among sites close to the dam, and for the highly mobile saltmarsh mosquito *Ae. vigilax*, among all sites.

For *Ae. vigilax*, there are indeed strong correlations between all pairs of sites, supporting

<sup>5</sup> Rae, D. 1983. The mosquito larvae of Ross Dam with particular reference to the ecology of *Culex annulirostris*. B.Sc. (Honors) thesis. Department of Biological Sciences, James Cook University of North Queensland, Townsville.

Table 1. Mosquitoes captured by CO<sub>2</sub>-baited EVS light traps at 7 sites<sup>1</sup> near the Ross River Dam between January 1984 and September 1985.

Taxon	BB	TT	RI	OV	Tp	Ke	St
<i>Aedes alboscuteUellatus</i>	0	1	0	2	12	19	0
<i>Aedes alternans</i>	1	0	1	4	5	3	0
<i>Aedes elchoensis</i>	10	3	9	4	99	2	0
<i>Aedes kochi</i>	1	1	0	2	34	9	3
<i>Aedes lineatopennis</i>	2	0	3	15	1,045	80	1
<i>Aedes mallochi</i>	0	0	0	0	3	0	0
<i>Aedes normanensis</i>	27	15	192	36	1,139	163	52
<i>Aedes notoscriptus</i>	0	0	0	49	28	15	22
<i>Aedes purpureus</i>	1	1	3	3	77	0	0
<i>Aedes quasirubithorax</i>	0	0	0	0	5	0	1
<i>Aedes vigilax</i>	379	230	94	313	373	323	444
<i>Aedes vittiger</i>	12	4	139	19	385	19	4
<i>Aedeomyia catasticta</i>	11	99	61	365	101	115	73
<i>Anopheles amictus</i>	97	37	548	42	65	44	133
<i>Anopheles annulipes</i>	8,850	2,919	6,381	1,273	1,175	317	293
<i>Anopheles bancroftii</i>	146	70	73	34	18	4	6
<i>Anopheles meraukensis</i>	107	68	366	20	227	23	33
<i>Coquillettidia crassipes</i>	78	324	47	78	83	58	11
<i>Culex annulirostris</i>	2,478	2,138	1,806	3,533	4,885	928	351
<i>Culex bitaeniorhynchus</i>	29	44	29	13	20	6	0
<i>Culex pullus</i>	0	0	0	0	2	2	0
<i>Culex quinquefasciatus</i>	36	36	55	173	122	81	1
<i>Mansonia uniformis</i>	2,188	2,316	354	39	72	21	5
<i>Mansonia septempunctata</i>	330	1,518	109	60	74	27	6
<i>Uranotaenia albescens</i>	2	0	2	5	3	0	0
<i>Uranotaenia nivipes</i>	2	4	0	1	0	0	0
Number collected	14,787	9,828	10,272	6,083	10,052	2,259	1,439
Number of species collected	21	19	19	23	25	21	17
Number of collections	146	143	145	149	146	149	120

<sup>1</sup> Zone 1 locations: BB—Big Bay; TT—Ti-Tree Bay; RI—Round Island. Zone 2 locations: OV—Oak Valley; Tp—Toonpan. Zone 3 locations: Ke—Kelso; St—Stanley.

the expectation that abundances of this species would vary in a similar way at all sites (see also Fig. 2). There was no particular tendency for correlations among sites within a zone to be stronger than correlations among sites in different zones.

For *Cx. annulirostris*, Zone 1 and 2 sites were strongly correlated within their groupings, but Zone 3 sites, on opposite sides of the reservoir, were only weakly correlated with each other. All pairs of sites gave significant correlations.

*Anopheles annulipes* shows a clear zonal pattern in its correlations. Zone 1 sites, closest to the dam, are all strongly correlated with each other. Correlations between Zone 1 and Zone 2 sites are less predictable: Zone 1 sites are all strongly correlated with one of the Zone 2 sites (Oak Valley) but not the other (Toonpan). Zone 3 sites are only weakly correlated or are not correlated with sites from other zones, or indeed with each other.

For the *Mansonia* species, the very low num-

bers of individuals collected at sites in Zones 2 and 3 in itself suggest that the reservoir produces all or most of these mosquitoes. Sites in Zones 1 and 2 tended to be well correlated with each other (except for Round Island, where few *Mansonia* were caught). Correlations involving Zone 3 sites are much less consistent.

*Seasonal and spatial variation in abundance:* Table 3 lists the significant factors influencing the major species (Figs. 2–5) and that of total mosquito abundance, as identified in analyses of variance. For total mosquito abundance there is, predictably, a range of effects. There are marked temporal changes in average abundance (as indicated by the significant effect of Month in the analysis of variance). There is also an overall difference between zones in average abundance, with Zones 1 and 2 having much higher total abundances than Zone 3 (Table 1), and there is a smaller, but still significant, difference between locations within zones (Table 1). There are also differences in the seasonal pattern of abundance

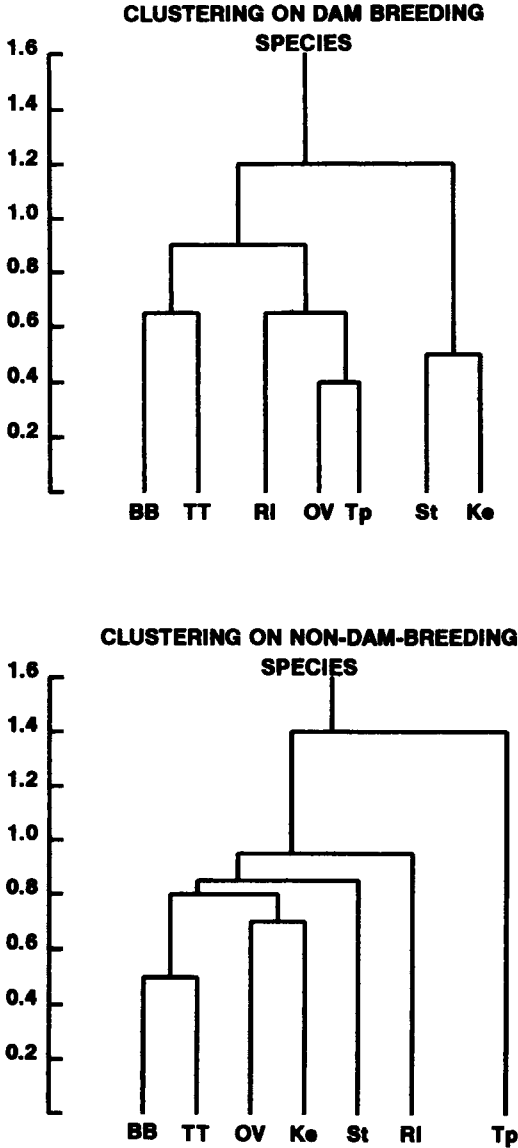


Fig. 1. Cluster analyses of mosquito species which do, and do not breed at the Ross River Dam, in relation to trapping sites. Abbreviations: BB—Big Bay, TT—Ti-Tree Bay, RI—Round Island, OV—Oak Valley, Tp—Toonpan, St—Stanley, and Ke—Kelso.

between zones, as indicated by the Zone  $\times$  Month interaction, and also between locations within zones. Thus the timing of peaks and troughs in abundance is spatially variable. Total mosquito abundance represents the aggregate of all the species sampled, and hence sums the behavior of different species whose seasonal and spatial patterns of abundance may be quite different from each other. Consequently, examination of factors

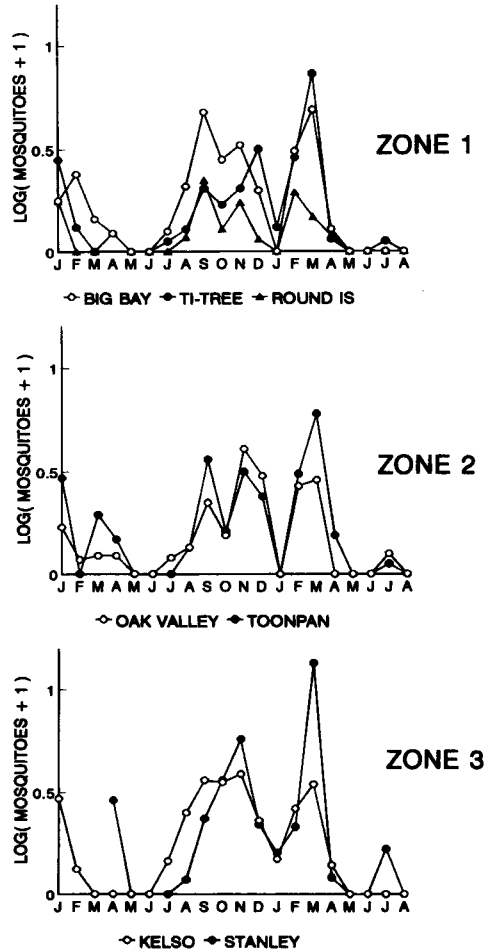


Fig. 2. Average nightly catches of *Aedes vigilax* by month in relation to sites and zones.

affecting each individual species reveals a simpler pattern in every case than that of total abundance.

- 1) *Ae. vigilax*: The pattern of abundance of this species clearly reflects that it does not breed in the dam (Table 2 and Fig. 2). There are very marked seasonal changes in abundance, with peaks occurring at similar times in all locations. There is a weak effect of Location on overall abundance, arising because catches at Round Island were lower than elsewhere, but there are no differences between zones.
- 2) *Culex annulirostris*: Two seasonal peaks were evident (Fig. 3): postwet season (March–May) in both years, and a spring peak in September–October, giving a strong effect of Month (Table 3). There are marked differences between zones, with substantially fewer individuals caught in Zone 3 than in the other 2

Table 2. Pearson's correlation coefficients, expressed as levels of significance, for logarithmically transformed catches of adult female mosquitoes from site to site (a =  $P < 0.0001$ , b =  $P < 0.001$ , c =  $P < 0.01$ , d =  $P < 0.05$ , NS = not significant).

Species/site	Zone 1			Zone 2		Zone 3		Species
	BB	TT	RI	OV	TP	ST	K	
<i>Anopheles annulipes</i>								<i>Culex annulirostris</i>
Big Bay (BB)		a	a	c	b	d	d	
Ti-Tree Bay (TT)	a		a	a	a	d	a	
Round Is. (RI)	a	a		a	a	a	a	
Oak Valley (OV)	a	a	a		a	a	c	
Toonpan (TP)	NS	NS	b	c		a	b	
Stanley (ST)	NS	b	b	NS	NS		d	
Kelso (K)	b	d	NS	NS	NS	c		
<i>Mansonia uniformis</i>								<i>Mansonia septempunctata</i>
Big Bay		a	a	a	a	b	NS	
Ti-tree Bay	a		b	a	a	NS	NS	
Round Is.	a	a		d	d	NS	NS	
Oak Valley	a	a	a		a	c	NS	
Toonpan	a	a	a	a		NS	NS	
Stanley	b	a	c	a	a		NS	
Kelso	NS	b	d	d	d	NS		
<i>Aedes vigilax</i>	all a							

Table 3. Significant effects on the abundance of mosquito species at 7 sites near the Ross River Dam, as detected by unbalanced mixed-model analysis of variance applied to log-transformed abundance data.

Taxon	Effect	df	F	P
<i>Culex annulirostris</i>	Zone	2 × 4	68.92	0.007
	Month	10 × 39	9.26	0.0001
	Month × Location	39 × 921	3.90	0.0001
<i>Anopheles annulipes</i>	Zone	2 × 4	15.85	0.01
	Month	10 × 39	5.65	0.0001
	Location	4 × 42	4.76	0.003
	Month × Location	39 × 921	4.86	0.0001
<i>Mansonia uniformis</i>	Month	10 × 39	10.79	0.0001
	Location	4 × 41	7.19	0.0002
	Month × Zone	20 × 39	4.60	0.0001
	Month × Location	39 × 919	6.00	0.0001
<i>Mansonia septempunctata</i>	Month	10 × 39	4.98	0.0001
	Location	4 × 41	11.19	0.0001
	Month × Location	39 × 921	7.96	0.0001
<i>Aedes vigilax</i>	Month	10 × 40	23.91	0.0001
	Location	4 × 61	2.59	0.05
Total mosquitoes	Zone	2 × 4	17.56	0.01
	Month	10 × 39	11.79	0.0001
	Location	4 × 42	2.94	0.03
	Month × Zone	20 × 39	1.86	0.05
	Month × Location	39 × 921	4.27	0.0001

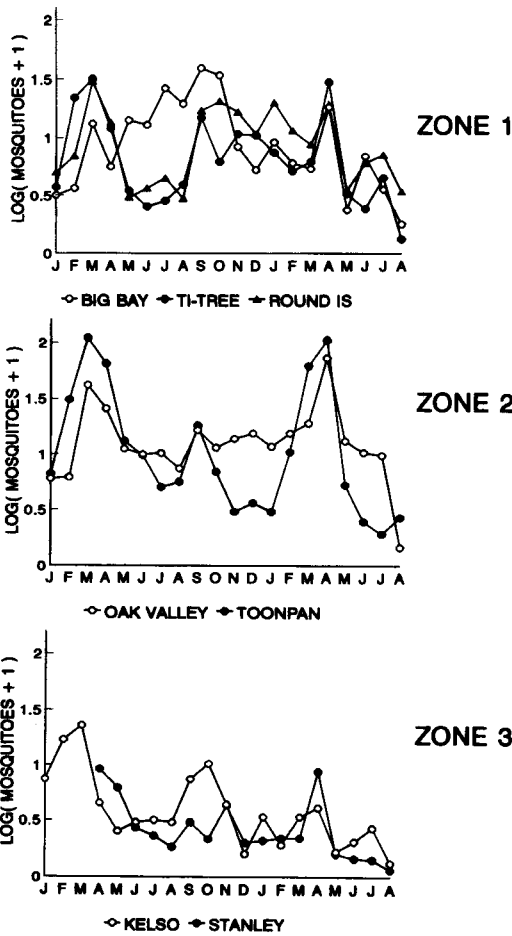


Fig. 3. Average nightly catches of *Culex annulirostris* by month in relation to sites and zones.

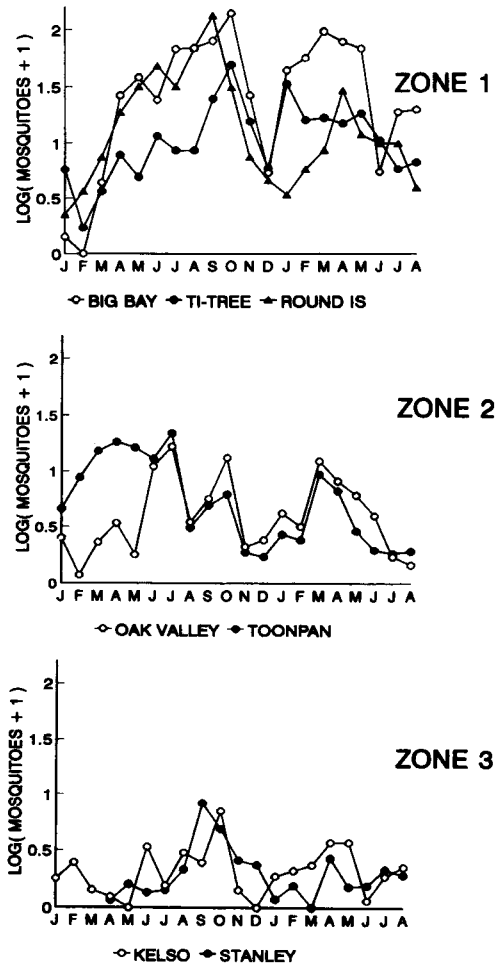


Fig. 4. Average nightly catches of *Anopheles annulipes* by month in relation to sites and zones.

zones. The average abundance was similar at locations within each zone, but the timing of abundance peaks varied between locations within Zone 1, giving a significant Month  $\times$  Location interaction: patterns of abundance at Big Bay were clearly different from those at the other 2 Zone 1 locations, showing a progressive winter–spring buildup similar to *An. annulipes* at Zone 1 sites (Fig. 4).

- 3) *Anopheles annulipes*: Here too, there were marked differences in abundance between zones. In this case Zone 1, on the shoreline, had by far the highest numbers of this taxon, and abundance decreased progressively with distance from the dam (Table 1 and Fig. 4). Especially in Zone 1, there were also differences between locations: peak abundances at Ti-Tree Bay were consistently lower than at the other 2 shoreline sites. In 1984 seasonal patterns of abundance differed from *Cx. an-*

*nulirostris* and were generally characterized by a buildup during the winter–spring dry season. However, in 1985 patterns of abundance were similar to that for *Cx. annulirostris* with an autumn peak.

- 4) *Mansonia uniformis*: This species achieved high abundance only at locations in Zone 1 (Fig. 5), but effects of Zone on average abundance throughout the period were swamped in the analysis of variance by the variability between locations within Zone 1 and by the strong Month  $\times$  Zone interaction. Abundances of this species in Zone 1 appeared related to water level of the dam, with a peak early in the study followed by a steady decline to 0 at all locations by the end of the study. The species was rare at any time in Zone 3.
- 5) *Mansonia septempunctata*: Patterns of abundance for this species are very similar to

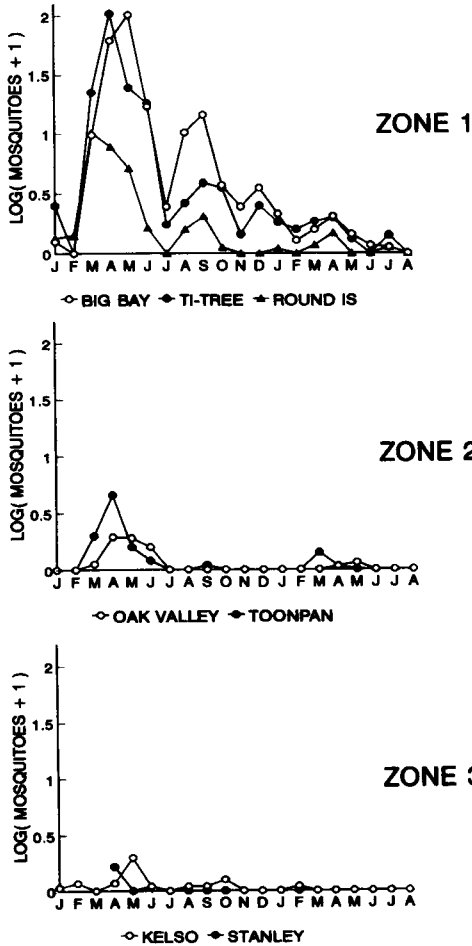


Fig. 5. Average nightly catches of *Mansonia uniformis* by month in relation to sites and zones.

those for *Ma. uniformis* (Table 2 and Fig. 5), except that at one Zone 1 location (Ti-Tree Bay), abundances remained relatively high for a longer period than elsewhere, giving a strong Month  $\times$  Location interaction. As for *Ma. uniformis*, high abundances only occurred at shoreline (Zone 1) locations, and even there declined to 0 as the study progressed.

**Arbovirus conversion rates:** HI-antibody conversion rates for the 4 years from 1983–84 to 1986–87 were analyzed for Zones 1, 2, and 3 with that for Pallarenda (Zone 4) from 1983–84 to 1985–86 (Table 4).

For the alphaviruses, there were significant differences in the proportion of the chickens converting by both zone ( $\chi^2 = 15.02, 3 \text{ df}, P < 0.005$ ) and year ( $\chi^2 = 69.19, 3 \text{ df}, P < 0.001$ ).

There was a significant interaction effect in that the differences seen over the 4-year period were

not consistent with zone ( $\chi^2 = 27.46, 8 \text{ df}, P < 0.001$ ). Proportions remained steady or dropped over time for Zones 1, 2, and 3 whereas they rose for Zone 4 (although the precision of these latter data is inferior to those for Zones 1–3).

For the flaviviruses, zone ( $\chi^2 = 30.93, 8 \text{ df}, P < 0.001$ ) and year ( $\chi^2 = 17.17, 3 \text{ df}, P < 0.001$ ) were both significant as was their interaction ( $\chi^2 = 30.93, 8 \text{ df}, P < 0.001$ ), once again indicating that differences depended on the site under consideration. Proportions remained consistently high for Zone 2 and proportions increased steadily at Zone 3 sites. Few conversions to flaviviruses occurred in Zone 4 (Pallarenda).

There was evidence of the activity of the following alphaviruses, Ross River (all years), Sindbis (all years), and Getah (1985–86, 1986–87). Barmah Forest virus was not included in the testing panel. Flavivirus activity was considerably less than that of the alphaviruses but the following were considered to have been present: Murray Valley encephalitis (1982–83, 1986), Kunjin (1984), Kokobera (1985–86), Alfuy (1984–86), and Edge Hill (1984).

**DISCUSSION**

From January 1984 to September 1985, 54,720 adult mosquitoes were collected by the EVS traps in Zone 1 on the Stage 1 water margin (average collection 80 mosquitoes/trap), in Zone 2 on the planned Stage 2 water margin (55 mosquitoes/trap), and in Zone 3, away from the dam (13 mosquitoes/trap). Mosquito prevalence at each of the 3 zones was to a large extent influenced by the proximity of suitable breeding habitats for 3 taxa, *An. annulipes*, *Cx. annulirostris*, and *Mansonia* spp., which comprised 81.2% of the total catch. Both *Cx. annulirostris* and *An. annulipes* breed extensively in the reservoir but also in permanent water holes, semipermanent swamps, and temporary rain-filled pools away from the dam. Both *Mansonia* spp. breed in association with aquatic plants, most common in the Big Bay and Ti-Tree Bay areas of the lake (Rae 1983<sup>5</sup>).

Examination of numbers of *Cx. annulirostris*, *An. annulipes*, and *Mansonia* spp. (dam-breeding species) by cluster analyses (Fig. 3) demonstrated the similarities between Zones 1 and 2, although reduced numbers of *Mansonia* spp. from Round Island resulted in this site being more closely linked to Zone 2. Round Island lacked floating vegetation in which *Mansonia* breed. Pearson's correlations of 115–149 separate collections of each of the above species from the 7 sites further illustrated this, but also indicated that *An. annulipes* catches from Toonpan were



Table 4. Percentage cumulative seroconversions in domestic chicken flocks to alphaviruses (Alpha) and flaviviruses (Flavi) tested by haemagglutination-inhibition test, Ross River Dam Zones 1 and 2 (on or nearby the reservoir) compared to Zones 3 and 4 (removed from the dam).

Year	Month of serum collection	Zone 1		Zone 2		Zone 3		Zone 4	
		Alpha	Flavi	Alpha	Flavi	Alpha	Flavi	Alpha	Flavi
1983-84	January	25.7	1.1	24.4	0.5	17.2	0	0	0
	March	45.7	1.1	31.3	1.2	48.2	1.3	20	0
	November	68.3	4.3	74.8	1.7	86.7	1.8	32.5	0
1984-85	January	0	0	34.9	1.0	17.2	1.5	44.4	0
	February	0	0	35.8	1.0	19.1	1.5	44.4	0
	August	25.8	1.1	59.2	7.3	43.1	3.1	88.8	11.1
	November	NA	NA	NA	NA	NA	NA	NA	NA
1985-86	February	18.4	0.4	34.2	4.5	15.7	0.4	75.0	0
	March	35.9	2.6	41.1	7.4	35.7	0.4		
	May	66.5	5.8	66.1	9.5	63.0	6.5		
	November	NA	NA	84.4	9.5	91.1	10.0		
1986-87	December	0	0	0	0	NA	NA		
	March	1.4	0.7	14.3	4.8	20.8	6.9		
	April	39.9	0.7	19.6	5.7	42.4	12.8		
	June	51.0	0.7	22.6	5.7	49.5	12.8		

Note: New sentinel flocks were established each year after the November bleeding.

dissimilar (Fig. 4) to those from Big Bay and Ti-Tree Bay.

In general within Zones 1 and 2, correlations between sites in species abundance were highly significant but this was not the case for Zone 3 sites, Stanley and Kelso, where within-group significance values were  $P < 0.05$ ,  $< 0.01$ , NS, and NS for *Cx. annulirostris*, *An. annulipes*, and the 2 *Mansonia* species, respectively (Table 2). Although both of these trapping sites were 3-4 km away from the dam and in similar topography, they also were the most distant from each other, being on opposite sides of the dam. Whereas numbers of *Cx. annulirostris* and *An. annulipes* would be affected by common factors such as generalized rainfall and flooding, we were not able to record localized falls or the various water management practices of residents on what predominantly are rural blocks. There was, however, limited suitable breeding habitat.

That all *Ae. vigilax* correlations were highly significant ( $P < 0.0001$ ) for all 7 sites suggests: 1) a common influence (i.e., saltmarsh inundated by tides), and 2) wide dispersal. *Aedes vigilax* is well known for movement of up to 64 km from its coastal saltmarsh breeding sites (Marks 1969) and therefore, an equitable distribution at all trapping sites would be expected. In contrast, the restricted distribution of *Mansonia* spp. to mainly the Zone 1 sites (Fig. 5) suggests that both *Ma. uniformis* and *Ma. septempunctata* do not fly far.

Along the low-lying flats of the Murray River in Victoria, Myers (1954) noted that biting of

*Cx. annulirostris* and *An. annulipes* was restricted to 150 m from water. More recently, Russell (1986) demonstrated that at Echuca, Victoria, some *Cx. annulirostris* dispersed at least 7 km, subsequently confirmed in southwestern New South Wales (O'Donnell et al. 1992). The relative contributions of the different breeding sites of *Cx. annulirostris* and *An. annulipes* in relation to the numbers collected in the 3 zones is difficult to interpret because of the diversity of their breeding habitats. However, the strong correlations for the major numbers of adult *An. annulipes* and *Cx. annulirostris* at Zone 1 and 2 sites, respectively, suggest that extensive breeding habitat in the reservoir extends an influence for at least 2 km. The more rapid falloff in numbers of *An. annulipes* compared to *Cx. annulirostris* is probably related to their relative dispersal powers.

The temporal patterns of *Cx. annulirostris*, *An. annulipes*, and *Ae. vigilax* collected at Big Bay, Oak Valley, and Stanley, and for *Ma. uniformis* and *Ma. septempunctata* at Big Bay, have been figured from April 1984 to September 1985 in Jones et al. (1991). A more extensive analysis with additional sites indicates that temporal patterns can differ within a particular zone (e.g., for *Cx. annulirostris* at Big Bay compared to Ti-Tree Bay and Round Island in 1984), from zone to zone (e.g., *Cx. annulirostris* at Ti-Tree Bay compared to Oak Valley and Toonpan), and from year to year (e.g., *An. annulipes* in 1984 compared to 1985). Although the average minimum

temperature for June 1984 was 2.8°C higher than for June 1985, July–August 1984 temperatures were 0.8–1.5°C colder. Hence, there does not seem to be an obvious explanation for this temporal difference.

Peak abundance of *Cx. annulirostris* toward or at the end of the wet season (March–May) is well known (e.g., Charleville and Kowanyama [Kay 1979], Darwin and Casuarina [Russell and Whelan 1986]). Increasing temperatures after winter generally produced a spring peak in September–October and numbers generally rose until heavy wet season rains arrived. At this time, extensive growth of *Hydrilla verticillata* formed extensive mats over up to 30% of the reservoir's surface due to decreased water depth, thereby providing extensive additional breeding habitat for both *Cx. annulirostris* and *An. annulipes* (Barker-Hudson et al. 1986). Although *Mansonia* spp. numbers decreased almost from the commencement of the trapping program, those for *Cx. annulirostris* and *An. annulipes* in Zones 1 and 2 generally did not.

The cluster analysis of non-dam-breeding species clearly separated Toonpan from the other sites, probably due to the suitability of its clay-based soils for *Aedes* oviposition and for creation of many temporary pools after rainfall. Thus, 76.6% of the 3,352 *Aedes normanensis* (Taylor), *Aedes lineatopennis* (Ludlow), and *Aedes vittiger* (Skuse) were collected in the CO<sub>2</sub>-EVS trap set at Toonpan.

Alphavirus activity was generally high and unpredictable at the 4 zones where sentinel chickens were maintained (Table 4). Flavivirus activity, including Murray Valley encephalitis and Kunjin, was detected but at low levels. However, sentinel chickens develop an HI antibody response more readily to flaviviruses than to Ross River virus (Kay et al. 1986), one of the common alphaviruses, so seroconversion rates to alphaviruses may be underestimated.

Although the abundance of both mosquitoes and vertebrate hosts was greatest in Zones 1 and 2 closest to the reservoir, arbovirus conversion rates did not necessarily follow this pattern. Thus in terms of the potential hazard posed by the Ross River Dam (Stage 1), the risk of arbovirus infection would seem to be no greater than in Townsville–Thuringowa suburbs such as Kelso, Stanley, and Pallarenda. In fact, high alphavirus activity in 1984–85 and 1985–86 at Pallarenda may be due to its proximity to both coastal salt-marsh and an environmental park where *Ae. vigilax* and *Cx. annulirostris* are plentiful.

The results of this study have several implications for the spatial design of a minimal surveillance program using EVS traps for mosquito vectors of arboviruses:

- 1) Trap locations to monitor *Cx. annulirostris*, *An. annulipes*, and the *Mansonia* species should be stratified according to their distance from major breeding sites. In this case, where the Ross River Dam is clearly of major importance, the minimum requirement would be for a trap close to the water's edge, chosen for proximity to aquatic vegetation, and a trap at least 3 km away. The existence of substantial variation between locations within a zone of some species suggests that several traps within each stratum would be a significantly better monitoring design if resources were available to support it. Any of the above combinations would be suitable for *Ae. vigilax*.
- 2) Eldridge (1987) has reviewed studies in which there was either a positive or no correlation between mosquito abundance and arbovirus transmission. Clearly in this case, the situation at the Ross River Dam falls into the latter category where other entomological and epidemiological factors require consideration.

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