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Surveillance of the mosquito *Aedes aegypti* and its biocontrol with the copepod *Mesocyclops aspericornis* in Australian wells and gold mines

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Abstract. A survey of the dengue vector mosquito *Aedes aegypti* was undertaken using funnel traps to detect immature stages (larvae and pupae) in flooded disused mine shafts and wells in Charters Towers, Queensland, Northern Australia. The town has a history of dengue fever since 1885 when goldminers were the first recorded victims. During the latest dengue epidemic in 1993, 2% of the population had laboratory-confirmed dengue virus Type 2, despite source reduction of *Ae.aegypti* breeding-sites at ground level or above. This led to suspicions that dengue vector *Ae.aegypti* breeding-sites might be below ground level. When surveyed in March 1994, *Ae.aegypti* immatures were found in 9/10 wells and 1/6 mine shafts. The water in wells and mines had similar characteristics – except that turbidity was higher in the mines, which more often contained predators of mosquito immatures.

The copepod *Mesocyclops aspericornis* was collected from water in 1/10 wells and 2/6 mine shafts. Laboratory predation trials resulted in 95.5–100% predation by 25 copepods/l on *Ae.aegypti* first-instar larvae up to 200 larvae/l. Five wells containing *Ae.aegypti* in the survey were inoculated with fifty indigenous *M.aspericornis*, and five wells (one positive and four negative in the survey) were left untreated as controls. Nine months later, in December 1994, *Ae.aegypti* had been eliminated from all five treated wells but all untreated control wells contained *Ae.aegypti*, except for one well that contained a natural population of *M.aspericornis*. The role of wells and mines as winter/dry season refuges of *Ae.aegypti* in northern Australia is reviewed, and we recommend the use of *M.aspericornis* as a cost-effective, environmentally acceptable and persistent agent for the sustainable control of *Ae.aegypti*, especially in inaccessible breeding sites.

Key words. *Aedes aegypti*, *Mesocyclops aspericornis*, breeding sites, dengue, gold mines, wells, mosquito surveillance, Australia, Queensland.

Introduction

Outbreaks of dengue fever virus transmitted by the container-breeding mosquito *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae) occur in tropical north-eastern Australia. The gold-mining town of Charters Towers, Queensland, has a history of dengue fever since 1885 (Hare, 1898) and the only reported case of dengue haemorrhagic fever in Australia (Row *et al.*, 1993). Source reduction of breeding-sites has been the mainstay of *Ae.aegypti* and dengue control, but it failed to prevent the most recent epidemic in 1993 during which 2% of the population had serologically confirmed dengue virus Type 2 (Queensland Health: G. Bielby, pers. comm.). Tun-Lin (1992) investigated surface breeding-sites of *Ae.aegypti* such as tyres, buckets and rainwater tanks in Charters Towers, and determined Breteau Indices of 22–103 in 1990. However, subterranean water bodies in abandoned mine shafts and wells were overlooked. Some mines contained permanent groundwater, and *Ae.aegypti* was reported (as *Stegomyia fasciata* Theobald) to be breeding further than 2000 ft below the surface of a working mine in 1913 (Elkington, 1913).

Charters Towers has a fully reticulating water supply, but restrictions on use often apply and back-yard wells provide water for gardens, livestock and cleaning. Such wells are significant breeding-sites of *Ae.aegypti* in India, where 20.5% of wells in two villages contained larvae (Panicker *et al.*, 1982), and in French Polynesia where 24% of wells in a village contained *Ae.aegypti* (Lardeux, 1992). In Charters Towers, persistent groundwater habitats could be important breeding-sites of *Ae.aegypti* as the winter months are dry (average rainfall 2 mm per month from

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April to October inclusive). The 1993 epidemic of dengue occurred during a severe drought. Subterranean water bodies may have been overlooked, because they are often inaccessible and difficult to find.

The aims of this study were to establish if abandoned mine shafts and wells in Charters Towers were actual or potential habitats for *Ae.aegypti* breeding and, if so, to formulate protocols for the control of breeding in these sites. Since cyclopoid copepods were discovered at some sites, we investigated the potential of these indigenous biological control agents as a sustainable, cost-effective means of controlling *Ae.aegypti*.

Materials and Methods

Study site. Charters Towers (20°03′S, 146°16′E) is situated 135 km inland from the coastal city of Townsville which also has been a focus of dengue fever outbreaks. During the 1800s gold rush, Charters Towers was the second largest city in Queensland, but today it is a rural and mining community of *c.* 9400 population. Part of the gold-mining legacy is about thirty abandoned mine shafts, mostly caved-in, and many wells scattered throughout the residential areas of Charters Towers. At the beginning of this study in January 1994 the area was drought-affected and only eight mine shafts contained water; six of them were surveyed as described below. Vegetation around the mine shafts was dominated by *Cryptostegia grandiflora* (rubber vine) and the thorned *Ziziphus mauritiana* (Chinee apple), both of which can survive long periods of drought. All but two of the shafts were vertical and very deep, and their perimeters were unstable. The shafts were surrounded by 2 m high wire fences to prevent public access. Ten old wells on private property were also surveyed. Wells had little or no collar exposed above the ground, making them difficult to detect.

Physicochemical sampling. The physical dimensions of shafts and wells were measured where possible. Temperature, pH, turbidity and conductivity of the water were measured at all sixteen sites using a Horiba U-10 water-quality checker. Further analyses (including heavy metals) were performed on water samples from three mine shafts and four wells by the Government Chemical Laboratory, Brisbane.

Climatic data recorded at Charters Towers airport were obtained from the Bureau of Meteorology's National Climate Centre for the study period January–December 1994. Well water temperature was measured each month throughout the study to compare with ambient air temperature.

Biotic sampling. The abandoned mine shafts and wells were sampled for mosquito larvae and predators using funnel traps during the summer (January–March) of 1994. The funnel trap has been used successfully to sample the aquatic fauna of wells in Brazil (Kay *et al.*, 1992) and Laos (Jennings *et al.*, 1995) and its construction was described by Kay *et al.* (1992). Funnel traps were lowered into the water in wells or flooded mine shafts for *c.* 24 h per sample, and samples were taken on 2–8 consecutive days. A fishing rod was used to lower the funnel traps into shafts that had unstable or inaccessible surrounds. Water samples from the funnel traps were put through a 2 mm sieve to remove debris and larger organisms. Mosquito immatures and other organisms collected in the funnel traps were taken to the laboratory for identification and counting. Standard plate counts of total bacteria and coliforms were performed by Queensland Health (Laboratory of Microbiology and Pathology) on water samples taken from three mine shafts and four wells.

Laboratory evaluation of indigenous copepods as biological control agents of Ae.aegypti. Cyclopoid copepods (Crustacea: Copepoda: Cyclopsida) were found at 3/16 study sites. Examples of copepods were identified by C. Jennings, Queensland Institute of Medical Research, Brisbane. Colonies of copepods from the three sites were established in the laboratory using standard protocols (Brown *et al.*, 1991). Predation efficacy was calculated as the percentage mortality of standard densities of first-instar *Ae.aegypti* by 25 adult copepods/l after 72 h (Brown *et al.*, 1991). Predation trials were conducted in 2 litre plastic pots containing 400 ml *Chlorella* solution, 200 ml protozoan culture, 5 μg powdered rabbit food, and 1400 ml distilled water to bring the total volume to 2 litres. Groups of fifty adult copepods were tested against batches of 25, 50, 100 and 200 first-instar *Ae.aegypti* larvae/l, each test replicated three times for each colony.

Field trials of indigenous copepods as biological control agents of Ae.aegypti larvae in wells. Ten wells were sampled in March 1994 by placing funnel traps for three consecutive 12 h periods. The numbers of third- and fourth-instar *Ae.aegypti* larvae and the presence or absence of the copepod *Mesocyclops aspericornis* Daday were recorded. The contents of the traps were replaced into the wells after recording trap catches. Five wells were inoculated with fifty adult indigenous *M.aspericornis* after the March sampling period and five wells were left as controls. The ten wells were sampled as above each month (except October) until December 1994. This period covered the usually dry winter season from May to October.

Results

Physicochemical and biotic sampling of mine shafts and wells

The physiochemical and biotic parameters of interest at the sixteen survey sites are given in Table 1. Only 1/6 (17%) disused mine shafts was positive for *Ae.aegypti*, whereas 6/10 (60%) of wells contained *Ae.aegypti* immatures. Cyclopoid copepods were collected from two mine shafts and one well. Overall, there was a negative association between the presence of *Ae.aegypti* immatures and the presence of copepods and/or other predators. Other species of mosquito larvae found were *Aedes tremulus* (Theobald), *Culex quinquefasciatus* Say and *Anopheles*.

Water from both shafts and wells was warm (25–29°C) and neutral to alkaline (pH 6.4–8.5). Turbidity was high in the shafts (24–134 NTU), indicating pollution, and this was the only physicochemical factor that was significantly different between shaft and well water (Mann-Whitney Rank Sum test, $T = 98$, $P < 0.001$). The conductivity (490–2630 μs/cm at 25°C) of shaft and well water indicated medium to high concentrations of cations at both habitats. Water from 3/3 shafts and 3/4 wells tested had heavy metal (iron and/or manganese) concentrations above Australian guidelines for potable waters (National Health and Medical Research Council), while water from 2/3 shafts and 2/4 wells had fluoride concentrations that exceeded the guidelines. The number of coliform bacteria in the water was high (>800 per 100 ml) at all sites that were tested.

Table 1. Surveyed features of gold-mine shafts and wells at Charters Towers, northern Australia, January–March, 1994: +, present; – absent; nd, not determined; td, too deep to determine; NTU, Nephelometric Turbidity Units.

	Disused mine-shafts						Wells									
	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10
Depth to water (m)	45	6.0	2.0	45	2.5	0	4.8	12	4.0	3.7	7.0	10	8.5	8.5	5.4	nd
Depth of water (m)	td	>24	td	td	td	1.5–td	2.4	2.0	0.8	1.4	1.0	1.3	0.9	2.3	1.5	nd
Temperature (°C)	27	27	27	26	29	28	29	26	29	26	27	27	26	25	27	26
pH	6.4	7.6	7.8	8.1	7.9	7.9	8.5	7.8	6.5	8.5	7.4	8.4	7.2	7.9	6.4	6.9
Turbidity (NTU)	66	76	134	25	24	79	2	0	0	0	2	4	0	3	3	0
Ae.aegypti	–	+	–	–	–	–	–	+	–	–	+	+	+	+	+	–
Copepods	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	+
Other predators[1]	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–

[1] Frog tadpoles, Odonata larvae, Notonectidae.

The temperature of well water remained almost constant throughout the year (25–27°C) despite falls in the ambient air temperature during winter (Fig. 1). Most rain fell during the summer (January–March and October–December), whereas the winter (April–September) was dry.

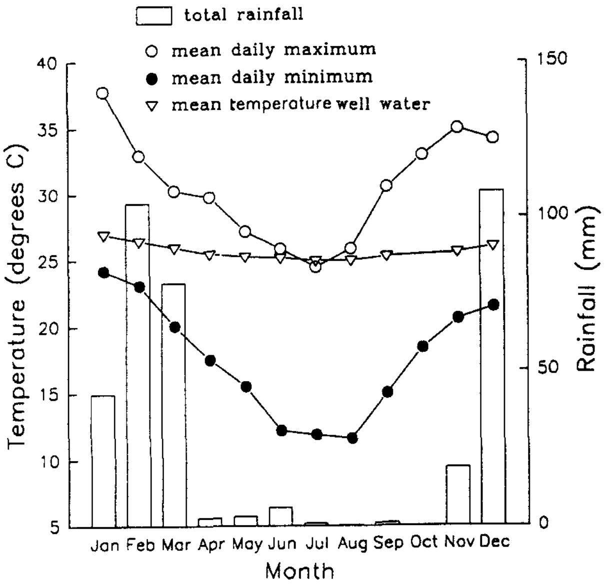

Fig. 1. Climatic data recorded at Charters Towers, northern Australia, during the study period January–December 1994.

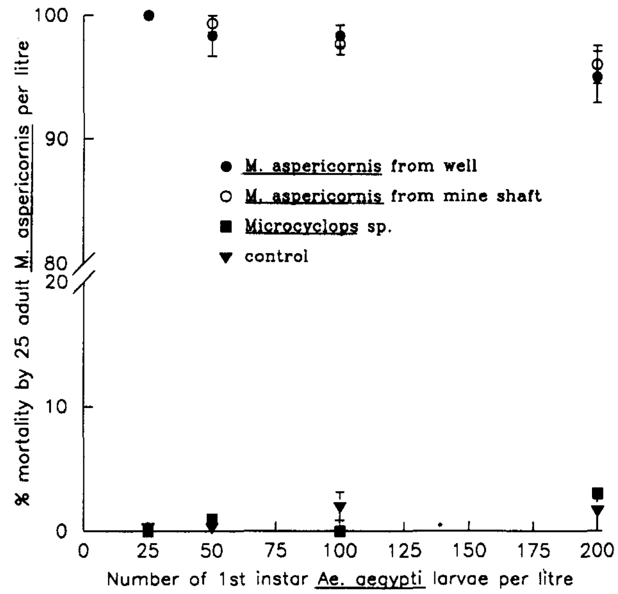

Fig. 2. The mean ($X \pm$ SE, $n = 3$) percentage mortality of first-instar *Aedes aegypti* exposed to twenty-five adult *copepods* per litre under laboratory conditions for 72 h.

Laboratory evaluation of indigenous copepods as biological control agents of Ae.aegypti

The copepods collected were identified as follows: from shaft 3, *Microcyclops* sp. indet.; from shaft 6, *M.aspericornis*; from well 10, *M.aspericornis*. Both colonies of *M.aspericornis* preyed on first-instar *Ae.aegypti* larvae (Fig. 2). The percentage of mortality inflicted by *M.aspericornis* decreased as the number of *Ae.aegypti* larvae/l increased ($r = -0.684$, $P < 0.001$, $n = 24$). The mean mortalities inflicted by both colonies of *M.aspericornis* at 25, 50, 100 and 200 larvae/l were 100%, 98.8%, 98.0% and 95.5% respectively. The indeterminate *Microcyclops* sp. was not a predator of the *Ae.aegypti* larvae, as mortality of larvae in the test was similar to that of the control.

Field trials of Mesocyclops aspericornis for biological control of Ae.aegypti in wells

Five of the six wells initially positive for *Ae.aegypti* (Nos. 5–9, Table 2) were inoculated with field-collected *M.aspericornis*, 50/well during March 1994. When surveyed the following month (April), *M.aspericornis* was found only in well 6, and they had become established in three further wells (nos 5, 8 and 9) by May. The remaining treated well (no. 7) was not recorded as positive for *M.aspericornis* until 6 months after inoculation. After *M.aspericornis* became established in each of the treated wells there was a decrease in the number of third- and fourth-instar

Table 2. The mean ±SE number of third- and fourth-instar *Aedes aegypti* (*Ae.ae.*) larvae per funnel trap sample ($n = 3$), and presence (+) or absence (–) of the copepod *Mesocyclops aspericornis* (*M.asp.*) for each month of a biological control trial in 10 wells in Charters Towers, northern Australia.

	Pre-treatment		Untreated															
	March		April		May		June		July		August		September		November		December	
Well	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*	*Ae. ae.*	*M. asp.*
1	0	–	6.0±3.5	–	10.3±2.0	–	12.0±1.2	–	1.0±1.0	–	5.7±1.2	–	14.0±2.5	–	0	–	0.7±0.7	–
2	35.7±3.8	–	7.3±0.9	–	20.7±2.3	–	35.7±5.4	–	43.0±5.1	–	35.3±8.2	–	13.3±1.8	–	7.7±1.5	–	9.7±1.5	–
3	0	–	7.3±1.9	–	18.7±4.7	–	33.3±3.5	–	26.7±4.1	–	12.7±2.4	–	31.0±3.6	–	48.3±3.0	–	35.3±5.2	–
4	0	–	0	–	7.0±0.6	–	11.7±2.3	–	44.3±5.0	–	31.3±0.9	–	38.7±2.4	–	27.7±1.5	–	23.0±3.2	–
Mean±SE	8.9±4.7		5.2±1.3		14.2±2.1		23.2±3.7		28.8±5.6		21.3±4.2		24.3±3.5		20.9±6		17.2±4.2	
	Pre-treatment		Treated with 50 *M.aspericornis* per well															
5	8.3±1.7	–	8.7±1.5	–	1.3±1.3	+	0	+	0	+	0	+	0	+	0*	–*	0*	–*
6	25.3±3.5	–	33.3±5.3	+	38.3±2.1	+	0	+	0	+	0	+	0	+	0	+	0	+
7	27.3±1.5	–	29.7±0.9	–	17.7±2.7	–	2.7±1.5	–	10.7±1.8	–	9.3±4.1	–	0	+	0	+	0	+
8	57.3±1.7	–	47.0±4.7	–	0.3±0.3	+	0	+	0	+	0	+	0	+	0*	–*	0*	–*
9	2.3±1.2	–	3.0±1.2	–	4.0±1.2	+	0	+	0	+	0	+	0	+	0	+	0	+
Mean±SE	24.1±5.2		24.3±4.5		12.3±3.9		0.5±0.4		2.1±1.2		1.9±1.2		0				0	
	Pre-treatment		Untreated, but indigenous *M.aspericornis* present															
10	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0*	–*	0*	–*

* Dry wells.

Ae.aegypti larvae collected. No *Ae.aegypti* larvae were detected in the treated wells from September to December, 6–9 months post-inoculation.

One of the untreated wells (no. 10) contained an indigenous population of *M.aspericornis* and this well remained negative for *Ae.aegypti* throughout the study period. All of the remaining untreated wells (nos. 1–4) contained *Ae.aegypti* by May and were positive at the end of the study period in December (Table 2).

Discussion

Wells in Charters Towers were found to be perennial breeding-sites for *Ae.aegypti*, as 9/10 wells in this study contained *Ae.aegypti* immatures during 1994. Wells have not been recognized previously as contributing to the ecology of this dengue vector in northern Australia. In contrast, disused mine shafts were not key *Ae.aegypti* breeding sites, perhaps because the flooded mines more often harboured indigenous predators. Only one shaft contained a small number of *Ae.aegypti*, whereas *M.aspericornis* was initially detected in 2/6 shafts but only 1/10 wells ($\chi^2 = 1.09$, $P > 0.05$).

Wells provided good oviposition sites for *Ae.aegypti* for several reasons. The water was accessible, clean, of suitable pH and salinity, and generally did not contain predators of mosquito larvae. The wells were clad with wood, iron or concrete, all of which provide a suitable solid substrate for oviposition around the water line. In addition, well water contained high levels of microbes for larval food, and it remained warm throughout the year.

From field studies in Charters Towers, the optimal temperature for development of *Ae.aegypti* immatures was 20–30°C (Tun-Lin, 1992). During our study, the average temperature of well water remained around 25°C, even during winter, whereas in previous studies (Tun-Lin, 1992) the average temperature of water in surface breeding-sites during winter ranged from 10°C in buckets to 22°C in rainwater storage tanks. Although the water temperature of surface breeding sites remained above the developmental threshold temperature of 8.3 ± 3.6°C for *Ae.aegypti* (Tun-Lin, 1992) and the monthly minimum air temperature during our study was 11.6°C, the ~25°C temperature of well water during winter would allow more rapid development of *Ae.aegypti* larvae than in surface sites.

Water in the mine shafts was similar to well water, except for its higher turbidity. Although *Ae.aegypti* has been reported as breeding in dirty and polluted water (Christophers, 1960), this species generally prefers to oviposit around clean water. The disused mine shafts therefore would not be optimal breeding sites but should be kept under surveillance during periods when other sites are not available (for example, following control programme). The turbidity probably results from rubbish thrown into shafts, and erosion from the rock and earth sides.

Locally collected *M.aspericornis* were shown to be voracious predators of *Ae.aegypti* first-instar larvae in laboratory trials, even at the unrealistically high densities of 100 and 200 larvae/l. Their potential impact as biological control agents against *Ae.aegypti* in wells was confirmed by the field trial involving addition of 50 *M.aspericornis* to each of ten wells in Charters Towers. In all treated wells, and in a well naturally harbouring *M.aspericornis*, the presence of this copepod coincided with the disappearance of *Ae.aegypti* larvae. Similarly effective results have been achieved with *M.aspericornis* against *Ae.aegypti* in French Polynesia by Lardeux (1992) and by Jennings *et al.* (1995) in Laos. In Charters Towers the copepod population persisted for the duration of the trial in all wet wells. Well water temperature and pH were conducive to the persistence of *M.aspericornis*, supporting the findings of other workers. Brown *et al.* (1991) found that 25°C was the optimal temperature for population growth of *M.aspericornis*, and Jennings *et al.* (1994) found that population growth occurred between pH 5.5 and 8.5 (pH 6–8 was optimal). All sixteen of our study sites contained water of pH ≤ 8.5, but three wells and one mine shaft contained water of pH > 8 which approached the copepods' upper limit of tolerance.

This study suggests that *Ae.aegypti* preferentially oviposited in wells during the dry winter season, as the relative abundance of *Ae.aegypti* larvae in the untreated wells was greatest during the dry months. Yasuno *et al.* (1977) found in villages in India that, during the rainy season, breeding of *Culex quinquefasciatus* shifted from wells to water-holding receptacles. During extended dry periods, which are common in northern inland Australia, wells could provide moisture refuges for *Ae.aegypti* populations. Subterranean habitats have been shown to act as moisture refuges for a number of insects in Australia's dry tropics (Weinstein, 1994). Populations of such insects expand into surface habitats during the wet summer months. *Ae.aegypti* populations would readily colonize ephemeral surface sites when the latter become available in summer. Such behaviour is consistent with the oviposition-driven dispersal of *Ae.aegypti* demonstrated by Reiter *et al.* (1995), so larval control of *Ae.aegypti* may therefore be carried out best in winter when populations are restricted. To clarify the relative importance of different types of breeding sites in relation to adult dispersal, egg diapause and continuous breeding of *Ae.aegypti*, a comprehensive study of mosquito ecology in wells and surface breeding sites during wet and dry seasons has begun at Charters Towers.

Our findings are important for several reasons. Primarily, the significance of subterranean habitats to the overall problem of *Ae.aegypti* in northern Queensland was highlighted. Wells have been often associated with *Anopheles* spp. breeding in Asia, the Middle East and in Africa (Batra & Reuben, 1979; Eshghy, 1977; Mattingly, 1969), with *Cx quinquefasciatus* in Asia and South America (Yasuno *et al.*, 1977; Kay *et al.*, 1992), and with *Ae.aegypti* in India (Panicker *et al.*, 1982) and Laos (Jennings *et al.*, 1995), as noted previously. Moreover, we expect that the importance of *Ae.aegypti* breeding-sites in wells and mines will become more apparent with further funnel trap studies. Surveys carried out by dropping a bucket into underground waterbodies underestimate the problem because they do not collect representative samples of mosquito larvae. Thirdly, this study shows that *M.aspericornis* can be a persistent, economical and environmentally sound means of *Ae.aegypti* control. The efficacy of this biological control agent may be greatest in winter, when the opportunity arises to attack *Ae.aegypti* larval populations restricted in subterranean refuges.

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