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# Release of *Mesocyclops aspericornis* (Copepoda) for Control of Larval *Aedes polynesiensis* (Diptera: Culicidae) in Land Crab Burrows on an Atoll of French Polynesia

F. LARDEUX, F. RIVIÈRE, Y. SÉCHAN, AND B. H. KAY<sup>1</sup>

ORSTOM, BP 529 and ITRMLM, BP 30, Papeete, Tahiti, French Polynesia

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**ABSTRACT** On Tereia Island, Rangiroa Atoll, 14,321 land crab burrows were treated with the copepod *Mesocyclops aspericornis* from January to June 1986, to control larvae of *Aedes polynesiensis* marks. In October 1987, the entire island of Tereia was retreated (17,300 burrows), and the neighboring island, Voisin, was left untreated as a control. From 5 to 15 mo after treatment, burrows with *M. aspericornis* contained an average of 2 *Ae. polynesiensis* immatures compared with 97 immatures from untreated burrows. Long-term larval control was successful in low-lying areas where burrows remained wet or were reflooded. Although there may have been other contributing factors, the major reason for lack of persistence of *M. aspericornis* in burrows over the entire island appeared to be poor resistance to desiccation. From all treated burrows in October 1987, *M. aspericornis* subsequently was found in 89.5, 39.1, and 24.1% of burrows sampled 5, 8, and 15 mo after treatment, respectively. The broad-scale results for Tereia indicated that there was no reduction of adult biting indices when compared with Voisin. Mark-release experiments on four occasions indicated that *Ae. polynesiensis* had a limited flight range and that the probability for interisland movement was low.

**KEY WORDS** Insecta, *Mesocyclops aspericornis*, *Aedes polynesiensis*, biological control

*Aedes polynesiensis* Marks, a vector of dengue viruses and *Wuchereria bancrofti* var. *pacifica*, breeds prolifically in a variety of artificial and natural biotopes (Jachowski 1954). Previous surveys of French Polynesian atolls (Klein & Rivière 1982) have demonstrated the importance of the gecardinid crab, *Cardisoma carnifex* (Herbst), in providing numerous flooded burrows, in which up to 1,000 immature *Ae. polynesiensis* may be found.

Gecardinid crabs occur throughout the world, and 140 species of mosquitoes have been associated with their burrows (Bright & Hogue 1972). The control of mosquitoes breeding in land crab burrows has never been addressed successfully (Bruce-Chwatt & Fitz-John 1951, Bonnet & Chapman 1958, Burnett 1959, Gardner et al. 1986) until the recent release of *Mesocyclops aspericornis* (Daday) at Avatoru, Rangiroa Atoll, French Polynesia (Rivière et al. 1987). However, only 2,432 burrows were treated and, as such, this was only a small-scale trial.

This article describes research from 1986 to 1989 on Tereia Island, Rangiroa Atoll, which evaluated the feasibility of transporting *M. aspericornis* to a remote island and of using them for the large-scale treatment of land crab burrows to control *Ae. polynesiensis*.

## Materials and Methods

**Study Area.** The experiment was carried out on Tereia and Voisin Islands (15°05'S, 147°57'W), part of Rangiroa Atoll. Rangiroa Atoll, part of the Tuamotu Archipelago, is ≈80 by 40 km in size and lies ≈300 km NE of Tahiti. Both islands are typical of many others in the Tuamotu group, some of which have been described in greater detail by Rivière (1979). Annual temperature varies from 24.5 to 29.3°C, and rainfall averages 1,753 mm, falling mainly from November to March. Most inhabitants (≈1,200) reside in the villages of Avatoru (Vaimate Island) and Tiputa (Tepaetia Island) but make regular visits to the other islands of the atoll to collect coconuts for the copra market. As a consequence, few damaged coconuts were available to provide breeding sites for *Ae. polynesiensis*.

Tereia is ≈32 ha in size and lies 25 km SW of Avatoru. The island is low and is comprised of accumulated coralline-shell materials and decomposed organic matter (Fig. 1). The ocean side of the isle is lined by a higher zone of coarse coralline materials and impenetrable scrub which is intermixed with coconut trees toward the middle of the island. The half of Tereia facing the lagoon is marked by an extensive coconut plantation, and the degradation products of these trees and other vegetation provide suitable habitat for burrows of the terrestrial crab. Within this

<sup>1</sup> Queensland Institute of Medical Research, Royal Brisbane Hospital P.O., Brisbane, Australia 4029.

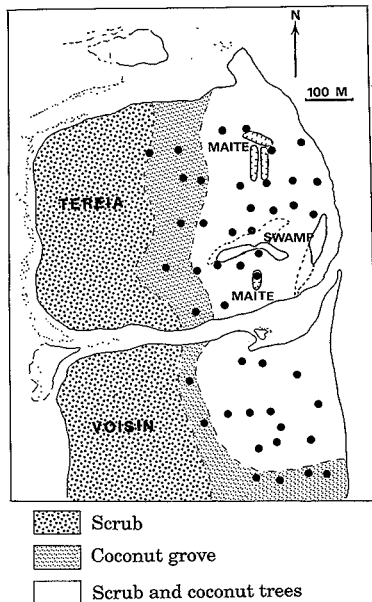


Fig. 1. Tereia and Voisin islands within Rangiroa Atoll, showing adult collection stations (•) and topographical features.

zone are two brackish swamps and four extensive depressions (maite) dug by early inhabitants for the cultivation of crops.

Neighboring Voisin Island, separated from Tereia by a 30-m channel to the south, was ecologically similar to Tereia and was used as an untreated check area.

**Pretreatment Survey.** Aerial survey maps of Tereia (treated site) and Voisin (untreated control) were obtained to facilitate estimation of the relative areas covered by the geographic strata (coconut grove, bush, scrub, maite, etc). To estimate the number of crab burrows (and consequently the number of copepods required for the treatment), quadrats (8 by 8 m) were systematically laid out at 100-m intervals over the entire island, and the mean number of burrows per quadrat was calculated. The total number of crab burrows for treatment then was estimated by summation of the area of each strata type multiplied by its mean burrow density.

**Treatments.** Because *M. aspericornis* do not occur naturally on Rangiroa Atoll, they were

transported by air from Tahiti and then by boat to Tereia. The *M. aspericornis* stock was reared outdoors at the laboratory on Tahiti in 8-m<sup>3</sup> concrete water tanks and was the same strain previously used by Rivière et al. (1987). Four releases of *M. aspericornis* were carried out in January 1986 (600 burrows), March 1986 (5,000 burrows), June 1986 (8,721 burrows), and October 1987 (total retreatment, 17,300 burrows). The first three treatments were done to establish mass-release methods and the feasibility of transporting copepods to a remote island, while providing the maximum period possible to study their persistence. In 1986, copepods were transshipped en masse in 20-liter jerry cans for application with a compression sprayer (Rivière et al. 1987), whereas in 1987, the *Mesocyclops* were transported as inocula of 50 in 20-ml tubes.

In October 1987, three teams of four people undertook a complete treatment involving the 17,300 crab burrows on Tereia. For each burrow, a hose (3 m long, 3 cm in diameter) was inserted into the basal chamber. With the aid of a funnel, 50 *M. aspericornis* were introduced into 1.3 liters water. This treatment took 1,440 man-hours.

**Evaluation of Treatments.** From January 1986 until the mass retreatment in October 1987, the presence of *M. aspericornis* and the abundance of *Ae. polynesiensis* was monitored in burrows by inserting a hose into the base of the burrow and pumping out the water into a 2-liter Erlenmeyer flask (Rivière et al. 1987). Burrows <4 cm in diameter were not sampled. The water was sieved through 100- $\mu$  mesh, and the contents were counted in a white tray. After counting, immature mosquitoes and copepods were returned to the burrow using the pumped water. The physicochemical characteristics recorded for each burrow were: length and diameter, water volume, temperature, pH, salinity, and dissolved oxygen (measured using a portable salinometer and an oxymeter) (Yellow Springs Inc., Yellow Springs, Ohio).

Upon completion of the mass treatment in October 1987, sampling emphasis shifted to the assessment of the effect of larval control on the adult *Ae. polynesiensis* population using Voisin as an untreated control. Adult mosquitoes were aspirated from human bait (Bonnet & Chapman 1958) before treatment (October 1987), and 5 (March 1988), 8 (June 1988), and 15 mo after treatment (January 1989). One person wearing repellent collected mosquitoes attracted to another who served as bait for 10 min at each station between 0700 and 1600 hours. Twenty-nine stations were sampled on Tereia and eight (later 17 from May 1988) on Voisin (Fig. 1). These stations represented each biotope where crab burrows occurred: open coconut grove, mixed scrub and coconut, and maite. Biting indices (mean catch per 10 min from all stations sampled) (Lardeux 1987) were calculated for each

Table 1. Mean numbers of *Ae. polynesiensis* larvae and pupae per *C. carnifex* burrow in the presence and absence of *M. aspericornis*

Period sampled	Island	<i>M. aspericornis</i> present			<i>M. aspericornis</i> absent		
		No. burrows sampled	No. larvae	No. pupae	No. burrows sampled	No. larvae	No. pupae
Jan. 1986	Tereia	NP	—	—	60	41.1	5.9
June 1986	Tereia	21	6.7	0.3	90	70.8	6.1
May 1987	Tereia	43	<1.0	0	11	>44	0
Oct. 1987	Tereia	15	0	0	—	—	—
March 1988	Tereia	19	0.7	0.2	4	44.0	7.0
	Voisin	—	—	—	10	14.4	5.2
June 1988	Tereia	62	1.0	0.5	8	190.7	26.6
	Voisin	—	—	—	13	4.3	13.6
Jan. 1989	Tereia	19	0.8	0.2	13	79.7	4.8
	Voisin	—	—	—	14	50.4	3.4

NP, none present (pretreatment).

island on the basis of 7–10 d of monitoring. Before statistical analysis, counts were transformed to  $\log(x + 1)$  to normalize the distribution and to stabilize the variance. Biting indices were analyzed by analysis of variance (ANOVA) to test for differences between the two islands (i.e., treatment effects) and among sampling periods (i.e., seasonal effects).

To test for dispersal of *Ae. polynesiensis* between Tereia and Voisin, four mark–release–recapture experiments were done in October 1987, March and June 1988, and January 1989. For each experiment, we used the model of Aronson (1972) to assess interisland dispersal and as such, *Ae. polynesiensis* females were marked with fluorescent powder with an insufflator and released on 3 consecutive d using different colored powders for each site (Tereia or Voisin) and day. Results were computed using the program developed by Lardeux & Loncke (1987) for Aronson's model.

## Results

**Characteristics of Crab Burrows.** As all the burrows (17,300) were treated on 15 ha of Tereia (the scrub was negative), the density of burrows was 1.15 per m<sup>2</sup>. This was close to the first estimation of 1.0 per m<sup>2</sup>, based on sampling 44 quadrats of 64 m<sup>2</sup> each. Burrow diameters ranged from 2 to 25 cm and burrow depths ranged from 15 to 130 cm, with median values of 5–10 cm and 30–70 cm, respectively. *Ae. polynesiensis* larvae were recovered from burrows containing from 0.2 to 3.6 liters of water. During the period of study, water temperatures in the burrows ranged from 26.2 to 30.6°C, salinity from 0.33 to 0.93 ppk, pH from 6.59 to 8.05 (range, usually 7.0–7.5), and dissolved oxygen 2.99  $\pm$  1.39 ppm ( $\bar{x} \pm$  SE). These values were consistent on both Tereia and Voisin. During 1987–1989, the numbers of wet burrows sampled on Tereia ranged from 79 to 166 and on Voisin from 24 to 40.

**Copepod Persistence and *Aedes* Immatures.** At each sampling period after treatment with *M.*

*aspericornis*, the numbers of immature *Ae. polynesiensis* on Tereia were compared in wet burrows positive and negative for copepods (Table 1). The 2.0  $\pm$  2.6 ( $\bar{x} \pm$  SE) larvae and 0.3  $\pm$  0.1 pupae (mean immatures, 2.03) in positive burrows were significantly less ( $P < 0.05$ ) than 85.8  $\pm$  60.7 larvae and 11.1  $\pm$  10.4 pupae (mean immatures, 96.9) in negative burrows when tested by the Mann–Whitney *U* test. When the mean numbers of *Ae. polynesiensis* larvae and pupae in positive burrows were expressed as a percentage of those in negative burrows (e.g.,  $2/85.8 \times 100 = 2.3\%$  and similarly for pupae), both stages were reduced by 97%. In contrast, the numbers of immatures per burrow from Voisin (larvae, 23.0  $\pm$  24.2; pupae, 8.5  $\pm$  7.2) were not significantly different from those in *M. aspericornis*-negative burrows on Tereia (Mann–Whitney *U* test,  $P > 0.05$ ).

Following the inoculation of burrows in the maite, the percentage of wet holes positive for *M. aspericornis* decreased to 50% from January to October 1986 (Table 2). After May 1987, the sampling program was extended to evaluate all of the areas treated. In June 1988, 52 of the 284 burrows sampled were in the maite. At this time,  $\approx$ 31 mo after their treatment in January 1986, 38.5% of these burrows were wet and 80% of these still contained *M. aspericornis*.

In May 1987 after a progressive total of 14,921 burrows had been treated in three steps, 53.8% of the wet holes sampled contained *M. aspericornis*. By January 1989, following the retreatment of the entire island in October 1987, only 24.1% of wet holes still contained *M. aspericornis* 15 mo after treatment.

In contrast to the 1987–1988 wet season (October–January, 1,383 mm), rainfall for the same period of 1988–1989 was below average (760 mm) at 528 mm. Consequently, by January 1989, only 8.4% and 13.2% of holes on Tereia and Voisin, respectively, were wet. Annual rainfall for 1987 and 1988 was 2,274 and 1,506 mm, respectively, in contrast to the 1,753-mm average.

As a consequence of the decreasing prevalence of *M. aspericornis* in burrows, *Ae. polynesiensis*

**Table 2.** Treatment schedule and percentage of wet crab burrows positive for *M. aspericornis* and *Ae. polynesiensis* immatures on Tereia and Voisin islands

Year	Month	Tereia						Voisin			
		No. burrows treated	No. burrows sampled	% Wet (no.)	% Positive for		No. burrows sampled	% Wet (no.)	% Positive for		<i>Ae. polynesiensis</i>
					<i>Mesocyclops</i>	<i>Ae. polynesiensis</i>			<i>Mesocyclops</i>	<i>Ae. polynesiensis</i>	
1986	January	600	172	28.5 (49)	0	71.9	—	—	—	—	—
	March	5,000	30 <sup>a</sup>	—	66.7	—	—	—	—	—	—
	June	9,321	30 <sup>a</sup>	—	56.7	—	—	—	—	—	—
	October	—	28 <sup>a</sup>	—	50.0	—	—	—	—	—	—
1987	May	—	284	28.2 (80)	53.8	15.0	—	—	—	—	—
	October	17,300	—	—	100	—	—	—	—	—	—
1988	March	—	569	23.4 (133)	89.5	6.0	154	18.2 (28)	0	35.7	—
	June	—	284	58.4 (166)	39.1	14.5	107	37.4 (40)	0	32.5	—
1989	January	—	941	8.4 (79)	24.1	27.9	182	13.2 (24)	0	54.2	—

<sup>a</sup> Burrows sampled from March to October 1986 were in maité. Data for January 1986 and from May 1987 to January 1989 refer to the entire island.

*siensis* prevalence increased. From March 1988 to January 1989, 6.0% (5 mo after treatment), 14.5% (8 mo) and 27.9% (15 mo) of wet burrows contained *Ae. polynesiensis* on Tereia, significantly less than 35.7, 32.5, and 54.2% on Voisin, respectively ( $\chi^2$  test with Yates' correction: March, 17.66; June, 5.95; January, 4.57; df = 1;  $P < 0.05$ ).

**Adult Mosquito Population.** There was limited interisland movement of *Ae. polynesiensis* (Table 3). Of 10,878 adults marked and released on Tereia during October 1987, March and June 1988, and January 1989, 692 (6.36%) and 47 (0.43%) were recovered on Tereia and Voisin, respectively. Of 9,762 adults released on Voisin, 662 (6.84%) and 49 (0.5%) were recaptured on Voisin and Tereia, respectively. After adjusting the raw data to the model of Armasson (1972), the calculated probabilities for intra- and interisland dispersal indicated that *Ae. polynesiensis* had little tendency for interisland dispersal. Of the two directions possible (i.e., Tereia to Voisin and vice versa), the probability of reinfestation of Tereia from Voisin was minimal.

The biting indices of *Ae. polynesiensis* for Tereia and Voisin remained similar from total retreatment (October 1987) through January 1989 (ANOVA:  $F = 0.22$ ; df = 1, 775;  $P > 0.05$ ),

indicating that there was no treatment effect (Table 4). There were, however, significant seasonal differences in biting indices with respect to time (ANOVA:  $F = 39.91$ ; df = 3, 775;  $P < 0.001$ ), and these main effects were consistent throughout because their interaction was not significant (ANOVA:  $F = 0.89$ ; df = 3, 775;  $P > 0.05$ ).

### Discussion

The research demonstrated that it was possible to carry out broad-scale introductions of *Mesocyclops* into land crab burrows on remote islands. Although Rivière et al. (1987) were able to inoculate 60–150 holes per man-hour using the compression sprayer, the treatment of Tereia was more arduous because of the low water levels in most burrows. When the entire island was retreated in October 1987, inoculation of *Mesocyclops* by tube was considered more reliable, but it reduced the treatment rate to 12 holes per man-hour.

Insertion of the tube into the main chamber of crab burrows was often problematic as *C. carnifex* is known to dig minor offshoots that are invariably dry (Goshima et al. 1978). Consequently, it was difficult to ensure that a totally effective treatment had been carried out, even

**Table 3.** Numbers of *Ae. polynesiensis* females marked, released, and recaptured on Tereia and Voisin islands, October 1987 to January 1989, and probabilities of interisland dispersal of *Ae. polynesiensis*

Collection site	Oct. 1987	March 1988	June 1988	Jan. 1989
Release on Tereia	3,061	937	3,973	2,907
Recapture on Tereia	180	66	386	87
Recapture on Voisin	15	1	31	0
Release on Voisin	3,390	1,312	3,366	1,694
Recapture on Voisin	239	29	349	51
Recapture on Tereia	14	7	27	1
Probability of daily dispersal <sup>a</sup>				
Tereia to Tereia	0.99	0.45	0.77	0.64
Voisin to Voisin	1.00	0.68	0.91	0.58
Tereia to Voisin	0.03	0.12	0.14	0.001
Voisin to Tereia	0	0	0.01	0

<sup>a</sup> Calculated by the model of Armasson (1972).

Table 4. Biting indices for *Ae. polynesiensis* collected Tereia and Voisin islands, October 1987–January 1989

Period	No. mo after total treatment	Island	No. stations	Index*	95% CI	
					Min	Max
Oct. 1987	0	Tereia	126	109.9	90.0	135.6
		Voisin	29	134.3	58.2	204.4
March 1988	5	Tereia	142	32.1	26.6	38.5
		Voisin	72	34.8	26.8	45.6
June 1988	8	Tereia	150	46.5	38.5	55.7
		Voisin	98	38.5	30.9	48.4
Jan. 1989	15	Tereia	72	37.3	28.8	48.4
		Voisin	74	32.5	25.3	42.5

\* Back-transformed mean number of females collected per bait per 10 min.

with the addition of extra water. This also applied to sampling *M. aspericornis* and *Ae. polynesiensis* immatures after treatment. Some new burrows were dug and others may have experienced periods of excessive salinity for *M. aspericornis*. However, because *M. aspericornis* became established in salinities up to 3.72 ppk at Avatoru, Rangiroa Atoll (Rivière et al. 1987), the recorded maximum salinity of <1 ppk on Tereia was not anticipated to present a problem. However, in view of the sporadic visits and relatively small sample size, short periods or discrete zones of high salinity may have been missed. Between visits, burrows also may have dried and then refilled. Thus, the data relating to wet burrows found negative for copepods could have been confounded by either inadequate treatment or subsequent copepod mortality.

The study on Tereia indicated that, in *M. aspericornis*-positive burrows, *Ae. polynesiensis* immature abundance was reduced from 85 to 2 per burrow and that prevalence was reduced from 71.9 to 6.0% of wet burrows. Although *Aedes* prevalence in wet burrows on Tereia was statistically lower than on Voisin for the 15-mo period, the trends in abundance (i.e., reduction of wet burrows, rise in *Aedes*-positive burrows) were the same for both islands. As pretreatment data for immatures were not available for both islands, this difference could not be ascribed completely to the effect of the copepod treatment. However, biting indices of *Ae. polynesiensis* remained statistically similar before and after treatment on both Tereia and Voisin, indicating that the treatment had no effect on the adult population. This was particularly surprising for March 1988, 5 mo after treatment, when 89.5% of wet burrows contained *Mesocyclops*. Perhaps partial larval control enhanced overall immature and adult survival, thereby resulting in comparable biting rates on the islands. The mark-release experiments with female *Ae. polynesiensis* confirmed that it is a sedentary species as in American Samoa (Jachowski 1954), and, as such, immigration from Voisin to Tereia was minimal.

In terms of broad-scale considerations, the treatments failed because *M. aspericornis* was not able to survive in dry burrows. Gecardinid

crabs also are sensitive to water deprivation (Herreid & Gifford 1963), but *C. carnifex* may move among burrows of different depths to ensure its survival. The results were most successful in the low-lying maite, where burrows most likely retained water for longer periods or *M. aspericornis* was reintroduced naturally when the maite were flooded by rain or by the upward movement of the water table. In June 1988 on Tereia, 80% of burrows in the maite contained *M. aspericornis* 31 mo after the initial treatment compared with 39.1% for the entire island 8 mo following treatment. At Kia-Ora maite across Rangiroa lagoon, 90% of burrows still contained *M. aspericornis* 47 mo after treatment (Lardeux et al. 1990).

Nevertheless, attempts with *M. aspericornis* to control larval mosquitoes in land crab burrows have had greater success and are more acceptable environmentally than other methods used in West Africa (Bruce-Chwatt & Fitz-John 1951), Tahiti (Bonnet & Chapman 1958), and Fiji (Burnett 1959, Gardner et al. 1986). The results on Tereia suggest that the persistence, and not the efficacy, of broad-scale control by *Mesocyclops* is in question, although the drought conditions following treatment of the entire island in 1987 made the test conditions more rigorous than planned. Lack of desiccation resistance most likely was the major problem, as observations in drying pools (S. Loncke, personal communication) and in car tires (M. D. Brown & B.H.K., unpublished data) indicated that *M. aspericornis* (French Polynesian and Queensland strains) do not survive in dry habitats. Copepods should still be considered in the control of crab hole-breeding mosquitoes, but only if accompanied by careful site evaluation that includes salinity measurements and the use of desiccation-resistant cyclopoid species.

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