ARBOVIRUS RESEARCH IN AUSTRALIA-PROCEEDINGS 5TH SYMPOSIUM

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## POTENTIALITIES OF MESOCYCLOPS ASPERICORNIS (COPEPODA) FOR BROAD SCALE CONTROL OF AEDES POLYNESIENSIS AND AEDES AEGYPTI IN FRENCH POLYNESIA /Loncke, Skiphane

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In French Polynesia, some haematophagous insects are vectors of human diseases. Ae polynesiensis (Diptera : Culicidae) is the main vector of Bancroftian filariasis, an endemic disease which still affects a great part of oceanian populations. This mosquito is also the vector of DEN fever in rural and urban areas.

Because of a lack of an efficient mass treatment against filariasis and protective inoculation against DEN fever, vector control remains the only measure to regulate these diseases. As preservation of environmental integrity is important to politicians, our efforts have focused on development of appropriate biological methods. Such methods have been based on adequate ecological knowledge of the vector and the belief that the larval stage is the most accessible and vulnerable.

Ae aegypti breeds in peridomestic artificial containers. They are typified by small collections of water e.g., cans, drinking water storage drums, tyres. However, because of the lack of running water in atolls, inhabitants collect rainwater in tanks. These tanks, which can contain several cubic meters of drinking water, are essential breeding sites of Aedes in villages of the Tuamotu archipelago. Breeding sites of Ae polynesiensis are identical to those of Ae aegypti in the villages where the 2 species coexist. However, Ae polynesiensis remains a typically rural species, and also breeds in natural biotopes such as coconut shells, tree holes, rock holes and particularly burrows of the land-crab Cardisoma carnifex. These crabs are numerous in low swampy zones of high islands and in atolls. Their burrows are composed of one or more tunnels which can lead to chambers in contact with the water table.

#### CONTROL AGENTS USED IN FRENCH POLYNESIA

Biological control trials have been realised in French Polynesia with different kinds of mosquito larval predators. Except for the mosquito Toxorhynchites amboinensis (Doleschall), only local predators were selected because of difficulties in importing exotic species. The species tested were (Riviere et al 1986): Tx amboinensis, introduced in Tahiti in 1975 from American

Samoa; Anisops tahitiensis Lundblad, the dragonflies Diplacodes bipunctata, Pantala *flavescens* and *Anax guttatus*; the coleopteran Rantus debilis Sharp; the planarian Dugesia tahitiensis Gourbault, and the mosquito-fish Poecilia reticulata Rosen and Bailey. Results were disappointing. Only the copepod Mesocyclops aspericornis was found to be a good candidate for efficient biological control.

*M* aspericornis is a cyclopoid crustacean with a female of about 1.2 mm in length. Its ability to ingest first stage Aedes larvae was discovered by Riviere and Thirel (1981). This copepod is cosmotropical and occurs naturally in streams and swamps of the Polynesian high islands. It is however not present in atolls and is generally not found in breeding sites of Aedes, so it has to be introduced for mosquito control. Only some breeding sites occasionally submerged by rivers, essentially rock holes and crab burrows, can be naturally colonised although it has been spread by villagers when collecting water.

Like other Cyclopoids, M aspericornis reaches the adult stage after 5 naupliar stages and 5 copepodid stages separated by moults. In good rearing conditions, it takes only a week to achieve complete development. A female can produce 40 to 80 eggs per batch and may lay more than 7 batches in her life. Mass-rearing of M aspericornis is easy and inexpensive. The copepod is polyphagous and can eat prey other than mosquito larvae. Hence, it can persist in breeding sites where mosquito larvae have disappeared. This ability avoids an obligate predator-prey relationship. This feature is very important for biological control applications and contributes greatly to its success. In addition to its food requirements the copepods may engage in non-nutritional killing behaviour. Twenty to 40 larvae can be killed in 24 h by an adult female or a fifth stage copepodid. Two limiting factors seem to inhibit the wide use of M aspericornis in broad scale experiments. The first one is a limited resistance to salinity with a  $LD_{50}$  of about 7% for sodium chloride. The second one is a limited resistance to desiccation. One should point out that this copepod is suspected as an intermediate host of dracunculosis and so should not be used in endemic countries.

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#### PRELIMINARY RESULTS WITH MESOCYCLOPS ASPERICORNIS

*M* aspericornis has been tested successfully in the laboratory and in small scale experiments (Riviere *et al* 1987). It can control first stage *Ae polynesiensis* and *Ae* aegypti for years if adequate conditions are maintained (mean temperature of about 27°C, salinity lower than 10%, no drying of the breeding site). In the original work by Riviere and Thirel (1981), *M* aspericornis was introduced into 27 ovitraps. Twice a week for 63 weeks, the fauna of these ovitraps was analysed, giving approximately 80% control of *Aedes* (Fig. 1). In the same manner, a batch of half tyres was inoculated with *M aspericornis* in 1981. Control of *Aedes* was always effective (Table 1). In 200 litre drums, the copepods persisted particularly well in shade (Paea), but not in those exposed to full sunlight (Tiputa). The increase of water temperature and the decrease of dissolved oxy-

### AEDES LARVAE IN OVIPOSITION TRAPS

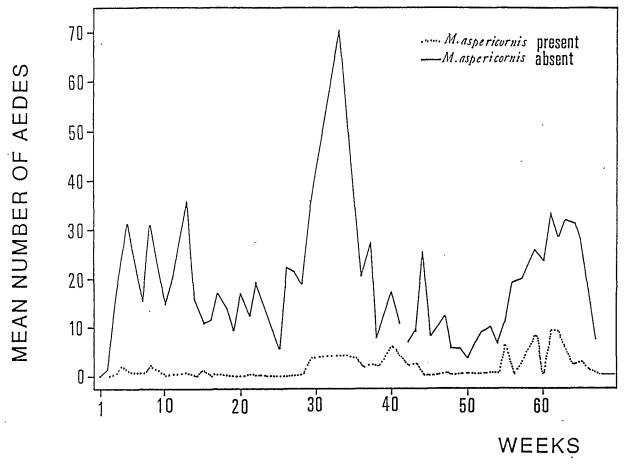


Figure 1. Abundance of Aedes larvae in oviposition traps in the presence or absence of M aspericornis

TABLE 1Persistence of M aspericornis following inoculation in several biotopes of Ae aegypti and Ae polynesiensis (f.Riviere et al 1987).									
									Biotope
]*	2	6	12	18	24	48	60		
Ovitraps	100	-	100	-	75	39	-		
Tyres	100	98	96	-	80	65		100	
Drums									
(Paea)	100	-	100	100					
Drums									
(Tiputa)	-	-	0	-	-	0			
Tree holes	92	-	68	-	-	19	-	17	

\* = Time in months.

#### TABLE 2

Comparison of numbers of Ae polynesiensis	breeding in treated	and untreated	crab holes, Kia-Ora-maite,
	Rangiroa.		

_	Months post treatment								
Zone	0	6	7	18	24	29	34	47	
Treated									
No. crab holes sampled	30	22	20	28	9	29	22	20	
% with M aspericornis	0	100	100	100	100	90	90	90	
% with Ae polynesiensis	100	35	15	36	33	17	14	15	
Not treated									
No. crab holes	20	24	20	38	11	60	_	-	
% with M aspericornis	0	0	Õ	°0'	Ō	Ő	0	0	
% with Ae polynesiensis	100	95	100	100	91	87	100	100	

gen explains the negative results obtained at Tiputa. The control of tree hole breeding *Aedes* is effective if these breeding sites do not dry up. The introduction of *M* aspericornis in 30 crab burrows provided effective control of *Ae* polynesiensis larvae for a period of >2 years (Table 2). On preliminary evidence, these burrows were chosen on their suitability to shelter *M* aspericornis.

#### BROAD SCALE CONTROL OF LAND-CRAB BURROWS

The previous small scale results prompted us to carry out a large scale experiment on land-crab burrows, one of the most numerous breeding sites in atolls. From 1985, we carried out experiments on Rangiroa atoll, Tuamotu archipelago (Lardeux 1987, Lardeux *et al* 1988 a, b). This atoll comprises several small isles, building the characteristic coral reef around a large lagoon. These small isles are traditionally exploited for coconuts for the copra market and coconut groves are the major biotopes of these islands. Land crab holes are numerous and are the only breeding sites for *Ae polynesiensis* outside villages.

Two isles were chosen, the first for treatment and the other as a control area. These 2 isles are next to each other and can be considered as similar in flora and fauna: both have an exploited coconut grove and impenetrable scrub areas. Crab burrow densities were similar on both islands as were Ae polynesiensis population densities as estimated by mark-release-recapture experiments according to the method of Arnason (1972), and showed that Ae polynesiensis had little tendency to disperse between the isles. The treated isle had an approximate surface of 30 ha. Crab-burrows were located on half of the isle, adjoining the lagoon. The treatment of these crab-burrows lasted 120 h and employed 12 workers (ie a total of 1440 man hours). M aspericornis were inoculated into more than 17,300 crab-holes. Treatment effects were analysed at 4 months, 8 months and 13 months after inoculation on the basis of the presence of mosquito larvae and copepods. Adult biting

densities were evaluated. On each occasion, the treated isle was compared with the control area.

#### RESULTS OF BROAD SCALE INOCU-LATION OF COPEPODS

#### Crab Burrows

During the experiment, the percentage of crab burrows containing water was constant between 20-30% on both isles, except in January 1989 (13 months after treatment) when this percentage fell to 9%, due to a period of dryness (Fig. 2). Four and 8 months after treatment, the percentage of crab burrows containing *Ae polynesiensis* was statistically lower on the treated isle than on the control area. This difference disappeared 13 months after treatment. During the same time, the percentage of crab holes with *M aspericornis* fell from 90% 4 months after treatment to 60% at 8 months and finally to 24% 13 months after treatment.

#### **Adult Biting Index**

Twenty nine mosquito capture stations were located on the treated isle and 8, then 17 stations (after the first control date) were located in the control area. These stations were chosen for their similarity regarding the different kinds of biotopes on both isles (clean coconut grove, scrub intermixed with coconut trees, scrub etc). The biting index was computed as the mean obtained during sampling over several days. Each sample was obtained by 2 persons at 1 sampling station for 10 min.

Counts were transformed to Log(x+1) to normalise them and stabilise the variance prior to carrying out an analysis of variants (ANOVA) (Fig. 3). There was no interaction between the "period" effect and the "isle" effect (ANOVA 1.18, 3df, NS). Hence, over the total period of 13 months, it can be concluded that (i) *Ae polynesiensis* adults undergo seasonal fluctuations as would be expected (period effect, 52.5, 3df, P<0.001) and (ii) the treatment had no long term effect on the adult mosquito population (isle effect, 0.22, 1df, NS.). To sum up, the



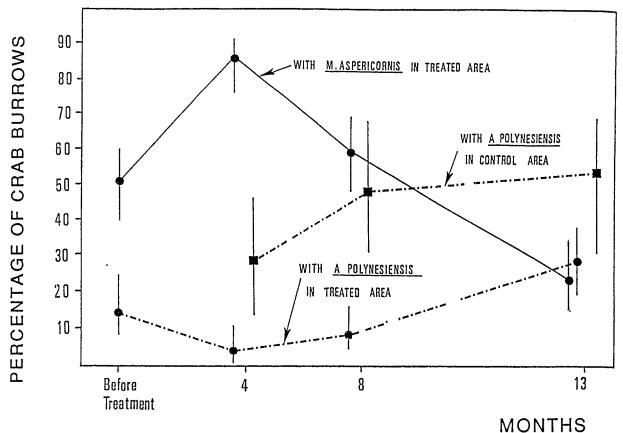


Figure 2. Percentage of crab burrows with *M* aspericornis and *Ae polynesiensis* in the treated area, compared with the control area (95% confidence intervals).

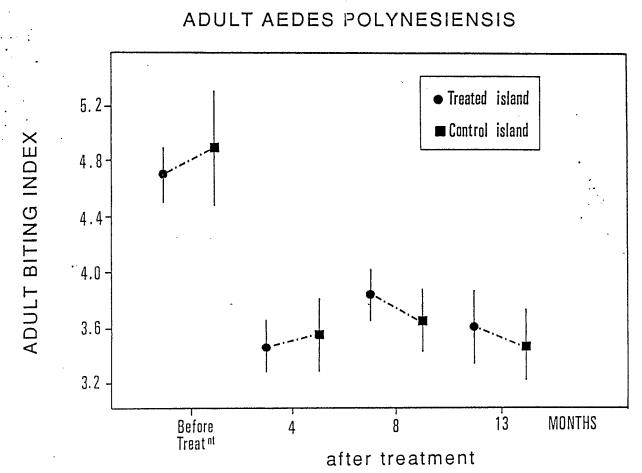


Figure 3. ANOVA comparison of adult biting index between the treated and untreated isle. (95% confidence intervals).

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inoculation of crab burrows with *M* aspericornis produced a signifiant fall of the percentage of burrows with *Ae polynesiensis*. However, this decrease did not last for more than 4 months. Nevertheless, each time *M* aspericornis were found, *Ae polynesiensis* were rarely present. Even when they were, the mean number of mosquito larvae was very low (1-2), while the mean larvae in untreated burrows often exceeded 200.

Although we were able to demonstrate that good control could be achieved in selected crab-hole zones, the broad scale results were disappointing. Although it is possible that some burrows missed initial treatment and it is known that *Cardisoma carnifex* builds new burrows, the reason was the inability of *M aspericornis* to manage the drying of the burrows. It should also be pointed out that only 24% of the burrows contained water at the time of inoculation.

Even if the mass rearing of M aspericornis is easy and inexpensive, large scale treatment of crab burrows is not feasible if dessication- and salinity-resistant stocks of copepods are not available. The salinity resistance should reach a level of 10%. The high cost of carrying out such treatments on remote islands is also another consideration; transport is expensive and logistics are difficult.

# POSSIBILITIES OF FIELD TREATMENT IN TUAMOTU VILLAGES

M aspericornis appears to be effective for large scale control only in easily controlled biotopes. Water tanks occur in every house of Tuamotu villages and would seem appropriate. These water tanks are breeding sites for Ae aegypti and Ae polynesiensis. A simulation experiment (Fig. 4) has been carried out in our laboratory. Three tanks of 8 cubic metres (2m x 2m x 2m) have been artificially inoculated with 10 M aspericornis. Another tank was used as an untreated control. Without M aspericornis, Ae aegypti proliferated although observations in the control tank were stopped prematurely due to a DEN outbreak in Tahiti. In the treated tanks, after an exponential phase of Ae aegypti population growth, M aspericornis suppressed this mosquito in 3 weeks. This experiment lasted 8 months and no Aedes larvae aged more than the first instar have been sampled since.

With this in view, the village of Tikehau (about 80 houses, Tuamotu archipelago) will be treated at the end of 1989. By then, 1 year of preliminary data concerning the population dynamics of Culicidae in this village will be available. The residents of this village are extermely proud and take care in keeping it tidy. With their continued

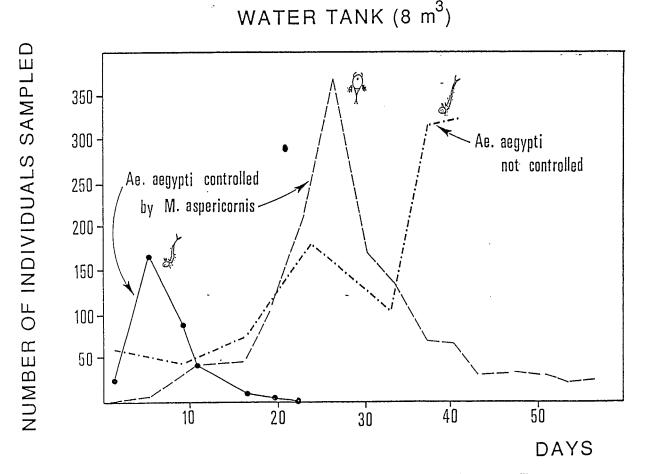


Figure 4. Simulation experiment of biological control with M aspericornis in water tanks of Tuamotu villages.

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support and especially that of the mayor whom we first approached in April 1987, we believe that introduction of *Mesocyclops* may be integrated into a sustainable 'bottom up' programme.

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