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Biological control of Culicidae with the copepod *Mesocyclops aspericornis* and larvivorous fish (Poeciliidae) in a village of French Polynesia

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Abstract. The copepod *Mesocyclops aspericornis* Daday and the larvivorous fishes *Gambusia affinis* (B. & G.) and *Poecilia reticulata* R. & B., were released into mosquito breeding sites in Tuherahera village, Tikehau atoll, French Polynesia, to control larvae of *Aedes aegypti* (L.), *Ae.polynesiensis* Marks, *Culex annulirostris* Skuse and *Cx quinquefasciatus* Say. Treatments were completed within a week, in January 1990.

Fish quickly eliminated mosquito larvae from the open breeding sites (ponds, wells). The impact of copepods in water tanks, drums and covered wells was inconsistent, apparently depending on the availability of microfaunal diet for growth of copepod nauplii. As the biting rate of adult *Ae.aegypti* seemed to be unaffected by the biological control of larvae, this village-scale experiment was judged to be unsuccessful as a means of vector control.

Key words. *Aedes aegypti*, *Aedes polynesiensis*, *Culex annulirostris*, *Culex quinquefasciatus*, *Gambusia affinis*, Copepoda, *Mesocyclops aspericornis*, *Poecilia reticulata*, Poeciliidae, biological control, larvivorous fish, mosquito control, Polynesia.

Introduction

The Tuamotu archipelago, French Polynesia, consists of seventy-six atolls with problems caused by mosquitoes (Diptera: Culicidae) in two ecological situations: (i) rural ecosystems characterized by small inhabited islands where the only agriculture is coconut production, and (ii) small villages with up to 300 people. Usually, there is only one village of this type per atoll, the rest of the island being used for coconut groves.

In addition to the serious nuisance problem caused by *Aedes* and *Culex* mosquitoes, dengue fever and Bancroftian filariasis are mosquito-borne diseases of importance in French Polynesia. As the local inhabitants are reluctant to use insecticides which might pollute lagoons, the only local source of animal protein, alternative methods of mosquito control are being investigated.

According to Rivière & Thirel (1981) and Rivière *et al.* (1987), the larvivorous copepod *Mesocyclops aspericornis* Daday, 1906, could be used in Polynesia as a biocontrol agent giving more than 95% reductions in larval densities of *Aedes polynesiensis* Marks and *Aedes aegypti* (L). As *M.aspericornis* is polyphagic, its populations may persist in the absence of mosquito larvae as prey. *M.aspericornis* is usually more effective against *Aedes* than *Culex* larvae, but it does not resist salinity over 7 parts per thousand and has only weak resistance to desiccation. The latter constraints contributed to the broadscale failure of *M.aspericornis* treatment against *Ae.polynesiensis* in a rural area (Lardeux *et al.*, 1989, 1991) where the main breeding-sites were land crab [*Cardisoma carnifex* (Herbst)] burrows extending below the water table.

On the other hand, atoll villages appear to lend themselves more readily to this biological control agent. Breeding sites of mosquitoes are easier to locate in villages, so that they may be reliably counted, treated and monitored. Results presented here come from the experimental introduction of predators into all mosquito breeding sites in a Polynesian village, using the copepod *Mesocyclops aspericornis* in combination with the larvivorous fishes

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Poecilia reticulata Rosen and Bailey and *Gambusia affinis* (Baird and Girard).

Materials and Methods

Study village. The village of Tuherahera, on Tikehau atoll, is situated about 250 km north-east of Tahiti. Most

organic nitrogen (D.O.N.), dissolved organic phosphorus (D.O.P.) and dissolved salts (NO_2, NO_3, NH_4, PO_4, SiO_2) were assayed by the techniques described in Aminot & Chaussepied (1983), using samples of water taken from various breeding sites and immediately analysed. Results were expressed in μmol l^{-1}.

Health education. As the inhabitants of Tikehau atoll are sensitive to environmental issues, especially pertaining

The data on densities of mosquito larvae and adults form time series, from January 1989 to June 1990, which [redacted]

presence vs absence of larvae in breeding sites is qualitative. The trends, which characterize mean mosquito abundance, was were compared before and after treatment to show the effectiveness of the treatment.

Results

Mosquito breeding sites and species associations

At the time of treatment in January 1990, the numbers and types of mosquito breeding sites available were thirty-six covered wells, thirty-five open wells and ponds, ninety covered water-tanks, ten open water-tanks and 126 drums (Table 1). Throughout the year, each house in the village [redacted]

all mean percentage of positive breeding sites was 49.3% for this species (Table 2). *Ae.aegypti* is associated with [redacted]

200-litre drums (56.5% positive) and covered water-tanks (62.3% positive) (Table 2).

Ae.polynesiensis is a more rural species. Although it can use the same artificial breeding sites as *Ae.aegypti*, it prefers natural sites such as tree-holes, rock-holes, half coconuts and land crab burrows. Hence *Ae.polynesiensis* is not seen as often as *Ae.aegypti* in the village breeding sites. However, it does choose some covered wells (17.9%) and drums (15.3% positive).

Cx annulirostris breeds in large collections of clear water: ponds, water holes, open wells (38.8%) and open water tanks (21.9% positive). *Cx quinquefasciatus* chooses the same breeding sites as *Cx annulirostris*, as well as sites containing much organic matter. In Tikehau, *Cx quin-* [redacted]

Table 3. Analyses of chemicals dissolved in water samples from wells, drums and tanks; mean values expressed as μmol per litre, standard deviation in parentheses.

	Wells	Drums	Water tanks
n	24	11	36
NO_2	0.8 (1.5)	0.3 (0.4)	0.06 (0.04)
$NO_3 + NO_2$	38.2 (27.1)	6.2 (13.4)	4.3 (4.0)
NH_4	1.7 (2.6)	0.6 (0.5)	0.09 (0.1)
D.O.N.	46.9 (26.0)	12.3 (18.7)	8.2 (6.7)
PO_4	3.1 (2.6)	2.1 (1.7)	0.7 (0.7)
D.O.P.	2.9 (2.1)	2.4 (1.7)	0.7 (0.6)
SiO_2	69.0 (43.8)	11.3 (30.0)	8.4 (14.1)

Five months after treatment, chemical analyses were made of thirty-nine breeding sites without *Mesocyclops aspericornis* and eight still positive for the copepod. These positive sites were all drums or water tanks containing rainwater. Statistically significant differences in mean level of salts were found for NO_2, NH_4, PO_4 and D.O.P., with higher levels in copepod positive rain water. Differences were not significant for SiO_2, ($NO_3 + NO_2$) and D.O.N. (Table 4). Temperature varied between 26 and 29°C, pH between 6.9 and 7.8, dissolved oxygen between 6.0 and 8.0 g l^{-1} but no significant difference was found between positive and negative sites. As expected, the salinity level was less than 0.03 p.p.k. for rainwater. In wells it ranged from 0.11 to 0.70 p.p.k, with a mean of 0.37 ± 0.15.

Survival of predators

After fish were put into thirty-five open breeding sites (wells, water holes and ponds) in January 1990, all the sites sampled still contained fish up to 5 months post-treatment (Table 5). Copepods were introduced to all the other breeding sites: drums, covered wells, cisterns and tanks (ninety open and ten covered). Covered wells remained positive for *M.aspericornis* (Table 5). In the covered water-tanks, only 1/68 sampled in March and 2/59 sampled in June still contained the copepod. Of the open water tanks, only 1/8 was still positive at the end of the experiment (Table 5). As for 200-litre drums, 10% still contained copepods in March and of ninety-two sampled in June 9% were still positive (Table 5).

Effects of treatments

Covered wells (Fig. 1A). Introduction of *M.aspericornis* in January 1990 reduced the prevalence of *Culex* spp. from 9–37% of covered wells to zero for *Cx annulirostris* in March and for *Cx quinquefasciatus* in June, 2–5 months post-treatment. The percentage of wells with *Ae.aegypti* larvae fell significantly from pre-treatment rates of 39–44% to post-treatment rates of 24% in March and 10% in June 1990. *Ae.polynesiensis* exhibited the same trend, falling from 31% in January to only 5% in March and June 1990.

Open wells and ponds (Fig. 1B). Larvae of *Culex* spp. outnumbered *Aedes* spp. in most open breeding sites before treatment. After introduction of fish, larvae dis-

Table 4. Comparison of mean μmol l^{-1} salt concentrations in copepod positive and negative rain-water (standard deviation, in parentheses). S, significant difference, $P < 0.05$; N.S., non-significant difference, $P < 0.05$.

M.aspericornis	*n*	NO_2	($NO_3 + NO_2$)	NH_4	D.O.N.	PO_4	D.O.P.	SiO_2
Present	8	0.40	8.66	0.66	15.69	2.15	2.36	15.80
		(0.07)	(2.49)	(0.04)	(3.65)	(0.37)	(0.37)	(6.58)
Absent	39	0.06	3.94	0.13	7.78	0.76	0.87	7.69
		(0.03)	(1.12)	(0.09)	(1.04)	(0.17)	(0.17)	(2.98)
Difference		S	N.S.	S	N.S.	S	S	N.S.

Table 5. Persistence of copepod and fish populations in various types of mosquito breeding sites.

Type of breeding site	Agent	January 1990 No. sites inocculated	March 1990 No. +ve/sampled	March 1990 %	June 1990 No. +ve/sampled	June 1990 %
Covered weels	Copepod	36	21/21	100	20/20	100
Open wells	Fish	35	24/24	100	31/31	100
Covered tanks	Copepod	90	1/68	1.5	2/59	3.4
Open tanks	Copepod	10	1/10	10.0	1/8	12.5
200-litre drums	Copepod	126	10/98	10.2	8/92	8.7

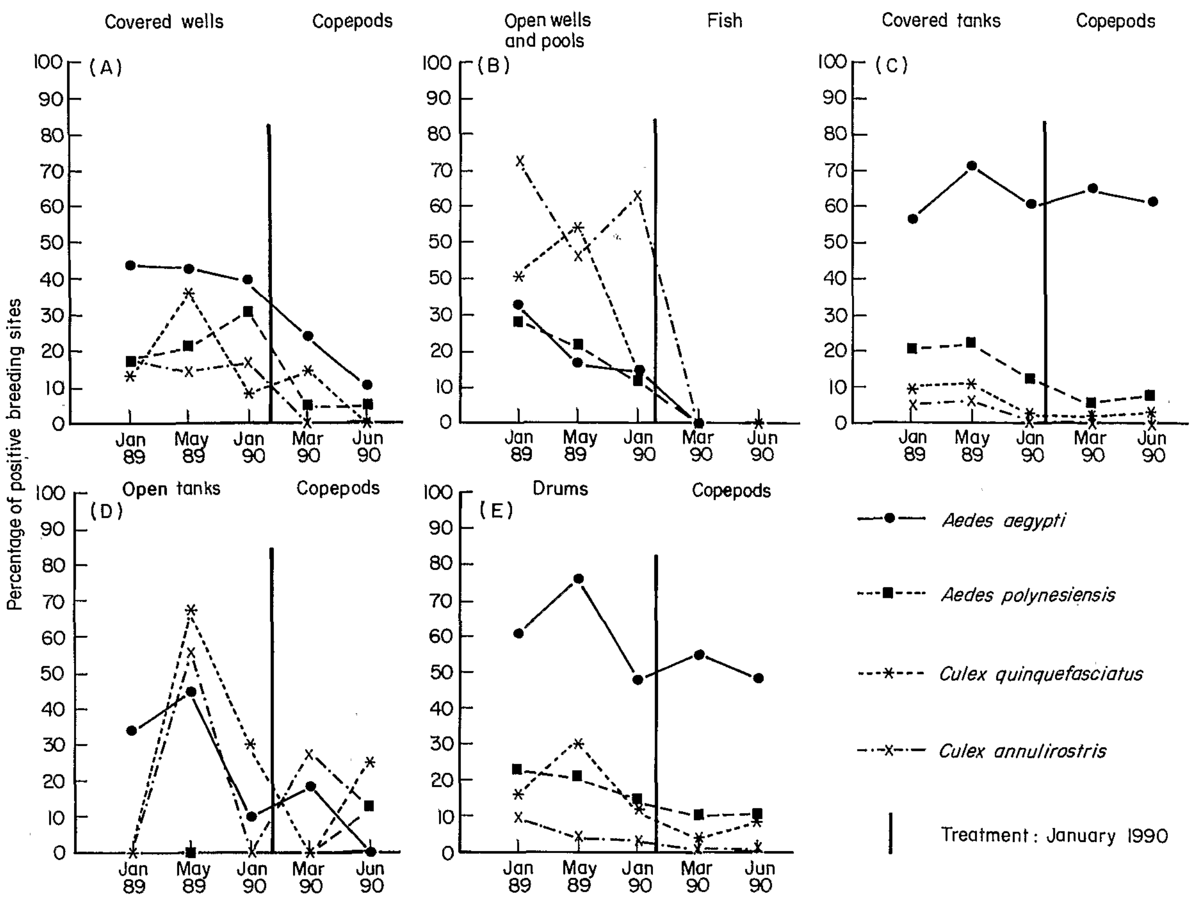


Fig. 1. Percentage of mosquito positive breeding sites, January 1989 to June 1990: (1) in covered wells, (2) in open wells and ponds, (3) in covered rainwater tanks, (4) in open rainwater tanks, and (5) in 200-litre drums. Just after the January 1990 survey, as indicated by the vertical line, fish or copepods were introduced as mosquito control agents.

appeared completely from all these former breeding sites.

Covered water-tanks (Fig. 1C). *Aedes* spp. outnumbered *Culex* spp. in covered tanks before introduction of copepods. Thereafter, the prevalence of *Ae.aegypti* and *Cx quinquefasciatus* remained unchanged (61–64% positive tanks for the former, less than 5% for the latter), whereas the prevalence of *Ae.polynesiensis* dropped below 10% and *Cx annulirostris* larvae disappeared completely.

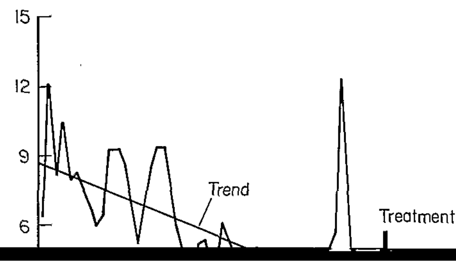
Open water-tanks (Fig. 1D). In the ten open tanks treated with copepods, the proportions with mosquito larvae did not reach the same levels as recorded in May 1989. No *Ae.aegypti* larvae were found in June 1990, 5 months post-treatment, but it is unclear whether reductions in all four species of mosquito larvae could be attributed to *M.aspericornis*.

200-litre drums (Fig. 1E). The percentage of positive drums before treatment was 48–76% for *Ae.aegypti*, much less for the other three species. After introduction of *M.aspericornis*, the prevalence of all four species of larvae remained stable throughout the study period.

Adult mosquitoes. Before the treatment of larval habitats, 1500 human bait collections of adult mosquitoes were done, followed by 666 catches post-treatment. These catches were averaged for each week period and the results form a time series with a strongly negative trend (Fig. 2). On no occasion were no mosquitoes caught in a daily collection. A significant reduction ($P < 0.05$) of the mean adult catches post-treatment could not be attributed to the treatment effect, since the downward trend in adult mosquito biting density occurred at the same rate throughout the pre-treatment as well as the post-treatment periods of assessment.

Discussion

Tikehau villagers are well aware of the nuisance caused by mosquitoes. Health education meetings served to develop their understanding of vector-borne diseases and biological control, so that this experiment was carried out with the full consent and help of the community. The village is kept free from potential *Aedes* breeding-sites in discarded tins, cans, old tyres and coconut shells by the municipal collection of rubbish and the instigation of the local religious associations. This conscientions cleaning of the village is an important contribution to the control of peri-



of *M.aspericornis* controlled the larval population of *Ae.aegypti* within 3 weeks (Lardeux *et al.*, 1989).

Some of the 200-litre drums were found to be negative for mosquito larvae and positive for *M.aspericornis*. When present in drums, the copepod acted as an effective control agent. Chemical analysis of water samples from drums showed that their nutrient content was intermediate between that of the rainwater from cisterns and well-water

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