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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 of the 300 inhabitants are grouped together in one village, built upon an island of about 240 ha. Eighty houses are spread along two roads extending 1.5 km; a few isolated houses are situated on the northern point of the island, several hundred metres away from the village. From all points of view, it is a typical village of the Tuamotu atolls.

The climate there is of the tropical humid type, with mean temperature varying between 24.5 and 29.3°C. The annual precipitation is about 1800 mm, with rainfall mainly between November and March.

Mosquito species. Only four species of Culicidae were recorded in the larval breeding sites at Tikehau village: *Aedes aegypti*, *Ae.polynesiensis*, *Culex quinquefasciatus* Say and *Cx annulirostris* Skuse.

Ae.aegypti is the main vector of dengue fever in French Polynesia. *Ae.polynesiensis* is the main vector of human Bancroftian filariasis caused by sub-periodic *Wuchereria bancrofti* (cobbold), canine filariasis caused by *Dirofilaria immitis* and is also suspected to transmit dengue fever. *Cx quinquefasciatus*, a minor vector of Bancroftian filariasis, is mainly a nuisance, along with *Cx annulirostris* which is also a good vector of *D.immitis*. The two species of *Aedes* have a diurnal activity while the two species of *Culex* have a nocturnal activity.

Mosquito breeding sites. On these atolls, unlike the higher islands of Polynesia, there is no running water, no streams or rivers. The only freshwater resources are: (i) wells and ponds dug approximately 2 m down to the water table, and (ii) rainwater collected from the roofs of houses and stored in various receptacles. Among these, large concrete cisterns, of up to 30 m^3 capacity. In general, rainwater is used for human consumption (drinking, cooking, showers, etc.), while water from the wells, which is more or less brackish or polluted, is used for other washing purposes and for watering gardens.

In Tikehau atoll, breeding sites of mosquitoes comprise wells, water holes, ponds, 200 litre drums and concrete water tanks, which may be well sealed, simply covered with sheet-metal or planks, or open to the sky. These distinctions may also be made amongst the wells. No classical peridomestic sites, such as discarded tins, cans, old tyres or coconuts, were recorded.

Breeding sites were classified into five categories, according to the kind of water they contained: rain water or groundwater; and how they were covered against external pollution. Thus we have distinguished between: (i) open wells and ponds, (ii) covered wells, (iii) open cisterns and tanks, (iv) covered cisterns and tanks, and (v) 200-litre drums.

Water quality. Temperature, pH, dissolved oxygen and salinity were measured in the breeding sites. Dissolved 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 to freshwater because of its scarcity, the introduction of biological control agents into mosquito breeding sites had to be carefully explained to the people. For this purpose, a series of health educational meetings were organized in order to explain our experiment and its relative harmlessness for humans and their environment.

Mosquitoes are regarded as a nuisance by the villagers and a dengue fever outbreak (serotype 1, then 3) hit French Polynesia in 1989–90. Both these factors have contributed to the idea of controlling mosquito populations. The information meetings enabled us (i) to attain treatment of all the known breeding sites in the village, and (ii) to obtain the participation of the inhabitants themselves in the treatment.

Treatment. Because *M.aspericornis* and Poecilid fish do not occur naturally on Tikehau, they were brought by air from Tahiti. Copepods were transported as inocula of fifty per 20 ml tube; fish were shipped in jerrycans of 10 litres capacity.

In one week in January 1990, *Mesocyclops aspericornis* were introduced into all the breeding sites where larvivorous fish could not be introduced. Basically, twenty to thirty fish were introduced into each open well, pond and water hole, whereas about fifty *M.aspericornis* were put into each drinking water container (cisterns, water tanks, 200-litre drums) and covered wells.

Evaluation. Mosquito larval densities were compared before (from January 1989 to January 1990) and after treatment (from January to June 1990). Human bait collections of mosquitoes were made from January 1989 to June 1990 in order to estimate any impact on the adult *Ae.aegypti* population.

Mosquito larvae were collected using a 300 μm mesh plankton net. Due to the difficulty of gaining access to closed water tanks and cisterns, only a qualitative assessment was done, based on presence or absence of larvae; the 'absence' parameter would have been overestimated wherever larvae were missed.

All breeding sites were sampled in January 1989 – 1 year before treatment; May 1989; January 1990 – a few days before treatment; March 1990 – 1 month after treatment, and June 1990 – 5 months after treatment.

Adult mosquitoes were collected on human bait by one collector using an aspirator between 16.00 and 18.00 hours, in groups of six houses per day, 15 min per house, representing a specific area of the village each working day. Adult mosquitoes were thus thus sampled from a total of thirty houses week after week, starting in January 1989, i.e. 1 year before treatment. After the treatment week in January 1990 they were sampled weekly until the end of the experiment in June 1990.

The data on densities of mosquito larvae and adults form time series, from January 1989 to June 1990, which oscillate on either side of a general trend. The time series for (human-bait catches) is quantitative whereas that for 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 had at least one breeding site producing mosquitoes. *Aedes aegypti* was present in most houses all year round.

The overall percentage of positive breeding sites (i.e. containing at least one larva) varied from 63–84% before treatment to 50–56% post-treatment. Proportions of positive wells were 57–71% pre-treatment falling to 15–29% post-treatment for covered wells, 88–96% pre-treatment dropping to zero after treatment of open wells. In the other types of breeding sites, i.e. water-tanks and drums, the percentage positive remained around 60 ± 10% (Table 1).

Among the four mosquito species encountered in Tikehau, *Ae.aegypti* was the most prevalent. The overall mean percentage of positive breeding sites was 49.3% for this species (Table 2). *Ae.aegypti* is associated with human activities, breeding mainly in artificial peridomestic containers. In Tikehau, its favourite breeding sites are 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 quinquefasciatus* has also been found alongside *Ae.aegypti* and *Ae.polynesiensis* in 200-litre drums (13.7% positive) (Table 2).

Water quality

There were higher levels of dissolved organic nitrogen (D.O.N.) phosphorus (D.O.P.) and more salts (NO_2, NO_3, NH_4, PO_4, SiO_2) in the samples of groundwater from wells than in rainwater stored in covered tanks. Rainwater in drums and open cisterns, exposed to external pollution, had an intermediate level of salts (Table 3).

Table 1. Numbers of mosquito breeding sites of five types assessed (n) and positive (+ve) during the five sampling periods.

	January 1989		May 1989		January 1990		March 1990		June 1990	
Type of breeding site	*n*	+ve	*n*	+ve	*n*	+ve	*n*	+ve	*n*	+ve
Covered well	23	13	28	20	36	24	21	6	20	3
Open wells, ponds	25	22	24	23	35	31	24	0	31	0
Covered tank	64	38	65	54	90	59	67	43	59	40
Open tank	3	1	9	8	10	3	10	5	8	4
200-litre drum	75	45	97	83	126	76	98	63	92	53
Total	190	119	223	188	297	193	220	122	210	105
Proportion+ve	63%		84%		65%		56%		50%	

Table 2. Percentages of positive breeding sites for each mosquito species in five types of breeding sites.

Type of breeding site	*n*	*Ae.aegypti*	*Ae.polynesiensis*	*Cx quinquefasciatus*	*Cx annulirostris*
Covered well	128	33.6	17.9	14.8	10.9
Open well	139	14.4	11.5	23.7	38.8
Covered tank	345	62.3	13.3	2.3	5.2
Open tank	40	19.5	2.4	21.9	26.8
200-litre drum	488	56.5	15.3	3.5	13.7
Total	1140	49.3	14.1	8.9	13.0

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 (0.07)	8.66 (2.49)	0.66 (0.04)	15.69 (3.65)	2.15 (0.37)	2.36 (0.37)	15.80 (6.58)
Absent	39	0.06 (0.03)	3.94 (1.12)	0.13 (0.09)	7.78 (1.04)	0.76 (0.17)	0.87 (0.17)	7.69 (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.

		January 1990	March 1990		June 1990	
Type of breeding site	Agent	No. sites inocculated	No. +ve / sampled	%	No. +ve / sampled	%
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

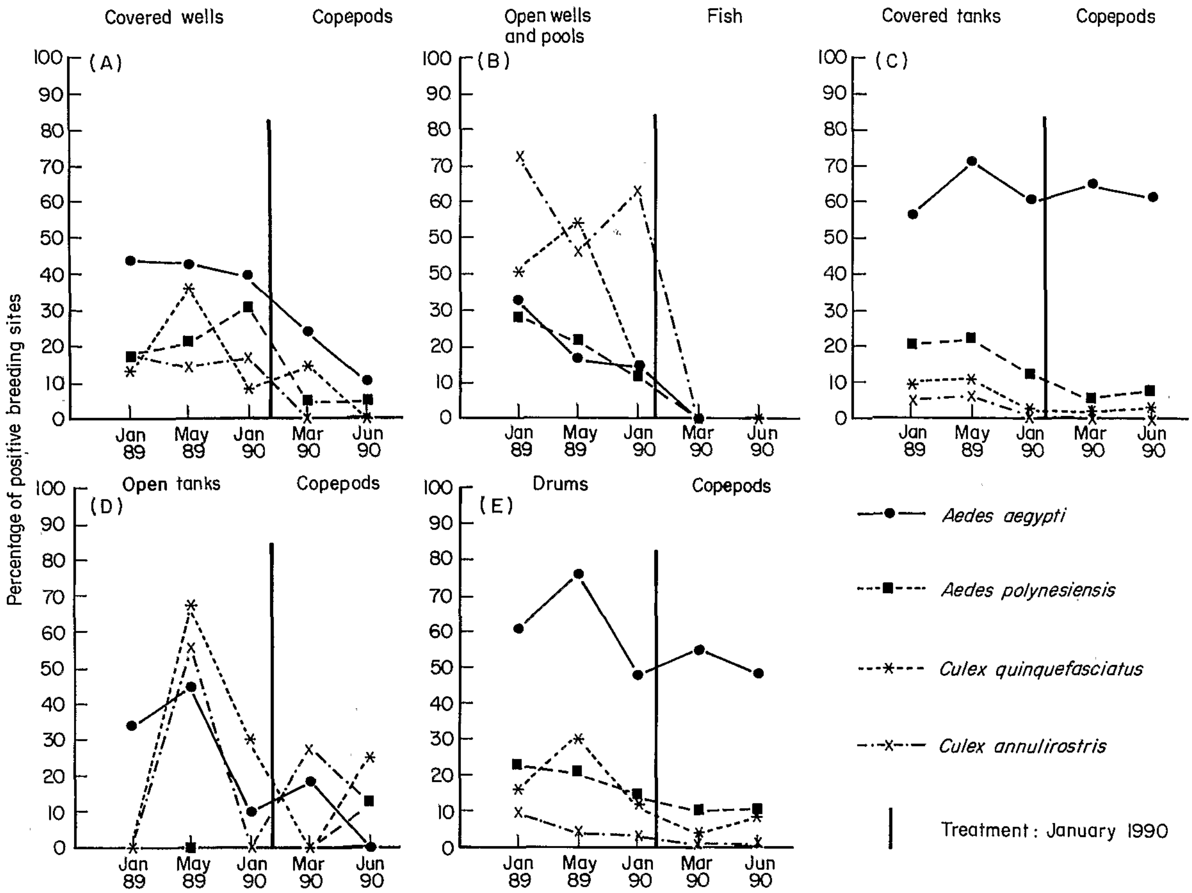

Fig. 1. Percentage of mosquito positive breeding sites, January 1989 to June 1990: (1) in covered wells, (2) in open wells and ponds, (3) in covered rainwater tanks, (4) in open rainwater tanks, and (5) in 200-litre drums. Just after the January 1990 survey, as indicated by the vertical line, fish or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

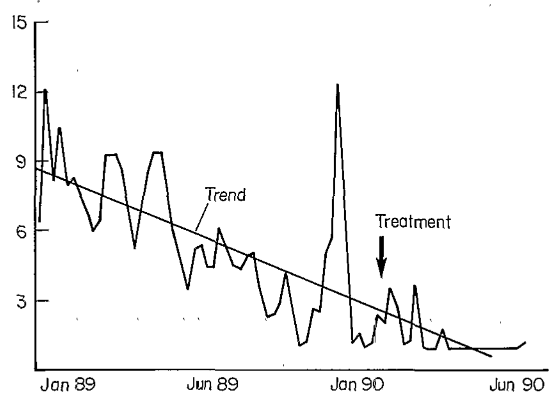

Fig. 2. *Aedes aegypti* biting density: time series of weekly human bait catches, 14.00–18.00 hours: mean number of *Ae.aegypti* females per man per 15 min indoors.

domestic *Aedes* in Polynesia, whereas disposal of such small breeding sites is still unsolved in many other parts of the world (Kay, 1986).

In sites which could not be eliminated, the introduction of larvivorous fish (*G.affinis*, *P.reticulata*) or copepods (*M.aspericornis*) was organized according to the characteristies of each breeding-site. Covered sites, preferred by *Aedes* spp., are unsuitable for fish, due to insufficient light, so were inoculated with *M.aspericornis*. Wherever fish would thrive in open wells and ponds, they were introduced as the predators of choice, and quickly eliminated larval populations of *Aedes* spp. and especially *Culex* spp. Five months after their introduction, large populations of larvivorous fish had developed in each selected site, showing good adaptation to their new environment. Hurlbert *et al.* (1972) and Hurlbert & Mulla (1981) noticed that the impact of *Gambusia affinis* on the invertebrate populations can lead to increased phytoplankton abundance and a change in the physico-chemical characteristics of the water, sometimes detrimental to the fish themselves. No such phenomenon has been noticed in Tikehau.

Unexpectedly, *Mesocyclops aspericornis* acted as a better biological control agent against *Culex* than *Aedes* larvae in covered wells, as the proportion of positive wells decreased to zero for both *Culex* spp. 2–5 months after treatment, but not for the *Aedes* spp. Unfortunately, copepod populations survived in only a few of the concrete water-tanks, most of which (open and closed) remained mosquito positive after treatment. Evidently copepods survived better in well water, containing mineral and organic salts, than in tanks of rainwater which contains few nutrients, as shown by chemical analysis. Presumably rainwater is unsuitable for development of planktonic micro-organisms on which copepods feed during their naupliar stage of development, whereas *Aedes* mosquito larvae feed by foraging on detritus and grazing on micro-algae on the cistern walls, a diet unsuitable for copepods in the early stages of their development. In a separate study, copepod nauplii grew better in tapwater (from a stream), so that an inocculum 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 (Table 3). Evidently water in drums may support sufficient microfauna to feed copepod nauplii which can then control mosquito larvae. However, the copepod fauna tends to be decanted from drums when villagers use and replenish the water.

As pointed out by Service (1983), it is necessary to assess the population density of adult mosquitoes in order to evaluate the success of any control method. In this case, the impact of biological control agents used against mosquito larvae gave unclear results with regard to the adult mosquito populations. The method of sampling (human bait collections indoors, 16.00–18.00 hours) preferentially collected *Ae.aegypti*, so we lack information on adult population densities of *Ae.polynesiensis*, *Cx annulirostris* and *Cx quinquefasciatus*. During the pretreatment sampling period, throughout 1989, a steady decline was observed in densities of *Ae.aegypti* adults. This was attributed to prolonged drought plus the results of increased public awareness of the need to discourage mosquito breeding. With seasonal rains at the end of 1989, the biting population density of *Ae.aegypti* females rose transiently to the initial levels of 9–12/man/15 min, but soon fell below 5/man/15 min to a level of no more than 1 bite per 15 min from May 1990 onwards. As the overall trend of declining density, as plotted in Fig. 2, is the same before and after treatment, we cannot define the degree of adult mosquito control attributed to larval predation by the biological control agents.

At the end of the experiment, 5 months post-treatment, most of the breeding sites were still positive, but with fewer larvae per site. So in spite of good control in some of the breeding sites, the abundance of mosquitoes emerging from other sites continued to cause nuisance problems. In this sense, biological control of mosquitoes in Tikehau has not proved successful on a large scale. However, the larval populations of both *Aedes* and *Culex* can be suppressed, and sometimes eliminated, by larvivorous fish and the copepod *Mesocyclops aspericornis* used in parallel to treat sites with appropriate characteristics for good survival rates of one or other of these biological control agents. A biological control agent capable of survival in tanks of fresh rainwater is urgently neded, for use against *Ae.aegypti* in particular. Dragonfly nymphs (Odonata) have been recommended by Sebastian *et al.* (1990) but would have to be imported for this purpose.

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