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Control of the *Aedes* vectors of the dengue viruses and *Wuchereria bancrofti*: the French Polynesian experience*

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In most of the 130 islands of French Polynesia, the stenotopic mosquitoes *Aedes aegypti* (the main local vector for the viruses causing dengue) and *Aedes polynesiensis* (the main local vector of *Wuchereria bancrofti*) share many breeding sites in water containers such as discarded cans, coconut shells, buckets and water-storage pots and drums. In addition to selective application of insecticides, non-polluting methods of controlling these mosquitoes have been evaluated during the last decade in two main ecological situations: (1) villages, where *Aedes* breeding sites are typically peridomestic; and (2) flooded burrows of land crabs, the major source of *Ae. polynesiensis* throughout the South Pacific region.

Large-scale trials of biological control agents, such as mosquito fish (*Gambusia affinis* and *Poecilia reticulata*) and copepods (*Mesocyclops aspericornis*), and of integrated-control strategies have demonstrated the efficacy of certain techniques and control agents against the target *Aedes* populations in some village situations. Generally, mechanical methods (the use of layers of polystyrene beads against mosquito larvae and pupae, and screening against adult mosquitoes) were more efficient than use of the biological control agents. By integrating several methods of control, mosquito densities (as measured by human-bait collections and larval surveys) were reduced significantly compared with the results of concurrent sampling from untreated villages, and control remained effective for months after the interventions ceased.

In land-crab burrows, the first attempts to control *Aedes* larvae used bacterial agents (*Bacillus thuringiensis*) and predatory copepods gave disappointing results. *Mesocyclops aspericornis* could be an effective control agent if the burrows were constantly flooded, but most burrows dry out and refill periodically, so copepod populations do not survive. As it proved difficult to reach all corners of the long sinuous burrows with any control agent, larvicidal (chlorpyrifos-methyl) baits were developed for foraging crabs to carry into their burrows. This novel technique proved to be effective and could become the method of choice for treating crab burrows. Further research is underway to find the optimum (biological or chemical) larvicidal ingredient for the crab bait.

Despite the ecological and logistical challenges of controlling the *Aedes* vectors of the dengue viruses and *W. bancrofti* in so many scattered islands, the French Polynesian experience indicates that relatively simple methods can be integrated and applied effectively and economically. Operationally, however, success also depends on a strong political commitment and on at-risk communities that are sufficiently motivated to maintain a good level of *Aedes* control.

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French Polynesia comprises some 130 volcanic islands and atolls scattered across 5 million km^2 of the South Pacific Ocean (135–145°W, 8–28°S). In this country, two human diseases are sustained by mosquitoes: bancroftian filariasis, caused by *Wuchereria bancrofti* transmitted by *Aedes polynesiensis* (Rosen, 1955); and dengue, caused by viruses transmitted by *Aedes aegypti* in urban environments and *Ae. polynesiensis* in rural areas (Maguire *et al.*, 1971). Both species of vector are diurnally active and *Ae. polynesiensis* is a serious human-biting nuisance on many islands. The common, night-biting, house mosquito, *Culex quinquefasciatus*, has spread to urban parts of the major islands, especially the capital city of Papeete in Tahiti. Although *Cx. quinquefasciatus* is a major vector of nocturnally periodic *W. bancrofti* in many other countries, it remains a relatively poor, secondary vector of the diurnally sub-periodic *W. bancrofti* found in Polynesia (Rosen, 1955).

In French Polynesia, dengue viruses circulate intermittently and cause irregular epidemics, most recently in 1971, 1975, 1979, 1988, 1989, 1996 and 2001 (see www.ilm.pf). As no vaccine is yet available to prevent dengue and the symptomatic treatment of dengue fever is not particularly effective, the most practical method of reducing the incidence and morbidity of the disease is vector control.

Bancroftian filariasis remains endemic in French Polynesia and many other Pacific-island communities (Burkot *et al.*, 2002). During the 1940s, the prevalence of human infection with *W. bancrofti* ranged from 30%–90% and transmission potentials varied widely with the archipelago involved (Lardeux and Cheffort, 2001). The control measures implemented between 1948 and 1980, which were primarily based on case treatment and mass administrations of diethylcarbamazine (DEC), successfully reduced the prevalence of the disease to <1%. After the mass-treatment programme stopped, however, prevalences rose to approximately 20% after 10 years, proving the efficiency of the local vectors. Recently, the Pacific Programme for the Elimination of Lymphatic Filariasis (PacELF) has re-introduced annual rounds of mass treatment to French Polynesia, although treatment is now based on a combination of DEC and albendazole (Burkot *et al.*, 2002). As past experience in French Polynesia has shown, chemotherapy alone is unlikely to eliminate bancroftian filariasis, and vector control should therefore still be undertaken (Esterre *et al.*, 2001). Indeed, control of *Aedes* is of particular importance because, in a process known as 'limitation', the efficiency of the potential vectors in this genus may increase as the mean intensity of microfilaraemia declines (Pichon, 2002). It is the very efficient transmission of *W. bancrofti* by local *Aedes* spp. which is largely responsible for the failure of mass treatment to stop transmission of this filaria in French Polynesia.

As *Ae. aegypti* and *Ae. polynesiensis* are both stenotopic species that breed in small containers, they are difficult to control with any single method. The ecology of *Ae. aegypti* is well understood and will not be presented here. *Aedes polynesiensis* is a typically rural species and breeds prolifically in a variety of artificial and natural biotopes (Jachowski, 1954), such as rock holes, coconut shells, tree holes, and particularly burrows of the land crab *Cardisoma carnifex* (Klein and Rivière, 1982). In more urbanized (but still 'rural') environments, such as villages, the breeding sites of *Ae. polynesiensis* are identical to those of *Ae. aegypti* (the two species can co-exist) and are typified by small collections of water in the peridomestic environment, in cans, storage drums for drinking water, tyres, wells etc.

The options for control of the vectors are limited. Personal-protection measures such as bednets are not very effective because the vectors are strongly anthropophilic and feed during the day, both indoors and outdoors. In French Polynesia, environmental protection is important to politicians and local populations alike. The long-term use of chemical insecticides is discouraged, and chemical applications (ultra-low-volume sprayings of malathion and permethrin) are only authorized during dengue epidemics. Research to discover better ways to control mosquitoes has therefore focused on the development of appropriate methods of biological control. Such methods have been developed on the basis of an adequate knowledge of the ecology of the vectors and the belief that the larval stage is the most vulnerable and accessible.

The data presented below were collected during attempts to improve the control of *Ae. aegypti* and *Ae. polynesiensis* in the insular environment of French Polynesia and particularly in two ecological situations: the rural environment, where land-crab burrows are the major breeding sites; and the village environment with its more 'classical' breeding sites. The results are probably applicable to all of the islands of the Pacific Region where similar situations exist and where *Ae. aegypti* and *Ae. polynesiensis* proliferate.

BIOLOGICAL-CONTROL AGENTS TESTED IN FRENCH POLYNESIA

Biological control has been attempted several times in French Polynesia, with various animals that feed on mosquito larvae. Except for the mosquito *Toxorhynchites amboinensis*, which was introduced into Tahiti in 1975 from American Samoa (Rivière *et al.*, 1979), only local predators (which were easier to acquire and considered safer in terms of environmental impact) have been investigated. Rivière *et al.* (1986) tested the following: the mosquito *Tx. amboinensis*, the backswimmer *Anisops tahitiensis*, the dragonflies *Diplacodes bipunctata*, *Pantala flavescens* and *Anax guttatus*, the coleopteran *Rantus debilis*, the planarian *Dugesia tahitiensis*, the mosquito fish *Poecilia reticulata* and a copepod, *Mesocyclops aspericornis*. Their results were disappointing apart from those for the mosquito fish and the copepod, which appeared to be good candidates as efficient agents of vector control. Since *M. aspericornis* was discovered and found able to feed on mosquito larvae (Rivière and Thirel, 1981), it and some other cyclopoid copepods have been tested successfully against *Aedes* and other mosquito larvae, both in the laboratory and in small-scale field experiments (Rivière *et al.*, 1987; Marten, 1990; Kay *et al.*, 1992, 2000; Marten *et al.*, 1994; Russel *et al.*, 1996; Vu *et al.*, 1998). When, in their original study, Rivière and Thirel (1981) introduced *M. aspericornis* into 27 ovitraps, they found that the copepods cut *Aedes* production in the ovitraps by about 80% over the next 63 weeks (Fig. 1). Rivière *et al.* (1987) showed that *M. aspericornis* persisted particularly well in 200-litre drums in the shade (exposure to full sun leading to temperatures that were too high and dissolved-oxygen concentrations that were too low for the copepod). The copepod could control tree-hole breeding *Aedes* effectively if these breeding sites remained wet (Rivière *et al.*, 1987). Similarly, a batch of water-filled half-tyres which were inoculated with *M. aspericornis* in 1981 (and have never dried out since) remain virtually free of *Aedes* larvae (Y. Séchan, unpubl. obs.). The introduction of *M. aspericornis* into 30 land-crab burrows provided effective control of *Ae. polynesiensis* larvae for a period of >2 years, although the burrows used may have been specifically selected for their suitability to shelter the copepod (Rivière *et al.*, 1987). Large-scale experiments in villages and crab burrows have now been carried out to see if the results of these pilot studies were as promising as they at first appeared.

CONTROL OF *Aedes* IN BURROWS

The control of mosquitoes breeding in land-crab burrows has never been addressed successfully, although >140 mosquito species are associated with land crabs throughout the world. In French Polynesia (and in similar South-Pacific islands), land-crab burrows have long been recognized as mosquito larval habitats (Bonnet and Chapman, 1958; Laird, 1988). They are probably the main, non-village sites of mosquito breeding on the

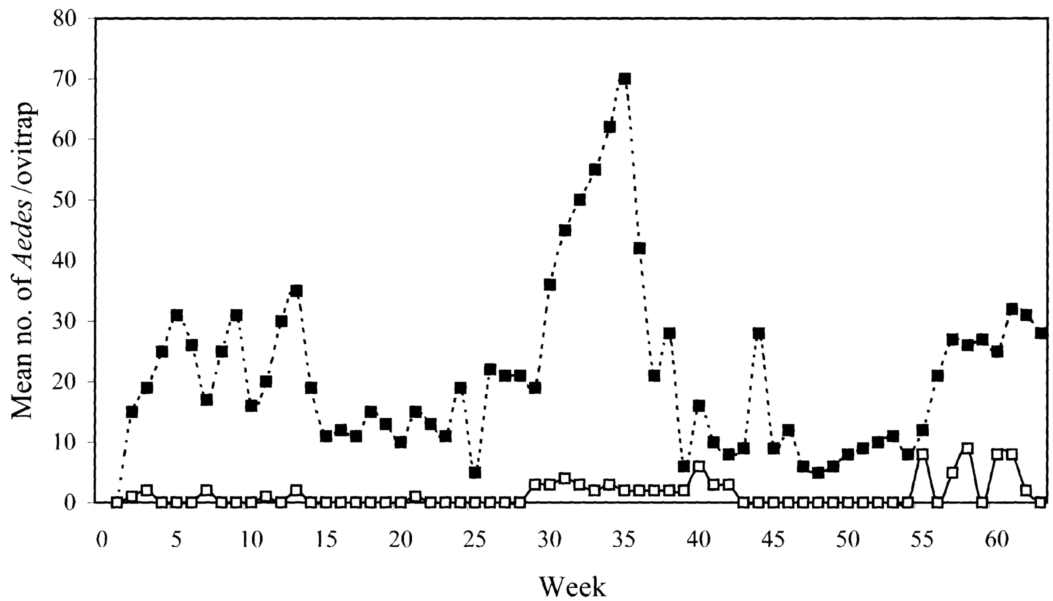

FIG. 1. Effect of the introduction of *Mesocyclops aspericornis* on *Aedes* larval abundance in small containers. The graph shows the mean numbers of *Aedes* larvae/ovitrap in 27 ovitraps which had been treated with the copepod on week 0 (□) or left copepod-free (■). After Rivière and Thirel (1981).

atolls (Lardeux *et al.*, 1992). From 1986 to 1989, large-scale experiments were carried out to control *Ae. polynesiensis* breeding in land-crab burrows on an entire islet, using the copepod *M. aspericornis* (Rivière *et al.*, 1987; Lardeux *et al.*, 1992). More than 17,000 crab burrows on the islet were each inoculated with 10–20 copepods, and the effects of treatment were followed for >1 year (using, as controls, burrows that had larvae but no copepods or copepods but no larvae). The impact of the intervention was assessed not only by checking burrows for copepods and mosquito larvae and pupae ('immatures') but also by monitoring the adult *Ae. polynesiensis* population (using landing catches on human bait).

The mean number of *Aedes* immatures collected from each burrow treated, 3 months earlier, with copepods (<2.0) was far lower than the corresponding value for the untreated burrows (approximately 100). Long-term control of the immatures was successful in low-lying areas, where the burrows remained wet, but the copepods failed to survive elsewhere, where burrows repeatedly dried up and filled with water again. When all the treated burrows were checked 3, 6 and 12 months after treatment, approximately 89%, 39% and 24%, respectively, still contained *M. aspericornis*. As the number of copepod-positive burrows declined, the number of mosquito-positive burrows increased, and eventually a treated burrow was as likely to be mosquito-positive as an untreated one (Table 1). The indices of adult biting were always similar to those recorded on an untreated, neighbouring islet. Thus, although good control was achieved in the crab burrows that were always wet, the overall results were disappointing. A few burrows recorded as 'treated' may not, in fact, have received any copepods. However, the main reason for the generally poor effectiveness of this control intervention was the inability of the copepods to survive the seasonal drying of the burrows.

A lack of efficacy and a lack of persistence may each contribute to treatment failure, as seen with insect growth regulators (Laird *et al.*, 1985), the bacterial toxin from *Bacillus thuringiensis* var. *israelensis* (Rivière *et al.*,

TABLE 1. *The results of a large-scale trial of the use of* Mesocyclops aspericornis *to control* Aedes polynesiensis *larvae in land-crab burrows (Lardeux* et al., *1992)*

Time	% of burrows on treated islet with:		% of burrows on untreated, control islet with *Ae. polynesiensis*
	M. aspericornis	*Ae. polynesiensis*	
1 week post-treatment	100	0	43
3 months post-treatment	89	6	38
6 months post-treatment	39	14	40
12 months post-treatment	24	28	42

1987), or the fungus *Tolypocladium cylindrosporum* (Gardner *et al.*, 1986). In the copepod trials, however, failure appears to be linked to a lack of persistence [and not the efficacy of broad-scale control by *M. aspericornis* (Loncke, 1991)]. It is also difficult to place copepods or any other control agents into the flooded chambers in the land-crab burrows in which the vector mosquitoes breed. These chambers are at the end of sinuous tunnels that can be >2m long. The various techniques used by Rivière *et al.* (1987) and Lardeux *et al.* (1992) to introduce the predatory copepods into these chambers were all time-consuming and not really adequate for routine, large-scale treatments. Lardeux *et al.* (2002*a*) recently developed a new technique in which the land crabs are used as couriers of the control agent. When dry, insecticide-impregnated food pellets, made by compacting various flours, were scattered near crab burrows, the crabs themselves carried the pellets (and insecticide) into the flooded chambers of their burrows. The insecticide used for the experiment was Reldan 40 EC (Dow AgroSciences, Indianapolis, IN), which contains 400 g of the active ingredient (chlorpyrifos-methyl)/litre. Assuming each flooded chamber contained 5 litres of water, each food pellet was impregnated with enough insecticide (approximately 0.5 mg of the active ingredient) such that one pellet taken into a chamber would release sufficient chlorpyrifos-methyl to kill any mosquito larvae present. By simply scattering the pellets, large areas could be treated in a short time. Results indicated that the treatment coverage was almost perfect (all the flooded chambers investigated contained insecticide), and no mosquito larvae could be found 48 h after treatment (although a few pupae still survived). The food pellets developed as bait could easily be impregnated with most insecticides and could easily be prepared in large quantities and stockpiled. Use of the food pellets is currently the method of choice for controlling the breeding of *Aedes* in land-crab burrows. Although this method uses a chemical insecticide, only the crab burrows are directly exposed to the chemical. It should be as easy to include a biological control agent in the pellets; a new strain of the bacterium *Bacillus circulans* which is toxic for *Aedes* species (Darriet and Hougard, 2002) will soon be tested.

CONTROL OF *Aedes* IN VILLAGES

Apart from crab burrows, *Aedes* are numerous in more urbanized environments, such as villages. There are two main types of village in French Polynesia: those on high islands [where running water is available, *Aedes* breed in small, peridomestic containers (tyres, cans etc.), and mosquito resting places (in the luxurious vegetation surrounding the houses) are numerous]; and those on the atolls [where no running water exists and most *Aedes* breed in ponds, wells and the various containers (cisterns, 200-litre drums etc.) used to store freshwater collected from wells or as rainwater run-off from roofs].

In the villages on high islands, cleaning around the houses (particularly the elimination of all small containers that can hold water) and the clearing of vegetation can significantly reduce mosquito biting densities, as *Aedes* spp. have a limited flight range (unpubl. obs.). Such mosquito control by 'source reduction' is feasible but is highly dependent on the motivation of the communities at risk and on strong local political support. A lack of motivation remains a problem in many communities. Health-education campaigns to mobilize communities, partly based on short messages on the television and radio, are currently being run by the Ministry of Health.

In a trial relevant to mosquito control in the atoll villages (Lardeux *et al.*, 1989), treatment of 8-m^3 concrete water cisterns with *M. aspericornis* (at just 10 copepods/cistern) eliminated *Ae. aegypti* from the cisterns in less than 3 weeks (Fig. 2). Whenever the treated cisterns were checked in the 8 months post-treatment, no *Aedes* larvae older than first instar were ever found (unpubl. obs.). This success led to the introduction of *M. aspericornis* or mosquito fish into all of the mosquito breeding sites in a village in the Tuamotu archipelago (Lardeux, 1992). These breeding sites consisted of wells, ponds and the large concrete or polyurethane cisterns (either open to the sky or covered) and drums that were used to collect rainwater from roofs. The fish quickly eliminated mosquito larvae from the open breeding sites (ponds, open wells; Table 2) but the impact of the copepods on mosquito breeding in the covered cisterns and 200-litre drums was inconsistent, apparently depending on the availability of the microfauna on which copepod nauplii feed. The 200-litre drums were often drained and refilled by the villagers, and this prevented the establishment of long-lasting colonies of the copepods. In the uncovered water cisterns and covered wells, the impact of *M. aspericornis* was good, particularly against *Ae. polynesiensis* (Table 2). Treatment with copepods not only led to a significant reduction in the proportion of

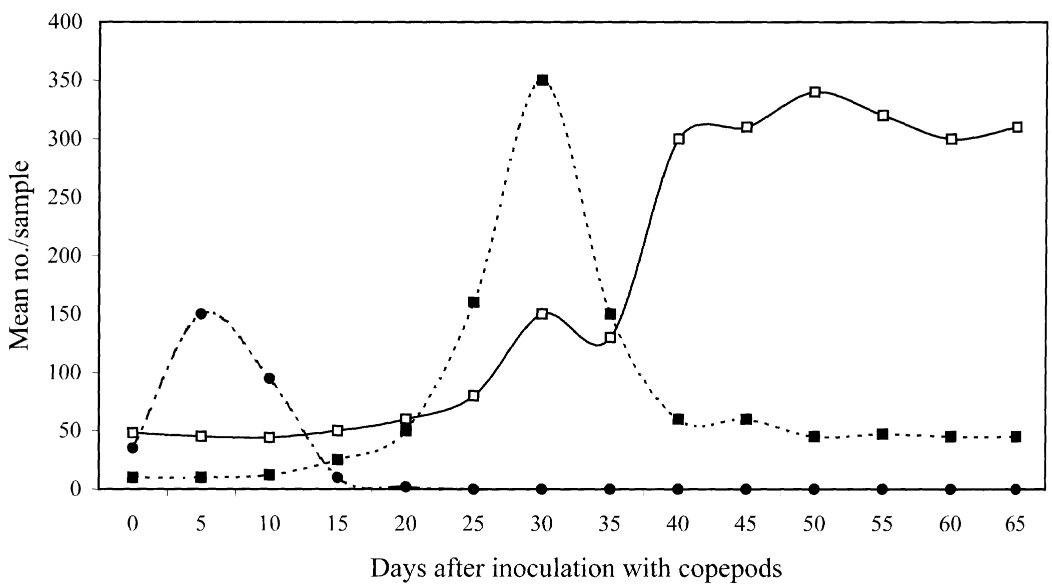

FIG. 2. The result of an experimental attempt at the biological control of *Aedes aegypti* in open-topped concrete water cisterns (each 8 m^3). Three of four cisterns that harboured *Ae. aegypti* larvae were each inoculated with 10 *Mesocyclops aspericornis*. The other tank was left copepod-free, as a control. The graph shows the mean numbers of *Ae. aegypti* larvae/sample in the cisterns with (●) or without copepods (□) and the mean numbers of *M. aspericornis*/sample in the copepod-inoculated cisterns (■). After Lardeux *et al.* (1989).

TABLE 2. *The efficacy of an attempt at biological control of* Aedes aegypti *and* Ae. polynesiensis, *using the copepod* Mesocyclops aspericornis *and the mosquito fish* Poecilia reticulata, *in peridomestic sites in French Polynesian villages (Lardeux, 1992)*

			% of breeding sites found mosquito-positive when checked:				
Type of breeding site	Control agent introduced	Target mosquito species	12 months pre-treatment	6 months pre-treatment	Immediately post-treatment	3 months post-treatment	6 months post-treatment
Covered wells	Copepod	*Ae. aegypti*	45	44	44	22	9
		Ae. polynesiensis	19	20	30	2	2
Open wells	Mosquito fish	*Ae. aegypti*	31	19	19	0	0
		Ae. polynesiensis	30	21	20	0	0
Covered tanks	Copepod	*Ae. aegypti*	55	70	60	63	60
		Ae. polynesiensis	20	20	15	5	6
Open tanks	Copepod	*Ae. aegypti*	32	45	10	15	2
		Ae. polynesiensis	–	–	–	–	–
Large (200-litre) drums	Copepod	*Ae. aegypti*	60	79	49	52	50
		Ae. polynesiensis	21	20	19	15	15

these sites in which mosquitoes were breeding but also led to a marked reduction in the mean number of larvae/positive site; these beneficial effects lasted several months. Sadly, as the number of *Aedes* biting each villager/week seemed to unaffected by the apparently successful biological control of the larvae, this village-scale experiment was judged to be unsuccessful as a means of vector control. Once again, *M. aspericornis* (and mosquito fish) were proved to be effective biological control agents in some well defined sites, but appeared insufficient to have a marked impact on vector biting when used alone. A trial of integrated control, in which all of the available control methods that were considered appropriate were used concurrently, was therefore conducted in a representative atoll village. Physical, biological and chemical control methods were applied to all the breeding sites, community participation was encouraged, and there was also a campaign of health education on basic mosquito ecology and the methods of control (Lardeux *et al.*, 2002*b*). Depending on the target species, the category and configuration of the larval habitat and its human use (i.e. whether a source of drinking or not), the abatement methods consisted of sealing the larval habitats with mosquito gauze (covering cistern overflows and drainpipes), treating them with 1% temephos, covering the water with a 10-cm thick layer of polystyrene beads, or introducing mosquito fish (*Poecilia reticulata*). All of the households in the study village were checked and treated as necessary. A community health agent was trained to continue the control programme at the end of the experiment. The entomological indices evaluated, from the results of human-bait collections and larval surveys, indicated that the mosquito populations were reduced significantly (compared with the concurrent results from an untreated, control village), and that mosquito control remained effective for (at least) 6 months after treatment. Larval indices indicated a halving of the number of mosquito-positive sites, and a significant reduction in the mean number of larvae/positive site (compared with the baseline values for the treated village and the concurrent values for the control village; Table 3). The mean number of mosquitoes per human-landing catch (Fig. 3) and the mean number of positive catches (i.e. catches with at least one mosquito; Fig. 4) were both reduced. The beneficial effects of the interventions were also noticed by the inhabitants, in terms of a reduction in the number of mosquito bites.

PRACTICAL CONSIDERATIONS

The French Polynesian experience has confirmed the general view (Zahar *et al.*, 1980) that *Aedes* mosquitoes are particularly difficult to control. The results of the recent experiments, however, offer more than a

TABLE 3. *The results of the integrated control of* Aedes *breeding sites in a village in French Polynesia (Lardeux* et al., *2002*b*)*

		Result:			
		7 months pre-treatment	1 month pre-treatment	1 month post-treatment	5 months post-treatment
Treated village	% of houses *Aedes*-positive	80	80	40	50
	Aedes larval density	+++	+++	0	+
Untreated, control village	% of houses *Aedes*-positive	70	70	70	70
	Aedes larval density	+++	+++	+++	+++

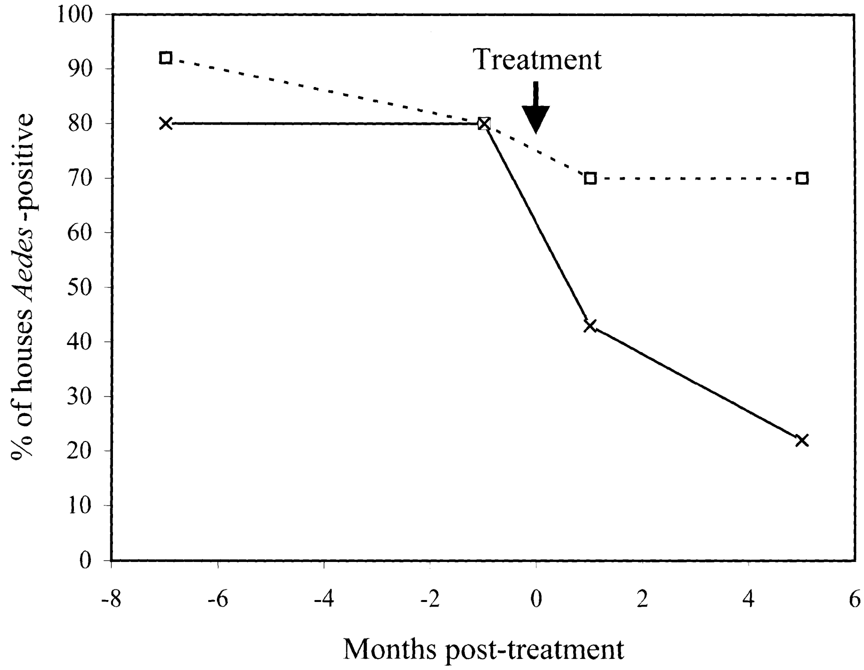

FIG. 3. The results of human-landing catches of adult *Aedes* mosquitoes before and after integrated control treatments in a French Polynesian village, compared with those of similar catches in an untreated control village. The graph shows, separately for the treated (×) and untreated village (□), the percentages of the checked houses that were found *Aedes*-positive (i.e. in which at least one *Aedes* mosquito was caught). After Lardeux *et al.* (2002*b*).

glimmer of hope. An integrated approach to the control of *Aedes*, including consolidation of the villagers' knowledge of mosquito ecology and motivation of the communities at risk (so that they reduce the numbers of mosquito-breeding sites and continue treatment in the absence of health professionals) seems the most promising. The presence of a community health agent specialized in mosquito control may be a necessity in some situations, to help and motivate other villagers (Lardeux *et al.*, 2002*b*). Biological control can be usefully incorporated into an integrated control programme: mosquito fish and, in some well defined situations, the copepod *M. aspericornis* have proved to be efficient. Physical methods, beginning with the elimination of unnecessary water containers and the clearing of vegetation, and ending with more sophisticated techniques such as the sealing of cistern overflows and the spreading of polystyrene beads on the water surface, appear to be the most efficient methods in villages **if** regular maintenance can be organized. The results of the pilot studies with the food pellets are encouraging and make the effective treatment of large areas of land and thousands of crab burrows a distinct possibility.

The 'limitation' phenomenon that occurs in filarial transmission by *Aedes* increases the efficacy of these mosquitoes as vectors and reduces the impact that vector control has on the prevalence of bancroftian filariasis. It might be appropriate to assume, as is often the case in malaria research, that any mosquito ingesting at least one parasite will become infective. The concept of 'vectorial capacity' used by malariologists is equally appropriate in filarial transmission. Lardeux and Cheffort (2001) have demonstrated that two of the parameters used to compute

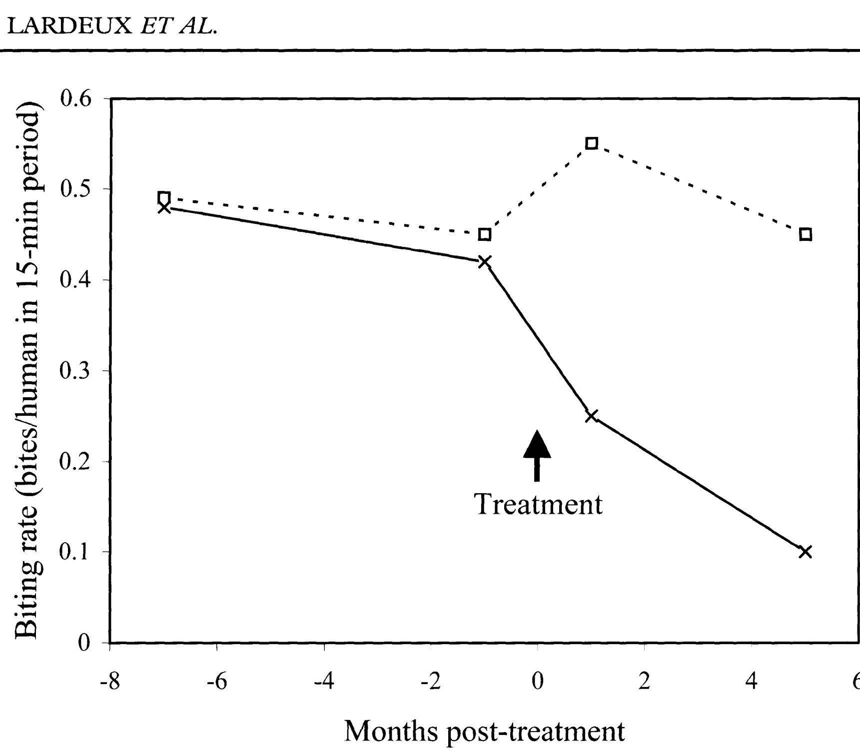

FIG. 4. The results of human-landing catches of adult *Aedes* mosquitoes before and after integrated control treatments in a French Polynesian village, compared with those of similar catches in an untreated control village. The graph shows, separately for the treated (×) and untreated village (□), the mean number of adult *Aedes* landing, in a 15-min period, on each volunteer used as bait. After Lardeux *et al.* (2002*b*).

vectorial capacity in malaria research — the duration of the extrinsic cycle of the parasite and the daily probability of the potential vector's survival — have an influence on filarial transmission by *Ae. polynesiensis*. As other components, such as the number of bites/human-day, can be modified by vector-control measures, such methods, if sufficiently effective, should reduce the prevalence of the human disease caused by the parasite. It is clear that transmission by the vectors that demonstrate 'facilitation' can be interrupted by vector control, in some cases quite easily (Pichon, 2002). Combatting the disease caused by parasites transmitted by vectors, such as *Aedes polynesiensis* in the Pacific Region, that exhibit 'limitation' is likely to be far more difficult. Under these conditions, large outbreaks of lymphatic filariasis may develop from just a few residual cases; total interruption of transmission may be impossible, although continuous vector control may still be sufficient to eliminate the disease as a public-health problem (Pichon, 2002). Vector-control techniques need to be improved and optimised if adult densities of the 'difficult-to-control' *Aedes* are to be markedly reduced!

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