

The Use of Predacious Copepods for Controlling Dengue and other Vectors

by

Brian H. Kay

Queensland Institute of Medical Research,
PO Royal Brisbane Hospital, Brisbane 4029 Australia

ABSTRACT

ALTHOUGH the predatory role of copepods was documented between the period 1930-1950, scientific evaluation was taken up only in 1980 in Tahiti, French Polynesia, where it was found that *Mesocyclops aspericornis* could effect 99.3% mortality in *Aedes (Stegomyia)* and 9.7% and 1.9% respectively of *Culex quinquefasciatus* and *Toxorhynchites amboinensis*.

Experimentation undertaken in crab burrows against *Aedes polynesiensis* and in water tanks, drums and covered wells met with mixed results. In Queensland, Australia, out of seven species evaluated in the laboratory, all but *M. notius* were found to be effective predators of both *Ae. aegypti* and *Anopheles farauti* but not of *Cx. quinquefasciatus*. However, field releases of *M. aspericornis* in both northern and southern Queensland showed mixed

responses. Results in Thailand were mixed but in Viet Nam, these were a great success. Predacious copepods have also had some success in the Americas, especially in discarded tyre piles. Although lack of nutrients and household cleaning of some containers can prevent their sustainability, they seem particularly suitable for large containers which are not cleared regularly (wells, concrete tanks and tyres) and merit consideration as a viable low-cost control option.

Introduction

Lindberg (1936)⁽¹⁾ and Hurlbut (1938)⁽²⁾ were the first to point out that some cyclopoid copepods were predators of mosquito larvae. Twenty years later, in Hawaii, Bonnet and Mukaida (1957)⁽³⁾ accidentally introduced *Mesocyclops*

obsoletus Koch (possibly a synonym of *M. aspericornis*) into their *Aedes albopictus* colony and found that a single copepod could kill 15-20 first and second instars per day. Strangely, it has taken yet another accidental discovery, approximately 20 years after Bonnet and Mukaida, to ignite the fuse of applied research with respect to predacious cyclopoids and mosquito control.

In 1976 in Tahiti, Riviere and Thirel (1981)⁽⁴⁾ discovered that ovitraps filled with stream water and tapwater differed in their positivity for *Ae. aegypti* and *Ae. polynesiensis* (20 vs 78%). In their ensuing work over 14 months, they concluded that *M. aspericornis* was responsible for up to 99.3% mortality of *Aedes* (*Stegomyia*) but only 9.7% and 1.9% respectively of *Cx. quinquefasciatus* and *Tx. amboinensis*. The important point about this work was that finally someone had recognized the excellent prospects of using predacious copepods as biological control agents of larval *Aedes* of the subgenus *Stegomyia*.

French Polynesia

From 1982, I became involved with the Institut Malarde/ORSTOM programme and the results are essentially summarized in Riviere, et al. (1987)⁽⁵⁾. *M. aspericornis* persisted indefinitely in a series of half tyres along the fenceline of the Paea laboratory and in a series of 200-litre drums under mango trees. However, at Tiputa on Rangiroa atoll, high temperatures up to 38°C precluded survival of *M. aspericornis* in sunlit drums. After five years, 17% of the inoculated tree holes (*Inocarpus fagifer*)

and 48% of the wells contained *M. aspericornis*. On average, larval *Aedes* had been reduced by 91-200% for at least 6-12 months and for up to five years.

During this time, we became preoccupied with burrows of the gecardine crab, *Cardisoma carnifex* which may extend at densities of 1/m² around low and volcanic islands. Gecardinid crabs occur throughout the world and 140 species of mosquitoes have been associated with their burrows, often extending a metre or more into the water table⁽⁶⁾. As (i) the problem of controlling mosquito larvae in land crab burrows has never been addressed successfully, and (ii) in view of the fact that *Ae. polynesiensis* was recognized as a major vector of dengue and Bancroftian filariasis in the Pacific, we commenced studies on Rangiroa atoll. Expectations had been heightened somewhat by a previous survey of Huahine island where *M. aspericornis* occurred naturally in crab burrows. The mean hourly biting rate of *Ae. polynesiensis* was 10 for the above situation compared to 154 on the nearby islets where *M. aspericornis* was absent. At Avatoru, Rangiroa atoll, 2432 crab holes were treated by a team in less than one week, which at one major site (Kia-Ora maite) resulted in 75.6% adult reduction after six months and *M. aspericornis* persistence for 47 months^(5,7). Stimulated by this result, from January 1986 - January 1989, the 32 ha low islands of Tereia and "Voisin" were studied. On Tereia, some 17 300 burrows were inoculated with *M. aspericornis*, thus proving that it was logistically possible to carry out broad scale field releases in isolated areas. From 5-15

months after treatment, burrows with *M. aspericornis* contained an average of two *Ae. polynesiensis* immatures compared with 97 in the untreated holes. However, long-term larval control was only successful in low lying areas where burrows remained wet or reflooded. Overall, the treatment was a failure as only 89.5, 39.1 and 24.1% of the burrows sampled 5, 8 and 15 months post-treatment contained copepods and there was no apparent reduction in *Ae. polynesiensis* biting densities⁽⁸⁾.

Lardeux (1992)⁽⁹⁾ released *M. aspericornis* into water tanks, drums and covered wells in Tuherahera village, Tiki hau atoll, with mixed results. Five months post-inoculation, *M. aspericornis* was present in 100% of the covered wells, 3.4% and 12.5% of the covered and open rainwater tanks and in 8.7% of the 200-litre drums. Where they survived, copepods proved effective biocontrol agents of *Aedes*. As we are also finding in Queensland, the low levels of nutrients especially in closed rainwater tanks, are unsuitable for the development of planktonic micro organisms on which copepod nauplii feed. Nutrient levels in wells and drums are higher than in tanks.

Australia

In Queensland, we have done laboratory evaluations of 7 *Mesocyclops* spp. up to larval densities of 200 per litre. All but *Mesocyclops notius* were effective predators of both *Ae. aegypti* and *Anopheles farauti* larvae but not of *Culex quinquefasciatus*^(10,11). We have now carried out field releases mainly of *M. aspericornis* on both northern (Darnley

Island, Charters Towers) and southern Queensland (Amity on Stradbroke Island, Brisbane) with mixed success. Although the presence of sufficient food reserves such as algae and protozoa is undoubtedly important (Jennings, et al. 1994)⁽¹²⁾ for sustained colonization by copepods, other factors such as human usage patterns, drying and possibly interspecies competition are also important. In trials in tyres during 1990-1992 in Brisbane, *M. aspericornis* with and in the absence of *Toxorhynchites* persisted for 18 months and gave effective control of *Aedes notoscriptus* throughout two summers but less so when winter water temperatures reached 11-15°C⁽¹³⁾. Trials at Darnley Island with *M. aspericornis* introduced into water tanks and 200-litre drums were less successful, mainly because these containers dried out in the dry season but also possibly owing to interspecific competition with an undescribed *Mesocyclops* Mb3 introduced naturally from the local reservoir. The replenishment of containers with reservoir water containing *Mesocyclops* offers some degree of sustainability for control run by indigenous islander people. *Mesocyclops* Mb3 controlled *Ae. aegypti* and *Ae. scutellaris* effectively over a six month period⁽¹³⁾. Although Australian and Asian *Mesocyclops* have been shown to have some degree of desiccation resistance⁽¹⁴⁾, water tanks and drums do not generally have sufficiently deep layers of sediment to facilitate this.

At Charters Towers, we have been successful in controlling *Ae. aegypti* in wells and have discovered that the funnel trap⁽¹⁵⁾ developed for our studies in Brasil, has

proved approximately four times more efficient in detecting *Ae. aegypti* than *Cx quinquefasciatus*. For *Ae. aegypti* in wells, there almost is a perfect correlation between trap catch and numbers present. This will facilitate identification of key breeding sites based on productivity. This issue is crucial to the development of sound control programmes. We also believe that because of the difficulties previously associated with the efficacious sampling from subterranean sites, their importance has been underrated.

Asia

Mesocyclops guangxiensis⁽¹⁶⁾, now synonymized as *M. woutersi* which has been collected from China, Lao and Viet Nam has proved to be an effective agent when used in wells⁽¹⁷⁾ and in large outdoor concrete tanks (Vu Sinh Nam, unpublished data). In fact the latter programme in northern Viet Nam at Phaiboi Village, Haihung province, has resulted in eradication of *Ae. aegypti* for over two years. In Thailand, Siriporn (1994)⁽¹⁸⁾ inoculated a variety of peridomestic containers with mixed success but also noted that some sites were being reinoculated during daily use from the village well and that household cleaning of some container types was a major impediment to success.

Americas

It would be remiss of me not to include the extensive work carried out by Gerry Marten and Marco Suarez in the USA, Mexico, Honduras, Puerto Rico and Colombia. In

the New Orleans area, 25 species were collected and seven were large enough to be effective predators of mosquito larvae⁽¹⁹⁾. These comprised *Mesocyclops* (*edax*, *rutneri*, and *longisetus*), *Macrocyclus albidus*, *Acanthocyclops vernalis*, *Diacyclops navus* and *Megacyclops latipes*. Interestingly *Homocyclops ater* was the largest species but did not prey on mosquito larvae and populations of *D. navus* were colonized, which did not always exhibit predatory behaviour. Much of their work has been reviewed by Marten, et al. (1994)⁽²⁰⁾.

Of the six species of cyclopoids tested in tyres around New Orleans as control agents of *Ae. aegypti*, *Ae. albopictus* and *Ae. triseriatus*, *D. navus*, *M. longisetus* and *M. albidus* reduced larval populations by .99%. The New Orleans Mosquito Control Board now routinely treats tyre piles about once per year with *M. longisetus* because of its superior resistance to desiccation and its tolerance of high summer water temperatures. As was shown in French Polynesia⁽⁵⁾, BTi can be applied in the inoculum sometimes using backpack sprayers to eliminate large mosquito larvae immediately.

In Honduras, Marten, et al. (1994)⁽¹⁹⁾ evaluated four copepod species found commonly through the region: *Mesocyclops longisetus*, *venezolanus* and *thermocyclopoides* and *Macrocyclus albidus*. *M. longisetus* survived well in outdoor concrete tanks, 200-litre drums, tyres and vases with live plants during the 20-30 week test period and gave excellent control of *Ae. aegypti*. As found with other community-based projects in Puerto Rico, Anguila⁽²⁰⁾ and in

Brazil⁽²¹⁾, cyclopoid persistence often depended on household water management practices. In all the trials carried out to date, householders readily accept cyclopoids and often are willing to take extra precautions to ensure that these biological control agents are not discarded when cleaning the containers.

To summarize what we know about copepods and their usage in biological control of dengue vectors (note that they are also effective *Anopheles* predators but considerably fewer kill large enough numbers of *Culex*):

- (1) Cyclopoids have been collected from freshwater bodies in the Pacific Australia, Asia and the Americas and applied for dengue control.
- (2) They can be easily mass produced at little cost, but the option of inoculating wells and large storage tanks in community settings would seem to be a better starting point for community-based projects.
- (3) Many cyclopoid species are likely to give .99% control (and even 100% when combined with other methods) of larval *Aedes* (and *Anopheles*).
- (4) Lack of alternate food resources and constant cleaning of some container types may present impediments to successful persistence and control.
- (5) They can be used with BTi.
- (6) They should not be used in countries where guineaworm is endemic.

To summarize, there are enough positive examples of using copepods for dengue

vector control that they have now gone past the 'promising' stage into operational reality.

References

1. Lindberg K, Crustaceans copepodes comme ennemis naturels de larves d'*Anopheles*. Bull. Soc. Pathol. Exot. 1936. 42:178-179.
2. Hurlbut HS, Copepod observed preying on first instar larva of *Anopheles quadrimaculatus* Say. J. Parasitol. 1938. 24:281.
3. Bonnet DD & Mukaida T, A copepod predacious on mosquito larvae. Mosq. News 1957 17:99-100.
4. Riviere F & Thirel R, La predation du copepode *Mesocyclops leuckarti pilosa* (Crustacea) sur les larves de *Aedes (Stegomyia) aegypti* and *Ae. (St.) polynesiensis*: essais preliminaires d'utilisation comme agent de lutte biologique, Entomophaga 1981. 26:427-439.
5. Riviere F, Kay BH Klein JM and Sechan Y, *Mesocyclops aspericornis* (Copepoda) and *Bacillus thuringiensis* var: *israelensis* for the biological control of *Aedes* and *Culex* vectors (Diptera: Culicidae) breeding in crab holes, tree holes and artificial containers. J. Med. Entomol. 1987. 24:425-430.
6. Bright DB & CL Hogue, A synopsis of the burrowing land crabs of the world and list of their arthropod symbionts and burrow associates. Los Angeles Co. Mus. Contrib. Sci. 1972. 220:1-58.
7. Lardeux F, S Loncke, Y Sechan, BH Kay & F Riviere. 1989. Potentialities of *Mesocyclops aspericornis* (Copepoda) for broad-scale control of *Aedes polynesiensis* and *Aedes aegypti* in French Polynesia. Arbovirus Research in Australia 5: 154-159.
8. Lardeux F, Riviere F, Sechan Y & Kay BH, Release of *Mesocyclops aspericornis* (Copepoda) for control of larval *Aedes polynesiensis* (Diptera: Culicidae) in land crab burrows on an atoll of French Polynesia. J. Med. Entomol. 1992. 29:571-576.
9. Lardeux FJR, Biological control of Culicidae with the copepod *Mesocyclops aspericornis* and larvorous fish (Poeciliidae) in a village of French Polynesia. Med. Vet. Entomol. 1992. 6:9-15.
10. Brown MD, BH Kay, & JK Hendrikz. Evaluation of Australian *Mesocyclops* (Cyclopoda: Cyclopidae) for mosquito control. J. Med. Entomol. 1991a. 28:618-623.
11. Brown MD, Kay BH & Greenwood JG, The predation efficiency of north-eastern Australian *Mesocyclops* (Copepoda: Cyclopoda) on mosquito larvae. Bull. Plankton Soc. Japan, 1991b., Spec. Vol. 329-338.
12. Jennings CD Greenwood, JG & Kay BH, Response of *Mesocyclops* (Cyclopoda: Copepod) to biological and physicochemical attributes of rainwater tanks. Envir. Entomol. 1994. 23:479-486.

13. Brown MD, Evaluation of *Mesocyclops* (Copepoda: Cyclopidae) for mosquito control in north-eastern Australia. Ph.D. thesis, Univ. Queensland. 1995.
14. Zhen TM, Jennings CD & Kay BH, Laboratory studies of desiccation resistance in *Mesocyclops* (Copepoda: Cyclopoida). J. Am. Mosq. Control Assoc. 1994. 10: 443-446.
15. Kay BH, CP Cabral, DB Araujo, ZM Ribeiro, PH Braga & AC Sleigh. Evaluation of a funnel trap for collecting copepods and imature mosquitoes from wells. J.Am. Mosq. Control Assoc. 1992. B:372-375.
16. Reid, JW & Kay, BH *Mesocyclops guangxiensis*, new species, and new records of four congeners (Crustacea: Copepoda: Cyclopidae) from China, Laos and Viet Nam, Proc. Biol. Soc. Wash. 1992. 105:331-342.
17. Jennings CD, Phommasack, BS Sourignadeth & Kay BH. *Ae. aegypti* control in the Lao People's Democratic Republic, with reference to copepods. Am. J. Trop. Med. Hyg. 1995. 53:324-330.
18. Siriporn Vihokto, Preliminary survey and experimental studies of *Mesocyclops* spp. as biological control agents of dengue vectors in a rural Thai community. M.Sc thesis, Mahidol University, Bangkok. 1994.
19. Marten GG, Issues in the development of cyclops for mosquito control. Arbovirus Research in Australia. 1989. 5:159-164.
20. Marten GG, Borjas G, Cush M, Fernandez E and Reid. JW, Control of larval *Aedes aegypti* (Diptera: Culicidae) by cyclopoid copepods in peridomestic breeding containers. J. Med. Entomol. 1994. 31:36-44.
21. Suarez M, *Mesocyclops aspericornis* as an alternative of *Aedes aegypti* control in Puerto Rico and Anguilla. Arbovirus Res. Australia. 1992. 6:115.
22. Vasconcelos AW, AC Sleigh, BH Kay, Cabral CP, Araujo DB, Ribeiro AM, Braga PH & Cavalcante, Jr. JS. Community use of copepods to control *Aedes aegypti* in Brazil. pp 139-144 in "Dengue a Worldwide Problem, a Common Strategy" (S.B. Halstead and H. Gomez-Dantes eds). Rockefeller Fdn/Min Health, Mexico. 1992.