CSIRO
Tropical
Animal
Science

Proceedings Fifth Symposium
August 28 — September 1, 1989
Brisbane
Australia

ARBOVIRUS RESEARCH IN AUSTRALIA

Q.I.M.R.

Editors: M.F. Uren, J. Blok and L.H. Manderson

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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ISSUES IN THE DEVELOPMENT OF CYCLOPS FOR MOSQUITO CONTROL

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Cyclopoid copepods are tiny crustaceans with unusual promise for the biological control of mosquito larvae. Cyclops have been used only for container-breeding *Aedes* and the introduction of cyclops to a container can lead to larvae control that lasts for months or even years (Marten 1984; Suarez *et al* 1984; Riviere *et al* 1987a; Marten 1989). Cyclops may be of use for controlling other species of mosquitoes (Mian *et al* 1986; Marten *et al* 1989).

Cyclops biology has been reviewed by Dussart (1969) and Wyngaard and Chinnappa (1982). Cyclops are one of the most common forms of freshwater zooplankton and are found in the breeding habitats of many species of mosquitoes. Most species of cyclops are too small to kill mosquito larvae; only the larger species are larvivorous. Like larvivorous fish, cyclops have a diet that is broad enough (including phytoplankton, protozoa and small aquatic invertebrates such as rotifers) to support large populations with a correspondingly high capacity for predation, independent of the supply of mosquito larvae. The larger species are known to prevent mosquito development in about 10% of breeding habitats (Marten 1984; Marten *et al* 1989).

Cyclops should be particularly useful in situations where larvivorous fish are not effective. Their small size allows free movement through aquatic vegetation where mosquito larvae can hide from fish. They can thrive in aquatic habitats, such as containers or temporary pools, which lack the macroinvertebrates that fish require as food. Many cyclops species can survive when temporary water bodies dry up. Mass production and transport of these tiny crustaceans is considerably less expensive than fish (Riviere *et al* 1987b).

There are numerous details to be worked out before cyclops are a routine part of mosquito control. Some of the main concerns are:

(1) selection of cyclops species that are most effective for a particular application;

(2) production, storage and distribution of cyclops on a large scale;

(3) integration of cyclops into practical mosquito control.

I shall comment on these concerns from the perspective of my recent experience in New Orleans, where I tested 5 local species of cyclops for biological control of *Ae albopictus* larvae by introducing the cyclops to ca 1000 discarded tyres (Marten 1989).

SPECIES SELECTION

There are ca 400 species of freshwater cyclops worldwide. More than 50 species should prey on mosquito larvae. Some criteria for species selection are:

(1) effectiveness as a larval predator;

(2) success in establishing a population when introduced to habitats where treatment is desired;

(3) long-term survival in a breeding habitat;

(4) convenience and cost of production, storage and distribution.

Effectiveness as a predator is a primary consideration. As a rule, effectiveness is in simple proportion to size. (I have observed one exception. *Homocyclops ater* is a large species that does not prey on mosquito larvae at all.) Without exception, ranking of the 5 larvivorous cyclops species in New Orleans as predators follows the same order as their size: *Macrocyclops albidus*, *Mesocyclops* sp. (*M leuckarti* species group), *Mesocyclops edax*, *Acnathocyclops vernalis*, and *Diacyclops navus*. *M albidus* is the largest; not only adults but also large copepodids prey on *Ae albopictus* larvae. Natural populations of *M albidus* in tyres seldom allow a single larva to survive, even if 1000 first instars are placed in a tyre at the same time. At the other extreme is *D navus*, the smallest larval predator. Only adult *D navus* prey on mosquito larvae, and their attacks are timid. They often abandon an attack as soon as they make contact with a larva. An average of 17% of *Ae albopictus* larvae survive *D navus* predation under field conditions.

How effective does larval predation have to be for biological control? The answer seems to be nearly 100% in the case of container habitats, particularly if more larvae hatch into the container than can be supported by available food resources (Fig 1). A first-instar predator like cyclops may thin a larval population without reducing the number of mosquitoes that emerge.

Cyclops can be useful only if they can establish a large population whenever they are introduced to a particular habitat. It is only necessary to introduce mature females, males being unnecessary because females are inseminated for life as soon as they reach maturity. I have found the probability for a single adult female to establish a population in a tyre is ca 50%. I routinely introduce 10 females.

Introductions appear to be most successful with species and strains that occur naturally in a given habitat. The success rate has been 96% or better when introducing *Macrocyclops*, *Acanthocyclops*, or *Diacyclops* to tyres. Natural populations of these species are found occasionally in tyres. The success rate is closer to 100% with strains of these species that are derived from natural tyre populations. The establishment rate has been only 80-90% for *Mesocyclops*, which does not occur naturally in tyres in New Orleans.

Cyclops can persist indefinitely in a container habitat as long as there is water and a food supply. Container habitats seldom have animals that prey on cyclops (*Toxorhynchites* larvae prey on cyclops, but they do not reduce cyclops populations significantly). Mosquito larvae are often the only significant competitors in containers.

In general, an aquatic habitat has sufficient food for cyclops if there is sufficient food for mosquito larvae. However, some containers are marginal for both mosquito larvae and cyclops because they lack organic matter inputs (eg leaf fall) that serve as an ultimate source of food. Introduction of cyclops to a marginal container can be facilitated by including a small quantity of

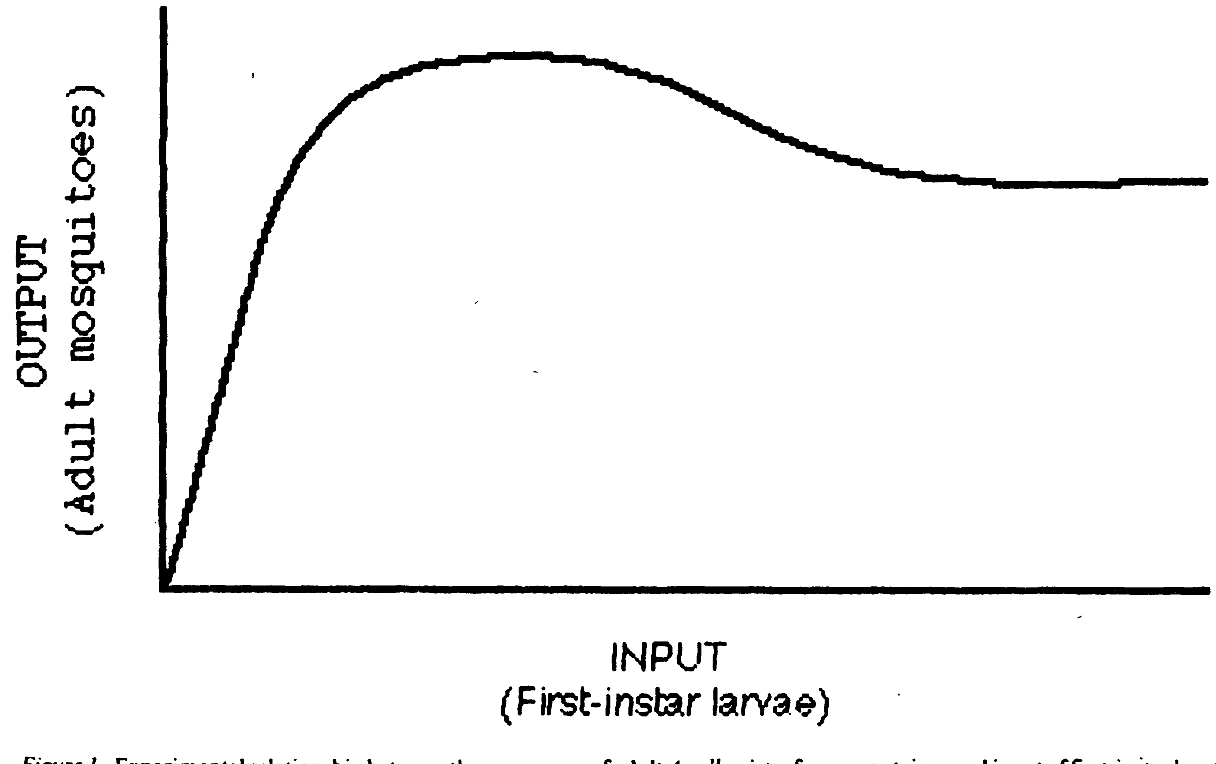

Figure 1. Experimental relationship between the emergence of adult *Ae albopictus* from a container and input of first-instar larvae to a container habitat (GG M, unpublished data).

organic substrate (eg wheat seed) and an inoculum of organisms to serve as food.

Desiccation resistance is the key to long-term survival in temporary breeding habitats. The 5 cyclops species examined in New Orleans differ significantly in their resistance to desiccation. Eggs and nauplii are not resistant to desiccation, but copepodids and adults of some species can survive for months when their habitat dries up, provided there is soil or litter to conserve a pocket of moisture around the animal. The cyclops are active within minutes after there is water once again. Copepodids survive best. I have observed variation in the desiccation resistance of different species, not only in controlled laboratory experiments but also in the field when tyres dry out. One species may survive in every tyre when another species survives in none.

Virtually nothing is known about introducing cyclops to groundwater habitats. The prospects for simple introduction seem best in temporary pools with a limited fauna. The major complication in permanent water is probably planktivorous fish, which shape the species composition of zooplankton by way of their feeding preferences (Hurlbert and Mulla 1981). It is conceivable that some species of larvivorous fish are creating mosquito breeding habitats because their predation on mosquito larvae is exceeded by the detrimental effect of their predation on larvivorous cyclops. On the other hand, some species of fish may encourage larvivorous cyclops. In general, we need a better understanding of the effect of plants and animals in the aquatic environment on the abundance of large cyclops species that prey on mosquito larvae.

One significant issue is whether exotic cyclops species should be used for biological control. A few cyclops species, most likely the largest, will be most effective as larval predators, and they could be of use beyond their natural geographic range. On the other hand, local species should be best adapted to local conditions and best able to establish and maintain a population when introduced to local mosquito breeding habitats. Because local species avoid risks of undesirable environmental impacts due to introducing an exotic, it seems prudent to use local species instead of exotics until we have more experience using cyclops for mosquito control.

Another issue is whether to use a single species of cyclops or a mix of species. Possible advantages of a mixture are:

(1) the food resources of 2 species may differ, providing a basis for the combined population to be greater than 1 species alone;

(2) the hunting habits of 2 species may differ, so 2 species cover a breeding habitat more completely than one;

(3) differences in tolerances to environmental stresses may allow one species to survive when another is wiped out.

My general experience in introducing 2 cyclops species together in the field is that the 2 species do tend to have a combined population greater than either alone. However, one of the species usually predominates in the long term. Larval predation is inferior in a mixture where an inferior predator predominates. Natural mixtures of *Macrocyclops* and *Diacyclops* in tyres reduced *Ae albopictus* larvae by 95% (Marten 1989), while *Macrocyclops* by itself consistently killed all the larvae. My field trials with mixtures of effective species have shown that they perform at least as well as either species alone, but not significantly better than the best species by itself. Species mixtures deserve further investigation, but so far they are not worth the effort.

Differences between strains can be extremely important. Morphologically indistinguishable cyclops — sibling species (Price 1958) or different strains of the same species — may differ substantially as larval predators. I have a strain of *D navus* that preys on *Aedes* larvae and one that does not. I have a strain of *M albidus* that survives drying in tyres and one that does not.

An important consequence of this variation in cyclops performance is the opportunity to breed desirable characteristics into colonies used for mosquito control. An equally important implication is the need to monitor cyclops colonies for deterioration. Selection under culture conditions may fail to maintain natural voracity as a predator or resilience to environmental stresses.

MASS PRODUCTION AND DISTRIBUTION

A basic issue for mass production of cyclops is whether or not the production should be centralised. An advantage of centralised production is its scope to employ any mode of production that is most effective. A disadvantage is the cost of transporting cyclops to where they will be used. Transport costs should be less when production is closer to the point of application; decentralised production can also be tailored to local needs. A limitation of decentralised production is that only simple production technologies would be feasible in most instances.

The range of possibilities for cyclops production can be illustrated by 2 very different approaches: (1) "industrial" production and (2) harvesting natural population from ponds. Industrial production is sophisticated and intensive. It uses select microorganisms as food in bottles, tanks, or vats under controlled conditions. Inputs are high and so are yields. I am presently using shallow fibreglass pans assembled in a rack. Food for the cyclops consists of bacteria, flagellates, ciliates and rotifers, using wheat seed as a substrate. The food organisms have been selected for their ability to withstand intense grazing by cyclops while providing nutritious food for each developmental stage. The system can produce as many as 50,000 copepodids or 10,000 adult cyclops per square foot of floor space per month (depending on the species). Capital and labour costs are relatively high.

Ideally the only labour required to harvest a natural cyclops population is dragging the pond with a plankton net. Millions of cyclops can be removed in a few hours if the cyclops are numerous. Prawn ponds are an example of ponds that are highly productive because food is provided for the prawns. Ponds without fish sometimes contain enormous numbers of cyclops. In the case of prawn ponds, capital and maintenance costs are assumed by the prawn producers. However, there are limitations to harvesting natural populations of cyclops. It is typical for a pond to contain several species of cyclops, not just the one desired for mosquito control purposes. Even if there is one species of cyclops, there may be a substantial labour requirement to separate the cyclops from other aquatic animals that are mixed with cyclops in the harvest. There is very little control. A pond with many cyclops 1 month may have few the next month. It may be necessary to draw on a large number of ponds to ensure that at least 1 will have a large cyclops population at a given time. Cyclops from a pond that is highly productive because of sewerage or other wastes may present a health hazard.

The best production system for most places will probably lie between the 2 extremes — a managed artificial pond. Details of management remain to be worked out. We can expect the depth should be no more than a few inches because production is limited by oxygen exchange when the water is deeper. An organic substrate (e.g. wheat seed) should be provided for intense production. An infusion of bacteria and protozoa should be sufficient food for most species of cyclops.

A major factor in pond management will be maintaining the fauna and flora to exclude animals that prey on cyclops or compete with them (eg cladocera or other species of copepods). Animals that are about the same size as cyclops can be a nuisance because they are difficult to separate from cyclops by straining. The role of phytoplankton could be an issue. Although phytoplankton are the principle food of cyclops (particularly juveniles) in nature, the nutritional value of phytoplankton for cyclops is much lower than protozoa (Brandl and Fernando 1975). My experience in the laboratory indicates that phytoplankton detract from intense production. This remains to be verified for ponds, but it could be difficult to exclude phytoplankton from eutrophic ponds that are out in the open.

I have found that cyclops can be stored for months at high densities in water at 5°C. A hundred thousand can be stored in a 1 L container, and no food is necessary. It is not possible to crowd cyclops at temperatures high enough for them to be active. Not only are there problems of oxygen supply, starvation, and fouling of the water, but some species of larvivorous cyclops prey upon one another when crowded.

Refrigeration may not be the most desirable way to store and transport cyclops on a large scale. Given the tolerance of many cyclops species to desiccation, it should be possible to store large numbers densely packed in solid material that is slightly damp. It may even be possible to encapsulate them in a dry granular preparation that falls apart when placed in water.

What is the best developmental stage for cyclops to be stored? Copepodids have a capacity to go into dormancy that enables them to survive environmental stresses better than nauplii or adults (Watson and Smallman 1971). They seem to survive storage best. Inseminated females are desirable because they make the introduction of males unnecessary. Females are normally inseminated during the last copepodid stage before becoming adults. It should be possible to store them immediately after insemination.

Work remains to be done on means of applying cyclops to breeding sites. Container breeding sites that are widely dispersed over residential areas require application container by container. Cyclops can be squirted without injury from a simple backpack sprayer with a 5 mm hole in the nozzle. Broadcast spraying may be more appropriate for aggregations of containers such as tyre piles. Broadcast spraying will entail considerable waste of cyclops, but it may be the only practical means of introducing cyclops to large tyre piles where introduction is necessary below the surface layer.

INTEGRATION INTO MOSQUITO CONTROL

For what kinds of mosquitoes can cyclops be employed? Their effectiveness for *Ae aegypti* and related species is well documented. They can also be effective predators of *Anopheles* larvae. *An albimanus* larvae are virtually absent from groundwater breeding sites in Latin America where *Mesocyclops* are abundant (Marten *et al* 1989). Introduction of *Mesocyclops longisetus* to small ponds reduced the survival of *An albimanus* larvae by 90% (GG M, MO Menendez and M Montufar-Garcia, unpublished data).

In general, cyclops are not effective predators of *Culex* larvae. It is common to see *Culex* larvae co-existing with larvivorous cyclops. The reason seems to be that bristles on *Culex* larvae frequently cause cyclops to abandon their attack. *Macrocyclops* and *Mesocyclops* prey on *Culex* larvae to some extent, and they may be effective enough with some species of *Culex* to be of use for biological control (Mian *et al* 1986). A search should continue for cyclops species that are particularly effective against *Culex*.

Larvivorous cyclops will be most effective when used in combination with other forms of larval control such as fish, indigestible algae (Marten 1986, 1987), *Toxorhynchites* (Focks *et al* 1986), larvicides, or adulticides. In Hawaii I observed *M aspericornis* to thrive in water with an abundance of indigestible green algae *Kirchneriella irregularis* that killed *Ae albopictus* and *Cx quinquefasciatus* larvae (Marten 1984).

Toxorhynchites larvae can be particularly helpful when combined with species of cyclops that are not strong enough predators to eliminate all the mosquito larvae by themselves. For example, incomplete predation by *Acanthocyclops* and *Mesocyclops* in the New Orleans field trials often thinned overcrowded populations of *Ae albopictus* larvae without reducing the production of adult mosquitoes very much (Table 1). Because *Toxorhynchites* are effective predators of third and fourth instar larvae, they complement first-instar predation by cyclops. Larvae of *T rutilus*, the native species of *Toxorhynchites* in New Orleans, were in about half the tyres in the study. Although *T rutilus* reduced *Ae albopictus* larvae and pupae only 74% when by itself, Table 1 shows that nearly all the larvae and pupae were eliminated when *T rutilus* was together in tyres with *Acanthocyclops* or *Mesocyclops*.

Cyclops are compatible with certain larvicides (W Che and GG M, unpublished data). They reproduce, complete their life cycle, and maintain normal populations at a permethrin concentration several hundred times necessary for a 100% kill of *Ae albopictus* larvae. The same is true at a *Bti* concentration several thousand times the lethal dose for *Ae albopictus*. The use of a larvicide with cyclops can accelerate the impact of cyclops treatment on mosquito production by 1 or 2 months. It can take a few weeks for a cyclops population to build up, and without a larvicide it takes several more weeks for the large larvae in a container to clear out. A larvicide can kill all larvae immediately and the cyclops can take over as the larvicide wears off. Ultimately larval predators must be evaluated in terms of their impact on adult mosquito populations. Does a 90% reduction in mosquito production reduce the mosquito population correspondingly? I am not aware of answers to this question in the scientific literature, but the answers are critical to achieving acceptable reductions in adult mosquito populations.

Secondly, how soon does an adult population of mosquitoes respond to a reduction or elimination of larvae? This depends on the natural mortality rate of the adult population. In New Orleans we conducted experiments with *Ae albopictus* populations at isolated tyre piles. The tyres were treated with *Macrocyclops* to assess the impact of cyclops not only on larvae but also their impact on the adult mosquito population. *Ae albopictus* larvae disappeared entirely from the tyre piles 8-10 weeks after introducing *Macrocyclops*. The decline in adults lagged about 2 weeks behind the decline in larvae, but once the adult population was small, it lingered longer than the larvae, disappearing about a month after the larvae. Elimination of the adult population could be accelerated by combining adulticiding with cyclops. Cyclops are sensitive to organophosphates, but pyrethroids should be compatible.

TABLE 1

Reduction of *Ae albopictus* larvae and pupae by *Toxorhynchites rutilus* in tyres with cyclops.*

	Acanthocyclops vernalis		*Mesocyclops* sp. (*M leuckarti* group)	
	Larvae	*Pupae*†	*Larvae*	*Pupae*†
Cyclops	90%	39%	95%	80%
Cyclops and *Toxorhynchites*	96%	97%	99%	99%

* Percentage reduction is in comparison with control tyres that contained neither cyclops nor *Toxorhynchites* (Source: Marten 1990). † The number of pupae can be considered an indicator of the production of adult mosquitoes.

Finally, there may be some medical issues associated with particular uses of cyclops. One of the most promising applications is *Ae aegypti* in drums, cisterns and vases used to store domestic water. Is there any harm in swallowing cyclops? Probably not, as long as they are not infected with guinea worm. While some species of cyclops are alternate hosts for this parasite, it is not a problem in most areas. Even where it is a problem, the use of cyclops should not present a hazard unless people bathe in the water. Another question concerning drinking water is whether cyclops that eat mosquito larvae infected with a human pathogen can themselves become infected and transmit the pathogen to humans. It appears highly unlikely but should be verified.

In conclusion, we appear to be on the threshold of a major new form of biological control for mosquito larvae. We now know that the right species of cyclops can completely eliminate certain species of mosquitoes from certain breeding habitats for extended periods. So far, this impressive degree of control has only been demonstrated for container-breeding *Aedes*, but there are numerous other possibilities to be explored, such as *Anopheles* and flood-water *Aedes*. Mass production, storage and application of cyclops should be inexpensive enough for routine use in the near future. While working out technical details of production and distribution, we should turn our attention increasingly to how cyclops can be integrated into practical mosquito control operations and combined with other methods of control to enhance their effectiveness.

Acknowledgments

I wish to thank Janet Reid for tutorials on cyclops biology, as well as continuing collaboration on species identification and systematics. The research behind this essay was conducted in collaboration with Edgar Bordes, Michael Carroll, Edward Freytag, Stephen Sackett, Mieu Nguyen and other staff of the New Orleans Mosquito Control Board. Thomas Monath, Bruce Francy, Jerome Freier, Donald Eliason and Wenyan Che of the Centers for Disease Control contributed in numerous ways. The author was supported by a senior research associateship from the National Research Council.

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CURRENT TRENDS, PROBLEMS AND WORKING ARRANGEMENTS IN A CALIFORNIA MOSQUITO ABATEMENT DISTRICT

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Alameda County Mosquito Abatement District provides mosquito control in an area of 815 square miles located along the eastern shore of San Francisco Bay in Central California, USA. The District includes all of the County of Alameda except for 1 small city on the northern boundary. It extends eastward from the bay to the Central Valley of California.

The mosquito abatement Act provides very powerful legal mechanisms to facilitate mosquito control. It defines larval sources as a public nuisance and provides legal procedures to abate the nuisance. It provides agencies with taxing powers and empowers them to "take all necessary or proper steps for the extermination of mosquitoes..." A relatively recent amendment to the act makes legal abatement procedures available to County government and dependent districts doing mosquito control.

MOSQUITO SOURCES IN ALAMEDA COUNTY

The early settlers of Alameda County were met by a full complement of mosquitoes produced in a variety of natural sources. Tidal waters ebbed and flowed over saltmarshes creating mosquito producing pools in the upper reaches of saltmarshes. Abundant winter and spring rainfall expanded these pools to vast floodlands as run-off waters backed up in the face of high tides. Rainfall created temporary mosquito sources by flooding lowlands, sinkholes and treeholes. Other temporary mosquito sources were formed when creeks overflowed their banks and subsided. Freshwater marshes, seepages and margins of lakes and streams formed permanent aquatic habitats for the production of mosquitoes.

Dramatic changes occurred in the number of variety of mosquito sources as the land was developed by settlers. Levee systems were constructed to keep tides off of the saltmarshes. Channels were excavated and banked to control flooding. Reservoirs were built to collect and store run-off, providing a manageable source of water for irrigation and domestic purposes. In recent times, the citizens have realised the value of freshwater and saltwater wetlands. The result has been efforts on the part of a number of governmental agencies to restore, enhance and maintain wetlands.