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Use of cyclopoid copepods for mosquito control

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Abstract

The New Orleans Mosquito Control Board mass produces *Mesocyclops longisetus* and *Macrocyclops albidus* for introduction to mosquito breeding sites as a routine part of control operations. *Mesocyclops longisetus* is used in tires that collect rainwater; *M. albidus* is used in temporary pools. Field trials in a *Spartina* marsh, rice fields, and residential roadside ditches in Louisiana suggest that *M. longisetus* and *M. albidus* could be of use to control larvae of *Anopheles* spp. and *Culex quinquefasciatus*. *Mesocyclops longisetus* has proved to be effective for *Aedes aegypti* control in cisterns, 55-gallon drums, and other domestic containers in Honduras.

Introduction

Although cyclopoid copepods have long been known to prey on mosquito larvae (Hurlburt, 1938; Lindberg, 1949; Bonnet & Mukaida, 1957; Fryer, 1957), the unique potential of these tiny crustaceans for mosquito control was first appreciated about a decade ago (Rivière & Thirel, 1981; Marten, 1984; Suárez *et al.*, 1984). Most large species of cyclopoids are voracious predators of first instar mosquito larvae, more effective for biological control than other predatory invertebrates because it is so common for cyclopoids to be numerically abundant. Cyclopoids are practical for large-scale use because most species are easy and inexpensive to mass produce (Rivière *et al.*, 1987a; Marten, 1990a; Suárez *et al.*, 1992).

The first large-scale field trials of cyclopoids for mosquito control were with *Mesocyclops aspericornis* (Daday) in crab holes that served as habitat for larvae of *Aedes polynesiensis* Marks (Rivière *et al.*, 1987b; Lardeux *et al.*, 1992). *M. aspericornis* has also been field tested in tires and domestic containers where *Aedes aegypti* (L.) breeds (Rivière *et al.*, 1987b; Lardeux, 1992; Suárez, 1992). Other species of *Mesocyclops* have been observed to prey on *Ae. aegypti* or *Anopheles* larvae in the laboratory (Marten, 1989; Marten *et al.*, 1989; Brown *et al.*, 1991; Kay *et al.*, 1992). *Mesocyclops* spp. were observed to have a pronounced negative association with larvae of *Anopheles albimanus* Wiedemann in ponds and other small bodies of water (Marten *et al.*, 1989).

Five years ago the New Orleans Mosquito Control Board started to explore the use of cyclopoids for mosquito control. Twenty-five species were collected from the New Orleans area (Marten, 1989; Reid & Marten, 1994), of which seven species were large enough to be effective predators of mosquito larvae (Marten 1990b): *Acanthocyclops vernalis* (Fischer), *Diacyclops navus* (Herrick), *Macrocyclops albidus* (Jurine), *Megacyclops latipes* (Lowndes), *Mesocyclops edax* (Forbes), *Mesocyclops ruttneri* Kiefer, and *Mesocyclops longisetus* (Thiébaud). The largest species, *Homocyclops ater* (Herrick), did not prey on mosquito larvae.

We mass produce cyclopoids in 2.5 m × 1.5 m fiberglass trays that are stacked in racks. The total capacity of the New Orleans Mosquito Control Board's production facility is a million adult cyclopoids per month. We use two species, *Mesocyclops longisetus* and *Macrocyclops albidus*, for operational mosquito control (Marten, 1993). This paper reviews the use of these cyclopoids in tires and temporary pools in New Orleans, as well as unpublished results from field trials with these and other cyclopoids in additional aquatic habitats where mosquitoes breed.

Table 1. Minimum and maximum temperatures survived by cyclopoids during one day of exposure in the laboratory.*

Cyclopoid species**	Temperature (°C)	
	Minimum	Maximum
Mesocyclops venezolanus (H)	8	42
Mesocyclops aspericornis (PR)	5	43
Mesocyclops thermocyclopoides (H)	4	42
Mesocyclops longisetus (H)	3	42
Mesocyclops ruttneri (NO)	1	42
Mesocyclops longisetus (NO)	1	41
Megacyclops latipes (NO)	0	39
Mesocyclops edax (NO)	0	38
Acanthocyclops vernalis (NO)	0	38
Macrocyclops albidus (H)	0	37
Macrocyclops albidus (PR)	0	37
Macrocyclops albidus (NO)	0	37

* Source: G. G. Marten & M. Nguyen, unpublished data
** Collection locations: H = Honduras, PR = Puerto Rico, NO = New Orleans.

Table 2. Predation by New Orleans strains of *Mesocyclops longisetus* and *Macrocyclops albidus* on mosquito larvae in the laboratory.*

Mosquito species	Number of larvae killed per day**	
	M. longisetus	*M. albidus*
Aedes albopictus	38.4±1.9	44.3±0.7
Aedes sollicitans	ND	29.4±2.4
Anopheles quadrimaculatus	23.5±2.0	23.1±3.9
Culex quinquefasciatus	28.7±3.6	26.8±1.8
Culex restuans	6.9±1.3	8.1±0.9
Culex salinarius	2.7±0.9	1.8±0.3

* Source: Marten (1990b) and unpublished data.
** Mean (±SE), based on experiments in which 50 newly hatched larvae were placed in small dishes with single adult female cyclopoids for 24 hours.
ND = no data.

Tires

Discarded tires are the most important breeding sites for *Aedes albopictus* (Skuse), *Ae. aegypti*, and *Aedes triseriatus* (Say) in New Orleans, mosquitoes that breed primarily during the warmer part of the year (April–November). We tested six species of cyclopoids in tires (Marten, 1990b, 1990c): *M. albidus*, *M. longisetus*, *M. edax*, *M. ruttneri*, *A. vernalis*, and *D. navus*. *M. longisetus* and *M. albidus* reduced *Aedes* larvae by >99%. The other cyclopoid species reduced the larvae by 80%–90%, which is not of practical use for mosquito control because overcrowded populations of larvae may only be thinned without substantially reducing the production of adult mosquitoes.

The New Orleans Mosquito Control Board now treats several thousand tires each year with *Mesocyclops longisetus* as a routine part of mosquito control operations. *M. longisetus* is the species of choice because it is more resistant to desiccation than *Macrocyclops albidus*, and it is best at surviving high summer temperatures (Table 1). If we introduce a mixture of *M. longisetus* and *M. albidus* to the same tire during the summer, *M. longisetus* takes over. *M. albidus* takes over in a mixture of the two species during the winter.

If *M. longisetus* is introduced to tires in April or May, before *Aedes* populations build up, it maintains a population of approximately 50–200 adults in a tire for as long as the tire contains moisture. It may be necessary to put a small amount of food (e.g., a few leaves or grains of rice) into clean tires to provide an initial food supply for the cyclopoid population. Cyclopoids survive longer in tires near trees or other vegetation because shade prevents the tires from drying and leaf-fall provides food and a reservoir for moisture. In New Orleans, it is seldom necessary to introduce *M. longisetus* to the same tires more than once a year.

Introduction of 50–100 adult *M. longisetus* per tire provides control of virtually all *Aedes* larvae that subsequently hatch into the tire. Thousands of *M. longisetus* can be transported in a small container to sites where they are poured or ladled into tires. They can also be transported in backpack tanks from which they are squirted into tires with a hand-held wand. Each tank carries enough cyclopoids to treat a thousand tires.

If large mosquito larvae are in a tire at the time cyclopoids are introduced, it is best to apply 10 000 ITU of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) per tire along with the cyclopoids (Marten *et al.*, 1993). The *B.t.i.* eliminates larvae that are already too large for *M. longisetus* to kill.

Cyclopoid predation on some species of *Culex* larvae is not as effective as their predation on *Aedes* larvae (Table 2). From laboratory observations, we know that *M. longisetus* and *M. albidus* kill virtually all *Aedes* larvae they attack. They abort most attacks on *Culex* larvae before actually killing them, possibly because the bristles on *Culex* larvae give cyclopoids the false impression they are too large to attack. Large numbers of larvae of *Cx. salinarius* Coquillet are sometimes observed in tires in New Orleans that contain *M. longisetus* (Marten, 1990b).

Temporary pools

Numerous species of mosquitoes breed in the pools that form in poorly drained areas of New Orleans during the cooler part of the year (November–May). Most common are the 'floodwater' *Aedes* – e.g., *Ae. vexans* (Meigen) and *Ae. sollicitans* (Walker) – which lay their eggs on damp soil to hatch as soon as a pool is formed. Also common are *Culex salinarius* and *Culex restuans* Theobald. Because *Culex* lay their eggs on the surface of the water, their larvae appear a week or more after a pool is formed.

Diacyclops navus and *Acanthocyclops vernalis* are common in these pools, surviving in the soil when pools are dry and appearing in large numbers as adults and late-stage copepodids within hours after a pool forms. *D. navus* and *A. vernalis* prey on mosquito larvae, but they do not reduce the larvae consistently enough to be of practical use for mosquito control. We use *Macrocyclops albidus* for mosquito control in these pools. During the cool season *M. albidus* functions better than *M. longisetus* because *M. albidus* tolerates temperatures down to 0 °C (Table 1). Although quantitative assessment is not complete, it appears that pools containing *M. albidus* seldom have enough mosquito larvae to require larviciding treatment. The main exception is *Cx. salinarius*, which sometimes is abundant in pools that contain *M. albidus*.

Although *M. albidus* is able to survive in damp soil, it dies if the soil dries completely. As a consequence, *M. albidus* occurs naturally in only a small percentage of temporary pools, ones connected to depressions or ditches that retain moisture when the pools are dry. Some pools appear suitable for *M. albidus* during the cool season because they retain moisture during that period, but they do not have a natural population of *M. albidus*. We introduce *M. albidus* to these pools in November or December, when they have enough water to last through the rest of the season. The introductions are usually successful for the duration of the cool season, though *M. albidus* subsequently disappears from pools that dry completely during the summer. We can make pools suitable for *M. albidus* by creating a depression to hold water when a pool dries. Tires, which retain rainwater throughout the year when shaded by trees, can provide a refuge for *M. albidus* in wooded areas where pools form.

Megacyclops latipes is a species that we are starting to investigate. We sometimes find natural populations of *M. latipes* in woodland pools that are dry much of the year. Pools with *M. latipes* contain few mosquito larvae.

Marshes

A typical *Spartina* marsh in Louisiana has a permanent layer of water under the grass mat. *Macrocyclops albidus* is often abundant under the mat. There also may be small numbers of larvae of *Anopheles crucians* Walker or *Anopheles quadrimaculatus* Say under the mat, particularly if *M. albidus* is not present. *Culex salinarius* larvae are sometimes present in very large numbers. Because the periphery of a marsh dries out completely at times, *M. albidus* populations disappear and reappear in that part of the marsh, which often has moderate numbers of *Anopheles* and sometimes very large numbers of floodwater *Aedes* larvae. *Acanthocyclops vernalis* is the common cyclopoid in peripheral areas.

We have not used cyclopoids for routine mosquito control in marshes, but we have conducted a field trial that suggests they have potential. We introduced *Mesocyclops longisetus* and *Macrocyclops albidus* to an isolated area of peripheral marsh near New Orleans (*ca.* 10 ha). Before the introductions the marsh had a natural population of *Acanthocyclops vernalis*, which was often abundant, as well as moderate numbers of *An. crucians* and *Ae. sollicitans* larvae. Within two months after the introductions, *M. longisetus* and *M. albidus* were abundant throughout the marsh, and *A. vernalis* disappeared. *M. longisetus* and *M. albidus* remained in the marsh for an additional four months, until it dried out, and the numbers of *Anopheles* and *Aedes* larvae were very low during that period. After being dry for several months, the marsh had water once again. *M. longisetus* and *M. albidus* were gone, *A. vernalis* was back, and the mosquito larvae returned to their prior abundance.

The results of this field trial suggest that periodic reintroduction of *Mesocyclops* and *Macrocyclops* when they are no longer present could reduce the production of *Anopheles* and *Aedes* mosquitoes in a marsh.

Rice fields

Most rice fields in Louisiana contain *Mesocyclops ruttneri* or *Acanthocyclops vernalis* in abundance, but it is unusual to find both species in the same field. In a field survey in Jefferson Davis Parish, Louisiana, all the rice fields contained first instar *Anopheles* larvae. Seventy-five percent of the fields with *A. vernalis* also had third and fourth instar *Anopheles* larvae in our samples, indicating that a substantial number of larvae survived predation by *A. vernalis*. In contrast, only 22% of the fields with *M. ruttneri*, a voracious predator of *Anopheles* larvae (Table 2), had third or fourth instar larvae in the samples, indicating that predation by *M. ruttneri* eliminated larvae from many fields before the larvae could finish their development.

To see if complete control of *Anopheles* larvae could be achieved, we introduced 1000 *Mesocyclops longisetus*, *Mesocyclops ruttneri*, *Mesocyclops edax*, and *Macrocyclops albidus* to a rice field in Jennings, Louisiana, shortly after flooding in April. Large numbers of *Acanthocyclops vernalis* and moderate numbers of *Anopheles quadrimaculatus* larvae were in the field when the *Mesocyclops* and *Macrocyclops* were introduced. It took nearly two months for the introduced cyclopoids to become abundant enough to appear in water samples from the field, but once that happened, there were approximately 10 adult cyclopoids/liter. *A. vernalis* and *Anopheles* larvae then disappeared from the treated field, even though *A. vernalis* and *Anopheles* continued to be present in adjacent (control) fields. All four introduced cyclopoid species remained abundant in the treated field until it was drained in August, and all four were still alive in the damp soil of the field in November. Only *M. albidus* survived the winter.

It appears that mosquito production from rice fields could be reduced by introducing *Mesocyclops* and *Macrocyclops*. The major practical problem lies in the fact that it is not possible to introduce the massive number of cyclopoids required to fully stock a field immediately; they require time to multiply to numbers that are large enough to impact mosquito larvae. Nonetheless, it should be possible to shorten the time it takes the cyclopoids to build up their numbers if fields contain ponds that serve as permanent reservoirs to stock each field with a large number of cyclopoids as soon as it is flooded.

Residential roadside ditches

Roadside drainage ditches in small towns of Louisiana provide breeding habitat for *Culex quinquefasciatus* Say. *Macrocyclops albidus* and *Gambusia affinis* (Baird & Girard) are common in many parts of these ditches, where they greatly reduce the number of *Cx. quinquefasciatus* larvae. However, because cyclopoids and fish are excluded from parts of the ditches that are highly polluted with effluent from septic tanks, the polluted water serves as a refuge for *Cx. quinquefasciatus* larvae, which are not harmed by pollution and thrive on the high concentration of bacteria in polluted water.

M. albidus disappears from most ditches during the summer, apparently because of high water temperatures and greater pollution due to reduced water movement in the ditches during that season. *M. albidus* starts to reappear in the ditches during the autumn, when pollution declines, and it is common in most unpolluted parts of the ditches by spring.

In October, we introduced *M. albidus* along 1200 meters of roadside ditches (5 adult female *Macrocyclops* per meter of ditch), to see if we could speed up the autumn-winter-spring invasion process. *M. albidus* was abundant in the treated ditches within a few weeks, and from December to March the number of sampling stations in treated ditches with *Cx. quinquefasciatus* larvae was approximately 90% less than in control ditches. *M. albidus* did not eliminate the larvae completely because the most highly polluted areas continued to serve as refuges for the larvae, but introduction of *M. albidus* substantially reduced the number of sites that required larviciding during the cool season.

Domestic containers (Honduras)

The New Orleans Mosquito Control Board has collaborated with the Honduran Ministry of Public Health to test and deploy cyclopoids for *Ae. aegypti* control in water storage containers at people's homes. Local strains of four cyclopoid species were tested in the city of El Progreso, Honduras (Marten *et al.*, 1992, 1994): *Mesocyclops longisetus*, *Mesocyclops venezolanus* Dussart, *Mesocyclops thermocyclopoides* Harada, and *Macrocyclops albidus*. All four species were effective predators of *Ae. aegypti* larvae, but *M. longisetus* was best for operational use because it survived in containers better than the other species.

M. albidus seldom survived more than a month in most containers, apparently because it was adversely affected by high water temperatures when containers were in the sun. *M. venezolanus* and *M. thermocyclopoides* did not last in 55- gallon drums that are used to store water for household cleaning; they swim in the water column, so they are removed from drums when water is dipped out for use. *M. longisetus* is not removed when water is dipped out because it concentrates its activity along the bottom and sides of a drum. *M. longisetus* can be lost from a drum when all the water is poured out and the drum is cleaned (Lardeux, 1992; Suarez, 1992), but the loss of *M. longisetus* can be avoided by removing the animals with a net before cleaning and returning them afterwards (Marten *et al.*, 1994).

M. longisetus can be used to control *Ae. aegypti* larvae in numerous containers besides drums. It is particularly effective in large cement tanks or cisterns, which often produce enormous numbers of mosquitoes if left untreated (Marten *et al.*, 1994). It has been used in water storage urns (Vasconcelos *et al.*, 1992), and it can be used in small aquatic habitats such as vases and bromeliads (Marten *et al.*, 1994; G. G. Marten & M. F. Suárez, unpublished data).

Biological research needs

The following biological research on cyclopoids would facilitate their use for mosquito control:

1. Taxonomy. Reliable species identification is essential because different cyclopoid species can be so different in their ability to survive in different kinds of mosquito breeding habitats.

2. Culture with prepared food. Cyclopoids can be mass produced only with live food at the present time (Suárez *et al.*, 1992). Production costs would be reduced if cyclopoids could be produced with prepared food.

3. Adaptation to temporary aquatic habitats. The loss of cyclopoid populations from sites that dry up is the most important biological factor limiting their use for mosquito control. Biological information on the population dynamics of cyclopoids in temporary aquatic habitats could help to identify (a) cyclopoid species that will survive when habitats dry, (b) criteria for recognizing specific habitats were cyclopoids will survive, (c) habitat modifications to augment survival, and (d) optimal timing of cyclopoid introductions with regard to seasonal cycles.

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