A SURVEY OF CYCLOPOID COPEPODS FOR CONTROL OF *AEDES ALBOPICTUS* LARVAE [1]

G. G. Marten[2]

ABSTRACT: Eighteen species of cyclops were collected from a variety of aquatic habitats in New Orleans. Six of the species prey on first-instar *Aedes albopictus* larvae: *Acanthocyclops vernalis, Diacyclops navus, Macrocyclops albidus*, *Mesocyclops edax*, *Mesocyclops longisetus*, and *Mesocyclops* sp. (*leuckarti* group). They generally thrive when introduced to tires. *Macrocyclops albidus* and *Mesocyclops sp.* appear most promising for biological control because introduced populations of these two species usually kill all larvae that hatch into tires. Naturally occurring mixtures of *D. navus* and *M. albidus* in discarded tires were observed to reduce the number of *Ae. albopictus* larvae by 95 percent.

INTRODUCTION

The invasion of continental United States *by Aedes albopictus* and its subsequent spread through much of the eastern portion of the country has stimulated a national effort for control. The Centers for Disease Control and the New Orleans Mosquito Control Board are collaborating on a study to develop integrated control for cities such as New Orleans. Part of the study is directed toward novel means of ecological and biological control.

The copepod *Mesocyclops aspericornis* (formerly called *Mesocyclops leuckarti pilosa)* is known to be an effective predator of first-instar *Aedes* larvae (Marten 1984, Suárez 1984, Revière et al. 1987). *Mesocyclops aspericornis* does not occur in the continental United States, but cyclops of one species or another are common among aquatic fauna virtually everywhere in the world. The state of Louisiana is no exception (Nasci et al. 1987).

Cyclops in New Orleans were surveyed during the summer of 1988 to determine which species could be of use for biological control of *Ae. albopictus* larvae in containers such as discarded tires.

METHODS

Species Survey

Cyclops were collected from about one hundred sites, covering a variety of aquatic habitats including freshwater lagoons, ponds, roadside ditches, swales, and containers such as discarded tires. To determine which species of cyclops prey on Ae. *albopictus* larvae, I placed 10 adults of each species in spot dishes with wells 21 mm in diameter, one cyclops in each well. Three first-instar *Ae. albopictus* larvae were placed in each well, and the number of remaining larvae was observed after 24 hours.

Tire Samples

Abandoned tires that have been around for several years often contain cyclops. A group of 80 such tires at the side of a dirt road in a wooded area of New Orleans was sampled in July for cyclops and mosquito larvae. Adult *Ae. albopictus* were abundant in the area, and many of the tires contained cyclops and/or *Ae. albopictus* larvae. The total volume of water in each tire was measured, and a 350-ml sample of water was removed from each tire. All animals were strained from the sample and preserved for identification and counting.

RESULTS

Species Survey

A total of 18 species of cyclops was found in New Orleans (TABLE 1). The 12 species without an asterisk in TABLE 1 did not prey on Ae. *albopictus* larvae when

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Division of Vector-borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522, U.S.A. Mailing Address: New Orleans Mosquito Control Board, 6601 Lakeshore Drive, New Orleans, Louisiana 70126, U.S.A.

tested in spot dishes. With one exception, the adult female body length of all these species is less than 1.0 mm (excluding caudal setae)—too small to prey on the larvae. Some of these species (e.g., *Eucyclops agilis)* were observed to initiate attacks on *Ae. albopictus* larvae, but they failed to carry them through. These species probably kill *Ae. albopictus* larvae on occasion, but not enough to be of use for biological control. *Homocyclops ater* is the only non-larvivorous species in TABLE 1 larger than 1.0 mm in body length: it does not appear to be a predator.

The six species of cyclops with asterisks in TABLE 1 killed *Ae. albopictus* larvae in the spot dish tests. Most killed all three larvae. They usually consumed everything except the head capsule, but sometimes they killed larvae without eating them. These six species have adult female body lengths in the range of 1.0 to 1.5 mm.

Tire Samples

Seventy percent of the sampled tires contained one or more species of Cyclops. Many of the tires contained more than one species. In fact, *M. albidus* and *D. navus* were almost always found together in the same tires: 44 percent of the tires contained both *D. navus* and *M. albidus* (TABLE 2). They appeared to have been in the tires for years. Other species of cyclops in the tires were *Paracyclops poppei*, *Paracyclops fimbriatus*, *Eucyclops agilis*, *Orthocyclops modestus*, *Ectocyclops rubescens*, and *Acanthocyclops vernalis.* The presence of these other species in tires seemed to be independent of whether D. *navus sad M. albidus* were in the tires, but populations of the other species were smaller in tires with *D. navus* and *M. albidus* (TABLE 2).

Culex quinquefasciatus and *Culex salinarius* larvae were abundant in the tires. Occurrence of the larvae of these two species of mosquitoes did not appear to be associated with any of the cyclops, but the numbers of *Culex* larvae were lower in tires containing *D. navus* and *M. albidus* (TABLE 2). *Macrocyclops albidus* was observed to prey on *Culex* larvae in the laboratory, but not nearly as frequently as *Aedes* larvae.

Aedes albopictus larvae were completely absent from nearly all tires that contained *M. albidus* and *D. navus*, even though *Ae. albopictus* larvae were found in 60 percent of the tires without *M. albidus* and *D. navus* (TABLE 2). Overall, nearly 20 times as many *Ae. albopictus* larvae were found in tires without *M. albidus* and *D. navus* as were found in tires that contained these two species of cyclops. *Macrocyclops albidus* was responsible for most of the predation in water samples (including detritus, cyclops, and all other organisms) that were removed from the tires and observed under a stereomicroscope while introducing first-instar larvae.

The few tires in which *Ae. albopictus* larvae were found together with *M. albidus* and *D. navus* are of particular interest. In contrast to most other tires, they contained a variety of zooplankton, including numerous cladocera. The abundance of alternative food appeared to reduce the intensity of cyclops predation on the mosquito larvae.

Paracyclops poppei was the only other species of cyclops in enough of the tires to assess its correlation with *Ae. albopictus* larvae. No correlation was observed.

DISCUSSION

Two major results are apparent from this study. First is the fact that several species and genera of cyclops prey upon *Aedes* larvae. Second is the intense predation by natural populations of *Macrocyclops* on *Ae. albopictus* larvae in tires. Additional evidence on

TABLE 1. Species of cyclops collected in New Orleans.

* *Acanthocyclops vernalis*	**Mesocyclops edax*
Apocyclops panamensis	**Mesocyclops longisetus*
**Diacyclops navus*	**Mesocyclops sp. (leuckarti group)*
Ectocyclops rubescens	*Microcyclops varicans*
Eucyclops agilis	*Orthocyclops modestus*
Eucyclops speratus	*Paracyclops fimbriatus*
Homocyclops ater	*Paracyclops poppei*
**Macrocyclops albidus*	*Thermocyclops inversus*
Metacyclops denticulatus	*Tropocyclops prasinus*

*Prey upon first-instar *Aedes albopictus* larvae.

the intensity of predation in tires comes from an experiment in which 100 first-instar *Ae. albopictus* larvae were introduced under field conditions to some of the same tires that were sampled in this study (J. E. Freier, pers. commun.). In most of the tires with *M. albidus* and *D. navus*, no larvae survived after 48 hours. (A few of the tires had one larva surviving.) In contrast, 45 to *55* percent of the larvae were still alive after 48 hours in tires that contained neither of these cyclops species.

The main reason that cyclops can be such effective predators of *Ae. albopictus* larvae is their broad food base—phytoplankton, protozoa, and small animals such as rotifers—which allows them to maintain large populations independent of the supply of mosquito larvae. A high reproductive capacity and a generation time of 2 to 4 weeks (depending upon temperature), plus the fact that a female is inseminated for life, ensure that a large population will be established within a few weeks after introducing only a few adult females to a container.

Cyclops populations can persist for long periods in a container, sometimes years. Many species can survive when a container dries up, as long as some soil or detritus remains. The eggs, which are carried in sacs on each side of a female's body until they hatch, are not resistant to desiccation. Cyclops survive drying as adults or copepodids, which are active within hours after there is water in the container again. The scientific literature contains very little about how cyclops cope with desiccation, whether a pocket of moisture is necessary for survival or whether there are physiological adjustments such as diapause.

Field trials have been initiated in New Orleans with four of the larvivorous species in TABLE 1. The trials are still in progress and details will be reported elsewhere, but some of the most conspicuous results are worth mentioning. When introduced to tires in a New Orleans woodlot, *Acanthocyclops vernalis*, *Diacyclops navus*, and *Mesocyclops* sp. established long-term populations of several hundred adults within a few weeks. *Macrocyclops albidus* established populations of 50 or more adults. *Macrocyclops albidus* and *Mesocyclops* sp. eliminated *Ae. albopictus* larvae as effectively as reported above for the naturally occurring mixtures of *Macrocyclops* and *Diacyclops*. *Acanthocyclops vernalis* appeared to be nearly as effective.

Considerable variation exists among the six larvivorous species of cyclops with regard to ecological characteristics that bear on their effectiveness as predators of *Ae. albopictus* larvae. The following remarks are based on observations in laboratory containers, using water and detritus from tires to simulate environmental conditions in tires.

Acanthocyclops vernalis occurs throughout the temperate regions of the world in a broad array of aquatic habitats—temporary and permanent, large and small. In containers, it spends most of its time actively hunting along surfaces where *Ae. albopictus* larvae

Table 2. Cyclops and mosquito larvae in tires on the edge of a New Orleans woodlot.

	Diacyclops navus and *Macrocyclops albidus* Present [a]	Absent [b]
Number of tires	35	40
Average number of *D. navus* [c]	66	—
Average number of *M. albidus* [c]	76	—
Percent of tires with other cyclops	49%	51%
Average number of other cyclops [c]	20	51
Percent of tires with *Ae. albopictus* larvae	8%	60%
Average number of *Ae. albopictus* larvae [c]	5.0	12.6
Percent of tires with other mosquito larvae [d]	35%	40%
Average number of other mosquito larvae [c,d]	3.5	6.8

[a] Both *D. navus* and *M. albidus* present in tire.

[b] Neither *D. navus* nor *M. albidus* present in tire.

[c] Averages are based only on tires where the indicated animal was present.

[d] *Culex* larvae.

graze. Experimental information on the effectiveness of *A. vernalis as* a predator is not yet complete, but predation in experimental containers is often 100 percent. Some larvae may escape predation if more than a few hundred are placed in the container at the same time.

Diocyclops navus is common throughout eastern North America in temporary water bodies such as small ponds and swales, where it is often highly abundant. *Diocyclops navus* swims throughout the water column and spends brief periods scanning the bottom, hunting behavior that puts it in frequent contact with *Ae. albopictus* larvae. It is the smallest of the six larvivorous species in New Orleans and the most timid as a predator of *Ae. albopictus* larvae. Predation is highly variable and is seldom 100 percent. For this reason, *D. navus* does not appear as promising for biological control as the other larvivorous species.

Macrocyclops albidus is a species of global distribution. It is common in New Orleans in permanent water bodies of all sizes. *Macrocyclops albidus* usually rests on the bottom of a container where it attacks *Ae. albopictus* larvae as they graze there. It sometimes hunts along the surface of the water, where it kills larvae hanging at the surface. It is a large and aggressive predator and often kills all larvae it encounters, even when it is too satiated to eat. It typically kills 100 percent of the *Ae. albopictus* larvae that are placed in a container, even if more than 1,000 are introduced at one time. *Macrocyclops albidus* is one of the most promising candidates for biological control, but it has some limitations. It generally has smaller container populations than the other species, averaging less than 100 individuals per tire. The *Macrocyclops* collected in New Orleans are less tolerant of high water temperatures that can occur in containers exposed to the sun, being unable to survive more than 37°C. They are less able to survive drying of the habitat.

Mesocyclops edax is the smallest species of *Mesocyclops* in New Orleans, but it is an aggressive predator. It is common in lakes throughout eastern North America, and it spends all of its time swimming in the water column. When introduced to containers, it usually establishes a population that is large enough to kill all *Ae. albopictus* larvae, particularly if phytoplankton are abundant in the water. However, *M. edax* appears to fare more poorly than the other cyclops species in containers where food is in meager supply.

Mesocyclops longisetus is the largest of the six species and a correspondingly aggressive predator. It is a neotropical species whose distribution in the United States is probably restricted to the Southeast. *Mesocyclops longisetus* swims throughout the water column, and it is known to eliminate *Anopheles* larvae in Latin America wherever it is naturally abundant (Marten et al. 1989). Only a few specimens of *M. longisetus* have been collected in New Orleans from a freshwater lagoon. There have not been enough to establish a culture or test *M. longisetus* in the field.

Mesocyclops sp. appears to be a new species of unknown distribution. It is a member of the *Mesocyclops leuckarti* species group, which is global in distribution; its morphology is most similar to *Mesocyclops aequatorialis prescei*, which is found in the Caribbean. In this study, *Mesocyclops* sp. has been collected only from freshwater lagoons in New Orleans but is probably common in other areas of southeastern United States. It thrives when introduced to container habitats. It is a large and aggressive predator that spends its time resting on submerged vegetation and leaves, where it grabs *Ae. albopictus* larvae as they graze. It appears to be nearly as effective as *M. albidus* at eliminating *Ae, albopictus* larvae, and it is more resistant to desiccation and more tolerant of high water temperatures. *Mesocyclops* sp. is a strong candidate for biological control.

In summary, *M. albidus* and *Mesocyclops* sp. are highly effective larval predators that usually kill 100 percent of Ae. *albopictus* larvae, even when more larvae are introduced to a container than would ever hatch into it under natural conditions. Sometimes lapses occur, however, which should be investigated further. These appear to be associated with an abundance of alternative food. Fortunately, this is not a common situation. Once a cyclops population builds up its numbers in a container, it grazes all food sources down to a very low level.

"The critical question for practical use of cyclops for biological control is how they can be produced in large numbers and how they can he stored and distributed to container habitats where *Ae. albopictus* breeds. Mass production of any of these species should be simple and inexpensive. I am using a culture system consisting of wheat seed, bacteria, protozoa, and rotifers. I have only produced them by the thousands so far, but it should be possible to expand the same production system to a larger scale. The materials are inexpensive, but procedures remain to be developed that are simple enough to have low labor costs.

Large numbers of cyclops can be stored for months in a small quantity of water at refrigerator temperature. The cyclops can pass without damage through a backpack spray nozzle with a simple hole several millimeters in diameter. The fact that cyclops can survive for months in soil or detritus that is only slightly damp suggests the possibility of storage and transport without large quantities of water. It may be possible to encapsulate them in a dormant state.

A significant advantage of the cyclops for biological control is their compatibility with many larvicides (Wenyan Che, pers. commun.). Cyclops can function normally in a concentration of permethrin several hundred times the lethal dose for *Ae. albopictus* larvae. *Bti* appears to have no detrimental effect, even at thousands of times the normal dosage for larviciding. If cyclops and a larvicide are applied together, the larvicide can produce an immediate kill of all larval stages, and the cyclops can take over as the larvicide diminishes in effectiveness.

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