Control of Larval *Aedes aegypti* (Diptera: Culicidae) by Cyclopoid Copepods in Peridomestic Breeding Containers

GERALD G. MARTEN,[1] GERARDO BORJAS,[2] MARY CUSH,[1] EDUARDO FERNANDEZ, AND JANET W. REID[3]

División de Enfermedades de Transmisión Vectorial, Ministerio de Salud Pública de Honduras, Tegucigalpa, Honduras

J. Med. Entomol. 31(1): 36–44 (1994)

ABSTRACT *Mesocyclops longisetus* (Thiébaud), *Mesocyclops thermocyclopoides* Harada, *Mesocyclops venezolanus* Dussart, and *Macrocyclops albidus* (Jurine) were tested for their effectiveness in controlling *Aedes aegypti* (L.) larvae in a variety of containers around homes in El Progreso, Honduras. All four cyclopoid species killed >20 larvae per cyclopoid per d under container conditions. *M. longisetus* was most effective, not only because it was the most voracious predator, but also because it survived best in the containers. *M. longisetus* maintained long-term populations in 200-liter drums, tires, vases, and cement tanks (without drains), providing the cyclopoids were not dried or poured out. *M. longisetus* reduced third- and fourth-instar *Ae. aegypti* larvae by >98% compared with control containers without cyclopoids. *M. longisetus* should be of practical value for community-based *Ae. aegypti* control if appropriate attention is directed to maintaining it in containers after introduction.

KEY WORDS Copepoda, *Aedes aegypti*, biological control

THE INTEGRATED DENGUE CONTROL PROJECT in El Progreso, Honduras, a city of ≈80,000 inhabitants, is concerned with community-based *Aedes aegypti* (L.) control (Fernández et al. 1992). The project uses mechanical methods of source reduction, such as cleaning water storage containers to interrupt larval development, storing tires and domestic containers so they do not collect rainwater, and eliminating tires or containers that are not needed.

Some breeding sites are not amenable to mechanical methods. A sporadic water supply may prevent the cleaning of water storage containers frequently enough to control *Ae. aegypti* larvae. Because of work outside the home, some housewives may not have the time to clean their containers frequently. Water storage tanks without a drain rarely may be cleaned, and some people have tires they are not willing or able to store out of the rain. All of these require an alternate method to eliminate *Ae. aegypti* production.

Cyclopoid copepods are a promising method of biological control. The larger species of these microcrustaceans, which prey on first- and second-instar mosquitoes, can maintain virtually 100% control of container-breeding *Aedes* for as long as the cyclopoids survive in the container (Rivière & Thirel 1981, Marten 1984, Suárez et al. 1984). *Mesocyclops longisetus* (Thiébaud) and *Macrocyclops albidus* (Jurine) currently are used to control *Aedes* larvae in tires (Marten 1990a,b,c), and *Mesocyclops aspericornis* (Daday) has proved effective against *Aedes* larvae in field trials with tires, water-storage drums, and cisterns (Rivière & Thirel 1981, Suárez et al. 1984, Rivière et al. 1987a).

Cyclopoids are more effective for *Aedes* control than other aquatic invertebrates that prey on mosquito larvae because their high reproductive capacity and broad diet (including phytoplankton, protozoa, and small animals) enable them to maintain abundant populations independent of mosquito larval abundance. Their small size and high reproductive capacity also make cyclopoids inexpensive and convenient for large-scale production and distribution (Rivière et al. 1987a, Marten 1990c, Suárez et al. 1992).

We conducted laboratory experiments and field trials to evaluate the effectiveness of cyclopoids for the long-term control of *Ae. aegypti* larvae in domestic containers in El Progreso. The purpose of the field trials was to determine which containers can be controlled by using cyclopoids and which species of cyclopoids are most effective. Four species common in Honduras were tested: *Macrocyclops albidus*, *Mesocy-*

[1] New Orleans Mosquito Control Board, 6601 Lakeshore Drive, New Orleans, LA 70126, and Department of Tropical Medicine, Tulane University, New Orleans, LA 70112.

[2] Departamento de Biología, Universidad Autónoma de Honduras, Tegucigalpa, Honduras.

[3] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560.

Table 1. Percentage of *Ae. aegypti* production from domestic containers in El Progreso, Honduras

Container	% Production
Laundry tanks	44
Tires	29
200-liter drums	12
Animal drinking containers	7
Buckets and pots[a]	3
Cisterns and pools[b]	2
Vases with live plants	1
Other[c]	2

Based on percentages of pupae in containers at 500 houses during September–October 1991. Breteau index, 38; total number of pupae, 1,764.

[a] More than 4-liter capacity.

[b] Cement construction, on or in the ground, no drain.

[c] Tin cans, bottles, car batteries, toilets, pipes, scrap iron, wheel barrels, and tree holes.

clops longisetus, Mesocyclops venezolanus Dussart, and *Mesocyclops thermocyclopoides* Harada. Because the first three species occur throughout much of Latin America, the results of these tests should apply broadly to that region.

Materials and Methods

Containers. Laundry tanks, tires, 200-liter (55 gal) drums, animal drinking containers, buckets, large pots, cisterns, ornamental pools, and vases are responsible for nearly all the *Ae. aegypti* production in El Progreso (Table 1).

Most homes in El Progreso have a cement laundry tank outside the house to store water for washing clothes and dishes. The tanks are needed because city tap water may be available for only a few hours a day. Clothes and dishes are not washed in the tanks themselves; water is dipped out for use and replaced from the tap when available. Most housewives clean their tanks about once a week by draining, scrubbing, and refilling them. As with all other water storage containers in this study, the water from these tanks is not used for drinking.

Water also is stored in 200-liter drums for household cleaning activities such as mopping floors and washing down the toilet in the latrine. Houses without a laundry tank may use water from the drum to wash clothes and dishes. The drums are always uncovered. Water is dipped out as needed and replaced with tap water. Because the water for floors or toilets does not have to be very clean, many drums are cleaned only once a month. Drums are cleaned by tipping to pour out the water, spraying the inside with a hose, and returning the drum to an upright position. Because the drums are outside and trees are numerous, even drums that are cleaned frequently often have sediment or leaves at the bottom.

Some people have discarded tires associated with a home business, and others keep old tires in their yard for eventual use or sale. Most of these tires contain leaves because they are shaded by trees, and they seldom dry out, particularly during the rainy season.

Animal drinking containers vary in size and shape. They include plastic bowls and tires cut in half lengthwise, but mosquito production is greatest from shallow cement pools that are embedded in the ground without a drain. Animal drinking containers seldom produce mosquitoes while in active use because the water is changed frequently and many animals (particularly ducks and geese) eat the larvae in their drinking water. However, *Ae. aegypti* production can be high from a drinking container in a yard without animals.

Buckets, 40-liter plastic drums, pots, and large plastic bowls are numerous in yards, because much of the cooking and cleaning is done outside. Most of these containers do not breed *Ae. aegypti* because they are in active use and larvae are eliminated before they can complete their development. However, they may breed mosquitoes if they are not in active use.

A few families use cisterns (cement tanks, several meters long, embedded in the ground) to store water. The cisterns have makeshift covers, and mosquitoes have easy access. Ornamental pools are similar to cisterns except they are shallower and not covered. The cisterns and pools are seldom cleaned and often contain enough detritus to provide abundant food for mosquito larvae. They can be a primary source of *Ae. aegypti* in neighborhoods where they are located.

Various containers—vases, tin cans, and plastic soft drink bottles tipped on their side with one side cut out—are used to hold live plants (e.g., philodendron) in water. Often the plant is a cutting with few roots, the water in the vase is clean, and the carrying capacity for mosquito larvae is low. Mosquito production from vases with a large quantity of detritus can be high.

Field Collection and Preliminary Screening. Cyclopoid copepods were collected from several hundred randomly selected aquatic sites in the vicinity of El Progreso. All species with an adult female body length >1 mm (from the front of the cephalothorax to the end of the caudal ramus) were considered large enough to be possible predators of mosquito larvae. They were placed in dishes (10 cm diameter) for 24 h with newly hatched *Ae. aegypti* larvae at a ratio of 10 larvae per cyclopoid. Larval head capsules and mangled dead bodies were considered to be a consequence of cyclopoid predation.

Following culture procedures described by Suárez et al. (1992), laboratory colonies of *Macrocyclops albidus* sensu stricto, *Mesocyclops longisetus* var. *curvatus* Dussart, *M. venezolanus*, and *M. thermocyclopoides* sensu lato were established to produce the numbers required for

the predation experiments and field trials described below.

Laboratory Predation Experiments. To assess the relative capacity of each of the four cyclopoid species to kill *Ae. aegypti* larvae, single adult female cyclopoids, previously held for 24 h without food, were placed in tissue culture plates (wells 35 mm diameter, 18 mm deep) with 50 newly hatched *Ae. aegypti* larvae. Surviving larvae were counted after 24 h. The temperature was 24–26°C.

To obtain a quantitative estimate of the maximum capacity of each species of cyclopoid to kill *Ae. aegypti* larvae under domestic container conditions, each of the four cyclopoid species was introduced to three 200-liter drums almost filled with water. The cyclopoids were given a month to build up their populations and equilibrate with whatever food was naturally available in the drums. Predation experiments then were conducted in five drums each day, one drum for each cyclopoid species plus a control drum without cyclopoids. The experiments were repeated on 3 different d using different drums each day. Drum water temperatures ranged from 21 to 28°C.

Each predation experiment was started by passing a net throughout the water to remove all adult and subadult cyclopoids in the 200-liter drum. Nets were emptied into a small dish of water, and the process was repeated until no additional cyclopoids were captured. Ten adult female cyclopoids then were returned to each drum, and 500 newly hatched *Ae. aegypti* larvae were added concurrently. Five hundred larvae were used because that number is enough to saturate the predatory capacity of 10 cyclopoids but not enough to cause excessive larval mortality from overcrowding (Marten 1990a). After 24 h, all the water from each drum was poured through a net to capture cyclopoids and larvae for counting. Each drum was rinsed until no more larvae were captured.

The same procedures were followed with automobile tires that contained rain water, except that only five cyclopoids and 250 *Ae. aegypti* larvae were used. The experiment was replicated four times for each cyclopoid species, using different tires.

Field Trials. The purpose of the field trials was to compare (1) the survival of each cyclopoid species in different types of containers under normal household use and (2) the effectiveness of each species in eliminating *Ae. aegypti* larvae. The trials were initiated in May–June 1991, at the beginning of the rainy season, by introducing adult females of each of the four species of cyclopoids to containers at houses scattered throughout several barrios in the city. There was no attempt to include all the containers in any neighborhood.

The rainy season and the field trials lasted 30 wk until the end of the year. *Ae. aegypti* is most abundant during this period. The field trials with vases started 2 mo later than other containers and lasted for 20 wk.

The containers used for the field trials included 29 laundry tanks, 34 200-liter drums (10 of which were not in active use), 37 tires, 12 animal drinking containers, 5 buckets, 10 40-liter plastic drums, 5 pots, 4 cisterns, 2 ornamental pools, and 26 plant vases (most of which were soft drink bottles on their sides). The numbers of cyclopoids introduced typically were 100–200 in laundry tanks and 200-liter drums; 50–100 in tires, animal drinking containers, buckets, plastic drums, and pots; 100–500 in cisterns or pools; and 10 in vases. Only *Mesocyclops longisetus* was tested in animal drinking containers, cisterns, and ornamental pools.

Most of the introductions involved one species of cyclopoid at a time, but a mixture of all four species also was tested in four 200-liter drums, five tires, three vases, and one cement pool that provided water for backyard animals. We thought a mixture might be superior to a single species because different species might hunt in different parts of a container, thereby covering it more thoroughly, or because one species might survive stress conditions that eliminate other species from a container.

Before each cyclopoid introduction, we explained to the residents the importance of mosquito larvae (many people did not realize larvae become adult mosquitoes) and the role of cyclopoids in *Ae. aegypti* and dengue control. The explanation included a demonstration of cyclopoids attacking and consuming *Ae. aegypti* larvae, aided by a magnifying glass. Small nets were issued to 15 families with 200-liter drums so they could capture cyclopoids before cleaning the drums and return them to the drums after cleaning. We demonstrated the procedure and helped housewives with its implementation the next two times they cleaned their drums. After that, they followed the procedure on their own.

Bacillus thuringiensis var. *israelensis* pellets (Bactimos [BTI], Summit Chemical, Baltimore, MD) were applied to 15 of the 37 tires (5 pellets per tire) and 15 of the 34 drums (15 pellets per drum) at the same time the cyclopoids were introduced. BTI, which is not harmful to cyclopoids (Rivière et al. 1987b, Marten et al. 1993), was applied to initiate control by killing all mosquito larvae too large for cyclopoids to kill.

All containers were inspected weekly during the first 4 wk after cyclopoid introduction and every 2–4 wk thereafter for as long as cyclopoids remained in a container. Control containers without cyclopoids also were monitored. In the case of tires, vases, and other smaller containers, the inspection procedure included removing all the water, passing it through a net to capture cyclopoids and mosquito larvae, counting the larvae as first and second or third and fourth instars

Table 2. Mean number ± SE of first-instar *Ae. aegypti* that died during 24-h laboratory predation experiments with adult female cyclopoids

Cyclopoid	Culture plates	Drums	Tires
Mesocyclops longisetus	46.8 ± 0.6 (41)	36.8 ± 1.7 (3)	36.5 ± 4.1 (4)
Macrocyclops albidus	41.0 ± 3.0 (20)	31.1 ± 3.9 (3)	33.4 ± 3.6 (4)
Mesocyclops thermocyclopoides	30.3 ± 1.9 (32)	31.6 ± 0.4 (3)	35.1 ± 4.6 (4)
Mesocyclops venezolanus	26.4 ± 2.3 (23)	36.4 ± 11.9 (3)	20.9 ± 6.3 (4)
Controls[a]	0.1 ± 0.1 (21)	18.8 ± 6.2 (3)	14.0 ± 3.1 (4)
No. *Aedes* larvae per replicate	50	500	250
No. cyclopoids per replicate	1	10	5

Values in parentheses are numbers of replicates.
[a] Mortality in controls (without cyclopoids) divided by 10 (in the case of drums) or 5 (in the case of tires) for comparison with drums and tires that contained cyclopoids.

and cyclopoids as adults or copepodids, and returning all cyclopoids and water to the container. With laundry tanks, drums, cisterns, and pools, cyclopoids and mosquito larvae were removed for counting by passing a net back and forth through the water and around the bottom and sides of the container, a technique verified to capture >95% of the cyclopoids and larvae in a container (G.G.M., unpublished data).

Field Predation Experiments. To test the ability of long-term *Mesocyclops longisetus* populations to kill *Ae. aegypti* larvae that might hatch into 200-liter drums, paper strips with 1,000 *Ae. aegypti* eggs were attached to the sides of five drums just under the water surface. The drums contained populations of 500–3,500 *M. longisetus* from introductions at least 3 mo earlier. The drums were in normal use before and during the experiment. After 5 d, all adult and subadult cyclopoids and *Ae. aegypti* larvae were removed with a net and counted. The same procedure was followed with a control drum that did not contain *M. longisetus*.

The same experiment was conducted with five tires containing long-term populations of *M. longisetus*, except that 500 *Ae. aegypti* eggs were added per tire. The number of *M. longisetus* in the tires at the time of the experiment ranged from ≈35 to 300. There was one control tire without *M. longisetus*.

Three mo after introducing cyclopoids to a series of plant vases, 20 newly hatched *Ae. aegypti* larvae were placed in three vases, each of which contained three *Mesocyclops longisetus;* three vases that contained two *M. venezolanus;* and three vases that contained three or four *Macrocyclops albidus*. Cyclopoids and surviving larvae were removed for counting at the end of 5 d by passing all the water from each vase through a net. The same procedure was used for 10 control vases without cyclopoids.

Cyclopoid Distribution Within Containers. The location of cyclopoids in a container can affect their frequency of contact with mosquito larvae and their vulnerability to removal if water is dipped out of the container for use. To observe cyclopoid location, 200-liter drums filled with water and containing ≈1,000 cyclopoids (a different species in each drum) were examined with a flashlight at various times of the day and night. Because it was difficult to see clearly to the bottom of the drums, cyclopoids also were observed in 20-liter glass bottles filled with water to within 10 cm of the top. Each bottle contained several hundred adult cyclopoids of a single species and was kept outside in the shade with a black plastic sleeve around its sides so light entered only at the top. The sleeve was removed at various times to observe the distribution of cyclopoids in the bottle.

Results

Field Collections and Preliminary Screening

Metacyclops cushae Reid, *Microcyclops anceps* (Richard), *Microcyclops ceibaensis* (Marsh), *Microcyclops dubitabilis* Kiefer, *Neutrocyclops brevifurca* (Lowndes), *Paracyclops chiltoni* (Thompson), *Thermocyclops inversus* Kiefer, and *Thermocyclops tenuis* (Marsh) were collected, but they were considered too small to be tested as predators of *Ae. aegypti* larvae. *Acanthocyclops* sp. *vernalis* (Fischer) group, *Diacyclops* sp., *Ectocyclops rubescens* Brady, *Eucyclops agilis* (Koch), *Mesocyclops pescei* Petkovski, and *Mesocyclops reidae* Petkovski were large enough to be tested (body length, 1.0–1.2 mm). Although these species killed *Ae. aegypti* larvae, none killed more than five larvae per cyclopoid-day. *Macrocyclops albidus* sensu stricto, *Macrocyclops albidus principalis* Herbst, *Mesocyclops longisetus* sensu stricto, *Mesocyclops longisetus* var. *curvatus*, *Mesocyclops thermocyclopoides* sensu lato, and *Mesocyclops venezolanus*, all of which had body lengths >1.2 mm, consistently killed all 10 larvae available to them.

Laboratory Predation Experiments

Mesocyclops longisetus, *M. venezolanus*, *M. thermocyclopoides*, and *Macrocyclops albidus* all killed a large number of *Ae. aegypti* larvae in

the tissue culture plates (Table 2). Although *M. longisetus* killed the most, the average of 46.8 larvae killed in 24 h does not reflect its full capacity as a predator because all 50 available larvae were killed in many of the replicates.

The cyclopoids killed almost as many *Ae. aegypti* larvae in the relatively spacious environments of drums and tires as they did in the more confined culture plates (Table 2). Because there were always at least 50 larvae surviving at the end of the experiments, the estimates shown in Table 2 represent the capacity of each cyclopoid species to kill *Ae. aegypti* larvae in a drum or tire environment where predation was not limited by the supply of larvae. *M. longisetus* killed the most larvae, and *M. thermocyclopoides* and *M. albidus* killed nearly as many. *M. venezolanus* usually killed the least, but results were erratic.

Field Trials

A common initial reaction of the public to cyclopoids was disbelief that such a tiny animal could kill mosquito larvae. People usually became enthusiastic after seeing the cyclopoids seize and eat mosquito larvae in the demonstration we provided. None objected to having cyclopoids introduced to their water storage containers. Acceptance of cyclopoids by the public seemed to depend primarily on acceptance of the individuals who promoted their use for mosquito control.

Cyclopoid Survival in Containers. The cyclopoids seldom survived for more than a week in small water storage containers such as buckets, 40-liter plastic drums, and pots, which were filled and emptied frequently. Nor did the cyclopoids last long in laundry tanks (pilas) with drains at the bottom, unless the tank was not in active use.

200-Liter Drums. Every housewife who was issued a net to remove cyclopoids from drums before cleaning followed the procedure without trouble. However, after 4–5 mo, some housewives tired of the process and terminated their participation, whereas others continued enthusiastically for the duration of the study.

Mesocyclops longisetus survived for the 30-wk duration of the field trials in 100% of the drums not in active use and in 89% of the drums in active use that were cleaned using the net rescue procedure. Whether or not the drum was in active use, the *M. longisetus* population usually multiplied to >500 adults within 2–4 wk after introduction and maintained that number for the rest of the field trials (Table 3). Population numbers were lowest in drums with frequent water turnover and clean conditions.

M. thermocyclopoides and *M. venezolanus* lasted for the duration of the field trials in 79% of the drums not in active use, but only for a few weeks in drums from which water was dipped on a regular basis (Table 3). *Macrocyclops albidus* disappeared from all drums within 3 mo after introduction, whether the drum was in active use or not.

Tires. Mesocyclops longisetus survived in tires better than the other species (Table 3). The *M. longisetus* population in automobile tires was usually 200–500 adults, but numbers were lower when a tire contained very little water. *M. albidus* survived in 72% of the tires for the duration of the field trials but disappeared from other tires (exposed directly to the sun but not dried out) within a week of introduction. Survival of *M. thermocyclopoides* was also highly variable, and *M. venezolanus* never lasted >2 mo in automobile tires.

All species of cyclopoids were killed if a tire dried out completely, but they were not killed as long as a small amount of moisture remained in the tire. Few tires dried out during our field trials, because it was the rainy season.

Truck tires provided a more spacious aquatic habitat than automobile tires. They never dried out because they contained a larger volume of water. *M. longisetus, M. venezolanus,* and *Macrocyclops albidus* survived in all truck tires for the duration of the field trials, their numbers often exceeding 1,000 adults. (*Mesocyclops thermocyclopoides* was not tested in truck tires.)

Animal Drinking Containers. Mesocyclops longisetus seldom lasted more than a few weeks in animal drinking containers in active use because the cyclopoids were lost when the water was changed. *M. longisetus* survived for the duration of the study in 75% of the cement animal drinking containers not in active use, disappearing only when the water was severely fouled by trash.

Cement Tanks and Pools. Mesocyclops longisetus sustained populations of several thousand adults in unused laundry tanks, cisterns, and ornamental pools, but on four occasions they disappeared for no apparent reason. On three of those occasions, a large population of ostracods or cladocerans appeared at the same time *M. longisetus* disappeared.

Vases. Cyclopoids were lost from vases if the water was changed without using a net. When not lost this way, all four species lasted for >3 mo on average (Table 3). Each of the cyclopoid species was still present in 65–80% of its vases when the trials were terminated 20 wk after introduction. Long-term cyclopoid populations were usually <10 adults in vases with plant cuttings, particularly if roots and detritus were sparse.

Species Mixtures. Mixtures of the four cyclopoid species never lasted more than a month in 200-liter drums, tires, vases, or the cement pool. *Mesocyclops venezolanus, M. thermocyclopoides,* and *Macrocyclops albidus* always disap-

Table 3. Performance of the four cyclopoid species in field trials

Cyclopoid	Cyclopoid survival, wk[a]	No. cyclopoids[b]	No. instars III and IV ± SE[c]
	200-liter drums in active use (n = 24)[d]		
Mesocyclops longisetus	27	90–4,000	0.01 ± 0.01 (85)
Macrocyclops albidus	7	16– 900	0.7 ± 0.3 (11)
Mesocyclops thermocyclopoides	3	20– 700	12.6 ± 9.6 (13)
Mesocyclops venezolanus	3	2– 500	5.3 ± 3.2 (17)
Controls[e]	—	0	55.0 ± 17.1 (35)
	200-liter drums not in active use (n = 10)[d]		
Mesocyclops longisetus	30	200–3,000	0 (12)
Macrocyclops albidus	6	1– 300	0 (12)
Mesocyclops thermocyclopoides	30	500– 700	1.0 ± 0.8 (12)
Mesocyclops venezolanus	30	300–1,300	0.3 ± 0.3 (12)
Controls[e]	—	0	10.5 ± 6.3 (12)
	Tires (n = 37)[d,f]		
Mesocyclops longisetus	26	25–1,600	1.1 ± 0.5 (61)
Macrocyclops albidus	21	25–2,000	3.3 ± 1.6 (32)
Mesocyclops thermocyclopoides	9	15– 600	ND
Mesocyclops venezolanus	5	2–3,000	6.8 ± 4.0 (28)
Controls[e]	—	0	59.5 ± 10.5 (84)
	Vases with live plants (n = 26)[d]		
Mesocyclops longisetus	18	3–120	0 (44)
Macrocyclops albidus	16	1– 55	0.3 ± 0.2 (58)
Mesocyclops thermocyclopoides	14	2– 65	0.1 ± 0.1 (38)
Mesocyclops venezolanus	14	1– 45	0 (22)
Controls[e]	—	0	4.8 ± 2.1 (25)

[a] Mean number of weeks that cyclopoid populations survived after introduction to containers. Maximum time for drums and tires was 30 wk; maximum time for vases was 20 wk.
[b] Range of cyclopoid numbers in containers (adults and large copepodids).
[c] Mean number of third and fourth instars of *Ae. aegypti* in all drums, tires, and vases while cyclopoids were in the containers (number of inspections in parentheses). Inspections during the first 2 wk after cyclopoid introduction are not included because cyclopoid and larval populations were not yet stabilized.
[d] n = number of containers.
[e] No cyclopoids introduced.
[f] All tires (automobile and truck).

peared, leaving *Mesocyclops longisetus* in the container; or all four species disappeared.

Effect of Cyclopoids on *Ae. aegypti* Larvae. The percentage of control containers (without cyclopoids) positive for first- and second-instar *Ae. aegypti* larvae is an indicator of the recruitment rate of larvae into the containers. During the field trials, 38% of the inspections of control containers of all kinds (n = 322) were positive for first and second instars.

Because it is conceivable for newly hatched larvae to remain in a container for a day or more before growing too large for cyclopoid predation, first and second instars are to be expected in some containers even when cyclopoid predation is completely effective. Seventeen percent of the inspections of containers with cyclopoids were positive for first and second instars (n = 468). Most of the first and second instars were in containers with <10 cyclopoids. Larval survival in containers with larger numbers of cyclopoids appeared to be so brief that first and second instars were seldom observed.

Third and fourth instars of *Ae. aegypti* were particularly useful indicators of cyclopoid effectiveness, because these larvae escaped cyclopoid predation. Thirty-one percent of the inspections of all control containers were positive for third and fourth instars, whereas only 6% of the containers with cyclopoids were positive.

Cyclopoids reduced the numbers of third and fourth instars even more than they reduced the number of positive containers (Table 3). *Mesocyclops longisetus* performed best, because it maintained large numbers in containers more consistently than the other cyclopoid species. The number of third and fourth instars of *Ae. aegypti* observed in 200-liter drums with *M. longisetus* was less than a thousandth the number in control drums. The number of third and fourth instars in tires with *M. longisetus* was 98% less than in control tires. The few third and fourth instars observed in tires with *M. longisetus* were associated with exceptionally low populations of the cyclopoid after the tires had nearly dried out. No third and fourth instars were observed in vases with *M. longisetus*.

Macrocyclops albidus reduced third and fourth instars of *Ae. aegypti* in drums by 99% and in tires by 93% compared with controls (Table 3). The performance of *Mesocyclops venezolanus* and *M. thermocyclopoides* in drums and tires was substantially poorer than the other tested species, because their populations often dropped

Table 4. Mean number (±SE) of third and fourth instars of *Ae. aegypti* larvae in 200-liter drums and tires during period immediately following introduction of *Mesocyclops longisetus*

	Weeks after cyclopoid introduction				Sample size[a]
	1	2	3	4	
Drums					
BTI not applied	27.0 ± 15.1	1.0 ± 1.0	0.2 ± 0.2	0	6
BTI applied[b]	0	0	0	0	7
Tires					
BTI not applied	54.1 ± 8.6	1.6 ± 1.6	0.6 ± 0.6	0	7
BTI applied[b]	0	0	0	0	7

[a] Number of drums or tires monitored.
[b] *BTI* was applied once (concurrently with *Mesocyclops* introduction).

to low numbers that did not eat all *Ae. aegypti* larvae. One or two third and fourth instars were seen occasionally in vases containing *Macrocyclops albidus* or *Mesocyclops thermocyclopoides* if there were fewer than five adults of these species in the vase.

It took up to 4 wk for third and fourth instars of *Ae. aegypti* to disappear completely from drums and tires after introducing *Mesocyclops longisetus* without applying BTI (Table 4). When Vectobac was applied concurrently with the introduction of *M. longisetus,* all mosquito larvae were dead within a day, and very few *Ae. aegypti* larvae were observed thereafter. BTI had no negative effect on the cyclopoids.

Thirty-six percent of the inspections of cement animal drinking containers, cisterns, or ornamental pools without cyclopoids were positive for third and fourth instars of *Ae. aegypti* (average number of third and fourth instars, 105 ± 32 [SE]; n = 33). Third and fourth instars were present in 7% of the inspections of these containers that had *M. longisetus,* and the numbers of larvae were low (average number of third and fourth instars, 2.1 ± 1.3; n = 57).

Culex larvae of all instars were observed frequently with all cyclopoid species in all containers used in the field trials.

Field Predation Experiments

In the experiments in which 1,000 *Ae. aegypti* eggs were placed in 200-liter drums that contained long-term populations of *Mesocyclops longisetus,* all *Ae. aegypti* larvae disappeared from all five drums. From the 1,000 eggs placed in the control drum, 860 larvae hatched and 248 larvae were still present after 5 d.

When 500 *Ae. aegypti* eggs were placed in each of five tires with long-term populations of *M. longisetus,* four tires had no surviving larvae after 5 d. The other tire (a small tire with a dense growth of algae and an unusually small population of *M. longisetus*) had 32 surviving larvae. In the control tire, 199 larvae survived.

When 20 newly hatched *Ae. aegypti* larvae were placed in plant vases 3 mo after cyclopoid introduction, *M. longisetus* always killed all the larvae. An average of 85 ± 14% of the larvae died with *M. venezolanus,* and 79 ± 5% of the larvae died with *Macrocyclops albidus.* During the 5 d of the experiment, 33 ± 8% of the larvae died in control vases without cyclopoids.

Cyclopoid Distribution within Containers

Mesocyclops thermocyclopoides and *M. venezolanus* almost always were swimming in the water column of 200-liter drums and 20-liter bottles. They were somewhat more concentrated toward the bottom. Less than 5% of these two species rested on the bottom or sides of the container.

Few *Mesocyclops longisetus* or *Macrocyclops albidus* were seen in the water column of drums. When observed in 20-liter bottles, 80–95% of the *M. longisetus* and *M. albidus* were within 8 cm of the bottom, some swimming and others on the bottom or sides. Almost all the *M. longisetus* and *M. albidus* that were not within 8 cm of the bottom of a bottle were clinging to the sides.

When the water in a bottle was agitated (as might happen when water is dipped out of a container for use), *M. longisetus* continued to cling to the sides or remained close to the side if loosened by the current. All four species were sometimes seen feeding at the water surface. If *M. longisetus* was at the surface, it moved to the side of the bottle when the water was agitated. The other species remained in the middle.

Discussion

All four species of cyclopoids in this study were voracious predators of *Ae. aegypti* larvae. The largest species (*Mesocyclops longisetus*) (adult female body length, 1.5 mm) killed the most larvae per cyclopoid per day, and the smallest species (*M. thermocyclopoides* and *M. venezolanus*) (adult body length, 1.2 mm) killed the fewest larvae. Kay et al. (1992) observed that *M. longisetus* killed more *Ae. aegypti, Anopheles farauti* Laveran, and *Culex quinquefasciatus* Say larvae than *M. aspericornis* (a slightly smaller species) under laboratory conditions in 2-liter beakers.

However, the effectiveness of each cyclopoid species in the field trials depended primarily on its ability to survive and maintain large numbers in a container. *M. longisetus* was clearly best in this regard, surviving well in all tested containers except laundry tanks, buckets, 40-liter plastic drums, and pots. *M. longisetus* usually remained in a container for the duration of the field trials unless it was dumped or dried out, although it sometimes disappeared from vases or cement

tanks (cisterns and animal drinking containers) when not discarded.

There are several explanations for the relatively poor survival of *Macrocyclops albidus*, *Mesocyclops thermocyclopoides*, and *M. venezolanus*. First, the distribution of *M. thermocyclopoides* and *M. venezolanus* throughout the water column exposed them to substantial losses when water was dipped from a storage container. Fewer *M. longisetus* or *M. albidus* were lost because most individuals of these species remained at the bottom or sides of a container when water was dipped out.

Second, overpopulation of containers may be a problem for *M. venezolanus*, and possibly for *M. thermocyclopoides*, which often disappeared after producing a large number of juveniles that may have eliminated their food supply. In contrast, *M. longisetus* and *M. albidus* populations consisted almost entirely of adults. The latter two species may prevent overpopulation by cannibalizing their own nauplii (G.G.M., unpublished data).

Third, intolerance to high temperatures may explain the low survival of *M. albidus*. Water temperatures in containers exposed to the sun in El Progreso may exceed 40°C. The maximum temperature that *M. albidus* can survive is 37–38°C compared with 42–43°C for the three species of *Mesocyclops* (G.G.M., unpublished data).

BTI should be a routine adjunct to cyclopoid introduction whenever there are mosquito larvae in a container at the time of introduction. Control of *Ae. aegypti* is immediate and long term when *M. longisetus* and BTI are applied together.

Mesocyclops longisetus is the species of choice for *Ae. aegypti* control, but the field trials demonstrated that a control program will not succeed if the cyclopoids are simply introduced to containers and forgotten. The effectiveness of *M. longisetus* for *Ae. aegypti* control will require community participation to maintain container conditions essential for its survival. For example, *M. longisetus* will last longer in tires that are shaded so they cannot dry out readily. If all the water in a drum is needed for use, a small amount should be left at the bottom to sustain the cyclopoids.

Community participation also is needed when containers are cleaned. Lardeux (1992) observed that *M. aspericornis* disappeared from 200-liter drums that were cleaned periodically. The net rescue procedure that we used when drums were cleaned was effective but required demonstration of the technique and substantial motivation on the part of housewives to continue the effort. Maintaining *M. longisetus* in drums takes an effort, but a selling point is that *M. longisetus* requires less effort than most other *Ae. aegypti* source reduction methods. *M. longisetus* eliminates *Ae. aegypti* larvae no matter how infrequently or incompletely drums are cleaned.

Finally, despite precautions, *M. longisetus* will sometimes disappear from containers, making reintroduction necessary. Although cyclopoids are small, they can be seen with the naked eye, and people need to make a point of observing them so they know when they are lost. When reintroduction is necessary, it can be done simply by transferring water containing *M. longisetus* from a nearby treated container.

Acknowledgments

Special thanks are extended to the following persons for their support and collaboration: Scott Halstead (Rockefeller Foundation); Andrew Arata (USAID Vector Biology and Control Project); José Gómez, Enrique Gil Ballorín, and Ramón Soto (Division of Vector-Borne Diseases, Honduran Ministry of Public Health); Carl Kendall and Elli Leontsini (Johns Hopkins University); Duane Gubler (Centers for Disease Control); Barnett Cline (Tulane University). Raúl Alvarado, Camila Galeas, Consuelo Gutiérrez, Reyna Lozano, Héctor Portillo, and Rosana Reyes (Integrated Dengue Control Project) participated in the field and laboratory work. The New Orleans Mosquito Control Board provided staff and facilities for experimental work in New Orleans. Edgar Bordes, Michael Carroll, Mieu Nguyan, and Giai Ngo (New Orleans Mosquito Control Board) contributed to the study. Funding for this study was provided by the Rockefeller Foundation (Health Sciences Division) through the Pan American Health Organization and the International Center for Community-Based Health Research (Johns Hopkins University); and by USAID's Vector Biology and Control Project through a contract with Tulane University.

References Cited

Fernández, E., I. Lagos, H. Portillo & G. Borjas. 1992. Community-based *Aedes aegypti* control programme in Honduras, pp. 279–282. *In* S. B. Halstead & H. Gomez-Dantes [eds.], Dengue—a worldwide problem, a common strategy. International Conference on Dengue and *Aedes aegypti* Community-based Control. Mexican Ministry of Health and Rockefeller Foundation, Mexico.

Kay, B. H., C. P. Cabral, A. C. Sleigh, M. D. Brown, Z. M. Ribeiro & A. W. Vasconcelos. 1992. Laboratory evaluation of Brazilian *Mesocyclops* (Copepoda: Cyclopidae) for mosquito control. J. Med. Entomol. 29: 599–602.

Lardeux, F.J.R. 1992. Biological control of Culicidae with the copepod *Mesocyclops aspericornis* and larvivorous fish (Poeciliidae) in a village of French Polynesia. Med. Vet. Entomol. 6: 9–15.

Marten, G. G. 1984. Impact of the copepod *Mesocyclops leuckarti pilosa* and the green alga *Kirchneriella irregularis* upon larval *Aedes albopictus* (Diptera: Culicidae). Bull. Soc. Vector Ecol. 9: 1–5.

1990a. Evaluation of cyclopoid copepods for *Aedes albopictus* control in tires. J. Am. Mosq. Control Assoc. 6: 681–688.

1990b. Elimination of *Aedes albopictus* from tire piles by introducing *Macrocyclops albidus* (Copepoda, Cyclopidae). J. Am. Mosq. Control Assoc. 6: 689–693.

1990c. Issues in the development of cyclops for mosquito control. Arbovirus Research in Australia 5: 159–164.

Marten, G. G., W. Che & E. S. Bordes. 1993. Compatibility of cyclopoid copepods with mosquito insecticides. J. Am. Mosq. Control Assoc. 9: 150–154.

Rivière, F. & R. Thirel. 1981. La prédation du copépode *Mesocyclops leuckarti pilosa* (Crustacea) sur les larves de *Aedes (Stegomyia) aegypti* et *Ae. (St.) polynesiensis* (Diptera: Culicidae): essais préliminaries d'utilisation comme agent de lutte biologique. Entomophaga 26: 427–439.

Rivière, F., Y. Séchan & B. H. Kay. 1987a. The evaluation of predators for mosquito control in French Polynesia. Arbovirus Research in Australia 4: 150–154.

Rivière, F., B. H. Kay, J. M. Klein & Y. Séchan. 1987b. *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. 24: 425–430.

Suárez, M. F., D. Ayala, M. J. Nelson & J. W. Reid. 1984. Hallazgo de *Mesocyclops aspericornis* (Daday) (Copepoda: Cyclopoidae) depredador de larvas de *Aedes aegypti* en Anapoima-Colombia. Biomédica 4: 74–76.

Suárez, M. F., G. G. Marten & G. C. Clark. 1992. A simple method for cultivating freshwater copepods used in biological control of *Ae. aegypti.* J. Am. Mosq. Control Assoc. 8: 409–412.

Received for publication 11 September 1992; accepted 6 July 1993.