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Honduras: Use of Cyclopoids for *Aedes aegypti* Control in El Progreso

October 1990 - December 1992

by

Gerald G. Marten, Ph.D.

VBC Report No. 81329

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Executive Summary

This study was part of the Honduran Ministry of Public Health's Integrated Dengue Control Project in El Progreso, Honduras. The objective was to evaluate cyclopoid copepods (cyclopoids), which are tiny crustaceans that prey on first and second instar mosquito larvae. The study supplemented conventional methods of *Aedes aegypti* source reduction in the Project. The study began in October, 1990, and continued to December, 1992.

Eighteen species of cyclopoids were collected from aquatic habitats in the El Progreso area and placed in laboratory dishes with first instar *Ae. aegypti* larvae to see if they killed the larvae. The four largest species (*Mesocyclops longisetus*, *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus*, and *Macrocyclops albidus*) killed the most larvae and were selected to be evaluated for *Ae. aegypti* control. Laboratory colonies of the four species were established to provide animals for laboratory experiments and field trials. Methods were developed for mass production of these animals in small artificial ponds at the laboratory.

The capacity of each of the four cyclopoids species to kill first instar *Ae. aegypti* larvae was evaluated in tires and 55-gallon drums at the laboratory. All four species killed approximately 20 larvae per day. *Mesocyclops longisetus* consistently killed the most larvae.

Each of the four cyclopoid species was introduced to laundry tanks (*pilas*), 55-gallon metal drums, tires, animal drinking containers, cisterns, ornamental pools, and vases at people's homes and monitored for 30 weeks. *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus*, and *Macrocyclops albidus* did not survive in the containers for more than a few months, but *Mesocyclops longisetus* maintained large numbers in all containers as long as it was not dumped out or dried out. *Mesocyclops longisetus* was able to survive in drums and other water storage containers that are cleaned periodically only if it was removed from the container with a net before cleaning and returned afterwards.

Mesocyclops longisetus was found to be effective for *Ae. aegypti* control in 55-gallon drums, tires, cement animal drinking containers, cisterns, ornamental pools, and plant vases. As long as *Mesocyclops longisetus* was in these containers, *Ae. aegypti* production was 98%-99% less on average than in control containers without cyclopoids.

Mesocyclops longisetus was selected for introduction to all appropriate containers in a barrio of approximately 160 houses. Distribution of the cyclopoids to the community and introduction to containers was easy, but *Mesocyclops longisetus* lasted only in containers such as cisterns and tires that were never cleaned and therefore required no special effort for maintaining the cyclopoids. Because the Project team had many activities in addition to *Mesocyclops longisetus*, it did not devote sufficient attention to instructing people on preventing the loss of *Mesocyclops longisetus* when they cleaned containers such as drums and vases, and *Mesocyclops longisetus* disappeared from those containers within a few months. Newly organized, the barrio lacked the active participation of community volunteers necessary to maintain a comprehensive array of *Ae. aegypti* control activities. *Mesocyclops longisetus* did not receive much attention from the volunteers, and *Mesocyclops longisetus* usually was not replaced when lost from drums or vases.

The Integrated Dengue Control Project plans to employ *Mesocyclops longisetus* in other barrios where public motivation, community organization, and active participation by community volunteers are sufficiently developed to ensure that the necessary attention will be devoted to maintaining cyclopoids in containers after introduction.

1. Introduction

In 1989, the Ministry of Public Health of the Republic of Honduras initiated the Integrated Dengue Control Project in El Progreso, a city of approximately 100,000 inhabitants. The main objective of the Project, which receives financial support from the Rockefeller Foundation, is *Ae. aegypti* source reduction through community participation (Fernández et al. 1992). The Project's public education team organizes a group of volunteers in each barrio, who in turn explain to their neighbors the significance of dengue fever, *Ae. aegypti*, and the containers in which *Ae. aegypti* breeds. They organize neighborhood campaigns to (a) get rid of discarded tires and containers that collect rainwater and (b) clean water storage containers frequently enough to prevent *Ae. aegypti* larvae from completing their development.

In 1990, the Project's director (Dr. Eduardo Fernández) invited the author to investigate the use of biological control as a supplement to other *Ae. aegypti* control methods in the Project. The objective was to evaluate how cyclopoid copepods (cyclopoids) could increase the effectiveness of the Project by eliminating *Ae. aegypti* production in containers for which existing methods were not functioning adequately. Cyclopoids are tiny crustaceans that prey on first and second instar mosquito larvae. They had already proved effective at eliminating *Aedes* larvae from tires in New Orleans (Marten 1990a), and they offered considerable promise for other kinds of *Ae. aegypti* breeding containers as well (Marten 1990b).

Cyclopoids were considered to be of possible use for all the above containers except "pots and buckets" and "miscellaneous." Aquatic animals like cyclopoids cannot survive for long in most pots or buckets because they are in such heavy use. Nor do cyclopoids last in miscellaneous small containers that dry out frequently.

The following containers are responsible for *Ae. aegypti* production in El Progresso:

Table 1
Percentage of *Ae. aegypti* Production

	Percentage
Laundry tanks (pilas)	44
Used tires	29
55-gallon drums	12
Animal drinking containers	7
"Pots and buckets"[1]	3
Cement tanks and pools[2]	2
Live plant vases	1
Miscellaneous[3]	2

[1] More than one gallon capacity.

[2] On or in the ground, no drain.

[3] Discarded tin cans, bottles, car batteries, toilets, pipes, and scrap iron; wheel barrels, tree holes, and coconut husks.

2. Study Procedure

The study followed the steps recommended by the World Health Organization for screening and evaluating biological agents for control of disease vectors (WHO 1992):

i. Search and laboratory operations
ii. Experimental laboratory operations
iii. Preliminary field trials
iv. Pilot production
v. Large-scale field trials

Phase I: Search and laboratory operations

Cyclopoids were collected from several hundred aquatic sites in the vicinity of El Progreso to obtain as many species as possible. Assistance with species identification was provided by a specialist in copepod taxonomy (Janet Reid, Smithsonian Institution, Washington, DC). Each species was tested by placing it in a laboratory dish with newly-hatched *Ae. aegypti* larvae to see if it killed them. For species that killed a large number of larvae, laboratory colonies were established to supply animals for the laboratory experiments and field trials in subsequent phases of the study. The colonies were maintained in 5-gallon plastic tubs using culture procedures similar to those described by Suárez et al. (1992).

Phase II: Experimental laboratory operations

Four species of cyclopoids (*Mesocyclops longisetus*, *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus*, *Macrocyclops albidus*) were selected for quantitative laboratory experiments to evaluate their effectiveness as predators of *Ae. aegypti* larvae. The experiments were conducted in tires and 55-gallon drums to obtain quantitative measures of cyclopoids predation in actual environments where they would be employed for *Ae. aegypti* control.

Phase III: Preliminary field trials

Mesocyclops longisetus, *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus*, and *Macrocyclops albidus* were introduced, one species at a time, to 29 laundry tanks, 34 55-gallon metal drums, 37 tires, 12 animal drinking containers, four cisterns, two ornamental pools, and 26 plant vases at people's homes. A net was given to many of the households with drums so they could remove most of the cyclopoids from their drum before cleaning and return them to the drum afterwards. The purpose was to avoid losing the cyclopoids when cleaning the drums. People were given help the first two times they cleaned their drums after the cyclopoids were introduced.

The containers were monitored for 30 weeks to observe (a) how long each species of cyclopoids lasted in each kind of container, and (b) the impact that each species had on *Ae. aegypti* larvae.

Phase IV: Pilot production

The key to mass producing cyclopoids is production of their food supply. Experiments were conducted with many different kinds of live and prepared foods. A scaled-up version of the culture system that we used for laboratory colonies was tested as well as small outdoor ponds in the yard behind the laboratory. Various construction materials were experimented with for the ponds and with different methods for storing, transporting, and shipping cyclopoids.

Phase V: Large-scale field trials

Mesocyclops longisetus was selected for large-scale field trials. It was introduced into all 55-gallon drums, tires, and other appropriate containers in Pénjamo barrio, which consists of approximately 160 houses.

The Project's biocontrol team began work in the barrio by coordinating its efforts with other activities of the Ministry of Public Health. A talk on dengue fever, *Ae. aegypti*, and the use of cyclopoids for *Ae. aegypti* control was presented to interested members of the community at the same time they came to a neighborhood clinic for children's immunizations and a talk on malaria and cholera prevention. The biocontrol

team then visited every home in the barrio, explained the use of cyclopoids to all households with appropriate containers, and introduced *Mesocyclops longisetus* to all those containers. They gave a net to people with drums and explained how to use it to avoid losing the cyclopoids when cleaning drums.

Several volunteers in the barrio were recruited to help ensure that people in the barrio did what was necessary to keep *Mesocyclops longisetus* in the drums and tires. One of the volunteers kept a supply of *Mesocyclops longisetus* at her home as a source of supply for persons who needed more. The Project's biocontrol team visited the homes periodically to confirm whether *Mesocyclops longisetus* was still in the drums and tires.

3. Findings

Phase I: Search and laboratory operations

The following species of cyclopoids were collected: *Metacyclops cushae*, *Microcyclops anceps*, *Microcyclops ceibaensis*, *Microcyclops dubitabilis*, *Neutrocyclops brevifurca*, *Paracyclops chiltoni*, *Thermocyclops inversus*, *Thermocyclops tenuis*, *Acanthocyclops* sp. *vernalis* group, *Diacyclops* sp., *Ectocyclops rubescens*, *Eucyclops agilis*, *Mesocyclops pescei*, *Mesocyclops reidae*, *Macrocyclops albidus* sensu stricto, *Macrocyclops albidus principalis*, *Mesocyclops longisetus* sensu stricto, *Mesocyclops longisetus* var. *curvatus*, *Mesocyclops thermocyclopoides* sensu lato, and *Mesocyclops venezolanus*.

Species less than one millimeter in body length (adult females, measured to the end of the caudal setae) were too small to prey on *Ae. aegypti* larvae. Species 1.0-1.2 mm in body length occasionally killed larvae, but only the species 1.2 mm or more in length killed large numbers. Those species were *Mesocyclops longisetus* (body length = 1.5 mm), *Macrocyclops albidus* (body length = 1.4 mm), *Mesocyclops venezolanus* (body length = 1.2 mm), and *Mesocyclops thermocyclopoides* (body length = 1.2 mm).

Phase II: Experimental laboratory operations

The following table presents mean numbers (± standard error) of *Ae. aegypti* larvae that died during 24-hour experiments in tires and 55-gallon drums with cyclopoids.

Table 2
Larval Mortality per cyclopoids

	Tires	Drums
Mesocyclops longisetus	36.5 ± 4.1	36.8 ± 1.7
Mesocyclops thermocyclopoides	35.1 ± 4.6	31.6 ± 0.4
Macrocyclops albidus	33.4 ± 3.6	31.1 ± 3.9
Mesocyclops venezolanus	20.9 ± 6.3	36.4 ± 11.9
Controls	14.0 ± 3.1	18.8 ± 6.2

The table shows that all four species of cyclopoids kill large numbers of *Ae. aegypti* larvae, but *Mesocyclops longisetus* kills the most.

Phase III: Preliminary field trials

No one objected to having cyclopoids introduced to their water storage containers. People are basically indifferent to these tiny animals. Acceptance of cyclopoids by the public seems to depend primarily on acceptance of the individuals who promoted their use for mosquito control.

None of the four species of cyclopoids in the preliminary field trials survived for long in laundry tanks because these tanks have a drain and the cyclopoids soon disappeared down the drain. *Mesocyclops longisetus* was the species that survived best in all other containers. *Mesocyclops longisetus* maintained a population of at least several hundred adults in tires as long as they did not dry out. It survived in large numbers in cisterns, ornamental ponds, and cement animal drinking containers. *Mesocyclops longisetus* survived in vases used to hold live plants, but there were often less than ten adults in a vase.

Fifty-five gallon drums were particularly important because they were so common. *Mesocyclops longisetus* numbered 500-2,000 adults in drums, and they stayed in the drums as long as they were not dumped out.

Housewives learned the procedure for rescuing cyclopoids with a net when cleaning the drum and applied it successfully without additional assistance from the team. Some women grew tired of the procedure after four or five months, while others are still following the procedure. They still have *Mesocyclops longisetus* in their drums, two years after *Mesocyclops longisetus* was introduced.

If the large number of *Mesocyclops longisetus* in most containers is multiplied by the equally large number of *Ae. aegypti* larvae that individual cyclopoids can eat in a day, the capacity to kill *Ae. aegypti* larvae is enormous. It is, therefore, no surprise that very few III/IV instar *Ae. aegypti* larvae or pupae were observed in containers with *Mesocyclops longisetus*. The following table presents the mean number of III/IV instar *Ae. aegypti* larvae observed in tires and 55-gallon drums during weekly inspections after introducing cyclopoids.

Table 3
Number of Larvae

	Tires	Drums
Mesocyclops longisetus	1.1 ± 0.5	0.01 ± 0.01
Macrocyclops albidus	3.3 ± 1.6	0.7 ± 0.3
Mesocyclops venezolanus	6.8 ± 4.0	5.3 ± 3.2
Mesocyclops thermocyclopoides	No data	12.6 ± 9.6
Controls (no cyclopoids)	59.5 ± 10.5	55.0 ± 17.1

The table shows that *Mesocyclops longisetus* reduced III/IV instar *Ae. aegypti* in 55-gallon drums by 99.9%, and it reduced the larvae in tires by 98%. The few III/IV instar larvae observed in containers with *Mesocyclops longisetus* were always at times when the *Mesocyclops longisetus* population was particularly low for some reason (e.g., a tire nearly drying out).

Phase IV: Pilot production

The best production system for *Mesocyclops longisetus* is an indoor system that operates at temperatures of 20-28° C. Two species of protozoa (*Chilomonas* sp. and *Paramecium caudatum*) serve as food for the cyclopoids, and the protozoa obtain their nutrition from rotting wheat seed. These protozoa however did not grow so fast under the hotter conditions that prevailed in the laboratory at El Progreso (up to 35° C), and no other production system was found that worked well under these conditions.

A system for producing cyclopoids in outdoor ponds was developed and it works well. The system includes the protozoa mentioned above, but it also includes a rotifer (*Pilodina* sp.), and fertilizer is applied to stimulate the growth of phytoplankton. The ponds are 6 feet x 3 feet x 8 inches and constructed with an inexpensive wood frame and plastic liner. It is essential to cover the ponds with window screen to prevent invasion by other aquatic animals.

It is desirable to have a way to store *Mesocyclops longisetus* if they are not needed right away. It is, however, difficult to store large concentrations of *Mesocyclops longisetus* in water because of their large appetite and the fact that they eat one another if they run out of food. Refrigeration was tried to slow their metabolism, but it did not work because *Mesocyclops longisetus* does not tolerate low temperatures. A system for storing cyclopoids on top of damp sponges in sealed plastic containers. They can be stored on the sponges at high densities for months and can also be transported or shipped on sponges.

Phase V: Large-scale field trials

Introduction of *Mesocyclops longisetus* to tires, 55-gallon drums, and other containers was accomplished quickly and easily. The cyclopoids were effective for controlling *Ae. aegypti* larvae in tires and cisterns where no special care was required for their survival. Because *Mesocyclops longisetus* was introduced to tires at the beginning of the rainy season (May), most of the tires contained water continuously until the end of the year.

The performance of *Mesocyclops longisetus* in vases and 55-gallon drums was not so good. It disappeared from most of these containers within two months, apparently because it was lost when the containers were cleaned. People did not "rescue" the cyclopoids properly when they cleaned their containers, and they did not request more *Mesocyclops longisetus* to replace their cyclopoids when they lost them. There appear to be several reasons why this happened:

1. *Mesocyclops longisetus* was introduced to the community (and their containers) within weeks after initiating the organizing effort in the barrio. Neither community volunteers nor the public had prior experience with *Ae. aegypti* source reduction.

2. The Project team took *Mesocyclops longisetus* directly to people's homes without including community volunteers as equals in the process.

3. Although each housewife was given an explanation of what she should do to maintain the cyclopoids in her drum, the Project team had many responsibilities in addition to cyclopoids and did not find the time to follow up with the housewives to make sure they were implementing the maintenance procedures correctly. Nor did the community volunteers follow through to make sure their neighbors maintained *Mesocyclops longisetus* in the drums.

4. Conclusions

1. *Mesocyclops longisetus* is effective at controlling *Ae. aegypti* larvae in most kinds of containers responsible for *Ae. aegypti* production in El Progreso (except pilas).

2. Introduction of *Mesocyclops longisetus* to containers is relatively easy, but maintaining them in the containers is more complicated.

3. To keep *Mesocyclops longisetus* in 55-gallon drums, it is necessary to remove the cyclopoids from the drums before cleaning so they will not be lost when the drums are cleaned.

4. The maintenance procedures for *Mesocyclops longisetus* are simple enough for housewives to learn quickly, but it is a challenge to communicate the necessary information to housewives on a large scale when the community organizing staff has numerous other responsibilities and tasks besides cyclopoids.

5. *Mesocyclops longisetus* will be lost from containers on occasion, even when proper maintenance procedures are followed. It is important to have a convenient way for people to replace *Mesocyclops longisetus* whenever they are lost.

6. *Mesocyclops longisetus* will survive in containers over the long term only where health volunteers and the general public are motivated to make the necessary effort.

5. Recommendations

The Integrated Dengue Control Project plans to use *Mesocyclops longisetus* in additional barrios of El Progreso during 1993, taking advantage of the lessons learned so far. Future use of *Mesocyclops longisetus* will incorporate the following recommendations:

1. *Mesocyclops longisetus* should be introduced to a community no less than six months after the community has been organized for *Aedes* control. By this time, community volunteers and the public have enough experience with the community participation process and *Ae. aegypti* source reduction to be able to (1) appreciate which households and containers will best be served by *Mesocyclops longisetus*; and (2) absorb the technical requisites for distributing *Mesocyclops longisetus* to appropriate containers and maintaining them there.

2. *Mesocyclops* longisetus should be deployed only in barrios whose health committees are strongly committed to improving *Mesocyclops longisetus* source reduction. After Project staff informs a barrio health committee about *Mesocyclops longisetus*, the cyclopoids should be introduced to containers only if the committee explicitly requests their use. Likewise, individual households should receive *Mesocyclops longisetus* only after requesting them.

3. Information to the public about *Mesocyclops longisetus* should emphasize the labor-saving advantages of using cyclopoids compared to other *Ae. aegypti* source reduction methods such as frequent cleaning of water storage containers.

4. Community volunteers in a barrio should employ *Mesocyclops longisetus* at their own homes for at least a month to gain experience before distribution of *Mesocyclops longisetus* to the rest of the barrio.

5. At the same time households receive *Mesocyclops longisetus*, they should receive simple written materials explaining how *Mesocyclops longisetus* functions and how it should be maintained in their container. (Appendix D specifies the information that the public needs to deploy *Mesocyclops longisetus* successfully for *Ae. aegypti* control.)

6. When *Mesocyclops longisetus* is used in 55-gallon drums, a net should be given to each housewife to rescue the *Mesocyclops longisetus* population before cleaning the drum. Project staff or community volunteers should work with the housewife on the cleaning and rescue process on at least two occasions after *Mesocyclops longisetus* introduction to make sure she is doing it properly.

7. Instructions on maintaining *Mesocyclops longisetus* in containers should urge people to make a point of "seeing" their cyclopoids. These tiny animals are visible to the naked eye despite their small size. It is important for people to check their containers periodically to make sure the cyclopoids are still there, so *Mesocyclops longisetus* can be reintroduced if lost.

8. All people who use *Mesocyclops longisetus* should understand that they can request replacement animals without embarrassment if they lose the *Mesocyclops longisetus* from their container. The procedure for obtaining additional *Mesocyclops longisetus* should be simple and easy so people do not hesitate to do it.

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Appendix A

Pilot Project On The Operational Use Of Cyclopoids For *Aedes Aegypti* Control in El Progreso, Honduras

Summary Report For The First Year (1991)

The overall goal for the first year of the project was to lay all the groundwork necessary for operational deployment of cyclopoids in Progreso during the second year. We had to determine which kinds of *Aedes aegypti* breeding containers can be treated effectively with cyclopoids, which local species of cyclopoids are best to use, and how it should be done. Our standard for effectiveness was complete elimination of all *Ae. aegypti* larvae from treated containers. Less than total elimination was unacceptable because it could merely be thinning overcrowded and food-limited larval populations without significantly reducing the production of adult mosquitoes.

Our starting point was the containers that we must deal with. The most important kinds of containers for *Ae. aegypti* production in Progreso are:

(1) cement laundry tanks (pilas) used to store water for washing clothes;

(2) used tires that collect water (often serving a function in the yard around the home);

(3) 50-gallon metal drums used to store water for household cleaning;

(4) containers such as buckets, pots, and basins, which have a capacity greater than one gallon;

(5) animal water supplies (typically constructed of cement); and

(6) vases for live plants.

The staff of the Integrated Dengue Control Project conducted a container survey covering approximately 500 houses in Progreso during September-October, the peak season for *Ae. aegypti*. The survey included a count of all *Ae. aegypti* pupae, the purpose being to use pupae as an indicator of adult mosquito production. Table 1 summarizes the distribution of pupae among the different kinds of containers. (The importance of laundry tanks may be exaggerated by the percentage of pupae in tanks in Table 1 because many of the pupae in laundry tanks appeared to be inviable.) Car batteries, toilets, pipes, wheel barrels, scrap iron, wells, tree holes, coconut husks, and all other containers not listed in Table 1 accounted for less than 2% of all pupae in the survey.

Table 1
Percentage of *Ae. aegypti* pupae in different kinds of containers in Progreso during September-October 1991*

	Percentage of pupae**
Laundry tanks (pilas)	44%
Used tires	29%
50-gallon drums	12%
"Buckets, pots, basins"***	8%
Animal water	3%
Vases for live plants	2%

* Based on a survey conducted by Enrique Gil, Gerardo Borjas, Eduardo Fernandez, and the staff of the Integrated Dengue Control Project.

** Percentage of all *Ae. aegypti* pupae encountered in the survey.

*** Containers one gallon or more in capacity.

The precise order of the containers in Table 1 should not be taken as an absolute indicator of their relative importance for a control program. While laundry tanks are at the top of the list in El Progreso, 50-gallon drums were the most numerous *Ae. aegypti* breeding containers in a survey conducted by the Ministry of Health in nearby Santa Rita, the control community for the dengue control project in Progreso. Each of the six kinds of containers in Table 1 produces a large quantity of adult *Ae. aegypti*, and each must be the object of a serious source reduction effort for *Ae. aegypti* control to be effective.

During 1991 we conducted comprehensive tests of cyclopoids in five of the six kinds of containers listed in Table 1, all but "buckets, tubs, and basins". Because buckets, tubs, and basins are in heavy use almost all the time, the turnover of water in these containers is so frequent it would not be feasible to maintain cyclopoids in them.

There were four specific objectives for the first year of the project. The first year's results are summarized below in terms of each of those objectives.

Objective 1: Identification of larvivorous species of cyclopoids

Field collections were made at several hundred sites in the vicinity of Progreso, and all cyclopoids were identified by a specialist at the Smithsonian Institution (Dr. Janet Reid). All species large enough to prey on mosquito larvae were tested in the laboratory, following a procedure that was particularly successful when we previously screened cyclopoids for *Ae. aegypti* and *Ae. albopictus* control in New Orleans. (This test was particularly informative because the relative scores of our New Orleans cyclopoids in the test were identical to their performance in field trials in New Orleans.) The procedure was to place an adult female cyclopoid in a small dish with 50 newly-hatched *Ae. aegypti* larvae. The number of surviving larvae was counted at the end of 24 hours.

Table 2
Predation on first instar *Ae. aegypti* larvae in small laboratory containers

	Larvae Killed*
Mesocyclops longisetus	46.8
Macrocyclops albidus	41.0
Mesocyclops thermocyclopoides	30.3
Mesocyclops venezolanus	26.4

* Average number of larvae killed per cyclopoid in 24 hours. Fifty larvae available to each cyclopoid. Based on 30 replicates for each species. Temperature: 26 C. An average of 0.1 larvae died during the 24 hours in control dishes without cyclopoids.

The smaller species of Honduran cyclopoids that we tested killed a few larvae on occasion, but the four largest species--*Mesocyclops longisetus*, *Mesocyclops venezolanus*, *Mesocyclops thermocyclopoides*, and *Macrocyclops albidus*--performed completely apart from the other species. They consistently killed large numbers of *Ae. aegypti* larvae (Table 2). We therefore considered all four of these species to be prime candidates for biological control. (Two of these species, *Mesocyclops longisetus* and *Macrocyclops albidus*, also occur in New Orleans and are in fact our two most effective cyclopoid species for mosquito control in New Orleans.) Laboratory colonies were established of the Hondurans strains of each of these four species to provide animals for extensive laboratory experiments and field trials in Progreso during 1991.

Objective 2: Evaluation of the effectiveness of each larvivorous cyclopoid species in different kinds of *Ae. aegypti* breeding containers.

Effectiveness for control requires high performance in two categories:

(1) maintaining a large population in the container over the long term;
(2) being able to kill any number of *Aedes aegypti* larvae that hatch into the container.

We evaluated the four species of cyclopoids with regard to both of these categories, by means of (a) field trials and controlled experiments at our laboratory and (b) field trials around peoples homes.

Field trials at the laboratory

We introduced *Mesocyclops longisetus* to the laundry tank in the yard behind the laboratory. (This tank is not typical because we are not using it to wash clothes.) *Mesocyclops longisetus* has maintained a population of several thousand individuals in the tank throughout the ten months since introduction. There have been no *Ae. aegypti* in the tank, though there have been *Culex* larvae from time to time.

We introduced each cyclopoid species to tires in the yard behind the laboratory, five replicate tires for each species. The tires have been left to themselves. The only source of water is rainfall, and no food has been placed in the tires. *Mesocyclops longisetus* has maintained populations in all tires, fluctuating between several hundred and several thousand individuals, as long as the tires have not dried out. *Mesocyclops longisetus* survive as long as some dampness remains in a tire, but they are wiped out if a tire dries out completely. The same has been true for *Macrocyclops albidus*, *Mesocyclops thermocyclopoides*, and *Mesocyclops venezolanus*, except their numbers have sometimes fluctuated below a hundred, and they have sometimes disappeared even when the tires have not dried out. We have never observed *Ae. aegypti* larvae beyond the second instar in tires containing any of the four cyclopoids species, though there have been substantial numbers of larvae of all instars in control tires (without cyclopoids) at the same location. Tires with cyclopoids sometimes contain *Culex* larvae.

One hundred individuals of each cyclopoid species were introduced to three replicate 50-gallon drums for each species in the yard behind the laboratory. They were reintroduced if they disappeared from a drum. *Mesocyclops longisetus* and *Mesocyclops thermocyclopoides* have thrived in all their drums throughout the six months of observation so far. Their numbers have fluctuated between extremes of approximately 500 and 4,000 cyclopoids per drum. *Mesocyclops venezolanus* and *Macrocyclops albidus* have established similar populations when introduced, but they have sometimes disappeared from the drums after a few months. *Mesocyclops venezolanus* disappeared after sudden bursts of population, fol-

lowed by a nosedive to extinction, whereas *Macrocyclops albidus* disappeared after gradual decline. We have never observed *Ae. aegypti* larvae in drums with any of the four species of cyclopoids, even though control drums (without cyclopoids) at the same location often have larvae.

We also tested a mixture of all four cyclopoid species together in the same drums. Sometimes *Mesocyclops longisetus* took over the mixture, but other times the mixture went to extinction before *Mesocyclops longisetus* took over.

We introduced each species of cyclopoid to vases containing live philodendron with their roots in water. There were four replicate vases for each species of cyclopoids, and they were kept in the insectary of our laboratory. Cyclopoids were reintroduced whenever they disappeared from a vase. The number of cyclopoids in the vases has fluctuated through time from only a few individuals to several hundred, the typical number being 5-30. *Mesocyclops venezolanus* has always displayed wide population fluctuations and gone to extinction within a few months. The other species have been more stable, and most of them are still in the vases five months after introduction, though each of them has gone to extinction in at least one of the vases. Populations of *Mesocyclops thermocyclopoides* in the vases have generally been larger than populations of the other species. There have occasionally been a few *Ae. aegypti* larvae in vases with *Mesocyclops venozolanus* or *Macrocyclops albidus*, but no *Ae. aegypti* larvae have been observed with *Mesocyclops longisetus* or *Mesocyclops thermocyclopoides*. Control vases in the same room often have 1-20 larvae.

Predation experiments in drums

We conducted a series of experiments in 50-gallon drums to provide quantitative assessment of the capacity of each of the four cyclopoids species to kill *Ae. aegypti* larvae in that environment. Fifty cyclopoids of a single species were introduced to each drum and left for one week. Five hundred newly-hatched *Ae. aegypti* larvae were then placed in the drums. (The ratio of ten larvae for each cyclopoids was intended to provide more larvae than the cyclopoids would kill, in order to compare the maximum predation capacities of each species under drum conditions.) Five days after introducing the larvae, any larvae that survived had grown too large to be killed by cyclopoids, so cyclopoids and larvae were removed and counted. Table 3 shows that the predation

capacity of *Mesocyclops longisetus* and *Macrocyclops albidus* was clearly greater than *Mesocyclops thermocylopoides* or *Mesocyclops venezolanus*, though even the latter two species were effective predators. We also conducted the same experiment with a mixture of all four species in the same drums, and the results were always inferior to those from any of the four species alone.

Table 3
Predation on first instar *Ae. aegypti* larvae in 50-gallon drums

	Number of larvae killed per cyclopoids*
Mesocyclops longisetus	9.7
Macrocyclops albidus	9.3
Mesocyclops thermocyclopoides	7.0
Mesocyclops venezolanus	5.6

* 500 larvae available to 50 cyclopoids (i.e., 10 larvae/cyclopoids). Based on 3 replicates for each species. Temperature: 26^{c}.

Because *Mesocyclops longisetus* and *Macrocyclops albidus* killed nearly all larvae available to them, the experiment was not able to estimate the maximum capacities for these two species. We are now repeating the experiments with a ratio of 50 larvae for each cyclopoids.

We conducted experiments in which we introduced 1,000 larvae to drums containing long-term cyclopoids populations. (We believe that 1,000 larvae is well in excess of the number that would normally hatch into a drum.) *Mesocyclops longisetus* always killed all the larvae. The number of survivors with *Macrocyclops albidus*, *Mesocyclops thermocyclopoides*, or *Mesocyclops venezolanus* ranged from zero to approximately fifty.

We conclude from the above results that *Mesocyclops longisetus* is the best species for *Ae. aegypti* control in drums because it is superior at maintaining long-term populations in the drums and it is superior at killing large numbers of *Ae. aegypti* larvae in the drums.

Field trials around peoples homes

Field trials were conducted in a variety of containers in five barrios of Progreso--several hundred containers in all. After cyclopoids introduction, each container was examined weekly for cyclopoids and mosquito larvae.

A water sample from each container was taken to the laboratory at the time of cyclopoids introduction, and 10 cyclopoids were observed in the sample for one week to check for toxicity. Toxicity is not a significant problem in any kind of container. Less then one percent of the containers had water toxic to cyclopoids.

We have not been able to achieve long-term survival of cyclopoids in laundry tanks. All four species of cyclopoids have usually lasted for a few weeks or months, but sooner or later they have gone down the drain despite our efforts to avoid it. All four species effectively eliminated *Ae. aegypti* larvae as long as they were in the tank.

50-gallon drums are different because they do not have a drain. We found that all species survived in a drum the 2-4 weeks until the drum's first cleaning. Then they were almost always lost. However, once we instituted the rescue system described below under objective #3, survival of *Mesocyclops longisetus* was excellent. We know that virtually no *Mesocyclops longisetus* are removed from drums when water is dipped out for household use. During the day, the cyclopoids are concentrated at the bottom, and people dip water from the top of the drum. (The cyclopoids are distributed from top to bottom of the water column at night.) *Macrocyclops albidus* and *Mesocyclops venezolanus* have generally disappeared from their drums a few months after introduction, even when not eliminated by cleaning. *Mesocyclops thermocyclopoides* may survive in drums as well as *Mesocyclops longisetus*, but we need a longer period of observation to be sure.

All four species have provided excellent control of *Ae. aegypti* larvae in drums (Table 4). The figure for *Mesocyclops longisetus* in Table 4 is based on nearly a hundred samples and indicates with a high degree of reliability that there are less than a thousandth as many *Ae. aegypti* larvae in drums with *Mesocyclops longisetus* compared to controls without cyclopoids.

We introduced the four species of cyclopoids to a total of about 50 tires around people's houses. During the first half of 1991, they survived for long periods only in large tires or smaller tires in the shade. Small tires in the sun dried out from time to time, and the cyclopoids were killed. In contrast, all species of cyclopoids have survived through the rainy season (the second half of 1991) in all tires to which they were introduced in the early part of the rainy season. Populations of *Mesocyclops longisetus* in the tires have had more stable numbers than populations of *Mesocyclops venezolanus* or *Macrocyclops albidus*. Mosquito control performance by *Mesocyclops longisetus* has been correspondingly better than the other two species (Table 4), because *Ae. aegypti* larvae survive in tires with cyclopoids only when cyclopoids numbers are very low (less than 10 cyclopoids), and *Mesocyclops longisetus* is never that low.

Table 4
Average number of *Ae. aegypti* larvae observed per container during weekly visits to tires and 50-gallon metal drums to which we introduced cyclopoids at people's homes in Progreso*

	Tires	Drums
Mesocyclops longisetus	0.8	0.02
Mesocyclops thermocyclopoides	---	0.3
*Macrocyclops albidus***	5.4	0
*Mesocyclops venezolanus***	7.7	0
Controls (no cyclopoids)	72.7	49.4

* Second to fourth instar larvae. For drums and tires not treated with *bti* at the time of cyclopoids introduction, the figures are based on larvae in the containers three weeks or more after cyclopoids introduction.

** The figures for *Mesocyclops venezolanus*, *Mesocyclops thermocyclopoides*, and *Macrocyclops albidus* in drums are based on a limited number of samples and can be expected to be revised when all the field trials are completed at the end of the year.

Overall numbers of II-IV instar larvae with *Mesocyclops longisetus* have been about one percent of what we have seen in control tires (Table 4). (We do not yet have enough data on *Mesocyclops thermocyclopoides* to make a statement about that species in tires.)

We have introduced cyclopoids to about fifteen live-plant vases in people's homes. *Macrocyclops albidus*, *Mesocyclops longisetus*, and *Mesocyclops thermoclopoides* are still going strong in six of the vases, and we have observed no *Ae. aegypti* in vases with these species of cyclopoids. Cyclopoids have disappeared from vases because they were dumped out or because of some other disaster such as a dog drinking all the water. The vases where cyclopoids are continuing successfully are ones that are out of harm's way.

For the most part we have not achieved long-term cyclopoids survival in animal drinking containers. Most people change the water frequently to keep it clean for their animals. One common form of drinking container is a tire cut in half, which has proved far too unstable for cyclopoids survival. The tire is easily tipped over or swished out with a broom. The other common form of container is a small cement pool, and this is the kind of drinking container where we have found nearly all *Ae. aegypti* pupae. We have achieved long-term survival of *Mesocyclops longisetus* in some of these, but we have not been able to do so in others.

Conclusions on the effectiveness of each cyclopoids species

Mesocyclops longisetus has been the most effective species in every type of container. *Mesocyclops longisetus* has survived reliably throughout our period of observation in 50-gallon drums and other household containers as long as it has not been dumped out, and *Mesocyclops longisetus* has survived equally well in tires as long as they have not dried out. *Mesocyclops longisetus* has consistently inflicted 100% mortality on *Ae. aegypti* larvae in our laboratory and field trials, exactly the performance we need for effective control. We can say with confidence that no complicating factor, such as spatial distribution of cyclopoids and *Ae. aegypti* larvae in a container or alternative food for cyclopoids in a container, interferes with the consistent and complete elimination of *Ae. aegypti* larvae by *Mesocyclops longisetus*. (We know that cyclopoids graze down the food in a container within a few days after the cyclopoids are introduced. After that the cyclopoids voraciously consume any food

that comes their way.) We can conclude that the effectiveness and duration of container treatment with *Mesocyclops longisetus* in operational control programs will depend exclusively on the ability of this cyclopoid to survive over the long term in different kinds of containers, and we can expect long-term survival to depend exclusively on the incidence of disasters such as drying the container or throwing the cyclopoids out.

Mesocyclops venozolanus and *Macrocyclops albidus* have been eliminated from further consideration in the project because of their poor performance compared to the other species. The performance of *Mesocyclops venozolanus* and *Macrocyclops albidus* has been inferior with regard to survival in containers and with regard to their capacity and reliability as predators of *Ae. aegypti* larvae.

We have less information on *Mesocyclops thermocyclopoides* because we got it into mass culture (and therefore started field trials) later than the other species. Although *Mesocyclops thermocyclopoides* has demonstrated a smaller predation capacity than *Mesocyclops longisetus* in predation experiments, *Mesocyclops thermocyclopoides* has performed well in the field trials so far. After a longer period of observation, we may find that long-term survival of *Mesocyclops thermocyclopoides* is better than *Mesocyclops longisetus* in vases.

Objective 3: Development of operational procedures for introducing and maintaining cyclopoids in *Ae. aegypti* breeding containers.

As we look toward full-scale operations next year, we will use *Mesocyclops longisetus* in tires and 50-gallon drums, as well as some of the small concrete pools that provide water for animals. We will use *Mesocyclops longisetus* or *Mesocyclops thermocyclopoides* in live-plant vases.

We know that we can achieve effective control by introducing 100 *Mesocyclops longisetus* to a 50-gallon drum, 50 *Mesocyclops longisetus* to a discarded tire, or 10 cyclopoids to a flower vase. However, it may take as long as four weeks for complete control to take effect (Table 5), because third and fourth instar larvae that were in the container at the time of cyclopoids introduction may take several weeks to clear out of the container. We can achieve immediate control by applying *bti* pellets

at the same time cyclopoids are introduced (Table 5), a practice we are now following on a routine basis. The *bti* kills all larvae immediately, and the cyclopoids kill all new larvae that enter the container.

When we started the project, we were concerned whether people would accept a small animal like cyclopoids in containers around their homes. Keeping in mind that drinking water is not an issue in Progreso because *Ae. aegypti* does not breed in water stored for drinking, we have found that people do not object to cyclopoids in water they use for cleaning. In fact, they have no objection to cyclopoids as long they are introduced to the cyclopoids by a woman. *Ae. aegypti* breeding containers are the domain of housewives and housewives trust other women.

Table 5
Average number of *Ae. aegypti* larvae in drums and tires during the weeks immediately following introduction of 100 *Mesocyclops longisetus*.*

	Weeks after cyclopoids introduction			
Drums	**One**	**Two**	**Three**	**Four**
bti not applied	21.4	1.0	0.2	0
bti applied**	0	0	0	0
Tires				
bti not applied	54.1	1.6	0.6	0
bti applied**	0	0	0	0

* Second to fourth instar larvae.
** *bti* applied only once, at the time of *Mesocyclops* introduction.

The most important way we need "acceptance" is in the cooperation that we need from housewives to maintain cyclopoids in their containers. Most of our effort this year toward maintenance of cyclopoids has been directed toward 50-gallon drums, where cyclopoids face the hazard of being dumped out during cleaning. Several months ago we initiated a procedure to collect cyclopoids from a drum by swishing a small net around the bottom for about half a minute before the water is dumped out. The cyclopoids are held in a small plastic food container while the

drum is cleaned, and afterwards they are returned to the drum. We achieved perfect long-term survival of *Mesocyclops longisetus* in drums with this procedure, and two months ago we turned the procedure over to housewives. The women have shown a keen interest, and so far every woman has maintained *Mesocyclops longisetus* successfully in her drum. We will continue to monitor for the rest of the year to see if the women maintain their enthusiasm, and we will continue to test modifications to the procedure to make it as convenient as possible for housewives.

We are continuing our efforts to establish long-term survival of *Mesocyclops longisetus* in laundry tanks. We are starting to test the insertion of strainers in the drains of the tanks to prevent loss of cyclopoids down the drain. The trick is how to clean the tank properly with such a fine strainer in the drain.

Objective 4. Development of techniques and procedures for mass production and distribution of cyclopoids.

We have set up seven small outdoor ponds (6 ft x 2.5 ft) at the Progreso laboratory for mass production of cyclopoids. The ponds are made with a wood frame and plastic lining, they are inexpensive, and they have functioned well. We will also build some cement ponds because construction with cement is inexpensive in Progreso. We have found it essential to cover the ponds with shade screen (as is used for plant nurseries) to keep out aquatic insects and prevent overheating of the water by the afternoon sun. We are experimenting with water depth, different kinds of food, and mineral fertilizers, to see if we can increase production and simplify maintenance. We believe we can improve the outdoor production system quite a bit, but we already have the techniques necessary to produce all the cyclopoids the project will need when it is scaled up to cover the entire city next year.

At the present time we use vials to transport cyclopoids for introduction to containers in the barrios. Cyclopoids can also be applied with a backpack sprayer. The technical side of distributing cyclopoids is well in hand, but more work needs to be done on organizational aspects with the community.

Work Plan For The Second Year

During 1991 we have completed the first three stages of the WHO scheme for screening and evaluating the efficacy of biological agents for the control of disease vectors: i.e., (1) search and laboratory operations, (2) experimental laboratory operations, and (3) preliminary field trials. We have nearly completed the fourth stage: pilot production by culture is complete; quantitative assessment of effectiveness is complete for *Mesocyclops longisetus*, and it will be complete for the other species within a few months. We have not yet prepared a formal assessment of safety and environmental impact (including impacts on nontarget organisms), but the work during 1991 has provided the information that we need to do it.

We are ready to begin the final stage of the WHO scheme, namely large-scale field trials. We know which species of cyclopoids we will use (i.e., *Mesocyclops longisetus*), in which kinds of containers, and how it should be done from a technical point of view. During 1992, we will develop two new directions for the project :

(1) scaling up to large-scale operation; and
(2) integrating cyclopoids with the community participation part of the *Ae. aegypti* control program in Progreso.

The way we distribute and maintain cyclopoids in *Ae. aegypti* breeding containers on a community scale during 1992 will be very different from the way we have done it with our field trials in 1991. Until now, everything we have done has been based on close personal contact with housewives, a quality of contact that will not be possible on a larger scale. Our greatest challenge will be how to realize the same degree of success that we experienced during 1991, but without the personal contact. Fortunately, the Integrated Dengue Control Project's development of public education, community organization, and community participation in Progreso should provide us with the vehicles we need to get cyclopoids out to the public.

The Integrated Dengue Control Project is now working with neighborhood associations, schools, churches, and other community resources in ten barrios of Progreso to refine its techniques for community-based

Ae. aegypti control using methods such as container cleanup and removal, proper container storage, covering containers, and dumping larvae out of containers before they complete their development. (Progreso has a total of 70 barrios.) Until now, we have conducted our cyclopoids field trials in barrios where the Integrated Dengue Control Project is not operating. Starting in January 1992, cyclopoids will join with the rest of the Integrated Dengue Control Project in one or more of the barrios where the Project is already operating. The task will be to treat every appropriate container in the barrio with cyclopoids, and to do so through the Project's community organizing process.

We will confront a whole new set of problems, completely different from the ones we have addressed during 1991. The following are examples of questions we shall be dealing with:

I. How do we organize mass production and distribution of cyclopoids for control operations on a community scale? Can we distribute cyclopoids to a large number of houses without going personally to every house? How do we tie the distribution of cyclopoids in with the community organizing and education activities of the community participation project?

II. What kind of information do we need to provide to people so they understand the purpose of cyclopoids and what they must do to maintain cyclopoids in their containers? What are the best ways to present the information (group meetings, pamphlets, videos, etc.)?

III. What is the compatibility of other *Ae. aegypti* control methods with cyclopoids? Which other methods lead to enhanced *Ae. aegypti* control when used in combination with cyclopoids? How do we combine cyclopoids with other methods to provide the best overall *Ae. aegypti* control?

IV. How do we structure a monitoring system to evaluate the effectiveness of cyclopoids for *Ae. aegypti* control?

The key to the effectiveness of *Mesocyclops longisetus* as a treatment for *Ae. aegypti* breeding containers is our ability to ensure that the cyclopoids are maintained in containers despite human activities that may eventually dump them out. We already have a simple routine to rescue the cyclopoids before cleaning a container so they can be returned to the

container after cleaning. Only experience will tell us how receptive the public is to such a routine, but we expect it to depend very much on general neighborhood feelings about mosquito control and whether people are motivated to take the extra effort. We will also need to develop, on a community scale, the means for a housewife to restock a container in her yard with *Mesocyclops longisetus* if something happens to the ones already in her container. This could be done from another container on the same property that has *Mesocyclops longisetus*, from a neighbor's container, or from a central source of supply in the neighborhood.

We are laying the groundwork for integration of cyclopoids with other methods of *Ae. aegypti* source reduction by field testing the other methods by themselves and in combination with cyclopoids. While retaining a primary focus on cyclopoids, our scope is expanding to how we apply cyclopoids in the context of a comprehensive menu of methods for treating various kinds of *Ae. aegypti* breeding containers. Table 6 shows an example of the kind of menu that we are considering. Wherever possible, we want to combine cyclopoids with other methods, so if one method breaks down in a specific container, the other methods will continue control. As we look at the methods that are most effective for each kind of container and decide on the role for cyclopoids, we have to consider the costs of each method, the physical compatibility of cyclopoids with other methods, and how the dissemination of cyclopoids and dissemination of other methods fit together in the community organizing process.

Table 6
Possible methods for integrated *Ae. aegypti* control

	Tires	Laundry Tanks	Drums	Buckets, etc.	Animal drinking	Vases
Cyclopoids	xxx	?	xxx		x	xx
Algae	?		?		?	?
Fish		?	?			
bti	x	x	x		?	x
Altosid	xx	xx	xx		?	xx
Abate	x	x	x		?	x
Salt						
Disposal	xxx					
Storage				xx		
Dumping*		xx	xx	xx	x	xx
Covers			?			

* Dumping *Ae. aegypti* larvae out of the container before the larvae complete their development.

? The method is a possibility, but not yet proved for the type of container indicated.

x The method can work in the indicated type of container, but it has some serious limitations.

xx The method should work well in the indicated type of container.

xxx The method should be particularly effective in the indicated type of container.

Appendix B

Pilot Project On The Operational Use of Cyclopoids for *Aedes aegypti* Control In El Progreso, Honduras

Summary Report For The Second Year (1992)

The project accomplished the following during 1992:

1. Published 1991's results.

2. Expanded cyclopoids operations to cover an entire barrio.

3. Developed a technique for long-term storage of large numbers of cyclopoids in small containers.

4. Developed a device to prevent the loss of *Mesocyclops longisetus* from drums when they are cleaned.

5. Began to use an *Ae. aegypti* computer model for strategic analysis of biological control.

6. Formulated plans for barrio-scale use of *Mesocyclops longisetus* during 1993.

1. Publication of results from 1991

We summarized our cyclopoids field data from 1991 during the first part of 1992. We also finished several experiments that we needed to include with the publication of our results from 1991. For example, we observed in the 1991 field trials that *Mesocyclops longisetus* survives much better in water storage containers like 55-gallon drums than the other species of cyclopoids that we tested. We then conducted experiments to show that the other species, which swim throughout the water column, are subject to removal when water is dipped from a drum for use. In contrast, *Mesocyclops longisetus* stays at the bottom, so very few are dipped out. The manuscript on the cyclopoids field trials in

Progreso--coauthored with Gerardo Borjas, Mary Cush, and Eduardo Fernández--was completed in August and submitted to the Journal of Medical Entomology.

2. Expanding cyclopoids operations to cover an entire barrio

All biological control operations in Progreso during 1992 were conducted by the Integrated Dengue Control Project's biocontrol team, headed by Héctor Portillo (and since August, his successor Catalina Sherman) under the direction of Eduardo Fernández.

The expansion of cyclopoids operations to cover an entire barrio had several objectives:

1. To gain experience with the logistics of *Mesocyclops longisetus* production and distribution on a barrio scale.

2. To explore the full range of containers for which *Mesocyclops longisetus* might work in a community participation program, including a view of the compatibility of *Mesocyclops longisetus* with people's use of the containers.

3. To prove the efficacy of *Mesocyclops longisetus* on a barrio scale before employing it in barrios organized by the Integrated Dengue Control Project.

4. To determine what kind of assistance or information the Project needs to provide to the public to ensure *Mesocyclops longisetus* is maintained in treated containers over the long term.

5. To see if the public would make the effort necessary to maintain *Mesocyclops longisetus* in treated containers.

6. To develop procedures for monitoring and evaluating the effectiveness of *Mesocyclops longisetus* in the context of community participation on a barrio scale.

Pénjamo was the barrio selected for the full-scale deployment of *Mesocyclops longisetus*. Organization of the community was implemented by biocontrol staff, supervised by Héctor Portillo. The goal was to

establish biocontrol in as many containers as possible. Preliminary container surveys and knowledge/attitude interviews were conducted during March and April. Then the biocontrol staff held meetings with the community to discuss cholera, malaria, dengue fever, *Ae. aegypti* control, and the use of biological control for *Ae. aegypti* control. *Mesocyclops longisetus* was introduced to tires and cisterns in April and 55-gallon drums in May.

The introductions were done directly by biocontrol staff without enlisting much participation from neighborhood volunteers. At the time of cyclopoids introduction to drums, each housewife was given a net and instructed on how to remove the cyclopoids from the drum before cleaning and returning them afterwards (the "rescue process"). Because the purpose of the work in this barrio was total coverage emphasizing biocontrol to the greatest extent possible, cyclopoids were introduced to every tire, cistern, and drum, whether the housewife was particularly interested or not.

We developed a standardized form for periodic inspections to check whether *Mesocyclops longisetus* was still in treated containers. Inspections were rapid and provided the information we needed. One of the health volunteers in the barrio kept a stock of *Mesocyclops longisetus* at her home, but most people did not take the initiative to get more *Mesocyclops longisetus* if they needed them, apparently because people did not receive a clear message that they should do so.

This procedure for distribution and maintenance of *Mesocyclops longisetus* was not what we originally planned. The original intention was to involve community volunteers very actively in the introduction of *Mesocyclops longisetus* to drums and all other containers. Volunteers were also intended to be the keystone for maintaining the cyclopoids in containers. However, the community organization process went slowly in this barrio, and *Mesocyclops longisetus* was introduced before the volunteers were ready to do their part. Moreover, because the biocontrol team was needed for other activities in the Integrated Dengue Control Project, the team did not immediately return to people's homes to ensure that housewives followed the cyclopoids rescue process correctly when they cleaned their drums, and the community volunteers did not follow up either.

The results in this barrio were successful in some ways and disappointing in others. *Mesocyclops longisetus* provided good control in tires. This is because no active cooperation was required from the people to maintain cyclopoids in their tires. The cyclopoids also did well in cisterns. The results were not so good with 55-gallon drums. The cyclopoids disappeared from most of the drums within two months.

There are some useful lessons to be gained from our results in this barrio, especially if we compare these results with results from other parts of the city last year, when all participating housewives successfully maintained *Mesocyclops longisetus* in their drums and other containers for long periods. There were three important differences:

1. All the housewives last year were volunteers, and they were all interested in what was happening. This year included a full spectrum of housewives, some interested and others not.

2. Last year the biocontrol team was able to concentrate considerable effort on the cyclopoids field trials. This year the biocontrol team had numerous other competing activities.

3. Last year the biocontrol team had a careful follow-up with each housewife to ensure that she was implementing the cyclopoids rescue process correctly when she cleaned her drum. That did not happen this year.

We can conclude from this year's results in Pénjamo that:

1. The production and distribution of *Mesocyclops longisetus* for large scale use is quite manageable. The problem is maintaining the cyclopoids in treated containers when it is not possible to provide a lot of individual attention to the public.

2. *Mesocyclops longisetus* works well in containers where the water is not changed (e.g., tires and cisterns), as long as there is a source of supply for replacement in case the cyclopoids are lost. To maintain *Mesocyclops longisetus* in containers such as drums or flower vases, which require the "rescue procedure" during cleaning, it is necessary to have a personal follow-up with each housewife to make sure she understands what she is doing.

3. We cannot demonstrate the efficacy of copepods for community-based *Ae. aegypti* source reduction on a barrio scale by promoting biological control in isolation. Copepods should be part of a comprehensive program of *Ae. aegypti* source reduction, which in turn should be part of a comprehensive program of environmental sanitation, which is what people really care about.

4. It is not realistic to introduce copepods to a community during the initial stages of organizing, because the volunteers are not ready.

5. It is not realistic to expect community organizers or health volunteers in a barrio to give much attention to copepods when they have many other activities as well. To be practical, the use of copepods must be structured so it makes as few demands as possible on everyone involved — promotion team, community health volunteers, and housewives.

3. A new cyclopoids storage technique

Until now we have stored *Mesocyclops longisetus* in the same tanks in which we produce them. However, we recently made a breakthrough at the New Orleans laboratory that could have far-reaching implications for the way we produce and distribute *Mesocyclops longisetus* in Honduras. The breakthrough was derived from learning that *Mesocyclops longisetus* can survive for long periods in a state of suspended animation in damp soil. We therefore tried to simulate the same conditions in a small container in the laboratory. After trying many different materials and container environments, we now have a design that allows us to store thousands of *Mesocyclops longisetus* for months in a pint container at room temperature.

Now that we can store large numbers of *Mesocyclops longisetus* for long periods in small containers, we will not have to use *Mesocyclops longisetus* as soon as we produce them. This will give us considerable flexibility with our production system in Progreso because we will not have to maintain a large number of cyclopoids in our production tanks to be ready at all times for whatever number of cyclopoids might be needed.

This breakthrough will also make the shipment of *Mesocyclops longisetus* very convenient, which in turn will increase the attractiveness of centralized production and distribution. Centralized production could be desirable in a country like Honduras where the professional resources to operate cyclopoids production facilities are in short supply.

4. A new device for maintaining cyclopoids in drums

A serious limiting factor for large-scale use of cyclopoids in 55-gallon drums is the fact that they can be dumped out when the drums are cleaned. Until now, we have been using a "rescue" procedure to maintain *Mesocyclopoids longisetus* in the drums in Progreso; i.e., housewives remove some of the cyclopoids from their drum with a net before cleaning and return the cyclopoids to the drum afterwards. The procedure is simple and effective, and last year's experience convinced us that most housewives can do it well. However, this year's experience demonstrated that housewives in a large-scale program may not receive the quality of instruction necessary for them to do it properly. We can still hope to improve the biocontrol promotion process so the "rescue" procedure works, but it would be much easier for everyone--community organizing team, volunteers, and housewives--if we could place a simple device in every drum when the cyclopoids are introduced, to ensure that the cyclopoids will not be lost from the drums when they are cleaned.

We have been working on a number of designs in the New Orleans laboratory this year, and we now have several that work very well. All of the devices capitalize on the fact that *Mesocyclops longisetus* is concentrated within two or three inches of the bottom of a drum. The devices are false bottoms that trap the cyclopoids in a drum when water is dumped out. The false bottom has a hole that allows the cyclopoids to move freely back and forth between the "refuge" beneath and the water column above. At any one moment, about 25% of the cyclopoids in a drum are in the "refuge", and they stay there when water is dumped out. These devices could have far-reaching consequences for the feasibility of maintaining *Mesocyclops longisetus* in drums in community participation programs where it is not possible to give intensive attention to every housewife.

5. *Ae. aegypti* computer model

We obtained ten years of daily weather records (temperature, humidity, and rainfall) for Progreso and put them into a computer file for use with Dana Focks' *Ae. aegypti* model. We also used field information from the Integrated Dengue Control Project's pupal survey to calibrate the model for the particular container conditions that we have in Progreso. The model is now up and running.

We have begun to use the model to address the following questions:

1. What is the relative importance of tires, pilas, 55-gallon drums, and other containers as sources of *Ae. aegypti*? Do they make their major contributions during different seasons? What is the impact of source reduction for each of these containers on the *Ae. aegypti* population?

2. How often is it necessary for housewives to clean their pilas and 55-gallon drums for *Ae. aegypti* control to be completely effective? What are the consequences if some of the housewives clean their pilas or drums less frequently (e.g. once every ten days or once every two weeks)?

The model's answer to the second question is particularly significant. According to the model, *Ae. aegypti* production should be completely eliminated from pilas and drums that are cleaned every seven days or less. However, control breaks down if the cleaning interval is longer; for example, there is not much reduction in the population of adult *Ae. aegypti* if the cleaning interval is increased to ten days. This result underscores how much the success of an *Ae. aegypti* control program based on cleaning pilas and drums depends on strict adherence of housewives to the recommended cleaning regimen. The result also highlights the importance of alternative methods of control (e.g., biological control) for housewives who are unable or unwilling to clean their pilas or drums so frequently.

6. Plans for barrio-scale use of *Mesocyclops longisetus* during 1993

Integration of *Mesocyclops longisetus* into the rest of the Integrated Dengue Control Project has been slower than I originally expected. Eduardo Fernández decided that it was best not to include biocontrol in the initial organizing process by the Project's promotion team in the barrios, and I accepted that decision. As a consequence, it has been necessary to wait until the promotion team organized the barrios. That has been done now, so we have recently been able to start this important part of the project — to deploy *Mesocyclops longisetus* in barrios where the community organization program is well established. Our expectations for success are high because these barrios have an active cadre of volunteers who can help sustain *Mesocyclops longisetus* in treated containers over the long term.

In barrios that have already been organized by the Integrated Dengue Control Project, biocontrol is viewed as a supplement to the conventional *Ae. aegypti* control methods already in use. The basic idea is to work with barrio-level health committees to identify containers that are still producing *Ae. aegypti*, ask why those containers are still producing mosquitoes, and decide what should be done. Biocontrol is presented as an option. If a committee decides to use biocontrol, the distribution of *Mesocyclops longisetus* will be implemented by health volunteers in the barrio with technical support from the biocontrol team of the Integrated Dengue Control Project. I have prepared a step-by-step procedure for implementation, as well as a compilation of information required by Project staff, community volunteers, and the general public to use *Mesocyclops longisetus* successfully for controlling *Ae. aegypti* in appropriate containers.

Appendix C

Publication of the First Year's Results on the Operational Use of Cyclopoids for *Aedes aegypti* Control in El Progreso, Honduras. Journal of Medical Entomology, In Press

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

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Abstract: *Mesocyclops longisetus*, *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus*, and *Macrocyclops albidus* were tested for their effectiveness at controlling *Aedes aegypti* larvae in a variety of containers around homes in El Progreso, Honduras. *Me. longisetus* was the most effective species because it not only was the most voracious predator but also survived best in the test containers. *Me. longisetus* maintained long-term populations in 55-gal drums, tires, vases, and cement tanks without drains (e.g., cisterns and animal drinking containers)--providing they were not dried or poured out. Compared to control containers without copepods, *Me. longisetus* reduced III/IV instar *Ae. aegypti* larvae by >98%. *Me. longisetus* should be of practical value in community-based *Ae. aegypti* control programs.

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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 employs 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 containers 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 that they are not willing or able to store out of the rain. All of these breeding sites 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 mosquito larvae, can maintain virtually 100% control of container-breeding *Aedes* for as long as the cyclopoids survive in a container (Rivière & Thirel 1981, Marten 1984, Suárez et al. 1984). *Mesocyclops longisetus* (Thiebaud) and *Macrocyclops albidus* (Jurine) are currently 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 rotifers) 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 common species in Honduras were tested: *Macrocyclops albidus*, *Mesocyclops longisetus*, *Mesocyclops venezolanus* Dussart, and *Mesocyclops thermocyclopoides* Harada. Because the first 3 species occur throughout much of Latin America, the results of these tests should apply to that region.

Materials and Methods

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

Almost every home in El Progreso has 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 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 55-gal 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 imbedded 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, 10-gal plastic drums, pots, and large plastic bowls (palanganas) 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, imbedded in the ground) to store water. The cisterns have makeshift covers, but mosquitoes still 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 the 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 greater then 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 tested placed in dishes (10 cm diameter) for 24 h with newly hatched *Ae. aegypti* larvae at a ratio of 10 larvae/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 *Me. albidus* sensu stricto, *Me. longisetus* var. *curvatus* Dussart, *Me. venezolanus*, and *Me. 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 4 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 diam and 18 mm deep) with 50 newly hatched larvae. The number of surviving larvae was 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 4 cyclopoid species was introduced to 3 55-gal drums almost filled with water. The cyclopoids were given a month to build up their populations amd equilibrate with whatever food (a broad spectrum of small organisms) was naturally available in the drums. Predation experiments were then conducted in 5 drums each day, one drum for each cyclopoid species plus a control drum without cyclopoids. The experiments were repeated on 3 different days, using different drums each day. Drum water temperatures on different days varied from 21°C to 28°C.

Each predation experiment was started by passing a net throughout the water to remove all adult and subadult cyclopoids in the 55-gal 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 much 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 5 cyclopoids and 250 *Ae. aegypti* larvae were used. The experiment was replicated 4 times for each cyclopoid species, using different tires.

Field trials

The purpose of the field trials was to compare (a) the survival of each cyclopoid species in different types of containers under normal household use and (b) 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 4 species of cyclopoids to containers at houses scattered throughout several *barrios in the city. The rainy season and the field trials lasted 30 wk until the end of the year. Ae. aegypti* is most abundant during this period. Only *Me. longisetus* was tested in animal drinking containers, cisterns, and ornamental pools. The field trials with vases started two months later than other containers and lasted for 20 wk.

Most of the introductions involved 1 species of cyclopoid at a time, but a mixture of all 4 species also was tested in 55-gal drums, tires, and vases. 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.

The containers used for the field trials included 29 laundry tanks, 34 55-gal drums (10 of which were not in active use), 37 tires, 12 animal drinking containers, 5 buckets, 10 10-gal 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 55-gal drums; 50-100 in tires, animal drinking containers, buckets, plastic drums, and pots; 100-500 in cisterns or pools; and 10 in vases.

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 55-gal drums, so that 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 implemention the next 2 times they cleaned their drums. After that, they followed the procedure on their own.

Vectobac[R] *Bacillus thuringiensis* var *israelensis* pellets were applied to 15 of the 37 tires (5 pellets/tire) and 15 of the 34 drums (15 pellets/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 weeks after cyclopoid introduction and every 2 to 4 wk after that 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 I/II or III/IV instars and cyclopoids as adults or copepodids, and returning all cyclopoids and water to the container. With 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.

Field predation experiments

To test the ability of long-term *Me. longisetus* populations to kill *Ae. aegypti* larvae that might hatch into 55-gal drums, paper strips with 1,000 *Ae. aegypti* eggs were attached to the sides of 5 drums just under the water surface. The drums contained populations of 500-3,500 *Me. longisetus* from introductions at least 3 months 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 *Me. longisetus*.

The same experiment was conducted with 5 tires containing long-term populations of *Me. longisetus*, except that 500 *Ae. aegypti* eggs were added per tire. The number of *Me. longisetus* in the tires at the time of the experiment ranged from ≈65 to 300. There was 1 control tire without *Me. longisetus*.

Three months after introducing cyclopoids to a series of plant vases, 20 newly hatched *Ae. aegypti* larvae were placed in 3 vases each of which contained 3 *Me. longisetus*; 3 vases that contained 2 *Me. venezolanus*; and 3 vases that contained 3-4 *Ma. 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, 55-gal 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 5-gal 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 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* group (Fischer), *Diacyclops* sp., *Ectocyclops rubescens* Brady, *Eucyclops agilis* (Koch), *Mesocyclops pescei* Petkovski, and *Mesocyclops reidae* Petkovski were large enough to be tested (body length = 1.01.2 mm), but they killed <1 *Ae. aegypti* larva/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, killed all 10 larvae available to them.

Laboratory predation experiments

Me. longisetus, *Me. venezolanus*, *Me. thermocyclopoides*, and *Ma. albidus* all killed a large number of *Ae. aegypti* larvae in the tissue culture plates (Table 2). Although *Me. 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 in Table 2 should reflect the capacity of each species to kill *Ae. aegypti* when saturated with larvae in a drum or tire environment. *Me. longisetus*

killed the most larvae, while *Me. thermocyclopoides* and *Ma. albidus* killed nearly as many. *Me. venezolanus* usually killed the least, but results with *Me. venezolanus* 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 people who promoted their use for mosquito contol.

Cyclopoid survival in containers. The cyclopoids seldom survived for more than a week in small water storage containers such as buckets, 10-gal plastic drums, and pots, which were filled and emptied frequently. Nor did the cyclopoids survive for long in laundry tanks with drains at the bottom, unless the tank was not in active use. Sooner or later all the cyclopoids went down the drain.

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

Me. 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 the drum was in active use or not, the *Me. longisetus* population usually multiplied to >500 adults within 2-4 wk after introduction and maintained that number for the rest of the field trials. Population numbers were lowest in drums with frequent water turnover and clean conditions.

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

Tires. *Me. longisetus* survived in tires better than the other species (Table 3). The *Me. longisetus* population in automobile tires was usually 200-500 adults, but numbers were lower when a tire contained very little water. *Ma. 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 *Me. thermocyclopoides* was also highly variable, and *Me. venezolanus* never lasted >2 mo in automobile tires. *Me. longisetus*, *Me. venezolanus*, and *Ma. albidus* survived in truck tires for the duration of the field trials, their numbers often exceeding 1,000 adults. (*Me. thermocyclopoides* was not tested in truck tires.)

All species of cyclopoids were killed if a tire dried out completely, but they survived as long as at least a small amount of moisture was in the tire. Few tires dried out during our field trials, because it was the rainy season.

Animal drinking containers. *Me. 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. *Me. 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. *Me. longisetus* sustained populations of several thousand adults in unused laundry tanks, cisterns, and ornamental pools, but on 4 occasions disappeared for no apparent reason. On 3 of those occasions a large population of ostracods or cladocerans appeared at the same time *Me. longisetus* disappeared.

Vases. Cyclopoids were lost from vases if the water was changed without using a net. When not lost this way, all 4 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 4 cyclopoid species never lasted more than a month in 55-gal drums (n = 3), tires (n = 3), or vases (n = 3). *Me. venezolanus*, *Me. thermocyclopoides*, and *Ma. albidus* always disappeared, leaving *Me. longisetus* in the container; or all 4 species disappeared.

Impact of cyclopoids on *Ae. aegypti* larvae. The percentage of control containers (without cyclopoids) positive for I/II 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 I/II instars.

Because it is conceivable for newly hatched larvae to remain in a container for a day or more before being killed by cyclopoids, I/II 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 I/II instar *Ae. aegypti* larvae (n = 468). Most of the I/II instar larvae were in containers with <10 cyclopoids. Larval survival in containers with larger numbers of cyclopoids appeared to be so brief that I/II instar larvae were seldom observed.

III/IV instar *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 III/IV instar larvae, whereas only 6% of the containers with cyclopoids were positive. However, cyclopoids reduced the number of larvae sustantially more than they reduced the number of positive containers (Table 3). (Inspections during the first 2 wk after cyclopoid introduction are not included in Table 3 because cyclopoid and larval populations were not yet stabilized.)

Me. longisetus performed best because it maintained large numbers in containers more consistently than the other cyclopoid species (Table 3). The number of III/IV instar *Ae. aegypti* observed in 55-gal drums with *Me. longisetus* was less than a thousandth the number in control drums. The number of III/IV instar larvae in tires with *Me. longisetus* was 98% less than in control tires. The III/IV instar larvae observed in

tires with *Me. longisetus* were associated with exceptionally low populations of the cyclopoid after tires nearly dried out. No III/IV instar larvae were observed in vases containing *Me. longisetus*.

Ma. albidus reduced III/IV instar *Ae. aegypti* larvae in drums by 99% compared to controls, and in tires by 93% (Table 3). The performance of *Me. venezolanus* and *Me. thermocyclopoides* in drums and tires was substantially poorer than the other tested species, because the populations of *Me. venezolanus* and *Me. thermocyclopoides* more often dropped to low numbers that did not eat all *Ae. aegypti* larvae.

No III/IV instar larvae were observed in vases with *Me. venezolanus*. One or 2 III/IV instar larvae were seen occasionally in vases containing *Ma. albidus* or *Me. thermocyclopoides* if there were <5 adults of these species in the vase.

It took up to 4 wk for III/IV instar *Ae. aegypti* larvae to disappear completely from drums and tires after introducing *Me. longisetus* without applying *Bti* (Table 4). When Bti was applied concurrently with *Me. longisetus* introduction, all mosquito larvae were dead within a day, and very few *Ae. aegypti* larvae were observed afterwards. *Bti* had no negative effect on the cyclopoids.

Thirty-six percent of the inspections of cement animal drinking containers, cisterns, or ornamental pools without *Me. longisetus* were positive for III/IV instar *Ae. aegypti* larvae (average number of III/IV instars = 105, SE = 32, n = 33). III/IV instar larvae were present in 7% of the inspections of these containers that had *Me. longisetus*, but the numbers of larvae were low (average number of III/IV instars = 2.1, SE = 1.3, n = 57).

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

Field predation experiments

In the experiments in which 1,000 *Ae. aegypti* eggs were placed in 55-gal drums that contained long-term populations of *Me. longisetus*, all *Ae. aegypti* larvae disappeared from all 5 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 5 tires with long-term populations of *Me. longisetus*, 4 tires had no surviving larvae after 5 d. The other tire (a small tire with a dense growth of algae and a small *Me. longisetus* population) had 32 surviving larvae. In the control tire, 199 larvae survived.

When 20 newly-hatched *Ae. aegypti* larvae were placed in plant vases 3 months after cyclopoid introduction, *Me. longisetus* always killed all the larvae. Eighty-five percent of the larvae (SE = 14%) died with *Me. venezolanus*, and 79% of the larvae (SE = 5%) died with *Ma. albidus*. During the 5 d of the experiment, 33% of the larvae (SE = 8%) died in control vases without cyclopoids.

Cyclopoid distribution within containers.

Me. thermocyclopoides and *Me. venezolanus* were almost always swimming in the water column of 55-gal drums and 5-gal bottles. They were somewhat more concentrated toward the bottom. Less than 5% of these 2 species rested on the bottom or sides of the container.

Few *Me. longisetus* or *Ma. albidus* were seen in the water column of drums. When observed in 5-gal bottles, 80-95% of the *Me. longisetus* and *Ma. albidus* were within 8 cm of the bottom, some swimming and others on the bottom or sides. Almost all the *Me. longisetus* and *Ma. 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), *Me. longisetus* continued to cling to the sides or remained close to the side if loosened by the current. All 4 species were sometimes seen feeding at the water surface. If *Me. 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 4 species of cyclopoids in this study were voracious predators of *Ae. aegypti* larvae. The largest species (*Me. longisetus*, adult female body length = 1.5 mm) killed the most larvae/cyclopoid/day, and the smallest species (*Me. thermocyclopoides* and *Me. venezolanus*, adult body length = 1.2 mm) killed the fewest larvae. Kay et al. (1992) observed that *Me. longisetus* killed more *Ae. aegypti*, *Anopheles farauti*, and *Culex quinquefasciatus* larvae than *Me. aspericornis* (a slightly smaller species) under laboratory conditions in 2-liter beakers.

However, the effectiveness of each cyclopoid species in this study depended most on its ability to survive and maintain large numbers in a container. *Me. longisetus* was clearly best in this regard, surviving well in all tested containers except laundry tanks (pilas), buckets, 10-gal plastic drums, and pots. *Me. longisetus* usually remained in a container for the duration of the field trials unless it was dumped out or dried out, though 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 *Ma. albidus*, *Me. thermocyclopoides*, and *Me. venezolanus*. First, the distribution of *Me. thermocyclopoides* and *Me. venezolanus* throughout the water column exposed them to substantial losses when water was dipped from a storage container. Fewer *Me. longisetus* or *Ma. 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 *Me. venezolanus*, and possibly *Me. thermocyclopoides*, which often disappeared after producing a large number of juveniles that may have eliminated their food supply. In contrast, *Me. longisetus* and *Ma. albidus* populations consisted almost entirely of adults. The latter 2 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 *Ma. albidus*. Water temperatures in containers exposed to the sun in El Progreso may exceed 40°C. The maximum temperature that *Ma. albidus* can survive is 37°-38°C, as compared to 42°-43°C for the 3 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 *Me. longisetus* and *Bti* are applied together.

Me. 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 *Me. longisetus* for *Ae. aegypti* control will require community participation to maintain container conditions essential for its survival. For example, *Me. 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 *Me. aspericornis* disappeared from water storage tanks with drains and from 55-gal drums that were cleaned. The net rescue procedure that we employed for cleaning drums was effective, but required demonstration of the technique and substantial motivation on the part of housewives to continue the effort. A selling point for *Me. longisetus* is that it can provide *Ae. aegypti* control no matter how infrequently a drum is cleaned.

Finally, despite precautions, *Me. 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 *Me. longisetus* from a nearby container that already has it.

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Appendix D

Compilation of Information Required by Integrated Dengue Control Project Staff, Community Volunteers, and the General Public To Use *Mesocyclops longisetus* for *Aedes aegypti* Control

Pautas tecnicas para el personal del Proyecto de Control Integrado del Dengue sobre el uso de *Mesocyclops longisetus* para el control de larvas de *Ae. aegypti*

Recipientes apropiados al uso de *Mesocyclops longisetus*

1. Drones de metal (55 galones). (No son apropriados los bariles más pequeños, en los cuales el agua se cambia frecuentemente.)

2. Llantas. Por ser animal acuático, *Mesocyclops longisetus* siempre necesita agua. Las llantas que colectan agua de lluvia y no se secan son apropiadas para *Mesocyclops longisetus*. La mayoría de llantas contienen agua durante toda la temporada de lluvia, pero muchas llantas se secan durante la estación seca.

3. Depositos construidos de cemento (sobre la tierra sin desagüe). Cualquier tamaño--pequeño o grande--puede ser propicio. (Los bebederos que se limpian de menudo no son apropriados.)

4. Floreros. Toda clase de construcción, puesto que el florero queda con agua.

Procedimientos para mantener *Mesocyclops longisetus* en recipientes.

1. Drones. Es necesario "rescatar" *Mesocyclops longisetus* de drones antes de limpiarlos. El procedimiento es (a) pasar una malla por todas partes del dron, en especial el fondo y junto a los lados y (b) guardar los *Mesocyclops longisetus* que se capturan en un pequeño recipiente de agua mientras el dron se limpia. (El proceso de pasar la malla por el dron deberá repitirse hasta no capturar más. Cada vez es necesario examinar lo que la malla capturó, para ver si hay

más *Mesocyclops longisetus*.) Los *Mesocyclops longisetus* pueden ser regresados al dron luego que se llena con agua después de llevar a cabo la limpieza.

El cloro mata *Mesocyclops longisetus*. Por lo tanto, si el dron se limpia con cloro, es necesario lavarlo bien con agua antes de regresarle los *Mesocyclops longisetus*.

Si todo el agua del dron tiene que ser utilizada, es necesario dejar un poco de agua (10 cm) al fondo, porque *Mesocyclops longisetus* necesita el agua para sobrevivir.

2. Liantas. Es preciso para la sobrevivencia de *Mesocyclops longisetus* que la llanta contenga agua todo el tiempo. El agua se conserva en la llanta mejor si la llanta se guarda en sombra.

Es deseable colocar cinco granos de arroz en cada llanta como fuente de alimento para *Mesocyclops longisetus* si la llanta está recien descartada y completamente limpia.

Una muestra de agua debe sacarse de cada llanta con un vaso (u otro recipiente) transparente cada dos semanas para averiguar que *Mesocyclops longisetus* todavía está en la llanta.

3. Depósitos. La mayoría de depositos sin desagüe casi nunca se limpian, pero si se limpian, es preciso rescatar los *Mesocyclops longisetus* con la malla, como descrito encima para drones.

4. Floreros. Si el florero se limpia o el agua se cambia, es necesario rescatar los *Mesocyclops longisetus* de la misma manera que se describió encima para drones. El agua del florero puede ser pasada por la malla para capturar los *Mesocyclops longisetus* si la malla no cabe dentro del florero.

Si el florero está muy limpia (sin raizes ni detritus), es deseable colocar 3 granos de arroz como fuente de alimento para *Mesocyclops longisetus*.

5. Presencia de larvas de mosquitos en recipientes con *Mesocyclops longisetus*. De vez en cuando habrán larvas de mosquitos en recipientes con *Mesocyclops longisetus*. Usualmente no significa la

falta de control de *Ae. aegypti* porque casi siempre las larvas son de Culex. La sobreviviencia de larvas de *Ae. aegypti* es rara cuando *Mesocyclops longisetus* está en el recipiente.

6. Inspección de recipientes para averiguar que siempre contienen *Mesocyclops longisetus*. *Mesocyclops longisetus* se pierde de recipientes de vez en cuando. Por lo tanto, es importante inspeccionar cada recipiente dos veces por mes. (Para poder hacerlo, es preciso que cada familia sepa ver y reconocer *Mesocyclops longisetus* a pesar de su tamaño pequeño.) Si *Mesocyclops longisetus* no se encuentra en recipientes que deben tenerlo, es necesario volver a introducirlo.

7. Reemplazamiento de *Mesocyclops longisetus* si se pierden de recipientes. Cada recipiente que contiene *Mesocyclops longisetus* para control de larvas también es una fabrica de producción de *Mesocyclops longisetus* y puede servir como fuente de estos animales para otros recipientes. Es fácil transportar *Mesocyclops longisetus* de un recipiente al otro. Los animales pueden sacarse del primer recipiente con malla, o se puede trasladar una cantidad de agua de un recipiente al otro después de verificar por ojo que hay *Mesocyclops longisetus* en el agua).

Información que el público necesita para utilizar *Mesocyclops longisetus* para el control de larvas de *Ae. aegypti*

1. Los camaroncitos (ciclops) comen las larvas de *Ae. aegypti*. Ningunas larvas de *Ae. aegypti* sobrevivirán con camaroncitos en el recipiente.

2. El cloro mata los camaroncitos.

3. Drones: Los camaroncitos no se perderán cuando el agua se saca del dron para uso, porque se concentran sobre el fondo del dron.

Usted puede larvar la pila cuando y de la manera que quiera, salvo que es necesario rescatar los camaroncitos con malla antes de botar el agua. De otra manera, los camarocitos se perderán. El procedimiento es pasar la malla por todas partes del dron, en especial al fondo, poner

los camaroncitos en un recipiente con agua y repitirlo hasta no capturar más camaroncitos con la malla. Guarde los camaroncitos y devuelvalos al dron después de ponerle más agua.

4. Floreros: Cuando se cambia el agua, hay que pasarla por la malla para capturar los camaroncitos y guardarlos en un recipiente con agua. Devuelva los camaroncitos al florero después de ponerle más agua.

5. Llantas y otros recipientes relevantes: Los camaroncitos morirán si se secan. Llantas que contienen camaroncitos deben guarderse en sombra donde la llanta no se secará. Es deseable agregarle más agua a las llantas si hay mucho tiempo sin lluvia y les parece que la llanta va a secarse.

6. Toda clase de recipiente: Si los camaroncitos no se ven al tratar de capturarlos con la malla, hay que avisar al voluntario para que se vuelven a intoducir al recipiente.

Información adicional que los voluntarios de la comunidad necesitan para utilizar *Mesocyclops longisetus* para el control de larvas de *Ae. aegypti*

1. Repientes apropiados para el uso de camaroncitos: drones de 55 galones, llantas que no se pueden eliminar ni almacenar, bebederos (piletas) sin animales, pilas ociosas, floreros, pozos y piscinas.

2. Al introducir los camaroncitos a un dron, hay que explicarle y demostrarle a la ama de casa cómo tiene que rescatar los camaroncitos cuando limpia el dron.

3. Cuando usted visite casas para inspección de solares, hay que verificar la presencia de los camaroncitos en todos los recipientes a los cuales se habían introducido. Si no están, hay que introducir más. Iqualmente, hay que introducir más si alguien le avise que sus camaroncitos habían desaparecido.

4. Hay que avisar a la promotora de CID cuando su suministro de camaroncitos se agote o si surgan problemas de mantenimiento en un recipiente al cual los camaroncitos se habían introducido.