Field Evaluation of *Mesocyclops longisetus* (Copepoda: Cyclopoidea) for the Control of Larval *Aedes aegypti* (Diptera Culicidae) in Northeastern Mexico

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ABSTRACT Field trials of the predacious copepod *Mesocyclops longisetus* Thiébaud were conducted in northeastern Mexico to determine the effectiveness of this species to control larval *Aedes aegypti* (L.) populations and to survive and reproduce in nature. Groups of 200, 50, and 50 ovigerous *M. longisetus* females were inoculated into 200-liter metal drums, discarded tires, and cemetery flower vases, respectively, which are 3 of the more important *Aedes* breeding sites in this area. Larvae were sampled at 15-d intervals, and total surviving cyclops were collected at the end of the study, 120 d later. Community participation was solicited through a simple training program on copepod rescue before drum cleaning and facilitated by the addition of a drum marker to remind residents of copepod presence. Results showed good cooperation and after 4 mo all peridomestic drums still supported variable numbers of cyclopoids. Average of larvae reduction was 37.5% for drums, 67.5% for flower vases, and 40.9% for tires. This study shows difficulties of using cyclopoids for tires and vases in areas where prolonged dry seasons dessicated these habitats and reduced copepod survival.

KEY WORDS *Aedes aegypti*, *Mesocyclops longisetus*, dengue, biological control, community participation

DENGUE FEVER IS a serious public health problem in Mexico and most Latin American countries where *Aedes aegypti* (L.) is recognized as the primary vector (Monath 1994). The association of vector and human densities is an important risk factor in dengue epidemiology (Rodriguez-Figueroa et al. 1995). Breeding of *Ae. aegypti* is facilitated in urban and rural areas by availability of artificial sources, such as discarded tires, tin cans, and plastic and glass bottles. The lack of piped-water forces people to store water in domestic containers such as cisterns, wash basins (pilas), and metal drums, which may become permanent vector breeding sites. Traditional *Aedes* control measures rely strongly on source reduction through community participation or chemical larvicides such as Temephos.

Predacious copepods are a promising alternate method to control larval *Ae. aegypti* populations under particular field situations. Laboratory evaluations of cyclopoid species have demonstrated high predation rates for *Ae. aegypti* larvae (Kay et al. 1992). Field releases have been successful, although limited to mosquito species thriving in land crab burrows in French Polynesia (Lardeux et al. 1992) and *Aedes albopictus* (Skuse) in tire piles in Louisiana (Marten 1990a, b). The success of copepods to control *Ae. aegypti* in most Latin American villages has been hampered by patterns of human behavior and specific characteristics of peridomestic artificial containers. To date, the most successful experience overcoming these problems was reported by Marten et al. (1994) in El Progreso, Honduras. These authors reported ≤30 wk survivorship of *Mesocyclops longisetus* Thiébaud released into 200-liter drums. Success was facilitated by participation of housewives. When drums were cleaned, these women used a net to collect copepods and return them to the cleaned drums. However, after 4–5 mo the housewives became tired of this process and ended their participation. Clearly, as noticed in Marten's study, when cyclopoids are released in artificial breeding sites around houses, community participation remains paramount to success.

As a part of the Integrated Dengue Control Project in Mexico, Monterrey was selected to evaluate the field effectiveness of copepods to reduce *Ae. aegypti* larval populations. Since its reappearence in Mexico after 1979, dengue has remained endemic in Monterrey, and in 1995, 167 dengue hemorragic cases were reported (Gomez-Dantes et al. 1996). The aim of the current study was to evaluate *M. longisetus* effectiveness in reducing *Ae. aegypti* larval densities in 3 breeding habitats (peridomestic 200-liter water drums, discarded tires, and cemetery vases), determine the persistence of reduction of *Ae. aegypti* larval and pupae stages by an established *M. longisetus* population, and to assess cyclopoid survival and population growth rates in these habitats when helped by community participation.

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Materials and Methods

Study Area. Located in the northeast of Mexico, Monterrey lies within the municipality of San Nicolas de los Garza in the Nuevo Leon Province (98° 17' E, and 23° N). Annual rainfall averages 600 mm, with a mean temperature of 28°C. This city of 4-million residents is characterized by semidry climate and contrasts with the tropical El Progreso, Honduras, site studied by Marten et al. (1994). Vegetation in this part of the state comprises bushes, cacti, and small to median height trees (Secretaria de Programacion y Presupuesto 1981). A group of dwellings in the "barrio" or "colonia" "El Mirador" was selected as the study site. Piped-water is not available and the residents store water for domestic use in 200-liter metal drums. The State Vector Control Program found this container to be a stable oviposition site for *Ae. aegypti*. Houses are built of cement with wooden walls, and most backyards have secondary larval sites such as discarded tires and plastic and glass bottles.

Treatment of 200 Liter Drums with Copepods. To maintain microorganisms and algae naturally produced in drums and to avoid hampering the daily water needs of the residents, a new drum was offered to each dweller who agreed to participate in the trial. Depending on the experimental design, some residents were permitted to use the water from the new drum, whereas others were asked not to use it. Treated and untreated drums were initially free of *Ae. aegypti* larvae and randomly selected. In total, 64 peridomestic drums owned by ≈40 families were used in field trials, with copepod treatment arranged in the following scheme: (1) 36 drums were treated with 200 ovigerous females of *M. longisetus*. People were allowed to use water routinely, but housewives in this group were instructed on several simple measures to preserve cyclopoids at the time of drum washing (i.e., to leave a small amount of water in the drums or to transfer water with copepods to a bucket during cleaning and then return the copepods to the drums after cleaning). To remind housewives about copepods, a fluorescent-painted and cement-filled beer can was placed in the drum. (2) Eighteen drums each were treated with 200 ovigerous females of *M. longisetus*, but housewives were requested not to use the water. Ability of cyclopoids to survive and their population growth rates under local conditions provided by the metal drums were evaluated, as well as their ability to control *Ae. aegypti*. (3) Ten drums were no treated with copepods and were exposed to cleaning and water management; they represented the larval *Aedes* population naturally found in El Mirador. (4) Ten drums were untreated and not used for water by residents and thereby served as a control for drums with cyclopoids, but with an undisturbed aquatic environment.

Discarded Tires and Cemetery Flower Vases. A group of 28 used tires were placed vertically in the shade at 24 houses. Water was added 1 wk before treatment to produce microorganisms and algae. Afterward, 50 ovigerous females of *M. longisetus* were introduced into each of 18 tires; 10 tires were untreated as controls. To avoid predation of cylops by 3rd and 4th *Aedes* instars, only larvae-negative tires and vases were used (Marten 1990). A city cemetery located close to El Mirador provided flower vases to evaluate predatory effectiveness and survival of cyclopoids. A week before treatment, flower vases were filled with water, and nearly 10% of the volume of each vase was filled with dry and fresh grass for protozoan and bacteria production. Most vases were made of marble or cement having a capacity of 4–8 liters. Forty-five flower vases in the shadow of graves were each inoculated with 50 ovigerous *M. longisetus* females, whereas 25 remained untreated. Only vases without *Ae. aegypti* larvae were used in this trial, whereas selection of tires and vases would was made by random numbers.

Larval and Copepod Sampling. Fortnightly during May–August 1994, mosquito larvae were sampled and recorded in treated and untreated containers. Plankton nets (0.3 and 0.1 m in diameter; mesh, 290 μm) were used to collect *Aedes* larvae; copepods were returned to the containers. Nets were dipped with circular movements from bottom to top. Approximately 30 attempts were made for drums, and 15 for tires and flower vases. At the end of the study, all containers were sampled thoroughly for cyclopoids. All copepods were preserved in 70% ethanol for later counting.

Data Analysis. Effectiveness of larval control by *M. longisetus* was defined as the percentage of reduction of untreated minus treated containers positive for at least 1 individual *Ae. aegypti* larva. Percent reduction formula was computed as [(% untreated container with larvae) − (% treated container with larvae)] ÷ (% untreated containers with larvae) × 100. Copepod control of each larval instar was compared by *t*-tests on arcsine-transformed data from treated against untreated containers (Zar 1984). Cyclopoid survival and population growth rates were assessed by the number of weeks they were still present in containers and by mean numbers of copepods per type of larval habitat at the end of the study.

Results

Field Effectiveness of Larval Control. Overall, the community was receptive to copepods introduction into their peridomestic water drums during our 120-d study period. Cyclopoid care procedures were followed during >90% of the study, and the drum bottom indicator remained to remind occupants not to wash away the copepods. This was shown by the presence of at least 1 individual *M. longisetus* in all treated drums at the end of the study. Nevertheless, a few participants tired of the copepod rescue procedure during the last weeks of the study.

Drums with Regular Water Use. Overall, the percentage of treated drums positive for *Ae. aegypti* was reduced 37.5% (Table 1). The percentage of positive drums with copepods was less than those without cyclopoids ($t = 3.37$, df = 7, $P < 0.05$). The greatest percentage of reduction (65.3%) was recorded 105-d postrelease, whereas the least (7.7%) was found on day 75. Control drums with water in use showed con-

Table 1. Percentage of reduction of drums positive for *Ae. aegypti* larvae that were treated and untreated with *M. longisetus*

Days	Drums positive for *Ae. aegypti* larvae (%)		
	Treated (no.)[a] $n = 36$	Untreated (no.)[a] $n = 10$	% reduction[b]
15	41.6 (15)	70.0 (7)	40.5
30	19.4 (7)	50.0 (5)	61.2
45	22.2 (8)	20.0 (2)	11.0
60	33.3 (12)	40.0 (4)	16.7
75	27.7 (10)	30.0 (3)	7.7
90	30.5 (11.5)	70.0 (7)	56.4
105	27.7 (10)	80.0 (8)	65.3
120	52.7 (19)	90.0 (9)	41.4
µ (±)[c]	31.8 (10.8)	56.6 (25.1)	37.5 (23.1)

Drum water was routinely used by residents who were instructed how to take care of copepods.

[a] Percentage of reduction and number of treated and untreated drums positive for *Ae. aegypti* larvae.

[b] Reduction = [(% untreated drums with larvae) − (% treated drums with larvae)] × 100/(% untreated drums with larvae).

[c] Arithmetic mean.

sistent larval populations, with a mean percentage of 56.6% (range, 20–90%) positive over the 4-mo study (Table 1).

Drums without Water Use. Overall, an average of 39.9% reduction in the number of these drums positive for *Ae. aegypti* larvae was obtained over the 120 d in the field (Table 2). Nevertheless, the percentage significant positive was reduced by compared with untreated drums ($t = 4.75$, df = 7, $P < 0.05$). The greatest percentage of reduction was 62.9% recorded 30-d after treatment, whereas the least was 4.7% of reduction 15 d after release. As expected, untreated and undisturbed water resulted in mosquito-positive drums averaging 78.7% mean over the 120-d period (Table 2).

Discarded Tires and Cemetery Flower Vases. Scarce rainfall and high evaporation rates forced us to refill these larval habitats every 10 d. Over 105 d of *M. longisetus* release, a mean of 40.9% larval reduction was obtained in discarded tires scattered in backyards (Table 3). The greatest larvae percentage of reduction (60.8%) was found on day 105 after treatment, whereas the least (7.0%) was recorded after 1 mo. Untreated tires were shown to be an excellent *Ae. aegypti* breeding habitat, and an average of 75.7% were positive over the 105-d study period. Larval positive tires were consistently recorded as high as 100% on day 105, and as low as 50% during day 30 after release (Table 3). However, fewer treated tires were positive for *Ae. aegypti* larvae than were those without copepods ($t = 4.46$, df = 6, $P < 0.05$).

Table 2. Percentage of reduction of drums positive for *Ae. aegypti* larvae that were treated and untreated with *M. longisetus*

Days	Drums positive for *Ae. aegypti* larvae (%)		
	Treated (no.)[a] $n = 18$	Untreated (no.)[c] $n = 10$	% reduction[b]
15	66.6 (12)	70.0 (7)	4.7
30	33.3 (6)	90.0 (9)	62.9
45	33.3 (6)	80.0 (8)	58.3
60	50.0 (9)	80.0 (8)	37.5
75	44.4 (8)	70.0 (7)	36.5
90	33.3 (6)	80.0 (8)	58.3
105	50.0 (9)	80.0 (8)	37.5
120	61.1 (11)	80.0 (8)	23.6
µ (±)[c]	46.5 (12.9)	78.7 (6.4)	39.9 (19.7)

House residents were not allowed to use drum water.

[a] Percentage of reduction and numbers of treated and untreated drums positive for *Ae. aegypti* larvae.

[b] % Reduction = [(% untreated drums with larvae) − (% treated drums with larvae)] × 100/(% untreated drums with larvae).

[c] Arithmetic mean.

Table 3. Percentage of reduction of discarded tires and cemetery flower vases positive for *Ae. aegypti* larvae that were treated and untreated with *M. longisetus*

Sites	Days	Positive for *Ae. aegypti* larvae (%)		
		Treated (no.)[a]	Untreated (no.)[a]	% reduction[b]
Tires				
		$n = 28$	$n = 10$	
	15	42.8 (12)	70.0 (7)	38.8
	30	53.5 (15)	50.0 (5)	7.0
	45	39.2 (11)	60.0 (6)	34.6
	60	46.4 (13)	80.0 (8)	42.0
	75	42.8 (12)	90.0 (9)	52.4
	90	39.2 (6)	80.0 (8)	51.0
	105	39.2 (11)	100.0 (100)	60.8
	µ (±)[c]	43.3 (5.2)	75.7 (17.9)	40.9 (17.4)
Flower vases				
		$n = 45$	$n = 25$	
	15	26.6 (12)	24.0 (6)	10.8
	30	15.5 (7)	32.0 (8)	51.5
	45	11.1 (7)	40.0 (10)	72.2
	69	8.8 (4)	24.0 (6)	63.3
	75	2.2 (1)	56.0 (14)	96.0
	90	4.4 (2)	60.0 (15)	92.6
	105	11.1 (5)	84.0 (21)	86.7
	µ (±)[c]	11.3 (8.0)	45.7 (22.1)	67.5 (29.8)

[a] Percentage of reduction and number of treated and untreated tires and vases positive for *Ae. aegypti* larvae.

[b] % Reduction = [(% untreated tires and vases with larvae) − (% treated tires and vases with larvae)] × 100/(% untreated drums with larvae).

[c] Arithmetic mean.

The best results of *M. longisetus* controlling *Ae. aegypti* larval populations were recorded in cemetery flower vases. Overall, there was a 67.5% reduction in the numbers of vases positive for *Ae. aegypti* over the 105-d study period (Table 3). Reduction increased from day 15 after inoculation (10.8%) to days 75 (96.0%) and 90 (92.6%) (Table 3). The number of treated vases without larvae was statistically lower than that of untreated vases ($t = 3.46$, df = 6, $P < 0.05$). This finding was remarkable when compared with the trend of increasing larvae populations in untreated vases from day 15 (24.0%) to day 105 (84.0%) (Table 3).

Instar-Specific Predatory Rates. Overall, the mean number of total larvae per instar was statistically lower in all types of treated containers than in untreated controls (Table 4). Cyclopoids significantly reduced total mean larval densities in drums with and without water management ($t > 3.34$, df = 7, $P < 0.05$). Similarly, mean density rates were significantly lower for treated tires and cemetery vases compared with those without *M. longisetus* ($t > 6.85$, df = 6, $P < 0.05$). Pupal densities were lower for all but drums used by resi-

Table 4. Means[a] and SDs of *Ae. aegypti* immatures in copepod-treated (T) and untreated (U) breeding habitats

Larval instar	Copepod	Drums with water in use	Drums with water unused	Tires	Flower vases
1st	T	8.2 (29.1)	4.9 (27.8)*	0.2 (1.4)*	2.3 (8.2)
	U	21.7 (48.6)	46.6 (43.1)	51.2 (33.0)	9.7 (30.0)
2nd	T	11.2 (45.4)*	13.8 (27.1)*	3.3 (4.1)*	1.1 (3.1)*
	U	36.5 (46.3)	92.7 (97.9)	63.6 (32.3)	14.2 (19.6)
3rd	T	14.7 (50.8)	10.8 (19.6)*	12.8 (7.2)*	4.1 (14.4)*
	U	30.8 (10.0)	79.7 (83.2)	56.7 (24.8)	16.9 (18.1)
4th	T	4.0 (5.1)*	6.1 (10.1)*	6.6 (4.5)*	3.6 (8.9)*
	U	15.0 (12.7)	45.3 (38.2)	37.9 (23.3)	11.9 (8.9)
Pupae	T	2.8 (1.7)	3.1 (3.6)*	3.2 (3.6)*	3.2 (8.9)*
	U	6.6 (6.7)	21.0 (34.7)	13.9 (13.4)	11.0 (24.5)
Total	T	44.2 (75.9)*	40.2 (35.3)*	26.8 (12.7)*	14.0 (33.1)*
	U	110.2 (42.5)	260.1 (239.6)	201.4 (62.4)	62.4 (83.5)

*, t-Test, $P < 0.05$.

[a] Arithmetic mean of *Ae. aegypti* larvae in breeding habitats at the end of the study.

dents ($t = 1.67$, df $= 7$, $P > 0.05$). Although predation by cyclopoids on older instars is infrequent, a significant reduction of the 4th instars was obtained for each of 3 breeding habitats. Similar significant results were obtained for mean densities of 3rd, 2nd, and 1st instars, except for 3rd and 1st instars in barrels with water use and 1st instars in flower vases.

Survival and Population Growth Rates. *M. longisetus* survived over the 105 and 120-d of the study periods in each of 3 different container types. Success of resident participation was also demonstrated by the presence of cyclopoids in drums that were used for domestic water and routinely cleaned. Clearly, such success was linked to our education program on how to take care of the copepods. Likewise, the ability of *M. longisetus* to survive in the physical and chemical conditions of the metal drum environment was documented for those drums without water management. Survival rates were similarly excellent in tires and flower vases in the city cemetery, although low rainfall forced us to refill the containers every 2 wk.

Population growth rates of copepods were variable (Table 5). For instance, we released 200 ovigerous females in both groups of drums at the beginning. After 120 d, a mean of 53.2 ± 53.4 copepods per drum of assorted ages was found, with a range of 2–244 individuals (Table 5). Releasing the same starting number in inactive drums, a greater mean of 179.3 ± 240.2 copepods per drum was obtained, with a range of 10–752 individuals. An initial amount of 50 females were inoculated into each discarded tire, and a mean of 120 ± 80.4 was obtained with a range of 1–394. The best results for *M. longisetus* growth rates were produced after releasing 50 female copepods into flower vases: a mean of 450.9 ± 622.8 copepods with a range of 6–4,097 (Table 5).

Discussion

If the community is properly motivated and instructed, copepods may survive and reproduce in domestic and peridomestic water storage containers. In our study, house residents were able to take care of cyclopoids released into their 200-liter drums for our 120-d study period. We provided simple directions regarding drum cleaning and placed an empty beer can in the drum as a reminder not to discard the copepod population. Conversely, failures with copepods inoculated into peridomestic drums occurred when community residents were not instructed and motivated (Jenning at al. 1995). These authors found that only 7.5% of the drums remained positive for the cyclopoid *Mesocyclops guangxiensis* Riviere & Thirel 1 mo after inoculation in Lao People's Democratic Republic. They argued that container cleaning in accordance with larval *Aedes* control policies prohibited copepod persistence in this country. Similarly, drum cleaning was the cause for *Mesocyclops aspericornis* Daday loss on Anguilla island (Suarez 1992). In contrast, Marten et al. (1994) reported up to 27 wk of survival of *M. longisetus* in peridomestic drums in El Progreso, Honduras, because copepods were rescued before cleaning drums and because drums were not the main water storage vessel and were washed infrequently.

Reduction of the number of containers positive for *Ae. aegypti* was variable for all container types. Over-

Table 5. Mean numbers of *M. longisetus* in different *Ae. aegypti* breeding habitats at the end of the study

Breeding habitat	n	Starting copepod no.[a]	Ending copepod no.				
			μ	±SD	Min.	Max	d
Drums, water in use	36	200	53.2	± 52.4	2	244	120
Drums, water unused	18	200	179.3	± 240.2	10	752	120
Tires	18	50	120.4	± 80.4	1	394	105
Flower vases	45	50	450.9	± 622.8	6	4,097	105

[a] Ovigerous *M. longisetus* females.

all, a modest reduction of ≈40% was achieved for active and inactive drums and discarded tires. The best reduction, of 67.5% was in flower vases. Other field trials releasing several copepod species showed variable results, ranging from cyclopoids being eliminated by drum cleaning (Rodriguez 1992, Suarez 1992, Jenning et al. 1995) to >90% larval *Aedes* reduction in drums through house-dweller participation in (Marten 1994).

The latter results were produced under different ecological settings and were linked to a richer nutrient environments than is discussed below . Nevertheless, in our study, mean density of all *Ae. aegypti* larvae was significantly lower in treated versus untreated containers; reduction ratios were 2.5-, 6.5-, 7.5-, and 4.5-fold for active and inactive drums, tires, and flower vases, respectively. Large 3rd and 4th instars which, according to Marten (1994) may represent actual predation effectiveness by copepods, were significantly lower in most treated habitats. These data indicate that larval reduction rates may be increased if more cyclopoids were released at the beginning of the intervention.

Despite successful community participation in copepod programs, successful reproduction of initial copepod numbers inoculated into larval habitats remains a key point to control larval *Aedes* densities. As documented in this study, *M. longisetus* was not able to thrive in high numbers in most of the containers, even in drums where the water was left undisturbed. Although 200 ovigerous females were released in peridomestic active and inactive drums, only 53.2 ± 52.4 and 179.3 ± 240.4 individuals were found 4 mo later, respectively. We felt that lack of protozoa and algae in this environment as a supplementary diet for *M. longisetus* affected population growth rates. Although organic matter was not analyzed, the scarceness of trees and general vegetation in this dry, semidesert region in northeastern Mexico was associated with poor nutrient content. Trials for food requirements have demonstrated the importance of this factor in the Lao Democratic Republic (Jenning et al. 1995). Clearly, an additional limiting variable to copepod releases as an *Ae. aegypti* control strategy in areas such as Monterrey city is the prolonged dry season. As seen in this study, tires and flower vases had to be refilled almost every 2 wk over the 4-mo period. Even though mechanisms of copepod resistance to dessication have been reported (Zhen et al. 1994), particularly if wet mud remains at the larval site bottom, we really do not know if such a surviving strategy would suffice with the high daily evaporation rates present in this part of Mexico. Therefore, copepod effectiveness on tires and vases has to be interpreted with this scenario in mind.

Finally, we conclude that sustainable community-based vector control programs may result in some degree of success, when copepod populations are released in the field to reduce larval populations of *Ae. aegypti* in northeastern Mexico. Additional ecological factors have to be met to achieve control if this strategy is decided for including,: a rich nutrient environment provided by breeding sites, a high copepod inoculation rate, and a regional rainfall pattern preventing dessication of temporary larval sites.

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